Article

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Repair of Rabbit Knee Cartilage by Bipolar Radiofrequency with Different Energy Settings and Recovery Periods

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Background: Arthroscopic bipolar radiofrequency energy (bRFE) is a common method for minimally invasive treatment of cartilage injuries. The benefits of bRFE are still controversial, and its safety has become the focus of attention.
Objective: This study aimed to reveal the effects of energy setting and recovery period on the efficacy and safety of bRFE.
Methods: The New Zealand white rabbit knee cartilage injury model was established, and bRFE was used to treat the cartilage with different energy settings, including 20 W and 40 W, and recovery periods of 0 and 1 month. By observing the immediate and late results on damaged cartilage, along with chondrocyte apoptosis, the effects of energy setting and recovery period on the efficacy and safety of bRFE were accessed.
Results: The pathological conditions, surface profile and chondrocyte viability in the bRFE treatment group produced greater late effects and were significantly better than those in the model group. Nevertheless, bRFE produced a timely injury that resulted in an increased rate of apoptosis ($p < 0.05$), which was alleviated in subsequent recovery ($p < 0.05$).
Conclusions: bRFE can effectively trim and improve the cartilage lesion area, and reduce cracks. Although bRFE produced timely chondrocyte damage, this was alleviated on subsequent recovery. Therefore, bRFE with appropriate energy is beneficial to the recovery of cartilage damage, proper attention should be paid to the recovery period.

Keywords: bRFE; cartilage injury; osteoarthritis; SEM; apoptosis

Introduction

Osteoarthritis is caused by degeneration of articular cartilage and subsequent secondary bone hyperplasia [1]. According to reports, osteoarthritis affects more than 500 million people across the globe [2]. Partial-thickness cartilage defects have been found to be the cause of articular cartilage degeneration, and there is still no effective treatment [3]. Due to the low tissue metabolism of articular cartilage, once it is damaged, auto-repairing results unlikely [4]. Therefore, the treatment of articular cartilage injury constitutes a challenge in the clinic. Articular cartilage lacks vascular tissue and has limited ability to regenerate after injury, often causing joint pain, stiffness, and dysfunction [5]. If it's not treated on time and effectively, it may develop into loss of joint function or physical disability [6]. From a medical point of view, the main goals of cartilage trauma treatment are to restore joint motion, relieve pain and eliminate or delay the onset of osteoarthritis [7]. Treatment can be conservative, such as drugs and arthrocentesis, surgical or subsequent promotion of repair and regeneration [8–10].

Arthroscopic bipolar radiofrequency energy (bRFE) is a common method for minimally invasive treatment of cartilage injuries in recent years [11]. Radiofrequency energy can provide a smooth and stable cartilage surface, using heat to contract or clear fibrotic cartilage, stopping further degeneration of the articular cartilage. bRFE can vibrate the electrolytes in the intracellular and extracellular fluid through energy, causing molecular friction and local tissue dissolution [11]. Such operation promotes a smoother cartilage surface and noticeable edge stabilization, resulting in better outcomes [12]. In addition, bRFE is simple to operate, relatively low cost, contains diversified radio frequency probes, provides precise conduction and positioning, and is more and more widely used in the treatment of articular cartilage injuries. However, the benefits of bRFE are still controversial. Its safety and long-term postoperative efficacy have become the focus of study [11].

A research conducted secondary arthroscopic observation on 15 cases of cartilage injuries treated with bRFE in 25 different locations [13]. They found that only three damaged cartilage degeneration remained after treatment; and more than 50% of the patients had partial or complete repair of the cartilage. They demonstrated that bRFE constitutes an effective method for treating partial cartilage damage. Another study compared the therapeutic effects of ra-

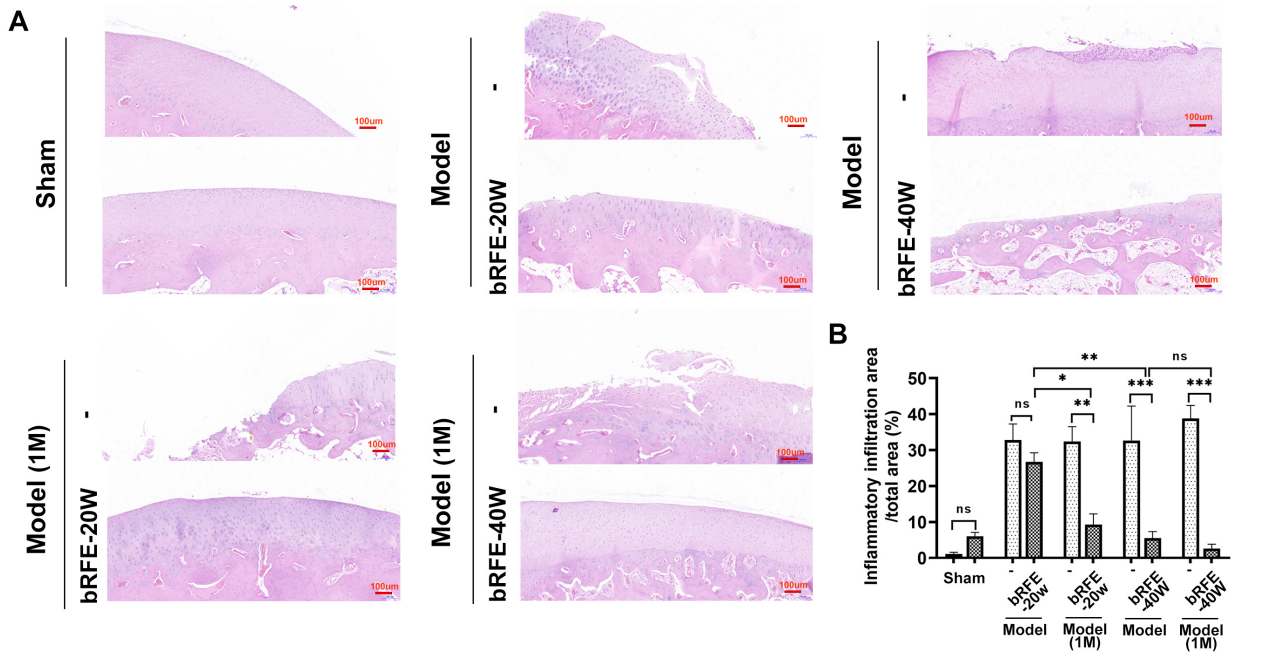

Fig. 1. Pathological conditions of articular cartilage in different groups. Rabbit articular cartilage was injured and treated with 20 W and 40 W arthroscopic bipolar radiofrequency energy (bRFE) ablation. The pathological analysis was performed immediately and one month later. (A) The cartilage pathology of different groups was studied by hematoxylin and eosin (H&E) staining, n = 3, scale bar = 100 µm. (B) According to the H&E staining results, the inflammatory infiltration and total area were quantified, and the proportion of the two values was calculated, n = 3. ns, no significance; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

diofrequency energy and mechanical debridement by creating a partial-thickness cartilage injury model on the patella or kneecap of 16 ponies; however, the mechanical properties of cartilage showed no significant differences [14].

Therefore, in this work, the New Zealand white rabbit knee cartilage injury model was established, and bRFE was used to treat the damaged cartilage under different energy settings. The effects of energy setting and recovery period on the efficacy and safety of bRFE were obtained by observing its immediate and late effects on damaged cartilage.

Methods and Materials

Animal Modeling

Male New Zealand white rabbits, aged 7 months and with n = 30, were adaptively fed for 2 weeks with natural light cycles. The cartilage injury model was constructed by the transection of the anterior cruciate ligament and medial meniscectomy performance. The joint was opened with a medial parapatellar incision, and the patella was dislocated to expose the articular surface of the femoral condyle. The anterior cruciate ligament was resected and the tibia was externally rotated to expose the medial meniscus and resected. After suturing, intramuscular injection of penicillin sodium with a dose of 40 $\times$ 10^4 U (CSPC, Shijiazhuang, China), was applied once a day for three consecutive days, to prevent infection. Cartilage damage was performed bilaterally.

bRFE and Grouping

According to the Arthrocare® system 2000 machine gear (Arthrocare Corporation, Austin, TX, USA) and the actual ablation energy pre-test, 20 W and 40 W were selected for the treatment, respectively. The Saphyre bipolar ablation probe (Smith-nephew, London, UK) was applied to treat the cartilage surface. Rabbits were randomly divided into five groups: sham, 20 W, 20 W (1 month), 40 W, 40 W (1 month). In the sham group, only the joint surface was incised without any treatment and then sutured. The other four groups underwent cartilage injury modeling. Two weeks later, bRFE was performed in order to repair wounded cartilage on the left knee, but not on the right side. The 20 W and 40 W groups were euthanized by applying an intravenous injection of pentobarbital sodium (100 mg/kg), immediately after the bRFE treatment. Then, the medial condylar articular cartilage was collected. The 20 W (1 month) and 40 W (1 month) groups continued to be fed for one month, and then the cartilage was collected following the same procedure described above.

H&E Staining

Articular cartilage was fixed overnight in 4% paraformaldehyde (BL539A, Biosharp, Hefei, China) and soaked in disodium EDTA solution (E118594, Aladdin, Shanghai, China) for decalcification. Thereafter, it was dehydrated with gradient alcohol, embedded in paraffin,

and cut into 4 µm thick sections. These sections were routinely deparaffinized and stained with hematoxylin and eosin (H&E) solutions (C0105S, Beyotime, Shanghai, China), during 5 min each, dehydrated with gradient alcohol and uncolored using xylene (10023418, Sinopharm, Shanghai, China). Specimens were observed under a microscope (CKX53, Olympus, Tokyo, Japan). The total and infiltrated area of inflammatory cells were analyzed taking into account the number of pixels using ImageJ software (version 1.8.0; National Institutes of Health, Bethesda, MD, USA). The ratio of the two renders the level of inflammation.

Scanning Electron Microscope (SEM)

Articular cartilage was immersed in 2.5% glutaraldehyde (P1126, Solarbio, Beijing, China) and postfixed with 1% OsO4 (115355, Aike, Chengdu, China) for 2 h. Samples were dehydrated with graded ethanol and then transferred to amyl acetate solution. Then, they were placed in a critical point dryer, soaked in liquid carbon dioxide, and heated to the critical point temperature to vaporize it for drying. Next, they were placed in a vacuum coating machine, and after gold was spayed into their surface, the morphology was observed using a SEM (SU8100, Hitachi, Tokyo, Japan).

Flow Cytometry

Cartilage fragments were digested with collagenase (40508ES60, Yeasen, Shanghai, China), and the mixture was filtered through a 70 µm cell sieve. Samples were centrifugated at 300 g for 5 min at 4 °C, the pellet was washed with pre-cooled medium, resuspended, and centrifuged repeatedly. Cells were suspended in binding buffer and incubated with Annexin V-fluorescein isothiocyanate and propidium iodide (CA1020, Solarbio, Beijing, China) for 15 min in the dark. Cell apoptosis was measured using flow cytometry (BD FACSVia, Bergen, NJ, USA).

Western Blotting

Proteins were harvested from the articular cartilage using lysis buffer (abs9229, absin, Shanghai, China) and quantified by Nano 300 spectrophotometer. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gels and hybridized polyvinylidene fluoride membranes (Roche, Shanghai, China) obtained through wet transfer. The membranes were blocked in 5% skimmed milk and incubated with primary antibodies against Bcl-2 Associated X protein (Bax, 50599-2-Ig), B-cell lymphoma-2 (Bcl2, 12789-1-AP), cleaved caspase 3 (19677-1-AP), β-actin (66009-1-Ig) and horseradish peroxidase-conjugated secondary antibody (SA00001-2 & SA00001-1, All from Proteintech, Wuhan, China). Blots were visualized with enhanced chemiluminescence (D601039, Sangon Biotech, Shanghai, China) and gray values were analyzed with ImageJ software.

Statistical Analysis

All data was presented as means ± SD. Comparisons were analyzed with two-way analysis of variance followed by Tukey's test in the SPSS software (Version 18.0, IBM SPSS statistics, Chicago, IL, USA). $p < 0.05$ is considered as statistically significant.

Results

Pathological Conditions of Articular Cartilage in Different Groups

The hematoxylin and eosin stain revealed that the cartilage surface of rabbits in the sham group was complete, smooth and without defects. The chondrocytes were neatly arranged and dense. The cartilage surface of rabbits in the model group without bRFE treatment had defects and cracks in different degrees, and some cartilage surfaces showed slight fibrosis. After 20 W bRFE treatment, most of the defects and cracks on the cartilage surface were ablated, and a small number of defects still remained. Following 40 W bRFE treatment, the cartilage surface turned out to be smooth. Subsequent to feeding for 1 month, the cartilage surface damage in the model group became more serious, showing partial cartilage surface loss, bone exposure, obvious fibrosis and large cracks on some cartilage surfaces. Regardless of the 20 W or 40 W in the bRFE treatment group, the cartilage surface was smooth after 1 month (Fig. 1A). The inflammation level was measured by the ratio of the infiltrated area of inflammatory cells to the total area. Compared with the control side, 40 W reduced intratissue inflammation ($p < 0.05$). After 1 month, both 20 W and 40 W treatments significantly diminished the inflammatory response ($p < 0.05$) (Fig. 1B).

The results of SEM revealed that the cartilage surface in the sham group was complete and smooth, and the tissue structure was uniform. In the model group, the cartilage surface was worn and the normal structure was destroyed. After 20 W and 40 W bRFE treatment, the defects on the cartilage surface were reduced, but there was still a slight uneven ablation. After 1 month of continuous feeding, the damage in the model group was more serious, with cracks and loose separation of the cartilage surface. Following 20 W bRFE treatment, the cartilage surface was slightly rough but without cracks; while in the 40 W bRFE treatment was basically smooth (Fig. 2).

Apoptosis Rate in Articular Cartilage Tissue

In the 20 W and 40 W group that underwent bRFE treatment immediately after modeling, the apoptosis rate of rabbit articular cartilage was significantly higher than that in the non-bRFE model group ($p < 0.05$), indicating that the bRFE treatment had immediate damage to the cells. After 1 month of continuous feeding, the apoptosis rates of articular chondrocytes after 20 W and 40 W bRFE therapy were significantly lower than those in the non-bRFE

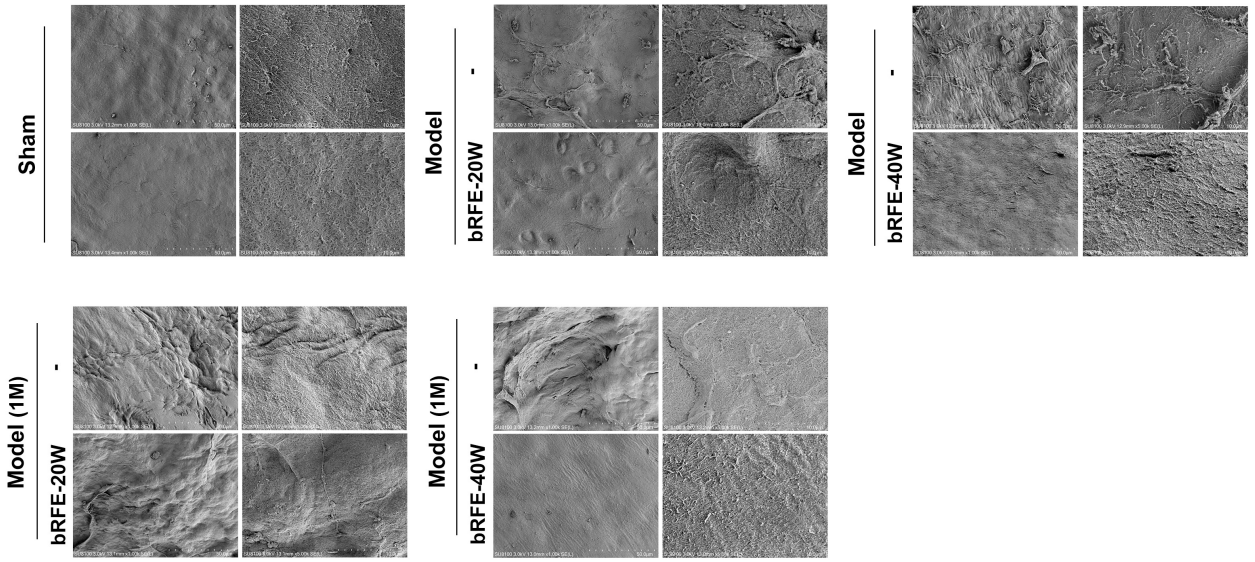

Fig. 2. Scanning Electron Microscope (SEM) results of articular cartilage in different groups. The cartilage surface profile is shown, n = 3, scale bar = 50 and 10 µm.

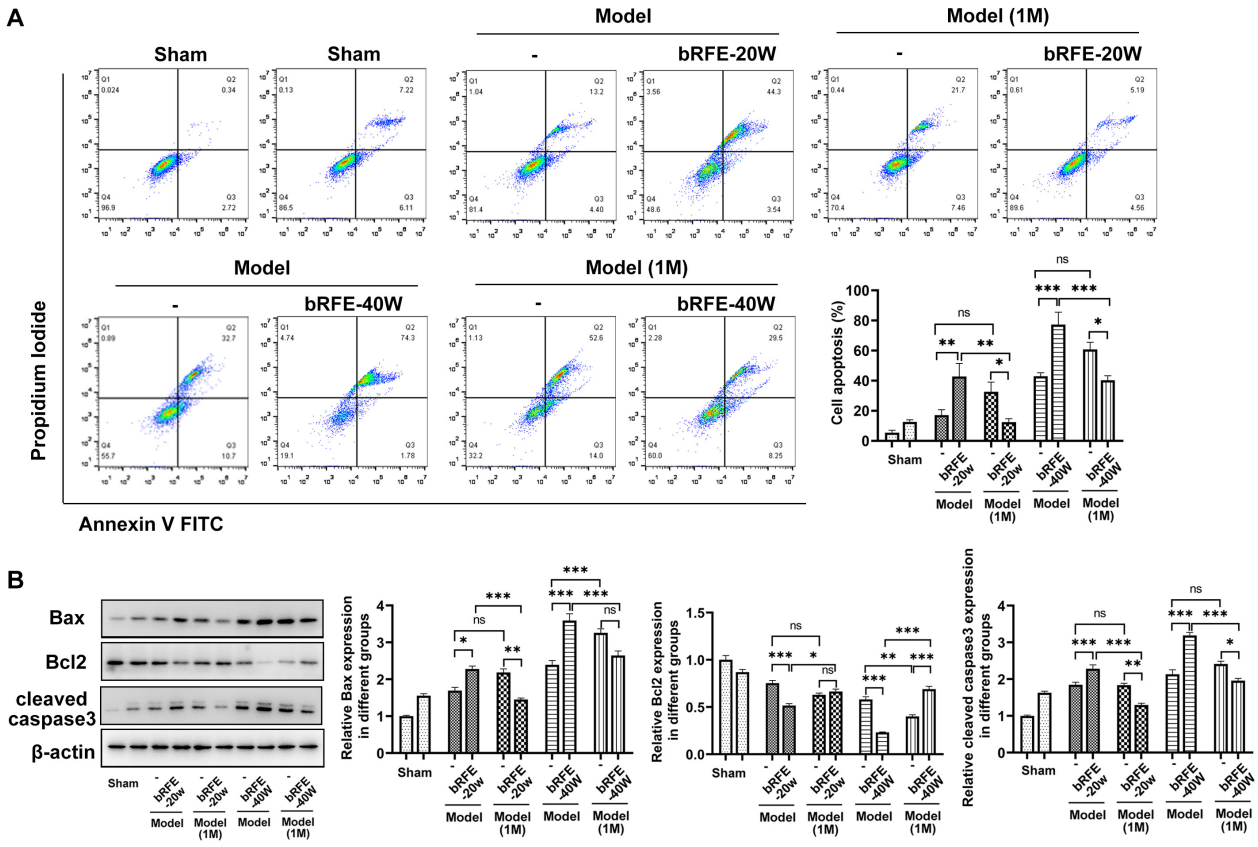

Fig. 3. Apoptosis rate in articular cartilage tissue from different groups. (A) The apoptosis rate of chondrocytes was determined using flow cytometry, n = 3. (B) The levels of apoptosis-related proteins were assessed using western blotting, n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Bax, Bcl-2 Associated X protein; Bcl2, B-cell lymphoma-2; FITC, fluorescein isothiocyanate.

group ($p < 0.05$), indicating that bRFE treatment can improve the damage of articular cartilage (Fig. 3A). Additionally, western blotting was applied to determine the levels of apoptosis-related proteins in articular cartilage tissue. Bax and cleaved caspase 3 levels significantly increased in tissues analyzed immediately after bRFE treatment, whereas

Bcl2 level decreased ($p < 0.05$). On the contrary, cleaved caspase 3 and Bax protein levels decreased significantly and Bcl2 protein levels increased in the tissues obtained after 1 month of bRFE treatment ($p < 0.05$). In the non-bRFE groups, after 1 month, levels of cleaved caspase 3 and Bax increased, while the ones of Bcl2 decreased (Fig. 3B).

Discussion

The treatment of articular cartilage defects remains one of the greatest challenges facing modern orthopedics. Notably, monopolar and bipolar radiofrequency devices have been accepted widely in clinical practice [15,16]. The results of radiofrequency are affected by several variables, including treatment regimen, power setting, exposure time, temperature and speed of fluid flow [17]. Our study reproduced the pathological damage through rabbit knee articular cartilage injury models in order to evaluate the safety and efficacy of bRFE with different levels and repair periods. Under a simulated arthroscopic environment and constant power transmission, it is convenient to assess the influence of bRFE settings on pathological conditions. According to the results of H&E staining, the surface of the cartilage in the model group showed fibrous changes, with cartilage debris and partial longitudinal cracks, which proved the successful establishment of the model. There were more alveolar cartilage lacunae and less inflammatory infiltrates in the cartilage after 40 W treatment, compared to 20 W treatment, which indicated that it promoted repair more favorably.

Cartilage smoothness may be overestimated under arthroscopic exploration because of lower magnification than SEM, resulting in insufficient fissure sealing at the end of the procedure [18]. In this study, cartilage smoothness can be clearly observed according to SEM. The results revealed that the pathological conditions, surface contour and chondrocyte viability in the bRFE treatment group were significantly better than those in the model group, and produced greater late effects. In contrast to unipolar RFE which operates primarily on heat generation, bRFE is primarily based on ablation. In addition to the function of vaporization and melting, it also has many functions such as forming, cleaning, tightening and hemostasis [11,19]. Therefore, in chondroplasty, it can not only provide a smooth surface for the injured cartilage, but also solidify the cartilage tissue, and the vaporized lesion cannot remain in the joint cavity, thus preventing the further development of cartilage damage.

Cartilage tissue is made up of chondrocytes and their matrix [20], and it is still controversial that RFE therapy may influence it for some time after treatment [21]. For the tissues collected immediately after bRFE, flow cytometry and western blot analysis showed that the apoptosis rate in the bRFE group was significantly higher than that in the model group. And then in the recovery period, the vitality of chondrocytes was restored. In terms of effectiveness, bRFE has obvious advantages over traditional mechanical cleaning [22]. Although, there is a risk of damage to chondrocytes, as long as the variables affecting these cells are adequately controlled, the current optimal therapeutic effect can be achieved [18]. A comprehensive evaluation of this technique requires long-term observation of chondrocyte viability. The present study focused on immediate and short-term impacts after surgery. In the future, long-term observation of cartilage changes to reflect the process of repair will constitute our research direction, since it is more valuable for clinical use.

Conclusions

bRFE used in chondroplasty can effectively trim and polish the cartilage lesion area, and reduce cracks. It also has the deleterious effect of inducing apoptosis timely. After a period of recovery, cellular apoptosis decreased. Therefore, when using bRFE to treat articular cartilage injury, special attention should be paid to energy selection and recovery period.

Availability of Data and Materials

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Author Contributions

SF, BC, and PZ contributed to the concept, methods and investigation. BC and GY contributed to the investigation, validation and analysis. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Animal experiments were supported by Laboratory Animal Ethics Committee of Huangpu Branch of the Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (No. IACUC-20211014-02).

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Mehana ESE, Khafaga AF, El-Blehi SS. The role of matrix metalloproteinases in osteoarthritis pathogenesis: An updated review. Life Sciences. 2019; 234: 116786.

[2] D'Amico D, Olmer M, Fouassier AM, Valdés P, Andreux PA, Rinsch C, *et al*. Urolithin A improves mitochondrial health, reduces cartilage degeneration, and alleviates pain in osteoarthritis. Aging Cell. 2022; 21: e13662.

[3] Sun D, Liu X, Xu L, Meng Y, Kang H, Li Z. Advances in the Treatment of Partial-Thickness Cartilage Defect. International Journal of Nanomedicine. 2022; 17: 6275–6287.

[4] Sakata R, Iwakura T, Reddi AH. Regeneration of Articular Cartilage Surface: Morphogens, Cells, and Extracellular Matrix Scaffolds. Tissue Engineering. Part B, Reviews. 2015; 21: 461–473.

[5] Dieppe PA, Lohmander LS. Pathogenesis and management of pain in osteoarthritis. Lancet (London, England). 2005; 365: 965–973.

[6] Rim YA, Nam Y, Ju JH. The Role of Chondrocyte Hypertrophy and Senescence in Osteoarthritis Initiation and Progression. International Journal of Molecular Sciences. 2020; 21: 2358.

[7] Żylińska B, Silmanowicz P, Sobczyńska-Rak A, Jarosz Ł, Szponder T. Treatment of Articular Cartilage Defects: Focus on Tissue Engineering. In Vivo (Athens, Greece). 2018; 32: 1289–1300.

[8] Zhang W, Ouyang H, Dass CR, Xu J. Current research on pharmacologic and regenerative therapies for osteoarthritis. Bone Research. 2016; 4: 15040.

[9] Evenbratt H, Andreasson L, Bicknell V, Brittberg M, Mobini R, Simonsson S. Insights into the present and future of cartilage regeneration and joint repair. Cell Regeneration (London, England). 2022; 11: 3.

[10] Li KH, Zhu Y, Zhang PH, Alini M, Grad S, Li Z. Osteochondral explants for diarthrodial joint diseases: bridging the gap between bench and bedside. EUROPEAN CELLS & MATERIALS. 2022; 44: 74–89.

[11] Lin C, Deng Z, Xiong J, Lu W, Chen K, Zheng Y, *et al*. The Arthroscopic Application of Radiofrequency in Treatment of Articular Cartilage Lesions. Frontiers in Bioengineering and Biotechnology. 2022; 9: 822286.

[12] Grieshober JA, Stanton M, Gambardella R. Debridement of Articular Cartilage: The Natural Course. Sports Medicine and Arthroscopy Review. 2016; 24: 56–62.

[13] Voloshin I, Morse KR, Allred CD, Bissell SA, Maloney MD, DeHaven KE. Arthroscopic evaluation of radiofrequency chondroplasty of the knee. The American Journal of Sports Medicine. 2007; 35: 1702–1707.

[14] Edwards RB, 3rd, Lu Y, Uthamanthil RK, Bogdanske JJ, Muir P, Athanasiou KA, *et al*. Comparison of mechanical debridement and radiofrequency energy for chondroplasty in an *in vivo* equine model of partial thickness cartilage injury. Osteoarthritis and Cartilage. 2007; 15: 169–178.

[15] Anderson SR, Faucett SC, Flanigan DC, Gmabardella RA, Amin NH. The history of radiofrequency energy and Coblation in arthroscopy: a current concepts review of its application in chondroplasty of the knee. Journal of Experimental Orthopaedics. 2019; 6: 1.

[16] Kim TH, Chang MC. Comparison of the effectiveness of pulsed radiofrequency of the suprascapular nerve and intra-articular corticosteroid injection for hemiplegic shoulder pain management. Journal of Integrative Neuroscience. 2021; 20: 687–693.

[17] Huang Y, Zhang Y, Ding X, Liu S, Sun T. Working conditions of bipolar radiofrequency on human articular cartilage repair following thermal injury during arthroscopy. Chinese Medical Journal. 2014; 127: 3881–3886.

[18] Peng L, Li Y, Zhang K, Chen Q, Xiao L, Geng Y, *et al*. The time-dependent effects of bipolar radiofrequency energy on bovine articular cartilage. Journal of Orthopaedic Surgery and Research. 2020; 15: 106.

[19] Rocco P, Lorenzo DB, Guglielmo T, Michele P, Nicola M, Vincenzo D. Radiofrequency energy in the arthroscopic treatment of knee chondral lesions: a systematic review. British Medical Bulletin. 2016; 117: 149–156.

[20] Charlier E, Deroyer C, Ciregia F, Malaise O, Neuville S, Plener Z, *et al*. Chondrocyte dedifferentiation and osteoarthritis (OA). Biochemical Pharmacology. 2019; 165: 49–65.

[21] Giddins G, Shewring D, Downing N. Articular cartilage and soft tissue damage from radiofrequency thermal ablation wands at wrist arthroscopy. The Journal of Hand Surgery, European Volume. 2021; 46: 632–636.

[22] Spahn G, Kahl E, Mückley T, Hofmann GO, Klinger HM. Arthroscopic knee chondroplasty using a bipolar radiofrequency-based device compared to mechanical shaver: results of a prospective, randomized, controlled study. Knee Surgery, Sports Traumatology, Arthroscopy: Official Journal of the ESSKA. 2008; 16: 565–573.