Original Article Clinics in Orthopedic Surgery 2025;17:710-720 • https://doi.org/10.4055/cios24502

The Effects of Irisin and Bevacizumab on Hyaline Cartilage Regeneration in Osteochondral Defects: An Experimental Study in Rats

Ahmet Emrah Açan, MD, Mert Emre Aydın, MD, Özgür Bulmuş, MD*, Emrah Özcan, MD†, Aslı Karakılıç, MD*, Gülay Turan, MD‡, Sevban Bayırlı, MD§

Department of Orthopedics and Traumatology, Faculty of Medicine, Balıkesir University, Balıkesir,
**Department of Physiology, Faculty of Medicine, Balıkesir University, Balıkesir,*
†Department of Anatomy, Faculty of Medicine, Balıkesir University, Balıkesir,
‡Department of Pathology, Faculty of Medicine, Balıkesir University, Balıkesir,
§Research Institute for Health Sciences and Technologies, Istanbul Medipol University, Istanbul, Turkiye

Background: The microfracture technique, a first-line treatment for full-thickness cartilage defects (FTCDs), involves perforation of the subchondral bone to allow bone marrow, including mesenchymal stem cells, to promote healing. However, microfracture often fails within 5 years due to the insufficient durability of fibrous cartilage and subchondral bone deterioration. To address this issue, augmenting bone marrow stimulation (BMS) with several systemic or local agents has been explored. This study evaluated the effects of irisin (IR) and bevacizumab (BEVA), individually and combined, as alternative intra-articular BMS augmentation methods compared with hyaluronic acid (HA) and platelet-rich plasma (PRP).

Methods: Forty-eight Wistar albino male rats were divided into 6 groups (n = 8 per group): control, PRP, HA, BEVA, IR, and BEVA-IR. An FTCD was created, followed by BMS in the control group. PRP, HA, and IR were injected intra-articularly on the day of surgery, with BEVA administered in the fourth postoperative week. All rats were sacrificed at 12 weeks. Distal femurs were analyzed via micro-computed tomography (micro-CT), and cartilage regeneration was assessed macroscopically and histologically using the International Cartilage Repair Society (ICRS) and Pineda scores, respectively.

Results: The Pineda score was significantly lower in the BEVA-IR group compared with the control ($p < 0.001$), PRP ($p < 0.05$), HA ($p < 0.05$), and BEVA ($p < 0.05$) groups. Significant differences in ICRS stages were observed between the control group and both the IR ($p < 0.05$) and BEVA-IR ($p < 0.01$) groups, as well as between BEVA and BEVA-IR ($p < 0.01$). Micro-CT analysis revealed that the defect width was significantly lower in the PRP ($p < 0.05$), IR ($p < 0.05$), and BEVA-IR ($p < 0.01$) groups compared with the control group. The defect depth in the BEVA-IR group was significantly lower compared with the control ($p < 0.01$), PRP ($p < 0.05$), BEVA ($p < 0.01$), and HA ($p < 0.05$) groups. The IR group also showed a significantly smaller defect area compared with the BEVA ($p < 0.01$) and HA ($p < 0.05$) groups.

Conclusions: To the best of our knowledge, this study is the first to investigate intra-articular IR in FTCDs. The IR and BEVA-IR groups, particularly the BEVA-IR group, demonstrated superior outcomes in all evaluations. These findings suggest that combining BEVA and IR has synergistic effects on cartilage healing in FTCDs.

Keywords: *Osteochondral lesions, Cartilage injuries, Irisin, Bevacizumab, Platelet-rich plasma*

Received December 21, 2024; Revised March 7, 2025; Accepted April 24, 2025
Correspondence to: Ahmet Emrah Açan, MD
Department of Orthopedics and Traumatology, Faculty of Medicine, Balıkesir University, Balıkesir 10145, Türkiye
Tel: +90-266-612-10-00, Fax: +90-266-612-13-09, E-mail: dremrahacan@hotmail.com

Full-thickness cartilage defects (FTCDs), mostly caused by trauma or degenerative processes, have a very limited capacity for regeneration due to the lack of blood supply to the cartilage.[1] In addition to causing significant morbidity, improper healing contributes to the development of osteoarthritis (OA), making it a continuously relevant topic in the field of orthopedics. Treatment modalities include debridement, osteochondral allograft or autograft transfers, autologous chondrocyte implantation and bone marrow stimulation (BMS) techniques, such as subchondral drilling and the microfracture procedure.[2] The microfracture technique, popularised in the 1990s, involves creating small perforations in the subchondral bone to allow bone marrow, including mesenchymal stem cells, to promote healing by bleeding into the defect.[3] Despite yielding inferior outcomes compared with other methods, it is considered a first-line treatment due to its simplicity, cost-effectiveness, and favourable short-term results.[3,4] However, the healing process typically results in fibrocartilaginous tissue, which predominantly contains type I collagen and exhibits inferior biomechanical properties compared with native hyaline cartilage, often leading to failure within 5 years due to the insufficient durability of the fibrous cartilage covering the defect and the deterioration of the subchondral bone.[5,6] Therefore, augmentation of the BMS technique with systemic or local agents is being explored. Although systemic agents such as losartan have shown positive effects on cartilage healing, their systemic administration carries potential side effects.[7] Consequently, intra-articular hyaluronic acid (HA) and platelet-rich plasma (PRP) have become widely used and accepted methods.[8-10] However, despite advancements in these augmentation techniques, hyaline cartilage regeneration has yet to be consistently achieved.

Irisin (IR) is a recently popular myokine released into the serum by skeletal muscles following exercise. In addition to its effects on the musculoskeletal system, studies have also reported its impacts on various metabolic diseases, brain functions, and Alzheimer disease.[11] The reported effects of IR on chondrocytes at the molecular level include promoting cell proliferation, differentiation, mitochondrial function, and anabolism by increasing type II collagen production, while reducing inflammation and pyroptosis, ultimately improving the survival rate of inflamed chondrocytes.[12,13] Similarly, a recent study highlighted that IR's chondroprotective mechanism helps protect against chondrocyte dysfunction and suggested the therapeutic potential of recombinant IR for OA.[14]

Angiogenesis plays a critical role in OA pathogenesis by promoting blood vessel invasion from the subchondral bone, which contributes to synovitis, inflammation, cartilage erosion, and osteophyte formation. Given the pivotal role of vascular endothelial growth factor (VEGF), studies on anti-VEGF treatments reported in the literature are garnering increasing attention. Bevacizumab (BEVA) is a monoclonal anti-VEGF antibody clinically used to inhibit angiogenesis, systemically in cancer therapy and locally as an intraocular injection for age-related macular degeneration.[15] The literature on the intra-articular use of BEVA is quite limited. However, 2 studies published in 2014 and 2016 reported positive effects of intra-articular BEVA injections on the progression of OA in rabbits.[16,17] Moreover, in 2021, Utsunomiya et al.[15] reported that intra-articular injection of 12.5 mg/0.5 mL BEVA at the fourth week after BMS improved the healing of osteochondritis dissecans lesions in rabbits. Remarkably, this treatment promoted both quantitative and qualitative increases in hyaline-like cartilage without disrupting subchondral bone formation.

The aim of this study is to compare the quantitative and qualitative effects of intra-articular injections of HA and PRP, widely used BMS augmentation methods in FTCD, on cartilage healing with intra-articular BEVA and recombinant IR injections, which have recently been reported in the literature. Additionally, the study hypothesizes that the combination of recombinant IR with BEVA might have a synergistic effect due to mechanisms that may create an optimal environment for cartilage repair by combining the anabolic and anti-inflammatory properties of IR with the anti-angiogenic and structural preservation effects of BEVA, potentially leading to both quantitatively and qualitatively superior outcomes that closely resemble native hyaline cartilage.

METHODS

This study was conducted with the approval of the lo-

cal Animal Ethics Committee, dated 28.09.2023, under the approval number 2023/8-5. It was carried out at our university's animal research center using 48 male Wistar albino rats (14–16 weeks old). All procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and adhered to international guidelines on animal research ethics. The minimum number of rats required for the 6 groups in the study was calculated using G*Power software (Heinrich-Heine-Universität Düsseldorf) analysis of variance (ANOVA), with an effect size of 0.50, a confidence level of 0.90, and a power of 0.8123, resulting in a total of 48 rats.

The rats were randomly divided into 6 groups (n = 8 per group), and the treatment protocols were as follows: control group (group 1): BMS technique applied to the osteochondral defect; PRP group (group 2): BMS + intra-articular PRP injection; HA group (group 3): BMS + intra-articular HA injection; BEVA group (group 4): BMS + intra-articular BEVA injection at the fourth postoperative week; IR group (group 5): BMS + intra-articular IR injection on the day of surgery; BEVA-IR group (group 6): BMS + intra-articular IR on the day of surgery + intra-articular BEVA at the fourth postoperative week.

Rat Osteochondral Defect Model

Under anaesthesia (intraperitoneal administration of 80 mg/kg ketamine hydrochloride and 8 mg/kg xylazine), the right knee joint of the rats was first shaved and then covered with a sterile drape.[9] Following this, an arthrotomy was performed using a medial parapatellar approach. A 0.8-mm diameter Kirschner wire was inserted into the centre of the trochlear region to a depth of 4 mm. Using a cannulated drill, a full-thickness osteochondral defect measuring 2.0 mm in width and 2.0 mm in depth was created at the centre of the femoral trochlear region (Fig. 1).[7,9,10] After withdrawing the Kirschner wire and confirming sufficient bleeding into the defect from the drill site to ensure proper execution of the BMS technique, the patella was carefully repositioned and the surgical layers were appropriately closed.[18] Then, augmentation materials, each with a volume of 0.1 mL for all groups, were injected using an insulin syringe with a 27-gauge needle through the lateral suprapatellar pouch under the guidance of a mini ultrasound device. (Vscan, General Electric Healthcare). In groups 2, 3 and 5, the injections were administered on the day of surgery after the layers were closed. In group 4, the injection was administered at the fourth postoperative week. In group 6, the IR injection was given on the day of surgery after the layers were closed, and the BEVA injection was administered at the fourth postoperative week. BEVA injections were scheduled for the fourth postoperative week, following the study by Utsunomiya et al.,[15] which reported enhanced cartilage repair when BMS was combined with an intra-articular BEVA injection at this time point using a dose of 12.5 mg/0.5 mL. As highlighted by Utsunomiya et al.,[15] this timing was chosen to prevent early inhibition of angiogenesis, which is essential for initial subchondral bone remodeling. By delaying the BEVA injection until the fourth week, the goal was to suppress excessive vascularization while preserving the subchondral bone necessary for cartilage repair. The dosages were selected based on the literature as follows: 100 μL of PRP; 0.1 mL of 25 mg/mL HA (Regenflex Bioplus, Regenyal), a formulation containing 2 different fractions: a cross-linked HA with a high molecular weight ranging from 1 to 2 million Da and a linear fraction with a lower molecular weight of 500 KDa; 10 μg/mL of recombinant IR protein (r-irisin; #11451: Cayman Chemicals) and 12.5 mg/0.5 mL of BEVA (Altuzan, 100 mg/4 mL vial, Roche).[9,12,15,19] A 100 μg IR vial (r-irisin; #11451: Cayman Chemicals) was completed by adding deionised water to a total volume

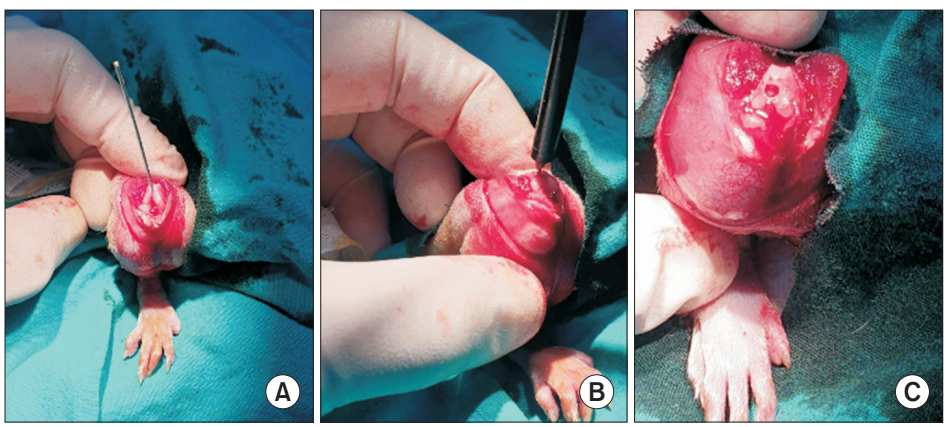

Fig. 1. Steps of the rat osteochondral defect model. (A) After arthrotomy via a medial parapatellar approach, a 0.8-mm Kirschner wire was inserted into the trochlear center to a depth of 4 mm. (B) A 2-mm wide and 2-mm deep femoral trochlear cartilage defect was created in the center of the trochlear region using a cannulated drill. A plastic sleeve on the drill ensured a standardized depth of 2 mm. (C) Bleeding from the Kirschner wire drill line into the defect confirmed adequate bone marrow stimulation.

of 10 mL. Thus, after reaching a concentration of 10 μg/mL, 100 microliters were injected intra-articularly into the knee joints of the rats in the IR group on day 0. For PRP preparation, blood was drawn into a tube containing Ficoll and processed using a cell extraction kit (Easy PRP Kit, Mesotech). Then, it was centrifuged at 1,200 RPM for 10 minutes in a standard laboratory centrifuge. The injections were administered within 1 hour of preparation. The rats were kept under laboratory conditions with unrestricted access to standard pellet feed and water. They were housed under a natural day/night cycle, with room temperature and humidity carefully regulated until the 12th week, when all groups were sacrificed via cervical dislocation performed under high-dose anaesthesia.

Radiological Imaging

The distal femurs of the rats were maintained in a 10% formaldehyde solution until they were transported for micro-computed tomography (micro-CT) analysis. Micro-CT scans were conducted by positioning the distal femurs in a plastic holder and scanning them using a U-CT system (MILabs MicroCT-OI) at 50 kVp and 45 mA, with a step angle of 0.75° and an exposure time of 40 ms for each projection. The scanned sections were reconstructed with voxel sizes of 60 micrometres. Following reconstruction in the coronal, sagittal, and 3-dimensional (3D) formats, the width and depth of the defect and the area not filled with regenerated cartilage were measured using the RadiAnt DICOM Viewer software (Medixant Company) (Fig. 2).

Histological Evaluation

Following micro-CT scanning, a macroscopic assessment of each resected distal femur was performed by a researcher blinded to the study groups (GT), using the International Cartilage Repair Society (ICRS) scoring system.[20] This system evaluates the degree of defect repair, integration with the border zone, and macroscopic appearance, assigning scores from 0 (worst) to 4 (best). A total score of 12 indicates normal cartilage, while scores of 8–11, 4–7, and 1–3 correspond to nearly normal, abnormal, and severely abnormal cartilage, respectively (Table 1). Following 2 days of 10% formalin fixation, each sample was placed in a 10% ethylenediaminetetraacetic acid (EDTA) solution, which was refreshed every 2 days during the 4-week decalcification period. The decalcified samples were embedded in paraffin and sliced into 5-micron-thick sections. After deparaffinisation, the tissue sections were stained with hematoxylin and eosin (Fig. 3). Subsequently, a blinded pathologist (GT) performed a histological evaluation of cartilage regeneration at the FTCD using the Pineda score, which ranges from 0 (best) to 14 (worst) (Table 2).[21]

Statistical Analyses

All statistical analyses were conducted using the SPSS software for Windows version 22.0 (IBM Corp.). The normality of the data distribution was assessed using the Shapiro-Wilk test. For comparisons involving 3 or more groups, quantitative variables were analyzed using one-way ANOVA, followed by Dunnett's post-hoc tests. For

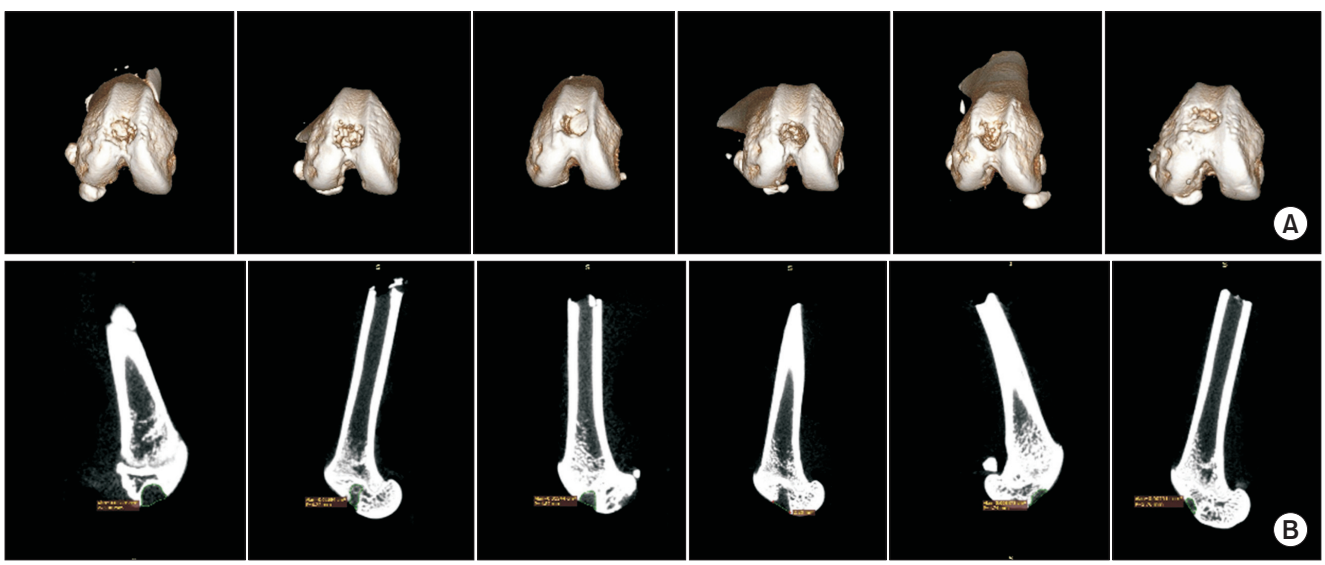

Fig. 2. Images represent, from left to right, the control, platelet-rich plasma, hyaluronic acid, bevacizumab (BEVA), irisin (IR), and BEVA-IR groups. (A) Three-dimensional reconstructions of micro-computed tomography images showing the distal femur with an osteochondral defect. (B) Osteochondral defect area measurements in the sagittal section were performed using RadiAnt DICOM Viewer software (Medixant).

Table 1. International Cartilage Repair Society Cartilage Repair Assessment Tool

Criteria	Points
Degree of defect repair	
Level with surrounding cartilage	4
75% Repair of defect depth	3
50% Repair of defect depth	2
25% Repair of defect depth	1
0% Repair of defect depth	0
Integration into the border zone	
Complete integration with surrounding cartilage	4
Demarcating border < 1 mm	3
3 / 4 Of graft integrated, 1 / 4 with a notable border > 1 mm width	2
1 / 2 of graft integrated with surrounding cartilage, 1 / 2 with a notable border > 1 mm	1
From no contact to 1 / 4 of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several small or a few large fissures	1
Total degeneration of the grafted area	0
Overall score	
Grade I normal	12
Grade II nearly normal	11–8
Grade III abnormal	7–4
Grade IV severely abnormal	3–1

categorical data, the Kruskal-Wallis test, the non-parametric counterpart to one-way ANOVA, was applied. Pairwise comparisons between groups where statistical significance was observed were performed using the Mann-Whitney *U*-test. Correlations between the variables were evaluated using Spearman's correlation analysis. Data are presented as mean ± standard deviation, and differences were considered statistically significant at $p < 0.05$.

RESULTS

On the day of the surgical procedure, 1 rat in the control group died during anesthesia. Additionally, 1 rat in the HA group and another in the control group died the day after surgery. To maintain the group sizes determined by the power analysis, the surgical procedure was repeated for these 3 rats the following day. A significant difference was found between the groups in terms of Pineda scores ($p < 0.001$). The mean Pineda scores of the groups were 12.6 ± 1 in control, 8.8 ± 1.2 in PRP, 8.8 ± 1.4 in HA, 8.4 ± 0.7 in BEVA, 5.3 ± 1.3 in IR, and 3.8 ± 1 in BEVA-IR groups.

Regarding the Pineda scoring system (Table 2), which ranges from 0 (best) to 14 (worst), the BEVA-IR group had a significantly lower score compared with the control ($p < 0.001$), PRP ($p < 0.05$), HA ($p < 0.05$), and BEVA ($p < 0.05$) groups. Additionally, the Pineda score of the IR group was lower than that of the control group ($p < 0.001$). No significant difference was observed between the Pineda scores of the IR and BEVA-IR groups ($p > 0.05$). Based on the morphological classification, a subcategory of the Pineda scoring system, mostly hyaline and fibrocartilage (score 1), was observed only in the BEVA-IR group, in 3 out of 8 subjects.

The mean ICRS scores of the groups were 9.2 ± 2.4 (6.9 ± 1.6 in control, 9.6 ± 2.4 in PRP, 9.6 ± 2.5 in HA, 7.8 ± 2.3 in BEVA, 10.1 ± 2.2 in IR, and 10.9 ± 1.1 in BEVA-IR). An analysis of ICRS stages indicated a significant difference between the groups ($p < 0.05$) (2.8 ± 0.7 in control, 2 ± 0.9 in PRP, 2 ± 0.8 in HA, 2.6 ± 0.7 in BEVA, 1.8 ± 0.9 in IR, and 1.6 ± 0.5 in BEVA-IR). Significant differences in ICRS stages were observed between the control group and the IR and BEVA-IR groups ($p < 0.05$ and $p < 0.01$, respectively). Additionally, a statistically significant difference was found between the BEVA and BEVA-IR groups ($p < 0.01$) (Fig. 4A and B).

Considering that ICRS scores of 7 and below (stage 3 and stage 4) are classified as "unfavourable" and scores of 8 and above (stage 1 and stage 2) are classified as "favourable," no significant association was found between the group variable and the ICRS category when analyzed according to groups ($p > 0.05$). However, all subjects in the BEVA-IR group were classified as favourable, whereas the proportion of favourable to unfavourable outcomes in the HA and IR groups (6 : 2, for both) was higher compared with the control, PRP, and BEVA (3 : 5, 5 : 3, and 4 : 4, respectively) groups (Fig. 4C).

Analysis of the CT images revealed significant differences between the groups in terms of width, depth, and the defect not filled with regenerated cartilage: F(5,42) =

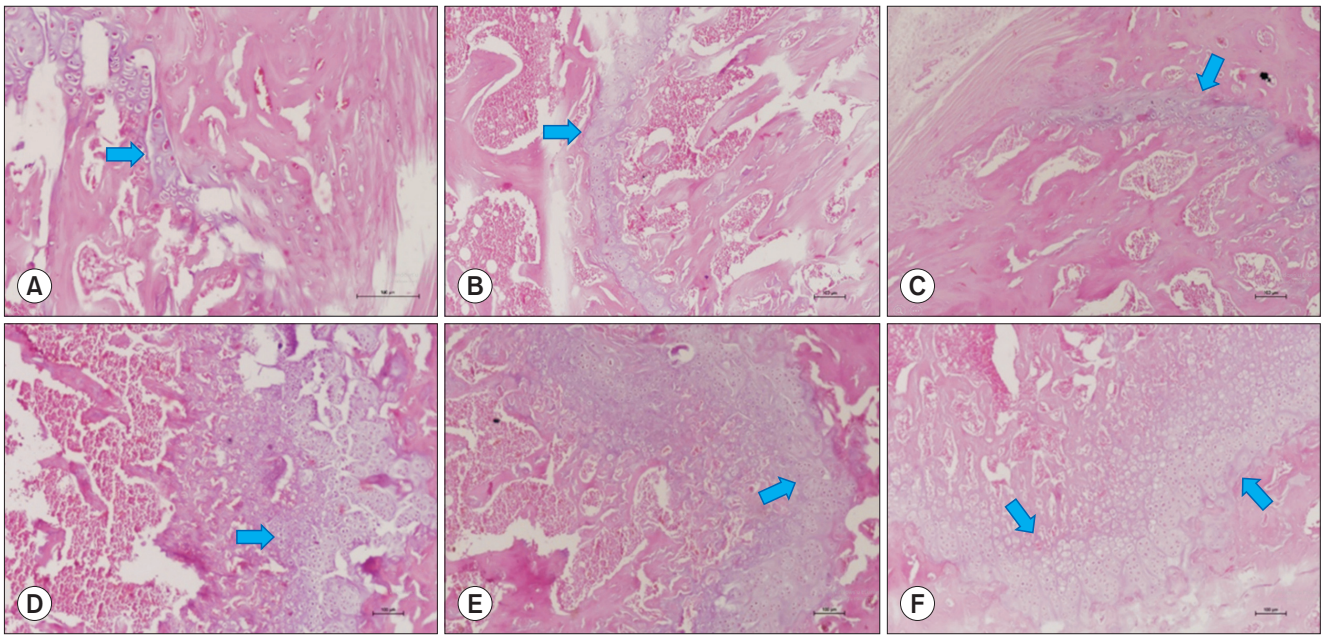

Fig. 3. Histological images (H&E, ×200) represent the control (A), platelet-rich plasma (B), hyaluronic acid (C), bevacizumab (BEVA; D), irisin (IR; E), and BEVA-IR (F) groups. Cartilage formation was more pronounced in the IR and BEVA-IR groups, particularly in the BEVA-IR group, as indicated by blue arrows.

Table 2. Pineda Cartilage Repair Score

Characteristics	Score
Filling of defect (%)	
125	–1
100	0
75	1
50	2
25	3
0	4
Reconstruction of osteochondral junction	
Yes	0
Almost	1
Not closed	2
Matrix staining	
Normal	0
Reduced staining	1
Significantly reduced staining	2
Faint staining	3
No staining	4
Cell morphology	
Normal	0
Mostly hyaline and fibrocartilage	1
Mostly fibrocartilage	2
Some fibrocartilage, but mostly	3
Nonchondrocytic cells	4

3.721, $p < 0.01$; $F(5,42) = 3.122$, $p < 0.05$; and $F(5,42) = 7.282$, $p < 0.001$, respectively (Table 3). The width measured by micro-CT was found to be lower in the PRP, IR, and BEVA-IR groups compared with the BMS group ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively) (Fig. 5A). Analysis of the micro-CT images revealed that the depth measured in the BEVA-IR group was lower compared with the Control, PRP, BEVA and HA groups ($p < 0.01$, $p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively) (Fig. 5B). Additionally, the depth measured in the IR group was lower than that of the BEVA group ($p < 0.05$). No significant difference was observed between the IR and BEVA-IR groups ($p = 0.477$).

The area of the defect not filled with regenerated cartilage, as measured by CT, was found to be lower in the

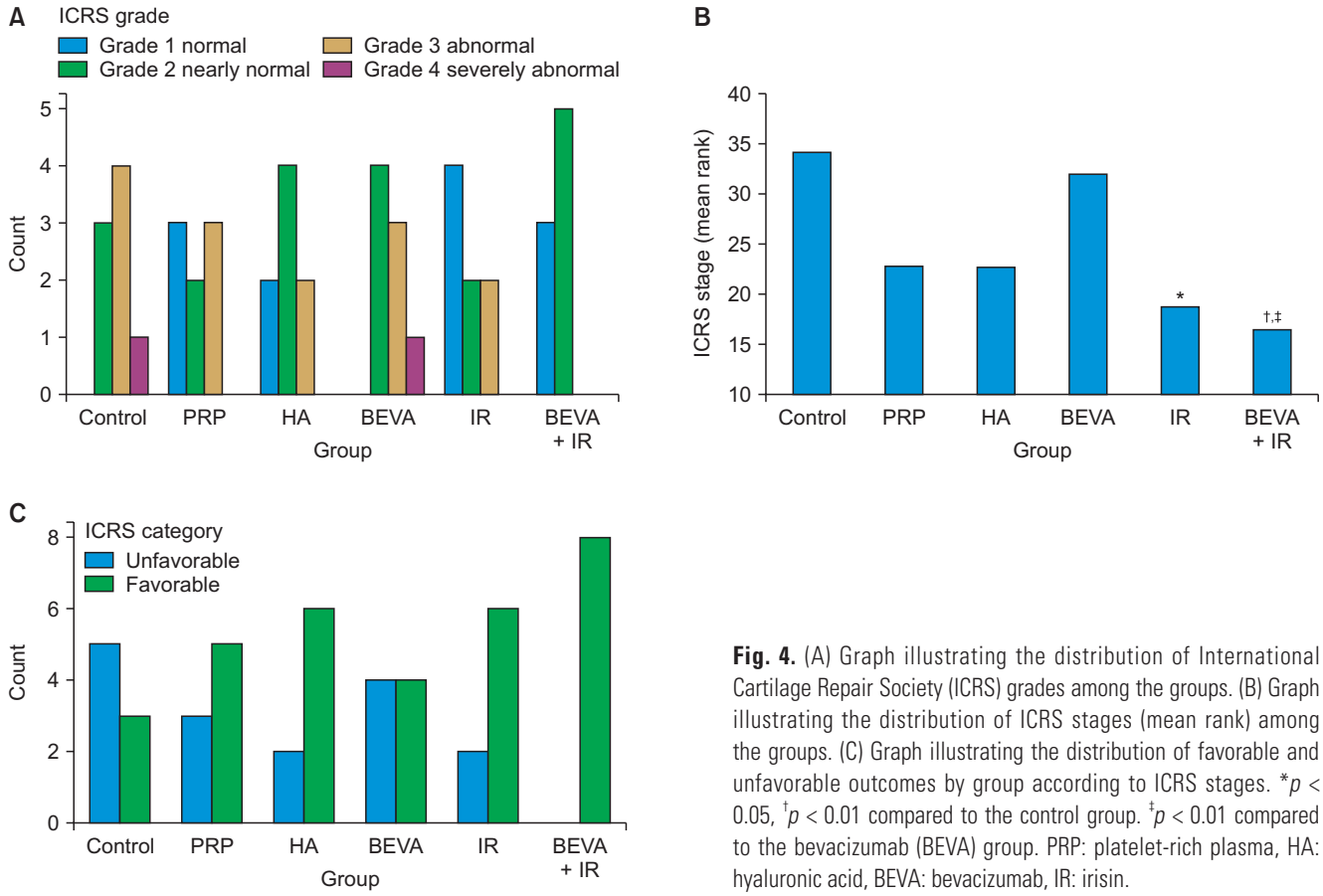

Fig. 4. (A) Graph illustrating the distribution of International Cartilage Repair Society (ICRS) grades among the groups. (B) Graph illustrating the distribution of ICRS stages (mean rank) among the groups. (C) Graph illustrating the distribution of favorable and unfavorable outcomes by group according to ICRS stages. *p < 0.05, †p < 0.01 compared to the control group. ‡p < 0.01 compared to the bevacizumab (BEVA) group. PRP: platelet-rich plasma, HA: hyaluronic acid, BEVA: bevacizumab, IR: irisin.

Table 3. Analysis of the Micro-CT Images

Group	Width (mm)	Depth (mm)	Defect not filled with regenerated cartilage (mm^3)
Control	2.09 ± 0.14	1.99 ± 0.27§	0.237 ± 0.004§
PRP	1.63 ± 0.46*	1.83 ± 0.3‡	0.224 ± 0.005‡
HA	1.75 ± 0.24	1.91 ± 0.3‡	0.251 ± 0.005‖,¶
BEVA	1.85 ± 0.29	1.94 ± 0.36§,¶	0.269 ± 0.003‖,**
IR	1.63 ± 0.24*	1.69 ± 0.31	0.019 ± 0.005
BEVA+IR	1.49 ± 0.38†	1.47 ± 0.31	0.016 ± 0.003
p-value	0.007	0.017	0.0001

Values are presented as mean ± standard deviation.
Micro-CT: micro-computed tomography, PRP: platelet-rich plasma, HA: hyaluronic acid, BEVA: bevacizumab, IR: irisin.
*p < 0.05, †p < 0.01 compared to control group. ‡p < 0.05, §p < 0.01, ‖p < 0.001 compared to BEVA + IR group. ¶p < 0.05, **p < 0.01 compared to IR group.

BEVA-R group compared with the control, PRP, BEVA, and HA groups (p < 0.01, p < 0.05, p < 0.001, and p < 0.001, respectively). Additionally, the IR group showed a smaller area of the defect not filled with regenerated cartilage compared with the BEVA and HA groups (p < 0.01 and p < 0.05, respectively) (Fig. 5C). However, no significant difference was observed between the IR and BEVA-IR groups (p = 0.411). A moderate positive correlation was found between the Pineda scores and the width (r = 0.431, p < 0.01), depth (r = 0.447, p < 0.01), and area (r = 0.409, p < 0.01) measured using micro-CT.

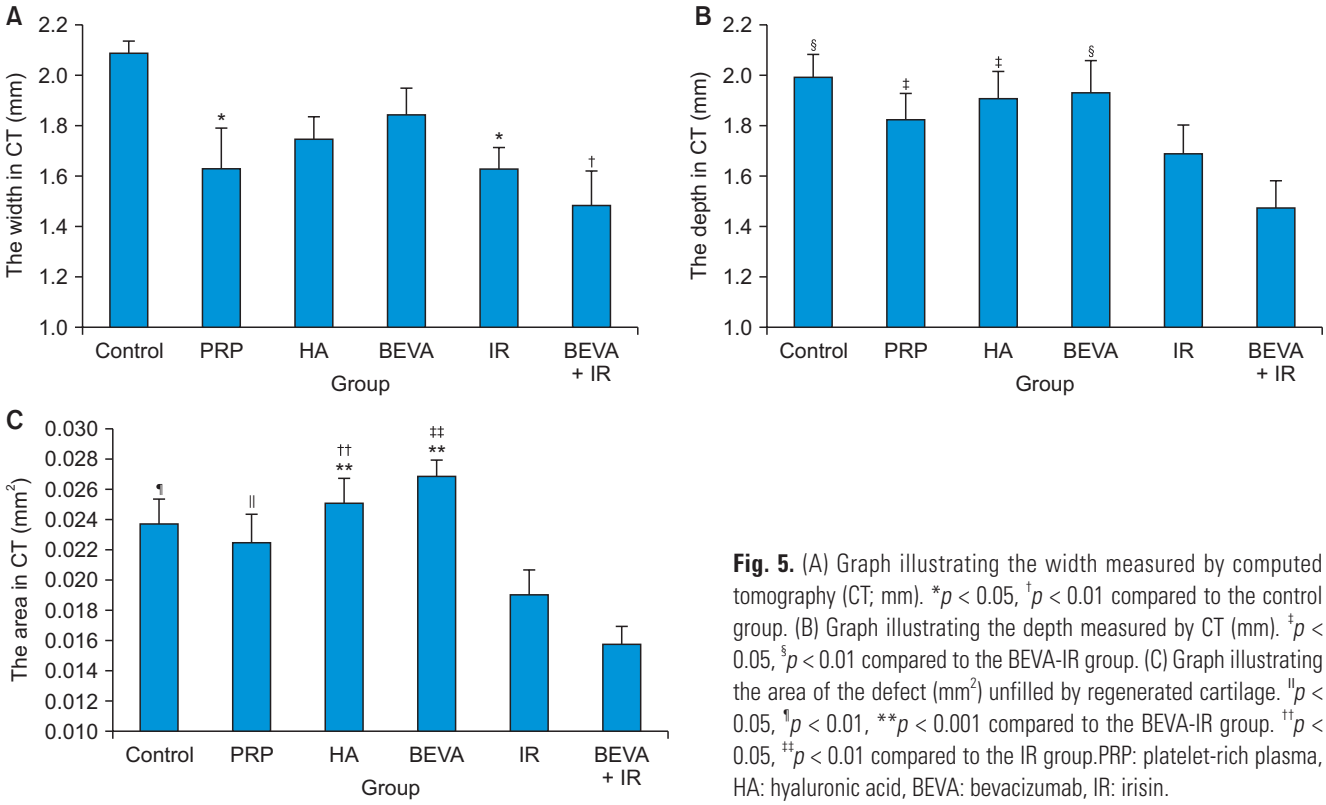

Fig. 5. (A) Graph illustrating the width measured by computed tomography (CT; mm). $^{*}p < 0.05$, $^{\dagger}p < 0.01$ compared to the control group. (B) Graph illustrating the depth measured by CT (mm). $^{\ddagger}p < 0.05$, $^{\S}p < 0.01$ compared to the BEVA-IR group. (C) Graph illustrating the area of the defect (mm^2) unfilled by regenerated cartilage. $^{\|}p < 0.05$, $^{\P}p < 0.01$, $^{**}p < 0.001$ compared to the BEVA-IR group. $^{\dagger\dagger}p < 0.05$, $^{\ddagger\ddagger}p < 0.01$ compared to the IR group.PRP: platelet-rich plasma, HA: hyaluronic acid, BEVA: bevacizumab, IR: irisin.

DISCUSSION

Based on the current literature, this study appears to be among the first to investigate the effects of intra-articular IR on cartilage healing in FTCDs. Additionally, IR was compared with BEVA alone and in combination with IR, as well as with PRP and HA, which are simple, commonly used BMS augmentation methods in clinical practice. In our study, based on the ICRS score used for macroscopic evaluation, a significant difference was observed between the IR and BEVA-IR groups and the control group ($p < 0.05$ and $p < 0.01$, respectively). Although the PRP and HA groups showed no statistically significant difference with the control group, they showed better scores than the BEVA group (Fig. 4A and B). However, when an ICRS score of 8 or above (stages 1 and 2) is considered favourable, it is noteworthy that although there was no statistical difference between the groups, all subjects in the BEVA-IR group were classified as favourable. Additionally, the favourable to unfavourable ratio in the IR and HA groups (6:2 in both) was better compared with the PRP and BEVA groups (5 : 3 and 4 : 4, respectively) (Fig. 4C).

Considering the Pineda scores used for microscopic evaluation, the BEVA-IR group demonstrated a significantly lower score compared with the control, PRP, HA, and BEVA groups ($p < 0.001$, $p < 0.05$, $p < 0.05$, and $p < 0.05$, respectively). The absence of a significant difference between the IR group and the BEVA-IR group further suggests that both groups were superior to the other groups, even at the microscopic level. The most noteworthy aspect of our study is that in FTCD healing, instead of a quantitative improvement in the defect, the desired outcome of healing with mostly hyaline cartilage, rather than fibrocartilaginous healing, was observed only in the BEVA-IR group (in 3 out of 8 cases). In contrast to the rabbit osteochondral defect study by Utsunomiya et al.,[15] the BEVA augmentation group in our study did not show a significant improvement in the quality and quantity of hyaline-like cartilage.

Radiological evaluation performed with micro-CT revealed that the depth and defect area not filled with regenerated cartilage were lower in the BEVA-IR group compared with the control, PRP, BEVA, and HA groups. The lack of difference between the BEVA-IR and IR groups may suggest that both the BEVA-IR and IR groups, particularly the BEVA-IR group, are superior to the other groups (Fig. 5).

The intra-articular use of BEVA was first reported by Nagai et al.,[17] wherein they observed a reduction in cartilage degeneration in a rabbit OA model created by resecting the anterior cruciate ligament. In 2016, Li et al.[16]

conducted a study on a rabbit OA model, comparing the progression of OA between a control group and groups treated with intra-articular BEVA (4 mg) administered twice at 3-week intervals and sodium hyaluronate administered weekly for 6 weeks. They reported that both BEVA and sodium hyaluronate slowed the progression of OA by reducing synovial hyperplasia and chondrocyte apoptosis, with the BEVA group showing better results. In another study, Lu et al.[22] demonstrated that intra-articular administration of BEVA (25 mg/kg/week), given 4 weeks before sacrifice, reduced subchondral H-type vessel formation and delayed OA development in a mouse OA model. Utsunomiya et al.[15] investigated the dose- and time-dependent effects of intra-articular BEVA on the healing of a rabbit osteochondral defect model. They reported that an intra-articular dose of 12.5 mg/0.5 mL BEVA, administered 4 weeks after surgery, significantly improved the quality and quantity of hyaline-like cartilage. They noted that subchondral bone penetration with BMS potentially induces undesired angiogenesis, leading to regeneration with fibrocartilage. However, this effect was prevented by BEVA, which, when injected at a later time point (4 weeks), likely did not affect subchondral bone formation, which is an important factor in supporting cartilage repair. Additionally, BEVA was found to reduce subchondral bone overgrowth. However, Utsunomiya et al.[15] reported that the same effect was not observed in the groups that received 25 mg/1 mL BEVA at 2 and 4 weeks after surgery or 25 mg/1 mL BEVA at 4 weeks after surgery. Therefore, the results obtained by Utsunomiya et al.[15] with 12.5 mg/0.5 mL BEVA administered 4 weeks after surgery do not seem to fully align with the findings of our study. This brings to mind the possibility that the dosing in rabbits might not be directly equivalent to that in rats. The current literature on intra-articular BEVA includes only a limited number of studies, and detailed pharmacokinetic information for scaling doses between rats and rabbits has unfortunately not been identified.[23]

The effects of IR, a myokine released into circulation from skeletal muscles following exercise, on bone were recognised earlier than its effects on cartilage, which has led to the availability of dose-dependent studies on its impact on bone healing.[24-26] However, it has been shown to attenuate defective autophagy, improve chondrocyte survival, protect against mitochondrial dysfunction, and promote cartilage proliferation and matrix gene expression, thereby maintaining intracellular homeostasis and metabolism through antioxidant and anti-inflammatory pathways, which have been increasingly recognised in recent years for their beneficial effects on cartilage regeneration.[12,14,27,28] Mao et al.[29] demonstrated an inverse relationship between the severity of OA and the levels of IR in serum and synovial fluid. Following this, an *in vitro* study conducted by Vadala et al.[27] was the first to report that IR could directly affect chondrocytes and potentially reduce OA-related cartilage degeneration. Then, *in vivo* studies by Wang et al.[14] and Li et al.[28] in mice demonstrated that intra-articular administration of IR reduced cartilage erosion and inflammation in the synovial membrane. Similarly, Posa et al.[12] reported the positive effects of IR on chondrocytes in an *in vitro* 3D culture of healthy human chondrocytes. In the study by Wang et al.,[14] 5 μL of an IR solution prepared at a dose of 1 μg/μL was injected into the knee joints of mice every 3 days for 8 weeks, whereas in Li et al.,[28] a single dose of 10 μL IR at a concentration of 20 mg/mL was administered in the first week after surgery. Except for these 2 studies, no other cases of intra-articular administration of IR in mice have been documented in the literature. In our study, 100 μL of IR solution at a concentration of 10 μg/mL was administered intra-articularly into the knee joints of rats. This dose is lower than the IR doses used in mice. The reason for selecting this dose in our study is that rats have a lower metabolic rate compared to mice.[30] However, the potential effects of IR on FTCD have not been investigated in any studies in the literature.

The major limitation of this study is that the BEVA treatment was only combined with IR. Although IR showed some macroscopic and microscopic advantages compared with PRP, HA, and BEVA alone, the fact that hyaline cartilage healing was primarily observed in the BEVA-IR group (in 3 out of 8 cases) underscores the need to evaluate BEVA in combination with PRP and HA. Among all treatment groups, the BEVA-IR combination demonstrated the most promising results, with superior outcomes in both macroscopic and microscopic evaluations. These findings suggest a potential synergistic effect between IR and BEVA, warranting further studies to investigate the underlying mechanisms and optimize treatment strategies. Additionally, the failure to achieve hyaline-like cartilage healing with BEVA alone, contrary to previous literature, and the similar outcomes in the IR group yet the successful healing observed only with the BEVA-IR combination therapy, is promising for FTCD treatment. Another key limitation of this study is the inability to assess the dose- and time-dependent effects of IR and BEVA in the rat model. Specifically, as demonstrated by Utsunomiya et al.,[15] creating subgroups within the BEVA group to explore the dose- and time-dependent effects of intra-articular BEVA could have potentially led to better outcomes in both the BEVA and BEVA-IR groups by iden-

tifying optimal dosing and timing. Additionally, this study utilized a single time-point analysis, with sacrifice occurring only at the end of the 12th week, thereby providing a single measure of total regeneration. This approach prevents insights into the dynamic process of cartilage regeneration over time and limits understanding of intermediate stages of healing. Moreover, this study did not include functional assessments such as weight-bearing analysis or joint mobility tests, which are crucial for evaluating the clinical relevance of cartilage regeneration. Furthermore, it is important to consider the impact of metabolic rate differences, as rats exhibit significantly faster tissue turnover compared to larger species and humans. This could affect the pace of regeneration or degradation observed in this study. Additionally, species-specific differences in cartilage composition, joint biomechanics, and inflammatory responses may further limit the extrapolation of these findings to human clinical applications. On the other hand, the absence of preliminary *in vitro* cytotoxicity studies to determine species-specific dosing may have impacted the translatability of the findings. Another limitation is that involving at least 2 independent blinded evaluators and performing inter-rater reliability analyses, such as intraclass correlation coefficient calculations, would enhance the reliability and validity of histological findings, addressing potential bias introduced by having a single evaluator. Finally, in this study, all osteochondral defects were created in the patellofemoral region of the knee, but other load-bearing areas were not examined.

In the augmentation of the BMS technique in FTCDs, although BEVA injection alone in the fourth week did not yield satisfying results, this study demonstrates that the combination of BEVA and IR not only enhances cartilage healing but also more closely approximates hyaline-like cartilage regeneration, making it a promising strategy for FTCD treatment. Similarly, although satisfying results could not be achieved with the injection of IR alone, the IR-BEVA combination resulted in hyaline-like cartilage healing. This suggests that combining BEVA and IR may have synergistic effects on cartilage healing, offering a promising strategy for achieving hyaline-like cartilage regeneration in FTCDs. Therefore, further dose- and time-dependent studies, incorporating functional assessments, long-term follow-up, and multiple independent evaluators for histological scoring, are required before clinical translation.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

The statistics were performed by Prof. Sinan Saraçlı, Department of Biostatistics, Faculty of Medicine, Balıkesir University.

This research received financial support from the Scientific Research Projects Unit of Balıkesir University (project No. 2023/233).

ORCID

Ahmet Emrah Açan https://orcid.org/0000-0001-7116-8773
Mert Emre Aydın https://orcid.org/0009-0006-6124-7842
Özgür Bulmuş https://orcid.org/0000-0001-7736-402X
Emrah Özcan https://orcid.org/0000-0002-6373-4744
Aslı Karakılıç https://orcid.org/0000-0001-7788-4949
Gülay Turan https://orcid.org/0000-0002-3702-8811
Sevban Bayırlı https://orcid.org/0000-0001-5428-1109

REFERENCES

1. Alford JW, Cole BJ. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med. 2005;33(2):295-306.
2. Falah M, Nierenberg G, Soudry M, Hayden M, Volpin G. Treatment of articular cartilage lesions of the knee. Int Orthop. 2010;34(5):621-30.
3. Gopinatth V, Jackson GR, Touhey DC, et al. Microfracture for medium size to large knee chondral defects has limited long-term efficacy: a systematic review. J Exp Orthop. 2024;11(4):e70060.
4. Vanlauwe J, Saris DB, Victor J, et al. Five-year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. Am J Sports Med. 2011;39(12):2566-74.
5. Gratz KR, Wong VW, Chen AC, Fortier LA, Nixon AJ, Sah RL. Biomechanical assessment of tissue retrieved after in vivo cartilage defect repair: tensile modulus of repair tissue and integration with host cartilage. J Biomech. 2006;39(1):138-46.
6. Sezgin EA, Atik OS. Are orthobiologics the next chapter

in clinical orthopedics?: a literature review. Eklem Hastalik Cerrahisi. 2018;29(2):110-6.

7. Utsunomiya H, Gao X, Deng Z, et al. Biologically regulated marrow stimulation by blocking TGF-β1 with losartan oral administration results in hyaline-like cartilage repair: a rabbit osteochondral defect model. Am J Sports Med. 2020;48(4):974-84.
8. Powers RT, Dowd TC, Giza E. Surgical treatment for osteochondral lesions of the talus. Arthroscopy. 2021;37(12):3393-6.
9. Ozdemir M, Birinci B, Haberal B, Ok Atilgan A, Demirkale I. In vivo study of the role of hyaluronic acid, N-acetyl cysteine, and deproteinized calf serum on injury-induced cartilage degeneration. Jt Dis Relat Surg. 2023;34(1):158-65.
10. Korucu IH, Kekec AF, Arslan A, Oltulu P, Korucu EN, Ozer M. Regenerative effects of hyperbaric oxygen therapy and platelet-rich plasma on the osteochondral defects of rats. Jt Dis Relat Surg. 2020;31(2):260-6.
11. Zerlotin R, Oranger A, Pignataro P, et al. Irisin and secondary osteoporosis in humans. Int J Mol Sci. 2022;23(2):690.
12. Posa F, Zerlotin R, Ariano A, et al. Irisin role in chondrocyte 3D culture differentiation and ıts possible applications. Pharmaceutics. 2023;15(2):585.
13. Chen T, Peng Y, Hu W, et al. Irisin enhances chondrogenic differentiation of human mesenchymal stem cells via Rap1/PI3K/AKT axis. Stem Cell Res Ther. 2022;13(1):392.
14. Wang FS, Kuo CW, Ko JY, et al. Irisin mitigates oxidative stress, chondrocyte dysfunction and osteoarthritis development through regulating mitochondrial integrity and autophagy. Antioxidants (Basel). 2020;9(9):810.
15. Utsunomiya H, Gao X, Cheng H, et al. Intra-articular ınjection of bevacizumab enhances bone marrow stimulation-mediated cartilage repair in a rabbit osteochondral defect model. Am J Sports Med. 2021;49(7):1871-82.
16. Li W, Jiang CY, Wang ZW, Xiao DM. Intraarticular injection of bevacizumab in treatment of osteoarthritis: a laboratory research on a rabbit model. Beijing Da Xue Xue Bao Yi Xue Ban. 2016;48(2):203-9.
17. Nagai T, Sato M, Kobayashi M, Yokoyama M, Tani Y, Mochida J. Bevacizumab, an anti-vascular endothelial growth factor antibody, inhibits osteoarthritis. Arthritis Res Ther. 2014;16(5):427.
18. Meng X, Ziadlou R, Grad S, et al. Animal models of osteochondral defect for testing biomaterials. Biochem Res Int. 2020;2020:9659412.
19. Tsai WY, Wu JL, Liu CC, et al. Early intraarticular injection of hyaluronic acid attenuates osteoarthritis progression in anterior cruciate ligament-transected rats. Connect Tissue Res. 2013;54(1):49-54.
20. van den Borne MP, Raijmakers NJ, Vanlauwe J, et al. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. Osteoarthritis Cartilage. 2007;15(12):1397-402.
21. Pineda S, Pollack A, Stevenson S, Goldberg V, Caplan A. A semiquantitative scale for histologic grading of articular cartilage repair. Acta Anat (Basel). 1992;143(4):335-40.
22. Lu J, Zhang H, Cai D, et al. Positive-feedback regulation of subchondral H-type vessel formation by chondrocyte promotes osteoarthritis development in mice. J Bone Miner Res. 2018;33(5):909-20.
23. Lee S, Nemeno JG, Lee JI. Repositioning bevacizumab: a promising therapeutic strategy for cartilage regeneration. Tissue Eng Part B Rev. 2016;22(5):341-57.
24. Kan T, He Z, Du J, et al. Irisin promotes fracture healing by improving osteogenesis and angiogenesis. J Orthop Translat. 2022;37:37-45.
25. Oranger A, Zerlotin R, Buccoliero C, et al. Irisin modulates ınflammatory, angiogenic, and osteogenic factors during fracture healing. Int J Mol Sci. 2023;24(3):1809.
26. Zhang X, Nan K, Zhang Y, et al. A novel injectable hydrogel prepared from phenylboronic acid modified gelatin and oxidized-dextran for bone tissue engineering. Int J Biol Macromol. 2024;261(Pt 1):129666.
27. Vadala G, Di Giacomo G, Ambrosio L, et al. Irisin recovers osteoarthritic chondrocytes in vitro. Cells. 2020;9(6):1478.
28. Li X, Zhu X, Wu H, et al. Roles and mechanisms of irisin in attenuating pathological features of osteoarthritis. Front Cell Dev Biol. 2021;9:703670.
29. Mao Y, Xu W, Xie Z, Dong Q. Association of irisin and CRP levels with the radiographic severity of knee osteoarthritis. Genet Test Mol Biomarkers. 2016;20(2):86-9.
30. Arias-Reyes C, Soliz J, Joseph V. Mice and rats display different ventilatory, hematological, and metabolic features of acclimatization to hypoxia. Front Physiol. 2021;12:647822.