To cite this article: Pepele Kurdal D, Akgun E, Baris Y, Midi A, Akpolat AO. Comparative efficacy of synthetic peptide, platelet-rich plasma, and hyaluronic acid alone or in combination in microfracture treatment of focal chondral defects. Turk J Clin Lab 2024; 3: 426-434

Research Article

Comparative efficacy of synthetic peptide, platelet-rich plasma, and hyaluronic acid alone or in combination in microfracture treatment of focal chondral defects

Fokal kondral defektlerin mikro kırık tedavisinde sentetik peptit, trombositten zengin plazma ve hyaluronik asidin tek başına veya kombinasyon halinde karşılaştırmalı etkinliği

Demet Pepele Kurdal*[1], Erkan Akgun[2], Yilmaz Baris[1], Ahmet Midi[3], Ahmet Onur Akpolat[1]

[1]Department of Orthopaedics Surgery and Traumatology, University of Health Sciences, Fatih Sultan Mehmet Training and Research Hospital, İstanbul, Turkey,

[2]Department of Orthopedics and Traumatology, Ankara Etlik City Hospital, Ankara, Turkey,

[3]Department of Pathology, Bahçeşehir University Faculty of Medicine, İstanbul, Turkey.

ABSTRACT

Aim: Although previous limited studies have evaluated the efficacy of adjuvants used alone or in combination to augment microfracture (MF) treatment for focal chondral defects, there are no studies comparing the outcomes of the synthetic peptide (SP) with other adjuvants such as platelet-rich plasma (PRP), hyaluronic acid (HA), or their combinations. This study aimed to evaluate whether the efficacy of MF treatment in focal chondral defects is influenced by the use of adjuvants either alone or in combination.

Material and Methods: Thirty-six rats were included in the study. Group 1 received MF alone, Group 2 received PRP after MF, Group 3 received HA after MF, Group 4 received a SP after MF, Group 5 received a SP plus PRP therapy after MF, and Group 6 received a SP plus HA therapy. The knees of the rats were assessed according to the International Cartilage Repair Society (ICRS) Cartilage Repair Assessment 1 (ICRS-1) and 2 (ICRS-2).

Results: The median ICRS-1 and ICRS-2 scores in Group 1 were lower compared to the other groups, while these scores in Group 2 and Group 4 were similar and higher than the other groups. Also, these scores in Group 5 and Group 6 were similar and lower compared to Group 3 (Group 1: 1 vs. Group 2: 12 vs. Group 3: 9 vs. Group 4: 11 vs. Group 5: 7 vs. Group 6: 7, $p < 0.001$ for ICRS-1 scores; Group 1: 0 vs. Group 2: 85 vs. Group 3: 70 vs. Group 4: 80 vs. Group 5: 45 vs. Group 6: 45, $p < 0.001$ for ICRS-2 scores).

Conclusion: In the MF treatment of focal chondral defects, SP, PRP, and HA injections have a beneficial adjuvant effect based on macroscopic and histopathological findings. However, the combination of these adjuvants is less beneficial than their individual usage.

Keywords: cartilage defects, hyaluronic acid, microfracture, plasma rich protein, synthetic peptide

Corresponding Author*: Demet Pepele Kurdal, Department of Orthopaedics Surgery and Traumatology, University of Health Sciences, Fatih Sultan Mehmet Training and Research Hospital, İstanbul, Turkey.
E-mail: drdmtppl@yahoo.com
Orcid: 0000-0003-2669-3020
Doi: 10.18663/tjcl.1544301
Recevied: 05.09.2024 accepted: 23.09.2024

TJCL Volume 15 Number 3 p: 426-434

ÖZ

Amaç: Daha önceki sınırlı çalışmalarda, fokal kondral defektlerin mikrokırık (MF) tedavisini güçlendirmek için tek başına veya kombinasyon halinde kullanılan adjuvanların etkinliği değerlendirilmiş olsa da, sentetik peptidin (SP) diğer adjuvanlar olan plazma zengin protein (PRP), hyaluronik asit (HA) ve bunların kombinasyonlarıyla sonuçlarını karşılaştıran bir çalışma bulunmamaktadır. Bu çalışmada fokal kondral defektlerde MF tedavisinin etkinliğinin adjuvanların tek başına veya kombinasyon halinde kullanılmasıyla etkilenip etkilenmediğini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Çalışmaya 36 sıçan dahil edilmiştir. Grup 1'e yalnızca MF uygulandı, Grup 2'ye MF sonrası PRP, Grup 3'e MF sonrası HA, Grup 4'e MF sonrası SP, Grup 5'e MF sonrası SP ve PRP kombinasyonu ve Grup 6'ya MF sonrası SP ve HA kombinasyonu uygulandı. Sıçanların dizleri, Uluslararası Kıkırdak Onarım Derneği (ICRS) Kıkırdak Onarım Değerlendirmesi 1 (ICRS-1) ve 2 (ICRS-2) kriterlerine göre değerlendirildi.

Bulgular: Grup 1'de medyan ICRS-1 ve ICRS-2 skorları diğer gruplara göre daha düşüktü, Grup 2 ve Grup 4'te ise bu skorlar benzer olup diğer gruplara kıyasla daha yüksekti. Ayrıca, Grup 5 ve Grup 6'daki bu skorlar benzerdi, ancak Grup 3'e kıyasla daha düşüktü (ICRS-1 skorları için: Grup 1: 1 vs. Grup 2: 12 vs. Grup 3: 9 vs. Grup 4: 11 vs. Grup 5: 7 vs. Grup 6: 7, $p < 0,001$; ICRS-2 skorları için; Grup 1: 0 vs. Grup 2: 85 vs. Grup 3: 70 vs. Grup 4: 80 vs. Grup 5: 45 vs. Grup 6: 45, $p < 0,001$).

Sonuç: Fokal kondral defektlerin MF tedavisinde SP, PRP ve HA enjeksiyonlarının makroskopik ve histopatolojik bulgulara dayalı olarak yararlı bir adjuvan etkisi vardır. Ancak, bu adjuvanların kombinasyonları, tek başına kullanımlarına göre daha az fayda sağlamaktadır.

Anahtar Kelimeler: hyaluronik asit, mikrokırık, kıkırdak defektleri, plazma zengin protein, sentetik peptit

Introduction

Focal chondral defects of the cartilage are currently an orthopedic problem with increasing incidence in young adults, especially due to the growing tendency to engage in contact sports [1-3]. According to the International Cartilage Repair Society (ICRS) classification, the frequency of grade 3–4 lesions (isolated cartilage lesion requiring repair) is between 16% and 41% [3, 4]. The low healing potential of the cartilage makes treatment very difficult. Treatment modalities vary according to the size of the lesion [5-8]. One of the most frequently used treatment techniques is the microfracture (MF) method, a bone marrow stimulation method which is cost-effective. It can be performed easily in a single session and yields satisfactory outcomes [9, 10]. This method aims to create tissue in the defect site that is closest to normal hyaline cartilage, although the tissue formed is not stable [11-13].

Previous experimental studies have shown that the use of adjuvants may enhance the efficacy of MF treatment, while there is limited research available on this subject [14-16]. These studies have evaluated the efficacy of MF treatment enriched with adjuvants such as platelet-rich plasma (PRP), hyaluronic acid (HA), collagen, and collagen-forming agents, which are believed to have positive effects on cartilage lesion regeneration. A few experimental models compare the individual and combined efficacies of adjuvant therapies in MF treatment with a control group. However, there are no studies comparing the effects of synthetic peptides and their combination forms.

We hypothesized that incorporating synthetic peptide (SP) adjuvant and its combinations into the MF technique could enhance treatment outcomes. Thus, this study aimed to assess the effectiveness of using synthetic peptide, either alone or in combination, in treating cartilage lesions using the MF approach.

Material and Methods

This prospective experimental study was approved by the Acıbadem University Experimental Animal Studies Local Ethics Committee (Jan 16, 2019 No: 2019/02) and was conducted in the research unit and operating rooms of Acıbadem University Experimental Animals Breeding and Research Center. Our study began with 36 experimental animals and was completed with the same number of rats without any loss. The sample size calculated for the study provided a sampling power of 0.90.

Study protocol

The study included 36 Sprague-Dawley female rats weighing 280–330 grams that attained skeletal maturity. The animals were

maintained at a controlled temperature (21°C) and illumination (12-hour day and night cycle) under conventional conditions in standard single cages that restricted their movement. The rats had free access to water and food ad libitum.

The rats were anesthetized by injecting ketamine hydrochloride (Ketalar®, Eczacıbaşı, İstanbul, Turkey) (90 mg/kg) and Xylazine® (Rhompun, Bayer, İstanbul, Turkey) (10 mg/kg) intramuscularly. Cefazolin 20 mg/kg was administered for prophylaxis of surgical site infection. Following skin preparation, the patella was dislocated laterally with medial arthrotomy by making a 2-cm medial parapatellar incision in the right knee region to access the joint. Using the method described by Kawasaki [17] and Yoshoika [18], a full-thickness chondral defect with a width of 1.2 mm and a depth of 1 mm (but not extending into the subchondral region) was created in the femoral medial condyle using a Burr with a diameter of 1.2 mm (Figure 1A-C). After the defects were created, the rats were observed with no additional procedure for 4 weeks to have chronic focal chondral defect. After 4 weeks, the rats were randomly divided into six groups, with six rats in each group. Each rat was kept in a separate cage to prevent carnivorism. The groups started the intervention treatment at the fourth week, and continued until the sixth week. At the end of the 6th week of the treatment rats were sacrified for the evaluation. The groups were organized as follows: Group 1 (control group) was applied MF only; Group 2 received PRP after MF; Group 3 received HA after MF; Group 4 received SP after MF; Group 5 received SP and PRP after MF; Group 6 received SP and HA after MF (Table 1).

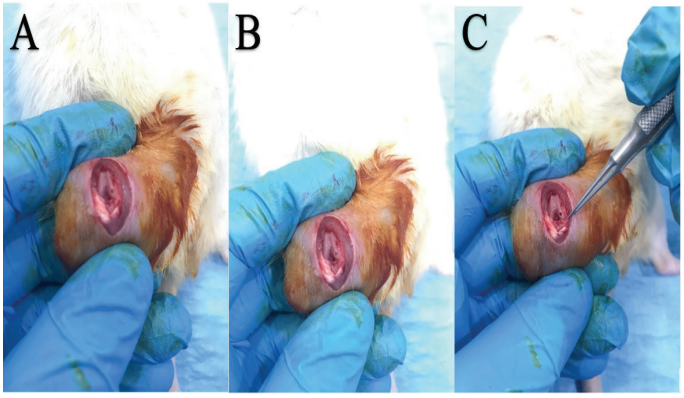

Figure 1. A: Creation of a subchondral defect with a 2-mm Burr in the medial femoral condyle, **B:** The view of the defect in the 4th week, **C:** The image after creating two MF with a 0.6-mm Kirschner wire at the defect.

Table 1. Surgical procedures and adjuvant therapies in the groups

Group	Procedure
1	Two MFs were created with a depth of 2 mm and a diameter of 0.6 mm using a 0.6 mm Kirschner wire (Figure 2)
2	MF + PRP prepared from autologous blood (1 cc) was performed. Half of the 250 µl PRP was injected into the microfracture site and the other half into the knee joint after suturation
3	MF + HA: One half of the 250 µl of HA was injected into the microfracture site, and the other half was injected into the knee joint after suturation
4	MF + SP: One half of the 250 µl SP was injected into the microfracture site, and the other half was injected into the knee joint after suturation.
5	MF + SP + PRP: One half of the 250 µl SP was injected into the microfracture site, and the other half was injected into the knee joint after suturation. Two hours later, 150 µl of PRP prepared from autologous blood was injected into the knee joint.
6	MF + SP + HA: One half of the 250 µl SP was injected into the microfracture site, and the other half was injected into the knee joint after suturation. Two hours later, 150 µl of HA was injected.

HA, hyaluronic acid; MF, microfracture; PRP, platelet-rich plasma; SP, synthetic peptide.

Preparation of adjuvants

PRP was prepared from autologous blood. For this purpose, 1 cc of blood was drawn from each rat. T-Lab tubes containing 3.2% sodium citrate per cubic centimeter were used. A single-motor Electromag centrifuge was utilized to process the blood, with tubes centrifuged at 2,600 rpm for 8 minutes. Approximately 250–300 µl of autologous PRP was obtained from each rat. The mean platelet value in the PRP sample was found to be 16.9 ± 5.6 x 10^3 platelets/µL. Half of the 250 µL of PRP was injected into the MF site, while the other half was injected into the knee joint.

The HA preparation (Altergon TDS) used in the study had a viscosity of 1.5–2.3 m^3/kg and a molecular weight of 1200 KDa. The intra-articular peptide preparation contained collagen tripeptide (IDEA drug).

Post-operative follow-up

No immobilization method was applied to the rats. They were monitored for 6 weeks, provided with comparable environments, nutrition, and care. At the end of this period, the animals were sacrificed using high-dose anesthesia.

Macroscopic and histopathological evaluation

The distal one-third diaphysis of the femur was incised with a saw in the right knee of all groups, and the defected areas were removed for evaluation. Macroscopic evaluation was performed first, using the scoring system of the International Cartilage Repair Society (ICRS-1) [19]. Both ICRS-1 macroscopic cartilage evaluation scores and ICRS-2 histopathological cartilage evaluation scores were applied, which are validated for use in Autologous Chondrocyte Implantation (ACI) and MF [20].

ICRS-1 and ICRS-2 evaluation scores are shown in Tables 2 and 3. The specimens in the groups were evaluated separately by blinded orthopedic surgeon and histopathologist. The main researcher was aware of the rats in the groups while the evaluation. There was a high inter-observer correlation ($r > 0.8$) in ICRS-1 and ICRS-2 scores. ICRS-1 and ICRS-2 were applied to the specimens for macroscopic and microscopic examination, and the average scores of the scientists (orthopedic surgeon and histopathologist) were recorded.

Table 2. Scores of the evoluation system International Cartilage Repair Society Cartilage Repair Assessment-1

Characteristic	Grading	Score
Degree of defect repair	Level of surrounding cartilage	4
	75% repair of defect depth	3
	50% repair	2
	25% repair	1
	0% repair	0
Integration to border zone	Complete integration with border zone	4
	Demarcating border < 1 mm	3
	3/4 of repair tissue integrated, 1/4 with notable border > 1 mm	2
	1/2 of repair integrated with surrounding cartilage, 1/2 with a notable border > 1 mm	1
	From no contact to 1/4 of repair integrated with surrounding cartilage	0
Macroscopic appearance	Intact smooth surface	4
	Fibrillated surface	3
	Small, scattered fissures or cracks	2
	Several, small or few but large fissures	1
	Total degeneration of defect area	0
Total, max		12

The specimens were fixed in 10% formaldehyde for 1 week and decalcified following fixation. Subsequently, they were divided into half longitudinally along the middle-line under the guidance of the defect area and taped for tissue follow-up. The specimens were washed in running water for 3 hours for deacidification. Then, a 13-hour follow-up was performed on an automatic tissue processor (Shanden Exelsior, ES). In this procedure, the tissues were subjected sequentially to formaldehyde twice for 30 minutes, alcohol six times for 60 minutes each, xylene three times for 60 minutes each, and paraffin twice for 60 minutes in the first and 80 minutes in the second cycle. Following tissue processing, 2 µm-thick sections of the paraffin-embedded tissues were stained with Hematoxylin & Eosin, Safranin O, and Toluidine Blue. The sections were evaluated under light microscope (Olympus Bx-50, Olympus Optical). The regenerative tissue thickness on the subcondral bone was measured with an oculometer. Some macroscopic and histopathological specimens of the groups were shown in Figures 2, 3.

Table 3. Scores of the evoluation system International Cartilage Repair Society Cartilage Repair Assessment-2

Histological parameters	Scores
Tissue morphology	0%: full-thickness collagen fibers, 100%: normal cartilage
Matrix staining (metachromasia)	0%: no staining, 100%: full metachromasia
Cell morphology	0%: no round/oval cells, 100%: mostly round/oval cells
Chondrocyte clustering (four or more grouped cells)	0%: present, 100%: absent
Surface architecture	0%: delamination, or major irregularity, 100%: smooth surface
Basal integration	No integration, 100%: complete integration
Formation of a tidemark	0%: no calcification front, 100%: tidemark
Subchondral bone abnormalities/marrow fibrosis	0% abnormal, 100%: normal marrow
Inflammation	0%: present, 100%: absent
Abnormal calcification/ossification	Present, 100%: absent
Vascularization (within the repaired tissue)	Present, 100%: absent
Surface/superficial assessment	0%: total loss or complete disruption, 100%: resembles intact articular cartilage
Mid/deep zone assessment	0%: fibrous tissue, 100%: normal hyaline cartilage
Overall assessment	0%: bad (fibrous tissue), 100%: good (hyaline cartilage)

Statistical analysis

All analyses were conducted using the Number Cruncher Statistical System (NCSS LLC, Utah, USA) software. Descriptive statistical methods (mean, standard deviation, median,

frequency, percentage, minimum, and maximum) were used to evaluate the study data. The suitability of the quantitative data to normal distribution was tested by Shapiro–Wilk test and histograms. Kruskal–Wallis test and Dunn–Bonferroni test were used to compare more than two groups of quantitative variables that did not exhibit normal distribution. P-value < 0.05 was considered statistically significant. The intra-class correlation coefficient was used to evaluate the agreement between the measurements (excellent, r > 0.8; good, r ≤ 0.8; moderate, r ≤ 0.6; fair, r ≤ 0.4; and poor, r ≤ 0.2). The sample size, power level, and effect size were calculated using G * Power Version 3.1.7.

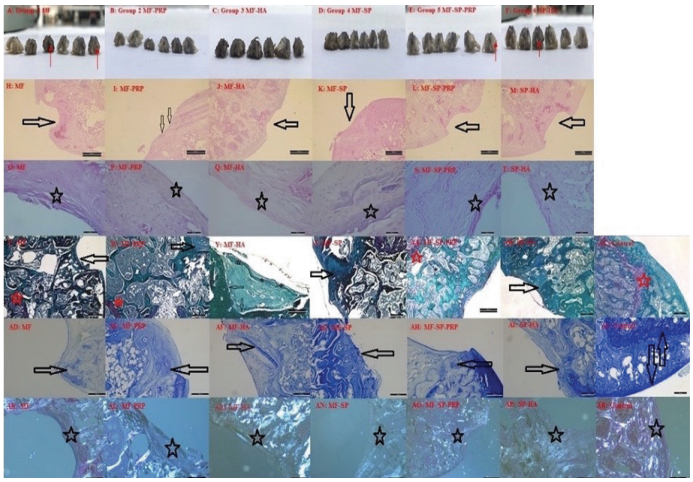

Figure 2. A: Defect area in MF group, **B, C, D:** Macroscopically healed in MF-PRP group, MF-HA group and MF-SP group. E: In MF-SP+PRP group and F: MF-SP+HA groups macroscopically, defect area was shown with arrow in some samples. H: Large defect area in MF Group H&EX40, I: Largely healed defect area in the MF-PRP group H&EX40. J: Partially healed defect area in MF-HA group H&EX40, K: In the MF-SP group, fibrous tissue on the surface and the defect area was healed with new bone formation under it. H&EX40. L: Partially healed defect area in MF-SP+PRP group and M: SP+HA H&EX40. O,S,T: fibrous tissue in MF, MF-SP-PRP and SP-HA groups, hyaline cartilage in P,Q,R:MF+PRP, MF-HA and MF-SP groups, Hematoxylin and Eosin X100. V-AC: Safranin stain appears to stain the growth plate pink (arrow), no surface staining (star) X40: Q: İnitial bone formation at the surface (star) in the SP Group, full integration at the basal (arrow), Safranin OX200. AD-AJ: Positive staining of cartilage surface and newly formed cartilage tissue. Toluidine BlueX100. AK-AR: Polarized light microscopic appearance, collagen fibrils are observed to be light in color.

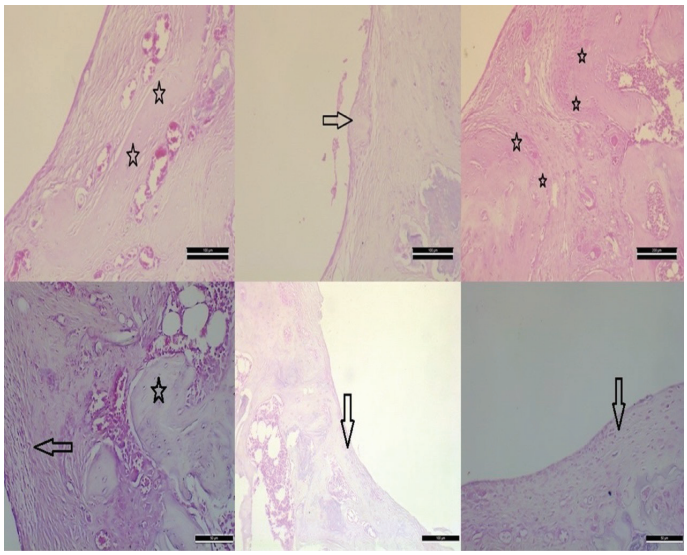

Figure 3. A: Focused view of the initial formation of the hyaline bone in the PRP Group H&EX200, **B:** Hyaline bone formation in the defect area on the surface in the PRP Group H&EX400, **C:** Focused view of new bone formation in SP Group H&EX200, D: Fibrous cartilage (star) and new bone formation (arrow) in Group 5, E: Fibrous cartilage formation on the surface in 5 groups H&EX100. F: Fibrous cartilage close-up view H&EX200

Results

The median ICRS-1 score in Group 1 was lower compared to the other groups, while the median ICRS-1 scores in Group 2 and Group 4 were similar and higher than the other groups. In contrast, the median ICRS-1 scores in Group 5 and Group 6 were similar and lower compared to Group 3 (Group 1: 1 vs. Group 2: 12 vs. Group 3: 9 vs. Group 4: 11 vs. Group 5: 7 vs. Group 6: 7, p < 0.001) (Table 4).

The median ICRS-2 score in Group 1 was found to be lower than in the other groups. In comparison, Group 2 and Group 4 had similar median ICRS-2 scores, which were higher than those in the remaining groups. The median ICRS-2 scores in Group 5 and Group 6 were similar and lower compared to Group 3 (Group 1: 0 vs. Group 2: 85 vs. Group 3: 70 vs. Group 4: 80 vs. Group 5: 45 vs. Group 6: 45, p < 0.001) (Table 5).

The subgroups of ICRS-2 were also compared statistically between the groups. Group 2's tissue morphology score was comparable to that of Group 4, and both groups had higher scores than the other groups. The tissue morphology score of Group 3 was higher compared to Group 5 and Group 6. Group 1 had the lowest score (Group 1: 0 vs. Group 2: 32.5 vs. Group 3: 17.5 vs. Group 4: 27.5 vs. Group 5: 10 vs. Group 6: 10, p < 0.001) (Table 5). While the inflammation scores in Group 2 and Group 4 were similar and lower than in the other groups, no significant differences in inflammation scores were found among the remaining groups (Group 1: 100 vs. Group 2: 55 vs. Group 3: 95 vs. Group 4: 50 vs. Group 5: 100 vs. Group 6: 100, p < 0.001) (Table 5) (Figure 4).

Table 4. Evaluation of the International Cartilage Repair Society Cartilage Repair Assessment-1 (ICRS-1) Scores across the Groups

ICRS-1	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	P-value
Normal, n (%)	0	3 (50.0)	0	2 (33.3)	0	0	0.001*
Close to Normal, n (%)	0	3 (50.0)	6 (100)	4 (66.7)	3 (50.0)	4 (66.7)	
Abnormal, n (%)	6 (100.0)	0	0	0	3 (50.0)	2 (33.3)	
Median (Q1–Q3)	1bcdef (0-2)	12acef (10-12)	9abcef (8-10)	11acef (9-12)	7abcd (6-8)	7abcd (6-8)	0.001*

The data are expressed as median (IQR) or number (%). * P <0.05 shows statistical significance. a: vs. group 1, b: vs. group 2, ac: vs. group 3, d: vs. group 4, e: vs. group 5, f: vs. group 6.

Table 5. Evaluation of the International Cartilage Repair Society Cartilage Repair Assessment-2 (ICRS-2) Scores across the Groups

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	P-value
Tissue morphology (under polarized light)	0^{bcdef} (0-0)	32.5^{acef} (20-35)	17.5^{abef} (10-20)	27.5^{acef} (20-30)	10^{abcd} (5-10)	10^{abcd} (5-10)	0.001 **
Matrix staining (Metacromazia)	0^{bcdef} (0-0)	15^{adef} (10-20)	20^{adef} (10-20)	25^{abc} (20-40)	25^{abc} (20-30)	25^{abc} (20-30)	0.001 **
Cell morphology	0^{bcdef} (0-0)	85^{acef} (70-90)	60^{abdef} (50-70)	75^{acef} (70-80)	42.5^{abcd} (30-50)	37.5^{abcd} (30-50)	0.001 **
Chondrocyte clustering	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	0.999
Surface architecture	0^{bcdef} (0-0)	80^{aef} (70-90)†	70^{aef} (60-70)	75^{aef} (70-90)	25^{abcd} (20-30)	20^{abcd} (20-20)	0.001 **
Basal integration	0^{bcdef} (0-0)	100^{a} (100-100)	100^{a} (100-100)	100^{a} (100-100)	100^{a} (100-100)	100^{a} (100-100)	0.001 **
Formation of a Tidemark	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.999
Subchondral bone abnormality/bone marrow fibrosis	50^{bef} (40-60)	70^{aef} (50-80)	45^{bef} (30-60)	45^{bef} (40-55)	30^{abcd} (25-40)	30^{abcd} (20-30)	0.001 **
Inflammation	100^{bd} (100-100)	55^{acef} (40-70)	95^{bd} (90-100)	50^{acef} (40-60)	100^{bd} (90-100)	100^{bd} (90-100)	0.001 **
Abnormal calcification/ossification	0^{bcdef} (0-0)	100^{a} (100-100)	100^{a} (100-100)	100^{a} (100-100)	100^{a} (100-100)	100^{a} (100-100)	0.001 **
Vascularization	0^{bcdef} (0-0)	80^{acef} (70-80)	55^{abd} (40-60)	75^{acef} (60-90)	45^{abd} (40-60)	50^{abd} (40-60)	0.001 **
Surface evaluation	0^{bcdef} (0-0)	85^{acef} (80-90)	65^{abdef} (50-80)	85^{acef} (80-90)	50^{abcd} (40-60)	45^{abcd} (40-50)	0.001 **
Deep zone assessment	0^{bcdef} (0-0)	20^{a} (20-30)	17.5^{a} (10-20)	25^{a} (20-30)	15^{a} (10-20)	15^{a} (10-20)	0.001 **
General assessment	0^{bcdef} (0-0)	85^{acef} (80-90)	70^{abdef} (70-80)	80^{acef} (80-90)	45^{abcd} (30-70)	45^{abcd} (30-60)	0.001 **

The data are expressed as median (IQR) or number (%). * P <0.05 shows statistical significance. a: vs. group 1, b: vs. group 2, ac: vs. group 3, d: vs. group 4, e: vs. group 5, f: vs. group 6.

Discussion

This study demonstrated that the use of PRP, SP, and HA alone in the MF treatment of focal chondral defects is more effective compared to the combined adjuvant therapy groups. There are a limited number of studies on the combined use of PRP and MF in osteochondrol defects. In a rat study, Hapa et al. reported that the histopathological outcomes of the MF + PRP Group at 6 weeks were better than those of the MF only group [14]. Using a sheep model with a follow-up duration of 12 months, Milano et al. found that the MF + liquid PRP and MF + gel PRP groups were superior to the MF only group with respect to the integration with the surrounding intact tissue, cartilage thickness, and chondrocyte clustering [21]. Our study found that the MF + PRP treatment group achieved better histopathological scores compared to the other treatment groups.

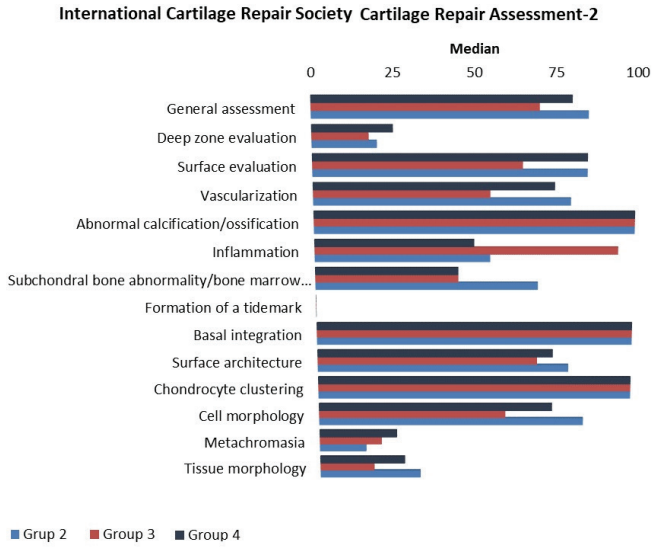

Figure 4: Distribution of the International Cartilage Repair Society Cartilage Repair Assessment-2 Scores across the Group 2, Group 3, and Group 4.

It was determined that intra-articular HA injection prevented cartilage degeneration, decreased synovial inflammation, and increased proteoglycan synthesis [22]. However, the effectiveness of HA in osteoarthritis and the effect of HA injection after MF on the outcomes of the MF technique remain controversial [16, 23-25]. Some studies showed that HA had positive therapeutic effects in the MF treatment of chondral lesions in terms of cartilage regeneration [16, 24]. However, another study demonstrated that the histopathological outcomes of the HA groups in both the early and late periods were not superior to those of the group that received saline injections. In this study, researchers discerned that integration into the surrounding intact cartilage tissue was poor in all groups [25]. In our study, the histopathological scores of the MF + HA treatment group were observed to be higher than those of the MF alone group and the combined treatment groups. Additionally, the ICRS-1 and ICRS-2 scores were similar in the MF + PRP and MF + SP treatment groups but were higher compared to the other groups. In the MF + HA group, these scores were higher than those in the MF-only group and the other combined treatment groups. On the other hand, the tissue morphology scores were higher in the MF + PRP and MF + SP treatment groups than those of the other groups.

In a clinical study, Kesiktaş et al. evaluated 52 patients with osteoarthritis. They divided the patients into HA, PRP, and SP injection groups. During a 3-month follow-up period, they showed a decrease in pain scores and an increase in functional scores in all groups [15]. However, no study evaluating the use of MF augmented with SP in focal chondral defects exists in the literature. The findings of this study not only support the work of Kesiktaş et al., but also show that the ICRS-1, ICRS-2, and tissue morphology scores were higher in the MF + PRP and MF + SP groups.

SP may offer an advantage in clinical practice compared to PRP adjuvant. SP has an advantage in treatment compared to PRP adjuvants. Unlike PRP preparation, it does not require blood collection from the patient or processing under sterile conditions. There are a few studies in the literature evaluating the combined intra-articular use of adjuvant therapies. Researchers have proposed that both adjuvants work through different mechanisms and augment each other's effectiveness in in vitro studies [26, 27]. Naraoka et al. observed that the combined use of SP and HA in the early period of osteoarthritis was more effective than using HA or SP alone and that the combined use increased cell clustering and type II collagen synthesis [28]. However, there are no studies in the literature evaluating the individual and combined use of SP and PRP to augment MF for the treatment of focal chondral defects. The results of our study differ from those in the literature. This study established that the combined use of different adjuvants to augment MF for the treatment of focal chondral defects is more beneficial than MF alone but less effective than the individual use of PRP, HA, and SP. We believe that this finding may be related to the effective dose adjustment.

There were several limitations in our study. First, there are structural differences between rat cartilage and human cartilage, which is a significant limitation. We were unable to enforce an immobilization period for the rats after the MF procedure, which was crucial for effective cartilage treatment. The short follow-up period of the procedure and the use of only female rats were other limitations. However, considering that the cartilage regeneration time of rats is 40–72 days [29-31], and our aim was to compare the efficacy of the adjuvants in augmenting MF for the treatment of focal chondral defects rather than to improve the recovery time, the short follow-up duration was not deemed a significant drawback. Furthermore, our study did not include detailed planning of the dosages of adjuvants and their combinations. In the literature, adjuvants were used at the same dosages in experimental studies [16, 23, 25]. Although the number of rats was limited, power analysis was found to be sufficient. Finally, this study did not evaluate the potential effects of repeated doses of the adjuvants. This could provide more comprehensive insights into the long-term efficacy and safety of these treatments. We believe that

our findings are likely to guide more comprehensive studies in the future. Long-term clinical studies are needed to obtain practicable information.

Conclusion

In the MF treatment of chondral lesions, PRP, SP, and HA injections stimulate regeneration based on the macroscopic and histopathological findings. However, the combined use of these adjuvants is less beneficial than their individual usage.

Funding

The authors declared that this study has received no financial support.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Ethics Approval

The study was approved by the Acıbadem University Experimental Animal Studies Local Ethics Committee (Jan 16, 2019 No: 2019/02).

Availability of Data and Material

The data that support the findings of this study are available on request from the corresponding author.

Authors' contribution

Concept – D.P.K., Design- D.P.K., Data collection and/or processing - D.P.K., E.A., Y.B., A.M., and A.O.A., Analysis and/or interpretation - D.P.K., E.A., Y.B., A.M., and A.O.A., Writing – D.P.K., Critical review- E.A., Y.B., A.M., and A.O.A. All authors read and approved the final version of the manuscript.

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