Original Article

Comparison of the Effects of Microfracture, Soft Callus Implantation, and Matrix-Supported Chondrocyte Implantation in an Experimental Osteochondral Defect Model in Rats

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Abstract

Background: The treatment of cartilage defects remains challenging due to the avascular nature of cartilage. **Aim:** This study investigates the therapeutic effect of soft callus in osteochondral defects and explores the ability of multipotent and pluripotent cells within the callus to form fibrous or hyaline cartilage in the defective area. **Methods:** Twenty-one rats were divided into three equal groups: Group 1 received only microfracture (MF), group 2 received microfracture with autologous chondrocyte implantation (MF+ACI), and group 3 received microfracture with soft callus implantation (MF+SCI). All rats underwent diaphyseal fracture in their left tibias, which was stabilized with a Kirshner wire. One week later, osteochondral defects were created in the right knees of all rats. For group 1, microfracture alone was applied to initiate healing in the defects. In group 2, heterologous chondrocytes, previously harvested from the lateral condyle of a rat's left femur and cultivated in a laboratory environment, were implanted into the microfracture site. In group 3, soft callus tissue obtained from the left tibial fracture was compressed and implanted into the defective area. All groups were sacrificed at the 6th week, and the healing status of the osteochondral defect areas was histopathologically evaluated. **Results:** Macroscopic examination at the end of the study revealed comparable ICRS-1 scores for MF+ACI (group 2) (11.28 ± 1.25) and MF+SCI (group 3) (11.14 ± 0.37), while MF alone (group 1) (4.28 ± 1.25) showed significantly lower results. Microscopic examination yielded similar outcomes. Regarding histological scores, ICRS-2 scores for MF (group 1) (35.30 ± 1.13), MF+ACI (group 2) (47.09 ± 1.63), and MF+SCI (group 3) (43.97 ± 1.49) were statistically significantly lower. **Conclusion:** Defects treated with soft callus implantation demonstrated comparable outcomes to the widely used and gold-standard autologous chondrocyte implantation. When compared to microfracture alone, better macroscopic and microscopic results were achieved with soft callus implantation.

Keywords: *Multipotent cell, osteochondral defects, pluripotent cells, rat, soft callus*

Introduction

Due to the avascular nature of cartilage, the treatment of cartilage defects poses a significant challenge. Mature cartilage is composed of different regions or layers exhibiting variations in extracellular matrix components and their orientations. Each region is provided by a unique combination of cellular, biomolecular, mechanical, and physical factors.

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Enzymatic degradation of the extracellular matrix, deficient formation of new matrix, cell death, and hypertrophic differentiation of cartilage cells contribute to knee osteoarthritis, negatively impacting individuals' lives.[1] Therefore, the treatment of cartilage damage holds a crucial position in orthopedic surgeries. Despite the utilization of various methods such as microfracture, osteochondral autograft transfer, mosaicplasty, and autologous chondrocyte implantation, each has its own morbidities and associated drawbacks.[2]

In recent years, autologous chondrocyte implantation within a tissue matrix has become the gold standard in treatment. Additionally, studies on stem cells and bone marrow cells in the osteochondral defect area have gained popularity. However, in the literature, there is currently no study that demonstrates the healing of cartilage with hyaline cartilage or the use of soft callus in cartilage treatment.[3] This gap is addressed by proposing that the mesenchymal stem cells, chondroblasts, fibroblasts, collagen fibers, and hypertrophic chondrocytes contained within the soft callus may generate hyaline cartilage when implanted into cartilage tissue.[4,5] Considering that mature soft callus does not mature in the same way as pseudoarthrosis tissue and forms a painless joint, it is hypothesized that it could be used in the treatment of osteochondral lesions to create a painless joint. Therefore, in this study, we aimed to experimentally investigate whether soft callus has a therapeutic effect on osteochondral defects and whether multipotent and pluripotent cells within the callus can create fibrous or hyaline cartilage in the defective area. Simultaneously, we sought to determine if soft callus could serve as an alternative treatment option for improving cartilage damage.

Materials and Methods

Ethical issues and animal preparation

The research project was scientifically and ethically approved by the local ethics committee. The numbers specified in the experimental groups were determined by a biostatistics expert through article reviews. According to the Pineda classification, the sample size was determined to be a minimum of $n = 7$ for each group, considering the effect size (0.80759) and SD (1.3) corresponding to the subclassification with the widest standard deviation, and achieving a power of 0.80 at a significance level of $\alpha = 0.05$.

Osteochondral tissue matrix

In our study, HYAFF® (Fidia Advanced Biopolymers, Abano Terme, Italy) was utilized as the osteochondral tissue matrix. This substance is an unaltered biologically degradable scaffold based on hyaluronic acid for hyaline-like cartilage regeneration. When implanted, it preserves its structure to support proliferation and differentiation, completely filling the lesion. As HYAFF® degrades over time, hyaluronic acid is released into the lesion, creating a microenvironment enriched with hyaluronic acid and embryonic-like properties.

Preparation of animals

A total of 21 rats from the Wistar Albino breed, with completed skeletal maturation and weighing 300–400 grams, were included in the study. The animals were housed in standard individual cages under controlled temperature and light conditions, allowing free access to water and food.

Group allocation

The rats were randomly divided into three groups of 7 rats each.

1. Group 1: Microfracture Only (MF)
2. Group 2: Microfracture+Matrix-Supported Chondrocyte Implantation (MF+MCI)
3. Group 3: Microfracture + Soft Callus Implantation (MF+SCI).

Surgical technique

Anesthesia was administered to the rats by intramuscular injection of ketamine hydrochloride (Ketalar®, Eczacıbaşı, Istanbul) and Xylazine® (Rhompun, Bayer, Istanbul). A longitudinal incision was made in the left crural region with saw, passing through the skin, subcutaneous tissue, and fascia to reach the bone. After safe osteotomies of the tibias, they were intramedullary fixed with a Kirschner wire [Figure 1a-c]. In the third group, this area was reopened in the first week to obtain a soft callus [Figure 1d and e].

All rats underwent shaving and disinfection of the surgical area under anesthesia, followed by a 2-cm medial parapatellar incision in the right knee region, reaching the knee joint by dislocating the patella laterally with medial arthrotomy. A full-thickness osteochondral defect of 1.2 mm width and 4-mm depth was created on the medial condyle [Figure 2a and b].

Microfracture was applied to the defects with a 0.6-mm-diameter Kirschner wire [Figure 2c]. Subcutaneous and skin layers were anatomically closed after surgical procedures [Figure 2d].

On the first day, the left tibias of the first group were osteotomized and fixed. During this, a random rat's incision was extended to the knee, and after arthrotomy, a cartilage graft was obtained from the lateral condyle. The harvested cartilage was delivered to the Bahcesehir University Histology Department.

Preparation of cartilage cells for implantation

Cartilage obtained under sterile conditions, as described in the surgical procedure below, was kept in physiological serum at room temperature and delivered to the Bahcesehir University Histology Laboratory. The cartilage was dissected into small pieces and incubated in type 2 collagenase for 48 hours. The separated cells were collected by centrifugation, and the pellet material was washed twice. The cells were incubated at 37°C under 5% CO_2 conditions. Cell counts were performed with each passage, and the cells were transferred to larger cell culture dishes. After ensuring that the cells remained uncontaminated during the approximately 4-week incubation period, the cells were detached using trypsin and transferred to the cartilage scaffold under sterile conditions. The graft was incubated in the laboratory until the surgical procedure, maintaining temperature under sterile conditions, and applied during surgery [Figure 3a–d].

In the first week, osteochondral defects were created in their right knees, followed by microfracture, and sacrificed at week 6.

On the first day, the left tibias of rats in the second group were osteotomized and fixed. In the first week, osteochondral defects were created in their right knees, followed by microfracture. The procedures described above were performed at Bahcesehir University, and chondrocytes soaked in the scaffold were compressed into the defects [Figure 3a]. Animals were sacrificed in week 6.

On the first day, the left tibias of rats in the third group were osteotomized and fixed. In the first week, osteochondral defects were created in their right knees, followed by microfracture, and a soft callus graft was obtained from the fracture line of the left tibia. The harvested soft callus was implanted into the defect by compression [Figure 4a-c]. Animals were sacrificed in week 6.

Evaluation

Macroscopic evaluation

All 21 rats used in the experiment, 7 from group 1 (*n*: 7), 7 from group 2 (*n*: 7), and 7 from group 3 (*n*: 7), were sacrificed at the end of the 6th week. The right knees of the rats were initially evaluated macroscopically according to the International Cartilage Repair Society (ICRS-1) scoring system. In this system, cartilage evaluation includes the following four parameters: degree of defect repair, integration to the border zone, macroscopic appearance, and overall repair assessment[6] [Figure 5].

Microscopic evaluation

Samples were randomized and sent to the pathology laboratory. The materials were fixed in 10% formaldehyde for 1 week. Following fixation, they were decalcified for 5 days using Shandon TBD-2. After the decalcification process, the tissues were subjected to routine tissue follow-up procedures in the pathology laboratory. Next, 2-micron thick sections were taken from paraffin-embedded tissues and stained with hematoxylin-eosin, Safranin O, and toluidine blue. Each sample was labeled with a pathology number and sent for evaluation. To ensure blind evaluation, the pathologist did not know which group the sample belonged to. Histopathological analyses were performed blindly by a pathologist. Sections were evaluated under a light microscope (Olympus Bx50, Olympus Optical). Mucosal thickness was measured with an ocular micrometer.

Several systems have been developed over time for macroscopic and microscopic evaluations in assessing osteochondral lesion treatment.[7,8] While developing these scoring systems, the International Cartilage Research Society (ICRS) introduced Articular Cartilage Repair Evaluation Scores 1 and 2.[6,9] Due to high variability among evaluators, a new histological scoring system consisting of 14 criteria, ICRS II, was developed to assess parameters related to chondrocyte phenotype and tissue structure.[9] ICRS II has been considered superior for reassessment by readers compared to existing histological cartilage repair grading systems. Therefore, in our study, ICRS-1 from macroscopic scoring systems and ICRS-2 from microscopic scoring systems were used [Figure 6].

Statistical evaluation

Statistical analyses were conducted using Number Cruncher Statistical System 2007 (NCSS 2007) Statistical Software (Utah, USA). Descriptive statistical methods such as mean, standard deviation, median, frequency, and ratio were employed for data evaluation. In addition, for intergroup comparisons of parameters that did not show a normal distribution, the Kruskal-Wallis test was utilized, and a *post-hoc* Dunn test was applied to identify the group causing the difference. The results were evaluated at a significance level of $P < 0.05$ with a 95% confidence interval.

Results

Macroscopic findings

The knees of the rats were macroscopically assessed, and ICRS-1 scores were evaluated [Figure 5]. Statistically significant differences were observed among ICRS-1 measurements according to groups ($P < 0.01$). Upon closer examination of the source of significance, the

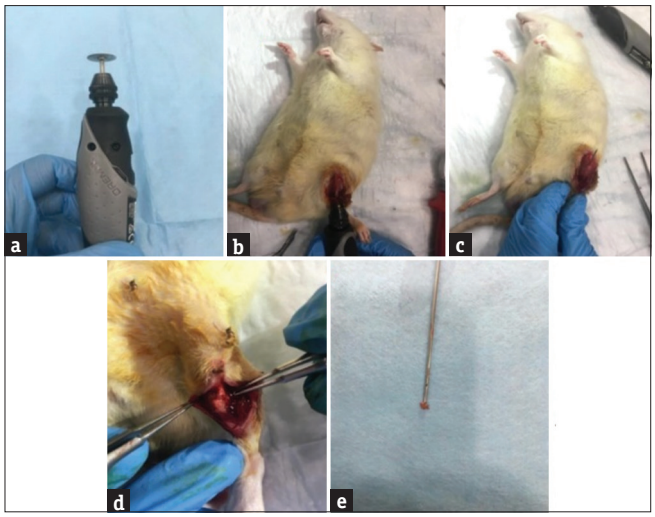

Figure 1: (a) Saw used to create the tibia fracture model. (b and c) Intramedullary fixation of fracture in rats. (d and e) Soft callus graft removal

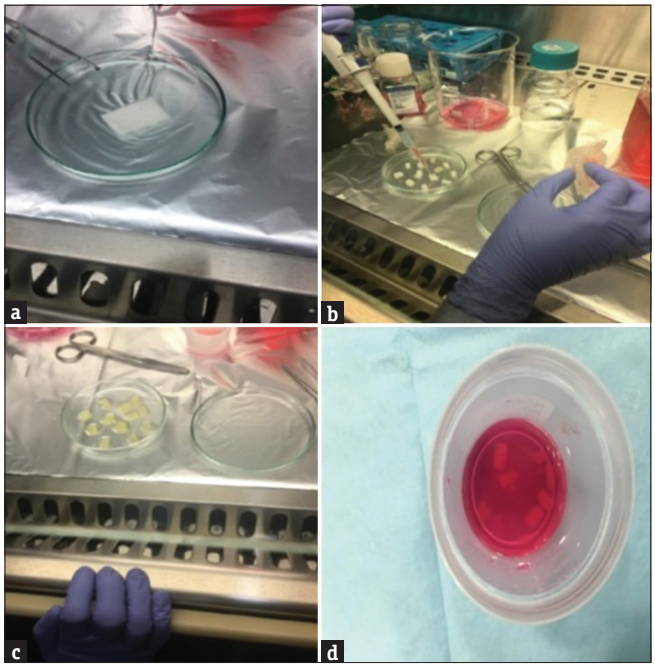

Figure 3: (a) Preparation of cartilage tissue matrix. (b) Chondrocyte transplantation into cartilage tissue matrix. (c) Cartilage tissue matrix seeded with chondrocytes. (d) Chondrocyte-seeded cartilage tissue matrix (day 1 - just before application)

Table 1: Evaluation of ICRS-1 measurements by groups

	ICRS-1		*P*	*Post-Hoc*
	Mean±SD	Median (Q1–Q3)		Dunn test
Group 1	4.28±1.25	5 (2–5)	0.001**	Gr 1-Gr 2
Group 2	11.28±0.48	11 (11–12)		*P*=0.001**
Group 3	11.14±0.37	11 (11–12)		Gr1-Gr 3
				P=0.003**

Kruskal-Wallis test and *post-hoc* Dunn test **$P<0.01$. Q1: First quarter, Q3: Third quarter

overall assessment criteria of group 1 were significantly lower than those of groups 2 and 3 (P = 0.001 and

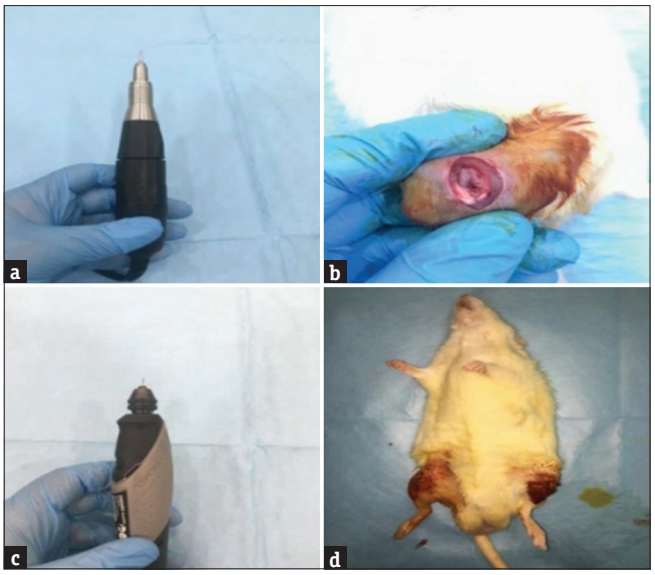

Figure 2: (a) 1.2 mm diameter drill used to create osteochondral defects. (b) Osteochondral defect created in rats. (c) Adjustable 0.6 mm diameter Kirschner wire used to create microcracks. (d) Bilateral closed incision

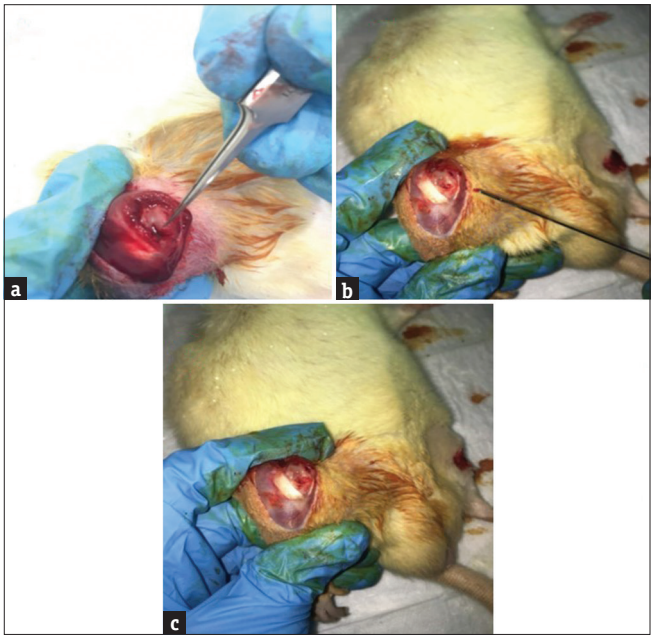

Figure 4: (a) Implantation of chondrocyte-seeded tissue matrix into the defect. (b and c) Implantation of soft callus into the defect

P = 0.003, respectively). No significant difference was found between group 2 and group 3 (P = 1.000 and $P > 0.05$, respectively) [Table 1].

A statistically significant difference was found between ICRS-2 measurements according to groups ($P < 0.01$); When the source of significance was examined, the general evaluation measurements of group 1 were found to be significantly lower than those of group 2 and group 3 (P = 0.001 and P = 0.0028, respectively). No significant difference was detected between group 2 and group 3 (P = 0.157 and $P > 0.05$, respectively) [Table 2].

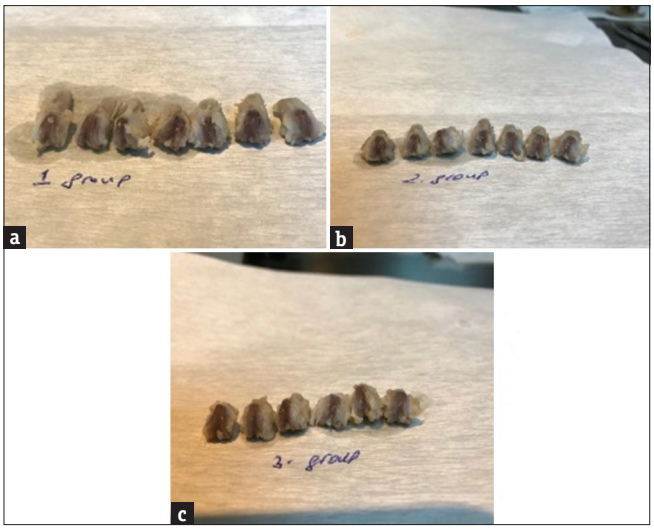

Figure 5: Macroscopic images of the defects. (a) group 1, (b) group 2, (c) group 3

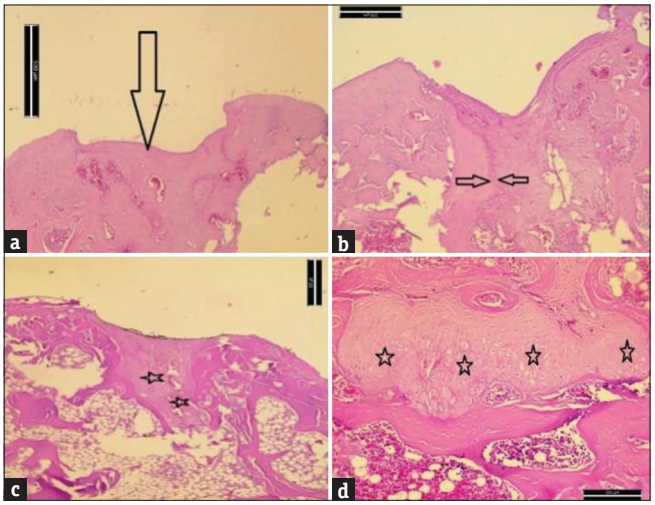

Figure 6: Histological examination of sections taken from knee joints by staining with hematoxylin eosin ((a) group 1, (b) group 2, (c and d) group 3)

Table 2: Evaluation of ICRS-2 measurements according to groups

	ICRS-2		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	35.30±1.13	35.4 (33.6–36.8)	0.001**	Gr 1-Gr 2 *P*=0.001**
Group 2	47.09±1.63	47.1 (44.6–50.0)		
Group 3	43.97±1.49	43.9 (41.4–45.7)		Gr1-Gr 3 *P*=0.028*

Kruskal-Wallis test and *post-hoc* Dunn test. *P<0.05 **P<0.01. Q1: First quarter, Q3: Third quarter

Table 3: Evaluation of surface architecture measurements according to groups

	Surface architecture		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	81.4±7.5	80.0 (75–90)	0.040*	Gr 1-Gr 2 *P*=0.021*
Group 2	92.9±6.4	95.0 (85–100)		
Group 3	82.9±9.9	80.0 (75–90)		Gr3-Gr 2 *P*=0.038*

*P<0.05

Table 4: Evaluation of cell morphology measurements according to groups

	Cell morphology		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	5.0±6.5	0 (0–10)	0.001**	Gr 1-Gr 2 *P*=0.008**
Group 2	29.3±6.7	30 (25–35)		
Group 3	34.3±12.4	30 (25–50)		Gr1-Gr 3 *P*=0.002**

Kruskal-Wallis test and *post-hoc* Dunn test **P<0.01. Q1: First quarter, Q3: Third quarter

Microscopic findings

Surface morphology measurements

Statistically significant differences were observed in surface morphology measurements among the groups (P < 0.05). On closer inspection, group 2 surface morphology measurements were significantly higher than those of group 1 and group 3 (P = 0.021, P = 0.038, and P < 0.05, respectively). No significant difference was found between group 1 and group 3 (P = 0.810 and P > 0.05, respectively) [Table 3].

Cell morphology measurements

Statistically significant differences were found in cell morphology measurements among the groups (P < 0.01). Upon examination, group 1 cell morphology measurements were significantly lower than those of group 2 and group 3 (P = 0.008, P = 0.002, and P < 0.01, respectively). No significant difference was observed between group 2 and group 3 (P = 1.000 and P > 0.05, respectively) [Table 4].

Chondrocyte clustering measurements

Statistically significant differences were found in chondrocyte clustering measurements among groups (P < 0.01). Upon examination, group 1 chondrocyte clustering measurements were significantly lower than those of group 2 and group 3 (P = 0.001 and P = 0.015, respectively). No significant difference was found between group 2 and group 3 (P = 1.000 and P > 0.05, respectively) [Table 5].

A statistically significant difference was found between inflammation measurements according to groups (P < 0.05). When the source of significance was examined, the inflammation measurements of group 1 were found to be significantly higher than that of group 2 (P = 0.033 and P < 0.05, respectively). No significant difference was detected between the other groups (P > 0.05). [Table 6].

Vascularization measurements showed statistically significant differences among the groups (P < 0.05),

Table 5: Evaluation of chondrocyte clustering measurements according to groups

	Chondrocyte Clustering		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	4.3±5.3	0 (0–10)	0.001**	Gr 1-Gr 2 *P*=0.001**
Group 2	33.6±8.0	35 (30–40)		
Group 3	29.3±6.1	30 (25–30)		Gr1-Gr 3 *P*=0.015*

Kruskal-Wallis test and *post-hoc* Dunn test *$P<0.05$ **$P<0.01$. Q1: First quarter, Q3: Third quarter

Table 6: Evaluation of inflammation measurements according to groups

	Inflammation		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	100±0	100 (100–100)	0.033*	Gr 1-Gr 2 *P*=0.030
Group 2	91.4±6.9	90 (90–100)		
Group 3	94.3±7.9	100 (90–100)		

Kruskal-Wallis test and *post-hoc* Dunn test *$P<0.05$. Q1:Birinci çeyreklik, Q3:Üçüncü çeyreklik

Table 7: Evaluation of vascularization measurements according to groups

	Vascularization		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Grup 1	3.6±6.3	0 (0–10)	0.010*	Gr 1-Gr 2 *P*=0.018*
Grup 2	15.0±4.1	15 (10–20)		
Grup 3	14.3±4.5	15 (10–20)		Gr 1-Gr 3 *P*=0.040*

Kruskal-Wallis test and *post-hoc* Dunn test *$P<0.05$. Q1: First quarter, Q3: Third quarter

Table 8: Evaluation of tissue morphology measurements according to groups

	Tissue Morphology		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	0±0	0 (0–0)	0.138	-
Group 2	5±6.5	0 (0–10)		
Group 3	4.3±5.3	0 (0–10)		

Kruskal-Wallis test and *post-hoc* Dunn test Q1: First quartile, Q3: Third quartile

Table 9: Evaluation of matrix staining (metachromasia) measurements according to groups

	Matrix staining (metachromasia)		*P*	*Post-Hoc* Dunn test
	Mean±SD	Median (Q1–Q3)		
Group 1	100±0	100 (100–100)	0.079*	-
Group 2	91.4±6.9	90 (90–100)		
Group 3	94.3±7.9	100 (90–100)		

Kruskal-Wallis test and *post-hoc* Dunn test *$P<0.05$. Q1: First quarter, Q3: Third quarter

with group 1 measurements being significantly lower than those of group 2 and group 3 (P = 0.018 and P = 0.040, respectively). No significant difference was found between group 2 and group 3 (P = 1.000 and $P > 0.05$, respectively) [Table 7].

Other microscopic evaluations

No statistically significant differences were found in tissue morphologies [Table 8], matrix staining [Table 9], abnormal calcification, basal integration, subchondral bone marrow fibrosis, or tidemark formation [Table 10] ($P > 0.05$).

As abnormal calcification, basal integration, and subchondral bone marrow fibrosis measurements were determined as 100 in all cases, evaluation according to groups cannot be made. Tidemark formation was found to be 0 in all cases.

In group 1, intense fibrocartilage tissue was observed in the defect area, but there was no formation of hyaline cartilage. In group 2, cartilage formation and integration with surrounding tissue were observed from the microcrack line toward the defect. Fibrous cartilage and some hyaline cartilage were present. In group 3, intense fibrocartilage was observed, with a small amount of hyaline cartilage, and in high magnification, fissures and chondroid matrix production were observed [Figure 6].

Discussion

Cartilage tissue, being avascular, aneural, and hypocellular, has a limited capacity to respond to trauma. Consequently, the characteristics of chondrocytes provide limited potential to regenerate cartilage, allowing it to transform into progressive damage.[10,11] In our study, the implantation of matrix-supported chondrocytes (MK+MCI) in addition to microfracture (MK) application yielded better results than MK application alone in the treatment of osteochondral lesions. However, the introduction of a novel method in our study, which involves soft tissue implantation in addition to MK application (MK+STI), resulting in outcomes similar to MK+MCI, distinguishes our study from the existing literature.

Various approaches exist in the diagnosis and treatment of osteochondral lesions in the literature. The target in the treatment of cartilage damage is the healing of tissue with hyaline cartilage. However, this is not as straightforward, especially when the cartilage damage is extensive. If cartilage damage is substantial, the repair capacity of chondrocytes becomes insufficient, and the damage is repaired with connective tissue.[12,13] Studies emphasize that the size and depth of the lesion are crucial factors. Partial healing can occur in full-thickness cartilage lesions that extend to subchondral bone, whereas spontaneous healing is not expected in surface

Table 10: Distribution of abnormal calcification basal integration, subchondral bone marrow fibrosis, and tidemark formation measurements by groups

	Median (Q1–Q3)			
	Abnormal Calcification	Basal integration	Subchondral Bone Marrow Fibrosis	Tidemark formation
Group 1	100 (100–100)	100 (100–100)	100 (100–100)	0 (0–0)
Group 2	100 (100–100)	100 (100–100)	100 (100–100)	0 (0–0)
Group 3	100 (100–100)	100 (100–100)	100 (100–100)	0 (0–0)

Q1: First quarter, Q3: Third quarter

defects that do not extend to the subchondral area. Mesenchymal stem cells in the bone marrow can contribute to the repair process only when the integrity of subchondral bone is compromised. In tissues other than cartilage, which have a vascular network, there is vascular entry, and cells migrate to the healing region to form tissue and matrix. However, there is no vascular tissue in cartilage healing; hence, cells must be supplied from another source. One way is to induce migration of mesenchymal stem cells from the bone marrow into the defect. Another way is through the implantation of chondrocytes into the defect from an external source.[12] Current surgical treatments for osteochondral lesions can be broadly categorized into five groups: reduction and fixation of osteochondral lesions, bone marrow stimulation, articular cartilage replacement, regenerative cell therapy, and metal implants.

The microfracture (MK) procedure in our study is one of the bone marrow stimulation methods, relying on the perforation principle from within the lesion to the bone marrow. The matrix-assisted chondrocyte implantation (MACI) procedure, on the contrary, is a second-generation autologous chondrocyte transplantation procedure considered within regenerative cell therapy.[14] The addition of soft tissue implantation (STI) to the MK procedure, a novel method not previously investigated in our study, suggests that the soft callus containing mesenchymal stem cells, chondroblasts, fibroblasts, collagen fibers, and hypertrophic chondrocytes, when implanted into the cartilage damage area, may potentially generate hyaline or hyaline-like cartilage. This is a unique aspect of our study.

Reviewing the literature up to the present day, there is a consensus that the results of cartilage defect healing are significantly positive after microfracture (MK) application alone. However, it has been acknowledged that it may become insufficient beyond a certain point.[15-19] In our study, we employed the most fundamental and simple method of applying microfracture to all groups to initiate cartilage healing. The results of our study indicate that, as a standalone procedure, MK application yields the lowest ICRS-1 and ICRS-2 scores among the three groups. While the functional impact of evaluating samples macroscopically and histopathologically is not precisely known, MK application alone demonstrated that it does not generate hyaline cartilage and cannot completely fill the defect.

At present, MACI is considered the gold standard for osteochondral defects.[20-25] However, the feasibility of applying MACI without the need for arthrotomy has been questioned by Zellner *et al*.[21] They measured osteochondral defects created in cadaver knees with a navigation system and applied them to cartilage scaffolds, which were subsequently arthroscopically implanted into the defects. Three different operators with geometrically different defects achieved flawless implantation. This study demonstrated that scaffolds could be applied arthroscopically for autologous chondrocyte transplantation. In conclusion, MACI treatment, which shows high satisfaction rates even in athletes, has become a reliable treatment today.

Ebert *et al*.[22] evaluated the clinical scores of 31 patients who underwent arthroscopic MACI prospectively at 3 months, 6 months, 1 year, 2 years, and 5 years. The results showed that arthroscopy-assisted MACI application demonstrated high clinical success and radiological improvement for up to 5 years.

Several comparative studies have been conducted in the literature to assess different methods. Desando *et al*.[26] compared the results of MACI and bone marrow concentrate applications based on AOFAS scores. Twenty-two patients, seven treated with MACI and fifteen with bone marrow concentrate, were evaluated according to AOFAS at the first, second, and third years. Additionally, histological evaluation was performed at the end of the second year. Although both groups showed a significant improvement in AOFAS scores at the end of the third year, the increase was more significant in the MACI group. Histologically, the bone marrow concentrate group showed signs of fibrous and hypertrophic changes, which were significantly lower in the MACI group. The primary finding was that, at the

end of the second year, both histologically and clinically, the results of the MACI group were superior.

In other studies, MACI was found to be superior to microfracture treatments.[27,28] However, due to the high cost and more invasive nature of MACI, microfracture technique was considered inevitable for patients with defects smaller than 3 cm^2.[27]

In a study conducted by Kon *et al*.[29] in 2011, they evaluated the results of treatments performed due to existing cartilage defects in 41 professional and semi-professional soccer players. The microfracture-treated group returned to sports at the end of the 8th month, while the MACI-treated group returned at the end of 12.5 months. Although both groups showed a significant improvement at the end of the second year, the ongoing follow-up of the microfracture group revealed a decline in scores over time. In conclusion, despite achieving similar results in terms of the success of returning to sports, the microfracture technique allowed faster return, but the MACI method provided more durable results in the long term.

Akgun *et al*.[30] divided 14 patients with full-thickness osteochondral defects into two groups in a case series. Seven patients were treated with MACI, and seven with matrix-guided autologous mesenchymal stem cell implantation (m-AMI). Clinical results were evaluated according to KOOS, VAS, and Tegner activity scores. The m-AMI group showed better results according to the KOOS score, with no significant differences in the Tegner and VAS scores. Although more patients and histological analyses are needed to support the data, they suggested that m-AMI could be effectively used and expedite improvement in the treatment of isolated osteochondral lesions.

In a study examining the histological results of microfracture, MACI, and cell-free scaffold applications, sheep knees with created chondral defects were sacrificed at 16 weeks for histological examination. The MACI group showed the highest amount of repair tissue in the defect, and the percentage of hyaline cartilage in the defective area was also the highest in this group. Although the microfracture group had a better defect-filling percentage compared to the untreated group, the improvement in terms of cartilage was weak.[31] In conclusion, while the microfracture method is a minimally invasive and easily applicable method, it falls short in terms of repair quality. Although MACI creates the highest quality repair tissue, these rates should be further improved.

In our study, MACI yielded the best results both macroscopically and histopathologically in animals. According to these results, we can say that MACI is a much better treatment option than microfracture alone. Our study concluded entirely in line with the literature at this point. However, the method our study focused on had not been addressed in the literature before. Indeed, the examination of the effects of soft tissue callus in cartilage defects, although not previously investigated, has been discussed in many studies regarding the role and importance of soft callus tissue, especially in fracture healing.[32-34] Murao *et al*.[33] examined mice in a tibia fracture model, sacrificing them on days 3, 5, 7, 14, 21, and 28 for histological analysis. They obtained a soft callus in the samples taken on the seventh day. In the soft callus, they observed cells that were predominantly mesenchymal progenitor cells. At this stage, adjacent periosteum thickened, and cartilage tissue appeared outside the bone; in fact, these osteochondral progenitor cells were found to be the main source of the soft callus.

Huang *et al*.[35] emphasized the necessity of progenitor cells for cartilage repair but mentioned that the most suitable cell source is controversial. In their review, they pointed out that mesenchymal stem cells produced from synovial joint tissues are superior to non-joint-derived cells. Until now, studies have accepted that joint-derived stem cells are the ideal source. They discussed that traditional chondrogenic induction protocols provide temporary cartilage formation, and new methods inducing stable cartilage need to be developed. Finally, they noted the lack of high-quality clinical studies and emphasized the need for reliable clinical data through stem cell-based, prospective, multicenter studies.

In our study, we aimed to address the existing gap in the literature by hypothesizing that soft callus-derived osteoprogenitor cells originating from the periosteum could provide stable cartilage. Although MACI showed low results compared to average scores for articular surface continuity, it showed similar results in one sample. In the literature, the cellular-level healing effects of soft callus tissue have been discussed in many studies. Our study investigated whether this cellular-level healing effect is effective in cartilage defects, and the results were found to be similar to MACI applications.

However, our study has some limitations. First, unfortunately, our study could only be conducted on animal cartilage, not on human cartilage. However, rats used as experimental animals are suitable for the study due to their biological resemblance to human cartilage and rapid healing properties because of their dense chondrocyte content. Another limitation is that the osteochondral defect area in experimental animals was created acutely, unlike most chronic human cases, and it shows less similarity to what occurs in humans. However,

when looking at the literature, it can be considered that such limitations are often overlooked, as similar studies tend to start and proceed in a similar manner.

Conclusion

In conclusion, statistically superior results were observed in both macroscopic and histopathological parameters with the application of soft callus implantation, which has the potential to generate hyaline cartilage as an alternative treatment to the current gold standard, matrix-induced autologous chondrocyte implantation. This alternative is considered due to its lower cost, the use of the patient's own tissue, and its reduced allergenic and infectious properties. The question may arise as to how soft callus can be obtained in the human body without fractures. For now, this question can be tentatively answered as applicable primarily in conjunction with cartilage defects associated with fractures. Additionally, recognizing this outcome at the cellular level could serve as a reference for future studies and the development of novel methodologies. Thus, we believe that our study holds significant importance in the literature in this regard.

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Conflicts of interest

There are no conflicts of interest.

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