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Histological Comparison of Nanocomposite Multilayer Biomimetic Scaffold, A Chondral Scaffold, and Microfracture Technique to Repair Experimental Osteochondral Defects in Rats

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ABSTRACT

Objective: We used biomimetic scaffolds, chondral scaffolds, and microfractures to repair experimentally created osteochondral defects in rat knees and then compared the results of each method.

Materials and Methods: We used a total of 56 female Wistar albino rats. The rats were grouped into 4 groups, with 14 rats each: biomimetic scaffold, chondral scaffold, microfracture, and control groups. Cylindrical full-thickness osteochondral defects 2.5 mm in diameter and 2 mm in depth were drilled into the right knees with the rats under general anesthesia. The knees of all rats were operated again after 4 weeks. Biomimetic and chondral scaffolds were classified into two groups. Microfractures 0.5 mm in diameter and 0.8 mm in depth were created in the rats of the microfracture group. The control group received no treatment. All the rats were observed for 6 weeks and then sacrificed, with samples subjected to macroscopic and histopathological examinations.

Results: The macroscopic and histopathological results in the biomimetic scaffold group differed significantly from those of the other treatment groups (p<0.05). When we compared the 3 treatment groups, the results of the chondral scaffold group were better than those of the microfracture group. The results of the microfracture group were somewhat better than those of the control group, but the result was not statistically significant (p>0.05).

Conclusions: Nanocomposite multilayer biomimetic scaffolds were better than chondral scaffolds and microfractures when used to treat osteochondral defects.

Keywords: Microfracture technique, osteochondral defect, pathology, rat, scaffold.

Introduction

Treatment of cartilage injury is one of the most popular topics in the field of orthopedics and traumatology [1-3]. As the cartilage lacks veins, its regeneration ability is low [3-5]. However, if the defect includes the subchondral bone, which is vascular, healing may be possible. Small defects can heal using fibrocartilaginous tissue from the subchondral area, while larger defects are more difficult to repair [6]. Some healing techniques seek to stimulate regeneration. However, tissue renewal is often incomplete and short lived.

If a strained or damaged synovial joint has to function normally, it is necessary to restore the hyaline cartilage [3]. Several treatment options are available. These include bone marrow stimulation techniques (abrasion, drilling, and microfracture), cell-based treatments (autologous chondrocyte transplantation or the use of bone marrow stem cells), defect-filling techniques (osteochondral autografts or allografts), the use of tissue engineering products (scaffolds and matrices), and the use of pharmacological agents [7-9].

The microfracture technique effectively treats full-thickness articular cartilage defects [7]. The cartilage differs from normal articular joint cartilage in terms of composition and mechanical characteristics. The advantages of the microfracture technique include the simplicity of the procedure, its low cost, and possibility of application in a single session with low morbidity [7].

Matrix-supported treatments retain chondrocytes within the defect. Nanocomposite multilayer biomimetic scaffolds have been used to repair type I collagen, hydroxyapatite, cartilage, and osteochondral defects [9]. Such scaffolds are three-dimensional and imitate osteochondral tissue. The cartilage layer has a smooth surface (formed by pure type I collagen) to ensure good articulation. The intermediate layer is formed of 60% type I collagen and 40% magnesium-hydroxyapatite (both w/w). The bottom layer consists of 30% type I collagen and 70% magnesium-hydroxyapatite; this stimulates subchondral bone production [10-12].

Another treatment option is to use a chondral scaffold (a bioabsorbable joint implant). The scaffold can be employed to facilitate cartilage repair after microfracture of a full-layer osteochondral defect. Bone marrow-derived mesenchymal and progenitor cells enhance chemotaxis toward the defect. The scaffold (infused with autologous serum and polyglycolic acid (PGA) is implanted into the full-layer defect.

Treatment of cartilage defects is a difficult and long process. In the long period, the aim is to obtain a durable, permanent, and cartilaginous tissue that resembles the original joint cartilage. In this study, we aimed to compare the results of biomimetic scaffolds, chondral scaffolds, and microfractures by creating full-thickness osteochondral defects in the knees of rats.

Materials and Methods

This study was approved by the Institutional Animal Experiments Local Ethics Committee.

In this study a total of 56 mature female Wistar Albino rats weighing 220-270 g, that were 5-7 months of age were grouped into 4 categories, each consisting of 14 rats. Some animals were lost during the experimental period. The final groups analyzed were as follows: biomimetic osteochondral scaffold group (n=12), chondral scaffold group (n=10), microfracture group (n=12), and control group (n=14). A power analysis was conducted before performing the
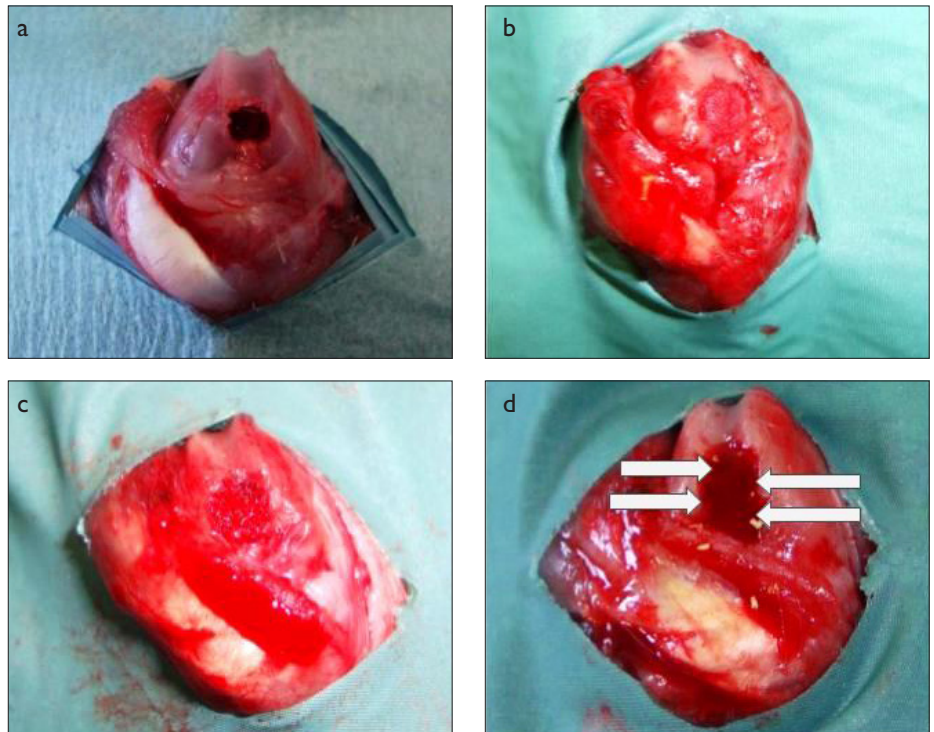
Main Points

- The biomimetic scaffold group improved better than the chondral scaffold group.
- The average extents of repair, proportion of overall defect filling, bone–cartilage interface appearance, and extent of bone-defect filling were lower in the biomimetic scaffold group than those in other 3 groups.
- The average extent of graft/recipient tissue integration was lower in the chondral scaffold group.

Figure 1. a-d. a) Knee with an osteochondral defect. b) Knee with a biomimetic scaffold. c) Knee with a chondral scaffold. d) Knee subjected to microfracture.

study experiment. The presence of at least 9 rats in each group was found to be sufficient.

Cylindrical full-thickness osteochondral defects 2 mm deep and 2.5 mm wide were drilled into the femoral intercondylar areas of the right knees via a medial incision and application of medial parapatellar arthrotomy (Figure 1A). The anatomical layers were restored, and the rats were observed for 4 weeks.

At that end of 4 weeks, osteochondral biomimetic scaffolds were placed in the defects of one group (Figure 1B), another group was treated via microfractures (0.5 mm wide and 0.8 mm deep) and placement of a chondral scaffold (Figure 1C), and microfractures alone was used to treat a third group (Figure 1D). The controls were not treated. The rats were observed for a further 6 weeks.

At 10 weeks after the first operation, all rats were sacrificed by inhalation of a lethal dose of ether. The lower right extremities were subjected to arthrotomy. Soft tissue was removed from the distal femur and the muscles cut. The anterior cruciate ligament and the meniscus were cleaned. Half of each distal femur was removed via the femoral diaphysis using medial transverse osteotomy. This study was carried out in accordance with the ethical standards provided in the 1964 Declaration of Helsinki and its later amendments.

Tissues were stored in 10% formalin for 5 days before routine tissue processing for histopathological analysis. Samples were decalcified at room temperature in DeCastro solution (30 mL 70% nitric acid, 50 g chloral hydrate, and 300 mL absolute ethanol) and returned to 10% formalin (pH 7.0) in phosphate-buffered saline.

Specimens were sliced with 2 mm thickness. An automated tissue tracking device was used for routine processing 13 h postfixation with 2 containers of formaldehyde, 4 containers of alcohol, 2 containers of xylene, and 2 vessels of paraffin. After performing proper fixation and decalcification procedure, the slices were embedded in paraffin, and serial sections of 4-μm in thickness were prepared (1 in 3 slices was retained) in a rotary microtome (MB 35 Premier; Shandon Inc., Pittsburgh, PA, USA) and coated with gelatin. The sections were stained using hematoxylin-eosin and safranin-O and examined under a light microscope, and the images were digitized.

Slices were evaluated according to 3 different systems: the macroscopic modified Fortier classifications (Table 1) [13], the Mankin scores (Table 2) [14], and the International Cartilage Repair Society (ICRS) scores (Table 3) [15].

In the modified Fortier scoring where the tissues were evaluated macroscopically, the parameters of the surface texture of repair tissue, percent area of defect filled, graft recipi-

Table 1. Macroscopic modified Fortier classifications [13]

Scoring system used for gross appearance (Fortier classifications)	Score
Surface texture of repair tissue	
Normal: Smooth hyalin	0
>75% normal	1
50-75% normal	2
<50% normal	3
Percent area of defect filled	
100%	0
>75%	1
50-75%	2
<50%	3
Graft recipient tissue integration	
100% of perimeter	0
>75% of perimeter	1
50-75% of perimeter	2
<50% of perimeter	3
Bone-cartilage interface appearance	
Regular	0
Slightly over/under lined	1
Mayor irregularity	2
Irregular or cystic	3
Bone defect filling	
100%	0
>75%	1
50-75%	2
<50%	3

Table 2. Mankin scores [14]

| I. Structure	
a. Normal	0
b. Surface irregularities	1
c. Pannus and surface irregularities	2
d. Clefts to transitional zone	3
e. Clefts to radial zone	4
f. Clefts to calcified zone	5
g. Complete disorganization	6
II. Cells	
a. Normal	0
b. Diffuse hypercellularity	1
c. Cloning	2
d. Hypocellularity	3
III. Safranin-O staining	
a. Normal	0
b. Slight reduction	1
c. Moderate reduction	2
d. Severe reduction	3
e. No dye noted	4
IV. Tidemark integrity	
a. Intact	0
b. Crossed by blood vessels	1
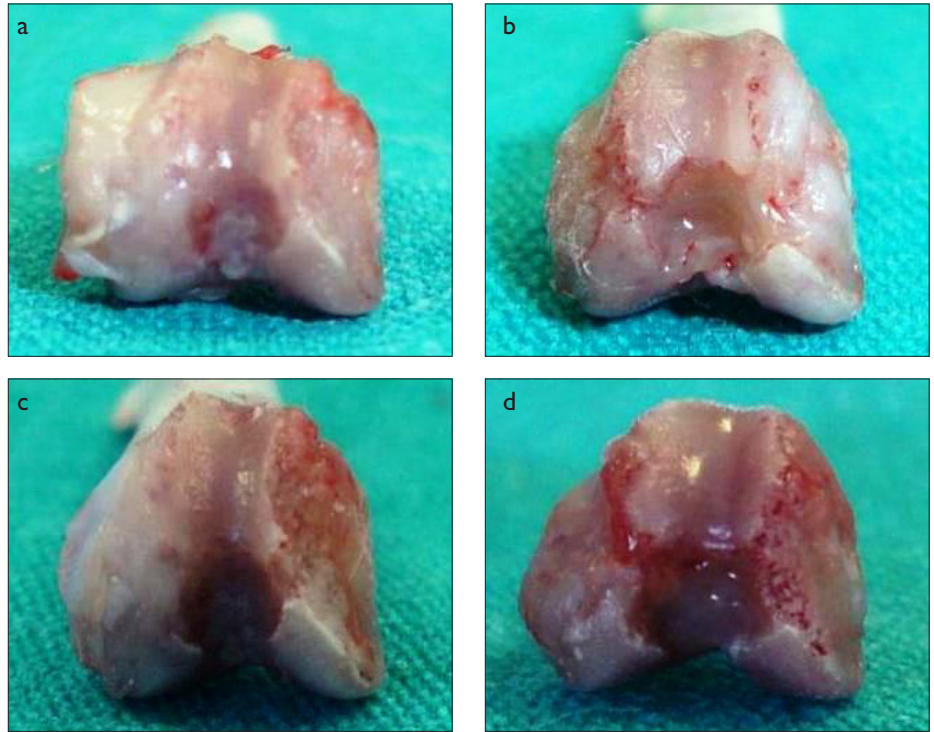
Figure 2. a-d. Macroscopic view of the a) biomimetic scaffold; b) chondral scaffold; c) micro fracture group (arrows show 4 microcircular holes in the microfracture); and d) control group.

Table 3. International Cartilage Repair Society (ICRS) scores [15]

Cartilage surface property	
Smooth, regular	3
Irregular	0
Cartilage Matrix structure	
Hyalin	3
Mix (hyalin/fibrocartilage)	2
Fibrocartilage	1
Fibrous tissue	0
Cell distribution	
Columnar	3
Mix (columnar/ Clustered)	2
Clustered	1
Dispersed disorganized cells	0
Cell population viability	
Dominant	3
Partially	1
<10%	0
Subchondral bone property	
Normal	3
Increased shape change	2
Bone necrosis/granulation tissue	1
Reserved / broken / callus	0
Cartilage mineralization	
Normal	3
Abnormal/inappropriate localization	0

ent tissue integration, bone-cartilage interface appearance, and bone defect filling parameters were examined (Table 1) [13].

The Mankin scoring evaluated cartilage structure, cell structure, the extent of the matrix stained with safranin-O, and the smoothness of articulation (Table 2) [14].

Cartilage characteristics, including the matrix structure, cell distribution, cell populations, and subchondral bone characteristics, were evaluated using the ICRS system, which grades cartilage mineralization (Table 3) [15]. Histopathological evaluation was done blinded by a pathologist.

Statistical Analysis

All macroscopic and histological data were recorded and compared using NCSS 2007 software (NCSS; Kaysville, UT, USA). Descriptive data are presented as averages with standard deviations and medians. As the data were not normally distributed, the nonparametric Kruskal-Wallis test and Mann-Whitney U-test were used for intergroup comparisons. A posthoc test (Dunn's test) was used to analyze the significance of differences between groups. A p value <0.05 was considered to reflect statistical significance.

Results

We used 48 rats (12 in the biomimetic scaffold group, 10 in the chondral scaffold group, 12 in the microfracture group, and 14 controls) and histopathologically studied the healing of
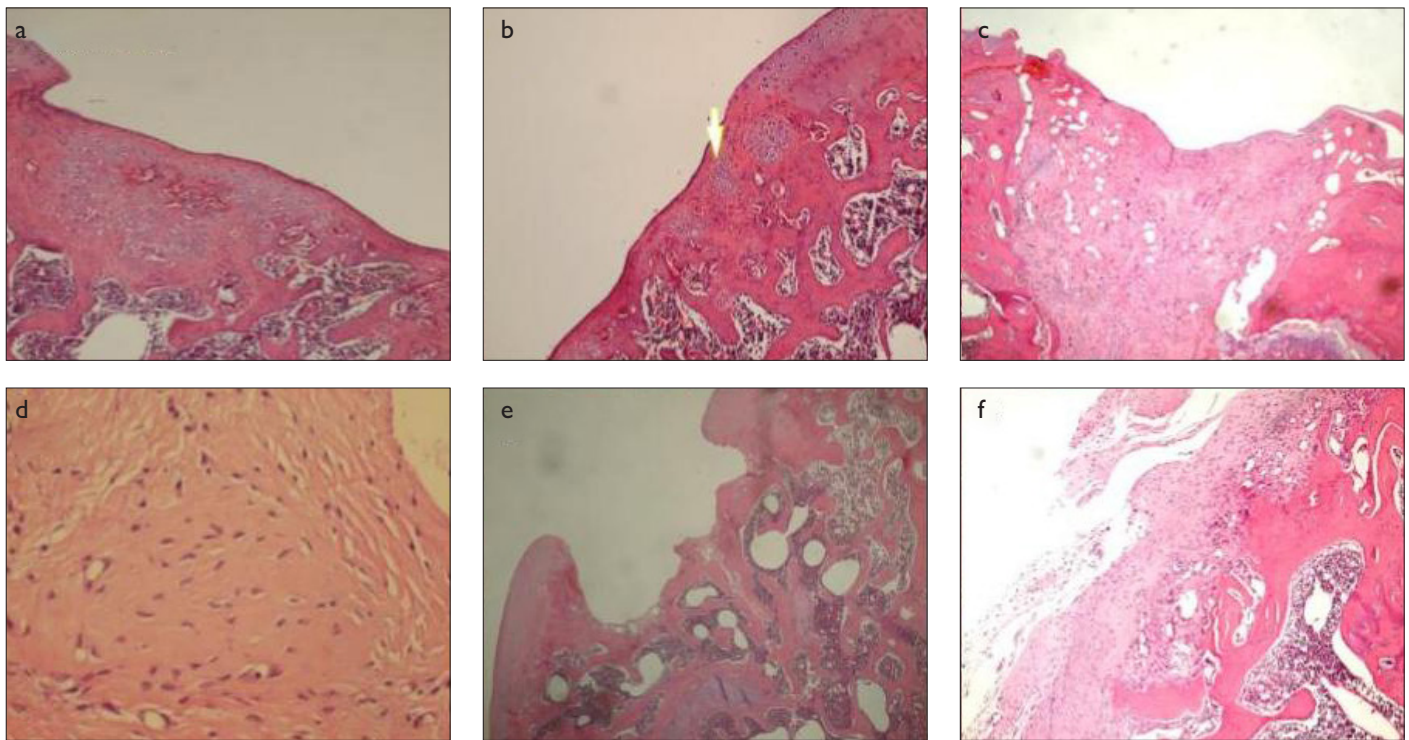
Figure 3. a-f. a) Fibrohyaline cartilage (×40). b) New islands of hyaline cartilage (biomimetic scaffold, ×100). c) Minimal filling defect (scaffold, ×40). d) Fibrous cartilage (microfracture, ×100). e) Filling defect (control, ×40). f) Fibrotic layer on the bony surface (control, hematoxylin and eosin ×100).

Table 4. Mean, median, and minimum and maximum values of the macroscopic scores (Modified Fortier scores)					
		Biomimetic scaffold group	Chondral scaffold group	Microfracture group	Control group	p
Surface texture of repair tissue	Mean	0.67±0.49	2.5±0.53	2.5±0.52	2.64±0.63	1 <0.001
	Median	1	2.5	2.5	3	
	Min-max	0-1	2-3	2-3	1-3	
Percent area of defect filled	Mean	0.25±0.45	1.5±0.85	1.7±0.67	1.57±0.85	1<0.001
	Median	0	1.5	2	1.5	
	Min–max	0-1	0-3	1-3	0-3	
Graft recipient tissue integration	Mean	0.33±0.49	0.2±0.79	-	-	2<0.017
	Median	0	0	-	-	
	Min–max	0-1	0-2	-	-	
Bone–cartilage interface appearance	Mean	0.92±0.67	1.9±0.74	1.7±0.67	2.36±0.93	1<0.001
	Median	1	2	2	3	
	Min-max	0-2	0-3	0-2	0-3	
Bone defect filling	Mean	0.42±0.67	1.4±0.97	1.4±0.70	1.71±0.83	1<0.004
	Median	0	1	1.5	2	
	Min-max	0-2	0-3	0-2	0-3	2<0.001
Total macroscopic score	Mean	2.58±1.62	8.5±2.41	-	-	
	Median	2	9	-	-	
1: Kruskal–Wallis test; 2: Mann–Whitney-U test						

knee defects (Figures 3A-F). The average macroscopic and histological scores of each group were calculated (Tables 4-6).

As the microfracture and control groups did not receive scaffolds, macroscopic data are not available for these 2 groups. The average macroscopic score of the biomimetic scaffold group was lower than that of the chondral scaffold group. According to the results, the biomimetic scaffold group improved better than the chondral scaffold group, and the result was statistically significant (Figure 2). Moreover, the average extents of repair, proportion of overall defect filling, bone-cartilage interface appearance, and extent of bone-defect filling were lower in the biomimetic scaffold group than those in other 3 groups. However, the average extent of graft/

Table 5. Mean, median, and minimum and maximum values of the Mankin scores

		Biomimetic scaffold group	Chondral scaffold group	Microfracture group	Control group	p
Cartilage structure	Mean	0.83±1.11	4.1 ± 1.1	4.1±1.10	4.71±1.54	
	Median	0.5	4	4	5	<0.001
	Min–max	0-3	2-6	3-6	1-6	
Cell abnormality	Mean	0.42±0.67	2±0.67	2.2±1.03	2.28±0.83	
	Median	0	2	2.5	2	<0.001
	Min–max	0-2	1-3	0-3	0-3	
Safranin-O staining	Mean	1.25±0.45	2.4±1.07	2.8±0.63	3.29±0.83	<0.001
	Median	1	2	3	3.5	
	Min–max	1-2	1-4	2-4	2-4	
Tidemark integrity	Mean	0.58±0.51	1±0	1±0	1±0	<0.001
	Median	1	1	1	1	
	Min–max	0-1	1	1	1	
Total Mankin score	Mean	3.08±2.07	9.5±2.41	10.1±2.23	11.28±2.78	<0.001
	Median	2.5	9	10	12	
Kruskal–Wallis test						

Table 6. Mean, median, and (minimum and maximum) values of the ICRS scores

		Biomimetic scaffold group	Chondral scaffold group	Microfracture group	Control group	p
Cartilage surface property	Mean	2±1.47	0.3±0.95	0.3±0.95	0.21±0.80	
	Median	3	0	0	0	<0.001
	Min-max	0-3	0-3	0-3	0-3	
Cartilage matrix structure	Mean	1.75±0.75	0.8±0.63	0.7±0.67	0.14±0.36	
	Median	2	1	1	0	<0.001
	Min-max	03	0-2	0-2	0-1	
Cell distribution	Mean	1.75±0.75	1±0.67	0.6±0.84	0.36±0.5	
	Median	2	1	0	0	<0.001
	Min-max	0-3	0-2	0-2	0-1	
Cell population viability	Mean	3±0	1.3±0.95	1.3±0.95	0.86±0.36	
	Median	3	1	1	1	<0.001
	Min-max	3	0-3	0-3	0-1	
Subchondral bone property	Mean	2.58±0.67	1.6±0.84	1.4±1.07	1±0.96	
	Median	3	2	1	1	<0.001
	Min-max	1-3	0-3	0-3	0-3	
Cartilage mineralization	Mean	2.75±0.87	0.3±0.95	0.6±1.26	0.43±1.09	
	Median	3	0	0	0	<0.001
	Min-max	0-3	0-3	0-3	0-3	
Total ICRS score	Mean	1-3.83±3.07	5.3±2.62	4.9±2.88	3±2.77	<0.001
	Median	15	5.5	5	2	
ICRS: International Cartilage Repair Society						

recipient tissue integration was lower in the chondral scaffold group.

Discussion

According to the results of this study, the biomimetic scaffold was significantly more effective than the chondral scaffold or microfracture.

The results of the chondral scaffold, microfracture, and control groups did not differ significantly. The chondral scaffold was somewhat more effective than microfractures, but statistical significance was not attained.

In this study, 3 different evaluations were made. The biomimetic scaffold group was found to be better in terms of 5 parameters (the surface

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texture of repair tissue, percent area of defect filled, graft recipient tissue integration, bone-cartilage interface appearance, and bone-defect filling), which were evaluated according to the modified Fortier scores.

The parameters such as cartilage structure, cell abnormality, safranin-O staining, and tidemark integrity were also found to be better in the biomimetic scaffold group than those in other groups.

Other parameters such as cartilage surface property, cartilage matrix structure, cell distribution, cell population viability, subchondral bone property, and cartilage mineralization used to evaluate the ICRS score were also found to be better in the biomimetic scaffold group than those in other groups.

Scaffolds ensure that cells or growth factors remain within a defect until recovery [16]. Various collagens, hyaluronan, fibrin, carbon fibers, porous polylactic acids, and polytetrafluoroethylene polyesters have been used as scaffolds [16]. An ideal scaffold must provide a temporary cap for the defect until the cells completely repair and must then be absorbed within the few months over which the chondrocytes self-renew [17].

Nanocomposite multilayer biomimetic scaffolds are highly porous, three-dimensional, hydrophilic, and imitate the osteochondral structure [8, 10, 18, 19].

Kon et al. [18] used biomimetic scaffolds to repair osteochondral lesions created in 24 femoral condyles of 12 sheep. The 3 groups were biomimetic scaffold group, biomimetic scaffold-plus-platelet-rich plasma group, and control group. Microradiographic, macroscopic, and histological data were collected. Only the biomimetic scaffold group exhibited significant bone regeneration and reconstruction of the cartilage surface.

Kon et al. [20]. treated 60 grade 3-4 osteochondral defects in the knees of 60 patients by osteochondral autologous transplantation, bone grafting (auto chondrocyte implantation), placement of biomimetic scaffolds, bone-cartilage grafting, and placement of hyaluronic acid scaffolds that had absorbed bone marrow cells. In another study, 30 patients with osteochondral lesions 1.5-6 cm^2 in area were treated using biomimetic scaffolds [19].

In a study on 2 horses, osteochondral lesions were created in medial condyles and chondral lesions in lateral condyles [9]. Both types of lesions were treated using biomimetic scaffolds. The animals were followed up for 6 months. Arthroscopy was performed under anesthesia 2 months after the operation to observe the scaffold structure and the extent of tissue recovery. The osteochondral and chondral lesions recovered well, the grafts were fibrocartilaginous in texture, and no inflammation was evident around any lesion.

We found that the efficacy of the biomimetic scaffolds was consistent with that reported in the literature. The biomimetic scaffold group scored significantly higher in terms of both macroscopic and histopathological parameters than the other groups.

The chondral scaffold (Swiss Biomed Orthopedics AG, Zürich, Switzerland), also termed as a bioabsorbable joint implant, has a molecular weight of 50,000 Da; this is a nonwoven absorbing material made of pure PGA [21]. Autologous serum is added and the scaffold is placed in a full-thickness osteochondral defect. The homogenous fiber structure endures until new cartilage is produced in a parallel arrangement at a controlled rate [12, 21]. This scaffold is bioabsorbable, soft, fairly flexible, and resistant to tearing [21].

Loken et al. [22]. created 4-mm-wide and 1.5-mm-deep osteochondral defects in the medial femoral condyles of both knees of 11 rabbits. A hyaluronan-based scaffold (HYAFF-11, Fidia Advanced Biopolymers Laboratories, Abano Terme, Italy) and mesenchymal stem cells were placed in one knee and the scaffold in the other. After 24 weeks, all defects had filled well; no significant differences were evident between the 2 groups.

Erggelet et al. used microfracture, alone or in combination with a PGA-based structured noncellular scaffold, to treat osteochondral defects created in sheep knees [12]. Three months later, the noncellular implant (containing autologous serum) had encouraged migration and differentiation of mesenchymal progenitor cells to/within the cartilage, associated with regeneration. Thus, the implant reliably enhanced cartilage recovery when placed after microfracture.

Gille et al. [23]. used autologous matrix-induced chondrogenesis (AMIC) to treat osteochondral lesions in the knees of 57 patients. A collagen-based structural scaffold (Chondro-Gide, Geistlich Pharma, Wolhusen, Switzerland) was placed after microfracture. Most patients followed-up for approximately 2 years reported reduced postoperative pain (mean visual analogue scale pain score preoperatively, 7.0; 1 year postoperatively, 2.7; and 2 years postoperatively, 2.0) and an increase in the average Lysholm score (mean score preoperatively, 50.1; 1 year postoperatively, 79.9; and 2 years postoperatively, 85.2). AMIC was efficient and reliable when used to treat symptomatic knee cartilage defects.

The literature indicates that scaffolds increased clinical scores, afford better cartilage repair, attract mesenchymal stem cells to the defect, provide mechanical protection, exhibit a general supportive function, render the articular surface smoother, encourage new bone formation in the subchondral area, and ensure better defect filling. We found that both of our tested scaffolds exhibited such properties.

Microfractures effectively heal full-layer lesions of knee cartilage [24-26]. Microfractures are a form of stem cell stimulation seeking to recreate a normal vasculature. The use of bone marrow stimulation techniques to treat chondral lesions reduces the strength of healed tissue. The tissue that forms after such procedures differs from normal cartilage in terms of structure, composition, and mechanical strength [24].

Heir et al. [27] created experimental 4-mm-wide osteochondral defects in the femoral knee condyles of 22-week-old New Zealand rabbits. Microfracture and mosaicplasty were compared in terms of defect filling, new bone generation, and bone mineralization. Mosaicplasty was optimal, being associated with high-level defect filling and new bone generation. However, subchondral bone mineralization was better after microfracture.

Güneş et al. [28] created 4-mm-wide full-thickness cartilage defects in the right medial femoral condyles of 40 mature rabbits. The control group was untreated. Two test groups underwent periosteal flap transplantation or microfractures; a third group was treated with both techniques. Twelve weeks later, the results were evaluated using the ICRS scale. The combined treatment (microfracture-plus-flap placement) afforded better repair than the other treatments.

Kuo et al. [29] histopathologically compared the results of 4 different treatments (microfractures, addition of bone morphogenetic protein 7 (BMP-7), microfractures combined with BMP-7 absorbed in a collagen sponge, and microfractures with the collagen sponge only) of full-thickness cartilage defects. Compared to controls, BMP-7 enhanced tissue repair (the

new tissue was of good quality). In the microfracture-plus-BMP-7 group, the extent of repair and the smoothness of the bony surface were enhanced; this combined treatment was thus favored. The cited authors considered that as BMP-7 induces cartilage differentiation, the addition of the protein after microfractures yielded a cartilage similar to hyaline in terms of both quality and quantity.

Xing et al. [30] treated full-thickness experimental cartilage defects in rat knees using both the microfracture technique and an adhesive osteochondral implant. Macroscopic, biochemical, histological, and genetic analyses performed 12 weeks later showed that the implant group exhibited good tissue repair and defect filling. Hyaline-like regenerative tissue and a high level of type collagen II (stained with safranin-O) were evident. In the microfracture group, the cartilage tissue was fibrotic, the extent of safranin-O staining was low, and the type II collagen structure was poor. Thus, the implant optimized repair and hyaline cartilage generation.

The literature indicates that scaffold techniques are significantly better than the microfracture technique [18, 22]. We also found that biomimetic (particularly) and chondral scaffolds were better than the microfracture technique.

Biomimetic scaffolds are now standard treatment options; such scaffolds effectively heal cartilage with osteochondral defects. Many different scaffold materials have been clinically used to treat such defects. However, no prior study simultaneously compared biomimetic and chondral scaffolds with the microfracture technique.

In conclusion, we have shown that both biomimetic and chondral scaffolds can be used to treat osteochondral defects. The formerly accepted microfracture technique is inadequate; biomimetic scaffolds fill defects better and yield a smoother cartilage surface. In the control group, defect filling was unsatisfactory and the cartilage surface was rough.

Biomimetic scaffolds can be reliably used to treat osteochondral defects; the scaffolds are three dimensional in structure and imitate articular cartilage and bone.

In this study, tissues were evaluated by a single pathologist. This is an animal study, which is unable to recapitulate exactly clinical osteochondral healing. Since there is no standard time in the literature, the time period of 6 weeks is questionable. The results of this study are not confirmed by biomechanical and radiological methods.

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