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The treatment of osteochondral defects with autologous osteochondral and apophyseal grafts in animal models

Otolog osteokondral ve apofiz greftleriyle osteokondral defektlerin tedavisi

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Objective

Apophyseal grafts were used as an alternative autograft source to restore osteochondral defects.

Patients and methods

Eighteen knees of 9 New Zealand rabbits with an average age of 4 months follow-up were included into the study. Osteochondral defects were created in the weight-bearing areas of the rabbits' medial femoral condyles. Six knees were repaired using 4 x 5 mm osteochondral autografts obtained from the minimal weight-bearing region of the femoral condyles at the level of the patellofemoral joint. Six knees were repaired using 4 x 5 mm apophyseal grafts obtained from the posterior part of the iliac-crest apophysis. Six knees with no treatment were used as the control group. At the end of week 12, the results were evaluated clinically, macroscopically and histologically.

Results

The defects filled by the grafts were smooth and glistening. A smooth hyaline cartilage-like tissue had formed. The untreated defect on the control group was incompletely filled with reparative tissue and levered below the articular surface. Statistical analysis presented that the osteochondral and apophyseal graft groups were significantly better than the control group in the surface area, cellular distribution, matrix composition and subchondral bone. There was no significant difference in the histological results between the two treatment groups.

Discussion

Apophyseal autografts could represent an alternative source to osteochondral autografts that were successfully employed for the treatment of osteochondral defects.

Key words: Osteochondral defect, osteochondral autograft, apophyseal graft, cartilage repair

Amaç

Bu çalışma, apofizyel greftlerin, kıkırdağa benzer yapılarından yola çıkarak osteokondral defektlerin tedavisinde alternatif bir otogreft kaynağı olarak kullanılıp kullanılamayacağını belirlemek için yapıldı.

Hastalar ve yöntem

Çalışmada, ortalama 4 aylık 9 Yeni Zelanda tavşanının 18 dizi kullanıldı. Osteokondral defektler, tavşanların mediyal femoral kondilin ağırlık taşıyan yüzeylerinde oluşturuldu. Altı diz patellofemoral eklem seviyesinde, femoral kondilin çok az ağırlık taşıyan yüzeyinden elde edilen 4x5 mm'lik osteokondral otogreft ile onarıldı. Altı diz iliak kanat arka kısım apofizinden elde edilen 4x5 mm'lik apofiz grefti ile onarıldı. Geriye kalan 6 diz kontrol grubu olarak kullanıldı. Sonuçlar klinik, makroskopik ve histolojik olarak 12 haftanın sonunda değerlendirildi.

Bulgular

Greftler ile doldurulan defektlerde hyalin kıkırdağa benzer, düzgün ve pürüzsüz doku oluştuğu gözlendi. Kontrol grubunda ise, defekt onarım dokusuyla kısmen dolu ve yüzeyi normal eklem kıkırdağından daha çökük olarak gözlendi. Yüzey, hücresel dağılım, matriks ve subkondral kemik incelemelerinde osteokondral ve apofiz greftleri ile onarılan grupların kontrol grubundan istatistiksel olarak anlamlı farklı olduğu gözlendi. İki tedavi grubu arasında ise histolojik değerlendirmede anlamlı farklılık yoktu.

Çıkarımlar

Apofizyel otogreft, osteokondral defektlerin tedavisinde bu ve başka çalışmalarda başarılı bir şekilde kullanılan osteokondral otogreftlere alternatif bir greft kaynağı olabilir.

Anahtar sözcükler: Osteokondral defekt, otogreft, apofiz otogrefti, kıkırdak onarımı

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Treatment of osteochondral defects are challenging. Clinical[1,2] and experimental[2-5] studies are limited. Comparative studies[2, 6-11] have only been very recently performed. However, a conclusion on the best treatment of osteochondral defects is not defined yet.

Articular cartilage has a limited healing potential.[1,12,13] Due to the low repair ability of articular cartilage, the treatment of osteochondral defects is a challenging issue in orthopedic practice.[5] Even a limited repair is obtained with conventional methods, the tissue formed is fibrous cartilage that does not possess the same biomechanical features as the original hyaline tissue. The purpose of treatment using surgical techniques is to ease the symptoms of the patient and to fully reconstruct the microstructure of the joint cartilage without losing its biomechanical and physiological properties. The main problems with the current methods employed to date are the inability to obtain original hyaline cartilage and the difficulty in securing the graft to be used in repair. This study was planned with the idea that apophyseal grafts could serve the same function as osteochondral grafts because of their similar cartilage structures.[14, 15]

PATIENTS AND METHODS

Nine skeletally immature New Zealand white rabbits with mean weights of 1750.0±144.9 grams (range between 1500 and 2000 grams) and mean ages of 4 months (3-5 months) were used. The study was approved by the local ethical committee of the university and local authorities. The growth plate fuses in the rabbit at skeletal maturity, between the ages of six and seven months,[14] therefore animals with a mean age of 4 months were used. Eighteen knees and 18 medial condyles of the 9 rabbits were operated. Three groups (osteochondral- Group 1, apophyseal- Group 2 and control- Group 3) consisting of 6 knees in each group were established.

Surgical Technique: Prophylactic antibiotic (100 mg of cefazolin sodium, Cefozin, Bilim İlaç, Turkey) was preoperatively administered intramuscularly. Anesthesia was induced by intramuscular injection of 5 mg/kg of xylazine chloride (Rompun, Bayer) and 30 mg/kg of ketamine hydrochloride (Ketalar, Pfizer). Following anesthesia, the knees were approached by a medial parapatellar incision. The femoral condyles were exposed by retracting the patella laterally. An osteochondral defect of a diameter of 3.8 mm and a depth of 5.0 mm was created on the weight-bearing femoral surface of the medial condyle using a consistent osteochondral repair (COR) system with a drill diameter of 3.8 mm. A cylindrical osteochondral graft measuring 4.0 mm of diameter and 5.0 mm in depth was obtained from the minimal weight-bearing periphery of the femoral condyles at the level of patellofemoral joint of the same knee using a 4.0 mm COR system osteochondral graft delivery tool. The graft was then implanted into the defect formed on the cartilage joint surface (Group 1) (Figure 1-A). Subsequently, following the reduction of the patella, the capsule and the skin were closed using 3-0 vicryl (Johnson & Johnson) and 3-0 silk sutures (Dogsan), respectively.

Six knees were approached by the same incision method as described above, and osteochondral defects measuring 3.8 mm in diameter and 5.0 mm in depth were formed on the weight-bearing femoral surfaces of the medial condyle using a COR system drill diameter of 3.8 mm. The ipsilateral iliac apophysis was approached through an oblique incision of 3.0 cm. Fibrocartilaginous and perichondral tissue was removed. A graft measuring 4.0 mm in diameter and 5.0 mm in depth was obtained from the posterior part of the iliac-crest apophysis using a 4.0 mm COR system osteochondral graft delivery tool. The grafts harvested were implanted into the defect created on the medial femoral condyle (Group 2) (Figure 1-B). The incision was closed in the same way as described above. Six knees with untreated cartilage defect were used as the control group (Figure 1-C).

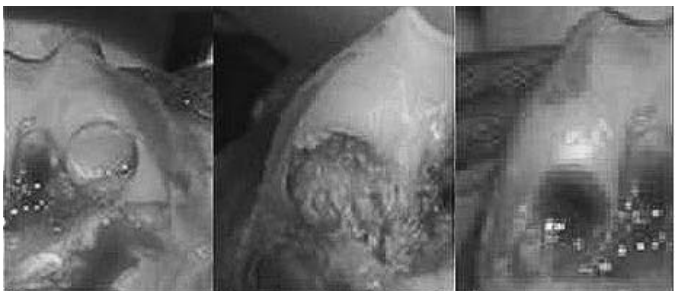

Figure 1.a **Figure 1.b** **Figure 1.c**

Figure **1-A** Intraoperative appearance of the osteochondral graft im plantation.
1-B Intraoperative appearance of the apophyseal graft implantation.
1-C Intraoperative appearance of the control group.

The rabbits were kept in standard cages measuring 60 x 40 x 30 cm. As a postoperative procedure, the rabbits were injected intramuscularly with a daily dose of 100 mg of cefazolin sodium for 3 days. The animals were allowed to move freely and were not immobilized. Skin sutures were removed on day 10 postoperatively.

The rabbits were terminated at week 12 after surgery and examined clinically, macroscopically and histologically. Clinical analyses were performed on the basis of range of motion, contracture and deformity. The knee joints after the muscles and surrounding soft tissues were removed, were analyzed macroscopically

in terms of adhesion, cartilage surface deformation and degeneration. Than, the medial femoral condyles were separated, fixed in 10% formalin for 2 days and decalcified in 5% nitric acid. Samples were fixed in 10% formalin for another day before they were finally embedded in paraffin. The osteochondral defect areas were sectioned at a thickness of 6 nm and stained with hematoxylin and eosin (HE). Histopathological analyses were performed using a light microscope by specialists with no knowledge of the sample groups.

International Cartilage Repair Society (ICRS) Visual Histological Assessment Scale (Surface, Matrix, Cellular Distribution, Cell Population Viability, Subchondral Bone, and Cartilage Mineralization) was used for histological analysis (Table 1).[16]

For statistical analysis, we used the Kruskal-Wallis test at a significance level of <0,05 for comparisons of groups. In case of significance, the Mann Whitney U test was employed to analyze pairwise differences between groups. The results were expressed as mean, standard deviation and median (min-max) (Table 2).

Table 1. Results of the groups according two ICRS Visual Histological Assessment Scale.[16]

	Feature	Score	Osteochondral otograft	Apophyseal graft	Control group
			Group-1	Group-2	Group-3
			Number	Number	Number
I.Surface					
	Smooth/continuous	3	6	5	-
	Discontinuities/irregularities	0	-	1	6
II. Matrix					
	Hyaline	3	3	2	-
	Mixture:hyaline/fibrocartilage	2	3	4	-
	Fibrocartilage	1	-	-	6
	Fibrous tissue	0	-	-	-
III. Cell distribution					
	Columnar	3	3	2	-
	Mixed/columnar-clusters	2	2	3	-
	Clusters	1	1	1	4
	Individual cells/disorganized	0	-	-	2
IV. Cell population viability					
	Predominantly viable	3	4	3	-
	Partially viable	1	2	3	5
	<10% viable	0	-	-	1
V. Subchondral bone					
	Normal	3	2	3	-
	Increased remodeling	2	4	3	2
	Bone necrosis/granulation tissue	1	-	-	4
	Detached/fracture/callus at base	0	-	-	-
VI. Cartilage mineralization (calcified cartilage)					
	Normal	3	4	4	5
	Abnormal/inappropriate location	0	2	2	1

Table 2. Statistical analysis according to ICRS Visual Histological Assessment Scale.

		Surface*	Matrix*	Cell Distribution*	Cell Population Viability†	Subchondral Bone*	Cartilage Mineralization
Control	Mean	,00	1,00	,67	,83	1,33	2,50
	Std	,000	,000	,516	,408	,516	1,225
	Deviation	,00	1,00	1,00	1,00	1,00	3,00
	Median	0	1	0	0	1	0
	Minimum	0	1	1	1	2	3
	Maximum						
Osteochondral autograft	Mean	3,00	2,50	2,33	2,33	2,33	2,00
	Std	,000	0,548	0,816	1,033	,516	1,549
	Deviation	3,00	2,50	2,50	3,00	2,00	3,00
	Median	3	2	1	1	2	0
	Minimum	3	3	3	3	3	3
	Maximum						
Apophyseal graft	Mean	2,50	2,33	2,17	2,00	2,50	2,00
	Std	1,225	,516	,753	1,095	,548	1,549
	Deviation	3,00	2,00	2,00	2,00	2,50	3,00
	Median	0	2	1	1	2	0
	Minimum	3	3	3	3	3	3
	Maximum						
P		0.001	0.002	0.007	0.038	0.012	0.770

*** Control versus osteochondral autograft and control versus apophyseal graft significant.**

† Control versus osteochondral autograft graft significant.

RESULTS

All animals tolerated the surgery well. The rabbits limped for 10-15 days after surgery. It was determined that joint movements were fully recovered and that there was no contracture or deformity. No infections or complication was observed during the study. At the time of dissection no signs of infection and arthrofibrosis were noted.

Macroscopically, neither adhesion nor patellar dislocation were observed. The defects were filled in the 2 study graft groups, and the grafts adhered firmly to the osteochondral defects. However, in the control group the defect was partly filled and the newly formed tissue was slightly depressed in appearance compared to that of the 2 study groups (Figure 2-A). The surface in the apophyseal graft groups was level with the normal cartilage (Figure 2-B), but still not as good as that of the osteochondral graft groups (Figure 2-C). In all 3 groups, the newly formed tissue exhibited a different appearance from the tissue on the surrounding cartilage and its borders could be clearly identified.

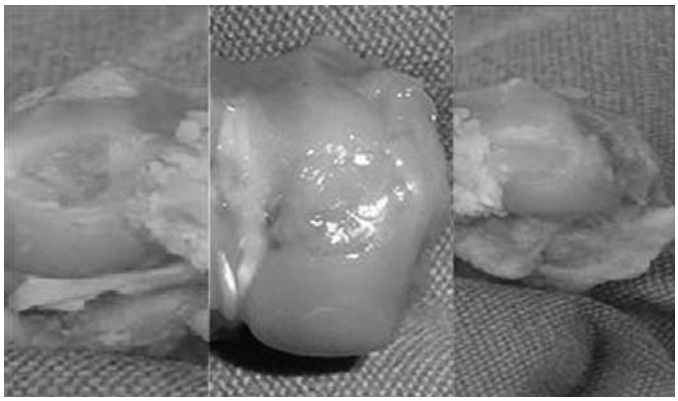

Figure 2.a **Figure 2.b** **Figure 2.c**

Figure **2-A.** Week 12 appearance of the control group.
2-B. Week 12 appearance of the apophyseal graft group.
2-C. Week 12 appearance of the osteochondral graft group.

Microscopically, in all cases in the control group the tissue had a thin fibrotic layer (Figure 3). In the osteochondral graft group, the tissue formed on the surface of the defect had a hyaline-like appearance in 3 animals and a mixed character in the other 3 animals (Figure 4). In the apophyseal graft group, the tissue formed on the surface of the defect was hyaline-like cartilage in 2 animals and had a mixed character in 4 animals (Figure 5). Results of the ICRS Visual Histological Assessment Scale (surface, matrix, cell distribution, cell population viability, subchondral bone, cartilage mineralization) are presented in Table 1.

In all groups, capillary formation was sparsely observed in patches beneath the defect area. In the osteochondral and apophyseal graft groups, the implanted grafts filled the defect site. The newly formed cartilage tissue bonded to the bone and the deeper part of the graft continued with the bone tissue. In the control group, the surface was depressed below the normal joint cartilage and a thin layer of disorganized fibrotic tissue could be detected on top of it. The tissue that formed on the defect area was clearly distinguished.

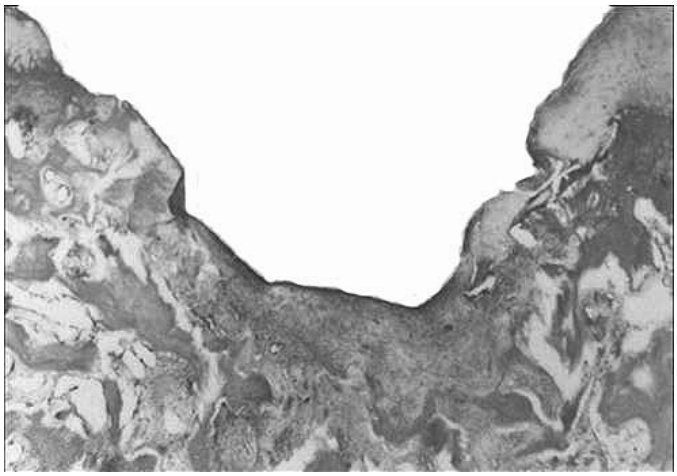

Figure 3. Histological appearance of the formation of irregular cartilage and lack of filling in the defect area in the control group (H. E. x 40).

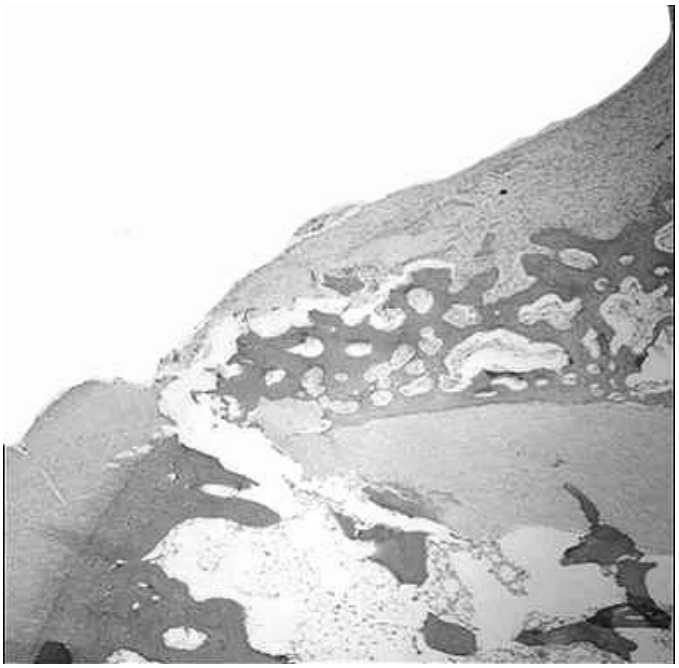

Figure 4. Histological appearance of the hyalin-like cartilage in defects repaired using osteochondral graft (H.E x 40). Repair tissue is on the right, and host tissue is on the left.

At statistical evaluation, with regard to such independent variables as surface area, matrix, cellular distribution and subchondral bone, the osteochondral graft and apophyseal graft groups differed significantly from the control group (p=0.001, p=0.002, p=0.007, p=0.012, respectively). Cartilage mineralization was not significant between these groups. Cell population viability was significant between these groups (p= 0.038), however this difference only emanated from autologous osteochondral graft group (p=0.018).

There was no significant difference between the 2 study groups according to all evaluation methods of ICRS Visual Histological Assessment Scale.

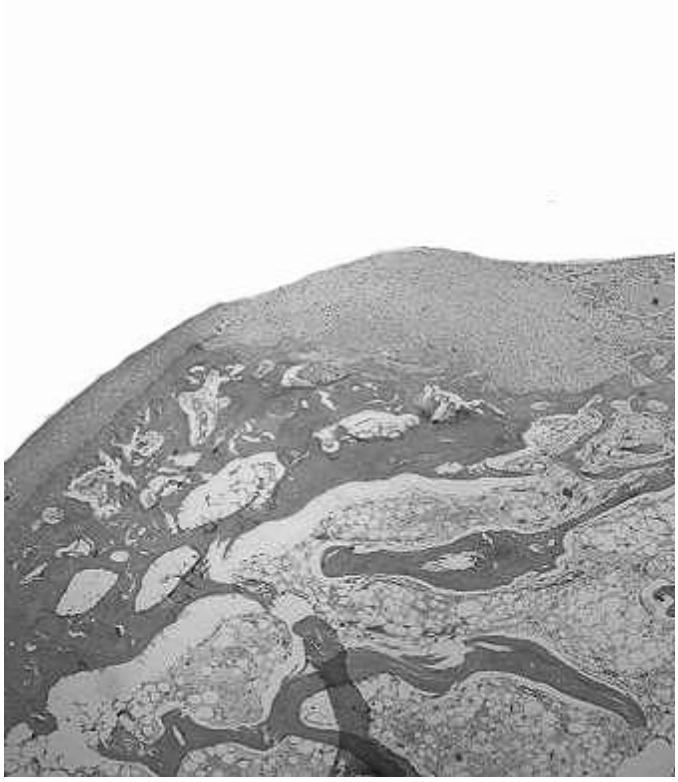

Figure 5. Histological appearance of the hyaline-like cartilage and normal joint cartilage in defects repaired using apophyseal graft (H.E x 40). Repair tissue is on the right, and host tissue is on the left.

DISCUSSION

Cartilage tissue has been acknowledged to have little regeneration and repair potential.[12, 13] When the cartilage is damaged, it is the pericondrium that provides cartilage regeneration as the perichondrial fibrocytes increase. This enables the defect to be filled with the granulation tissue which later becomes less vascularized and turns into fibrotic tissue. Rarely, the cells in such tissues may be chondroblasts and may turn into cartilage tissue under the effect of mechanical factors.[12] Defects in cartilage do not heal unless the subchondral area is involved in the process.[17] Being affected by mechanical and surrounding factors such as pressure, friction power and movement, the bone marrow cells from the subchondral area try to fill the defect. Repair tissues such as fibrosis, fibrocartilage or hyaline cartilage develop from the subchondral bone, and this repair tissue is biochemically and biomechanically different from the hyaline cartilage.[18, 19]

In previous studies marrow stimulating techniques, periosteum, pericondrium, and osteochondral autograft, allografts and autologous chondrocyte implantation were used to treat local cartilage defects.[9, 11, 20]

The best method to repair articular cartilage defects is open to discussion.

Osteochondral grafts are widely used in osteochondral defects. In our study the apophysis was compared to osteochondral grafts. Histologic structure of the iliac apophyseal graft is similar to the articular cartilage.[14, 15] The iliac apophysis is composed of a fibrocartilaginous layer; cartilage similar to epiphyseal cartilage with a physis.[14] Apophyseal grafts seldom have been used as a graft source in the literature. We found a few experimental studies in the literature in which apophyseal grafts were used in the repair of osteochondral defects. Benum[3, 4] used experimentally to transplant of autogenous osteochondral apophyseal grafts from the iliac crest to the defects of the femoral condyles. These findings suggested that the cartilage survived. Wu[15] transplanted experimentally autogenous iliac crest apophyseal graft into the defect created in the epiphysis of the femoral head. He pointed out that it was possible to reconstruct a joint surface and to repair the epiphyseal defect of the joint. Iliac apophyseal cartilage was also used in the treatment of the deficient piriform rim and maxilla in alveolar cleft grafting.[21]

The reasons for using apophyseal grafts in the treatment of osteochondral defects in the rabbit were that; like osteochondral autografts, apophysis bear a structural similarity to osteochondral grafts, resemble cartilage[14, 15] and can be easily obtained. With the aim of obtaining well filled defects, we tried to obtain a level joint surface. Since the grafts were firmly fixed, the animals were allowed to bear weight after surgery. At 12 weeks after implantation, the defects were filled with shiny, smooth, white, semitransparent tissue that resembled articular cartilage and the margins of the defects were remained. Macroscopic and histological evaluations indicated that the defects treated with autogenic implants were all filled with a hyaline-like tissue, while those in the control group were not filled. In addition, histological evaluation showed osseous integration of the transplants in all cases. Gross morphology and histology of untreated cartilage defects at 12 weeks revealed primarily disorganized and dense fibrous tissue filled with a white jelly-like substance. However, better histological results were obtained in the osteochondral and apophyseal groups at statistical analysis. Early success was achieved in the osteochondral autograft group. Survival of the transplanted cartilage and integration of the grafts have been shown. The similar results were obtained with apophyseal graft. We think that apophyseal graft having a smoother, convex surface and being well suited to osteochondral defect influenced this result.

Articular cartilage is comprised of a relatively small number of cells embedded in an abundant extracellular matrix.[16] It consists predominantly of type-II collagen, proteoglycans and water, along with smaller amounts

of other collagen types and noncollagenous proteins. Fibrocartilage, characterised by a high concentration of collagen type-I, rather than hyaline cartilage, which comprises collagen type II, is usually formed when reparative techniques are used.[22] Fibrocartilage is unable to restore the biomechanical properties of normal articular cartilage. In the experimental group of a study similar to our study, newly formed cartilage was found more intense for type II collagen in the matrix with immunohistochemical staining.[9] We could not performed histologic studies demonstrating the properties of type II collagen and glycosaminoglycans due to technical difficulties. Biomechanical tests were not conducted. Radiology was not assessed. A single time point and short follow up (3 months) were used for evaluation. These constituted the limitations of the study. There may be a risk of calcification of the grafted area in apophyseal grafts in a longer follow up. Such phenomena have been described in longer time surveys in the literature.[23,24] In our study, calcification of grafted area could not be described because the time of the follow up was short (3 months) for such an evaluation.

Two major surgical procedures, autologous chondrocyte implantation (ACI) and osteochondral autograft (OA) have recently become the most popular methods in the field. OA is different from others. In osteochondral defects, osteochondral graft plugs are first obtained from the non-weight bearing surface of the knee and then transferred to the defect area.[25] OA is one method that can be used to create hyaline or hyaline-like repair in the defect area.[2] In this way, not only are osteochondral defects filled with original joint cartilage but cartilage healing is also stimulated. Another advantage of the procedure is that it contains cartilage matrix and that with the restoration of the subchondral bone the joint contour is also reconstructed. The most important disadvantage of the method, however, is that the donor area is limited. It can be used to treat large lesions, but the ideal is to perform it on lesions with diameters measuring 1-2 cm, 3-4 cm being the maximum limit.

Another method used in the treatment of cartilage defects is ACI based on purifying and increasing the number of cartilage cells in vitro.[10, 11] Experimental work has shown that chondrocytes and undifferentiated mesenchymal cells placed in articular cartilage defects survive and produce a new cartilage matrix.[25] It can be performed on symptomatic 2-10 cm^2 osteochondral lesions and defects that are not very deep. It is not always advisable for defects deeper than 8-10 mm, for which osteochondral graft is recommended.[20] ACI has several disadvantages: it is a challenging procedure and is not used in deep defects. In addition, it is a two-stage procedure which poses fixation problems and maturation of the implanted cartilage takes a very long time. Finally, it does not allow early weight-bearing and is not cost-effective.

Healed cartilage in animal models is usually evaluated by gross, histology, and mechanical properties.[9] In our study, gross morphology and histology was used for the evaluation of the cartilage. Experimental and clinical investigations show that penetration of subchondral bone with microfractures leads to formation of fibrocartilaginous repair tissue on the articular surfaces.[25] In the previous experiments similar to our study which were performed with rabbits and using osteochondral autograft and chondrocyte implantation, biopsy specimens of the tissue in the grafts sites showed hyaline-like cartilage repair.[9,26] Gross morphology and histology of untreated cartilage defects at 12 weeks in rabbits revealed primarily disorganized and dense fibrous tissue;[9,26] these findings were similar to the both control and experimental groups of our study. In our experimental study, we used osteochondral and apophyseal autograft. In macroscopic findings, the surface of the joint of the newly formed tissue was smooth and transparent in appearance in the 12th week. In the same week, in histologic findings, it was seen that the defect was replaced by the hyaline-like or mixed character cartilage.

Although there have been many experimental and clinical studies, the literature shows no consensus on osteochondral defect repair. Comparative clinical studies involving the most extensively used methods like OA, ACI and microfracture have only recently been conducted.[6-8, 10] These recent comparative studies revealed various differences. In another study osteochondral lesions were treated using a combination of ACI and OA.[22] It was concluded that the hybrid ACI/OA technique provides a promising surgical approach for the treatment of patients with large degenerative osteochondral defects. In our experimental study we used and compared 2 grafts. The results that we obtained from apophyseal grafts at the end of 3 months were similar to those of osteochondral grafts. The results from these 2 groups were superior compared to those of the control groups.

OA has gained in clinical popularity because of its technical feasibility and cost-effectiveness. The advantages of OA are that it is economical and easily available, the immediate availability of viable osteochondral units, reliable osseous in-growth and the fact that it is a single-stage procedure. Donor site morbidity, limitations in graft size and number and difficulties in reconstructing an even cartilage surface are disadvantages. OA can only be used for smaller lesions because of the limited availability of donor plugs. As an alternative to OA in cases

in which the apophysis is still open, we think that apophyseal grafts can be used in the treatment of osteochondral defects with regard to the issue of donor site morbidity. Ossification of the apophysis is part of aging. Apophseal graft may then only be used in young to adolescents and not in adults if one wants to use as an alternative autograft source in the osteochondral defects. When adequate transplants can not be harvested from the condyle, in large defects, and in repeated surgery, apophysis may constitute a source of additional transplants.

In conclusion, we believe that, like the OA used extensively today , apophyseal autografts can be used in the treatment of osteochondral defects. Its major advantages are that it is easy to obtain, it allows early weight-bearing, it does not pose the risk of diseases transmitted by tissue transfer and poses no fixation problem. In addition, it is a single-stage operation, and no resorption occurs. The study not only requires further experimentation with animals for longer periods but also to be conducted on larger defects. Our study may be illuminating donor site morbidity when a limited amount of donor-graft tissue is available for transfer. Although a large number of treatment modalities have been used, ours is the first experimental study, to the best of our knowledge, to compare osteochondral and apophyseal grafts.

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