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Combination of microfracture and periosteal transplantation techniques for the treatment of full-thickness cartilage defects

Tam kalınlıkta kıkırdak defektlerinin tedavisinde mikrokırık ve periost transplantasyon tekniklerinin birlikte kullanımı

Taner GUNES,[1] Cengiz SEN,[1] Mehmet ERDEM,[1] Resid Dogan KOSEOGLU,[2] Nurper ONUK FILIZ[2]

Gaziosmanpasa University, School of Medicine, [1]Department of Orthopaedics and Traumatology, [2]Department of Pathology

Amaç: Kıkırdak defekti tedavisinde mikrokırık ve periost transplantasyon teknikleri birlikte kullanılarak tamir kalitesinin artırılması amaçlandı.

Çalışma planı: Kırk adet Yeni Zelanda türü olgun tavşanın sağ femoral medial kondillerinin yük binme yüzeyinde 4 mm çapında tam kalınlıkta kıkırdak defekti oluşturuldu. Denekler rastgele yöntemle eşit sayıda dört gruba ayrıldı. Kontrol grubunda sadece defekt yaratılıp, herhangi bir işlem uygulanmadı. İki gruba periost flebi transplantasyonu veya mikrokırık uygulandı. Son grupta ise mikrokırık ve periost flebi birlikte uygulandı. Ameliyat sonrasında tüm tavşanlara iki hafta immobilizasyon uygulandı. Ameliyat sonrası 12. haftada tüm hayvanların yaşamı sonlandırılarak, çıkarılan örnekler ICRS (International Cartilage Repair Society) skalasına, yeni oluşan kıkırdak alanına ve canlı kondrosit sayısına göre değerlendirildi.

Sonuçlar: Kombine tedavi grubu, subkondral kemik ölçütü dışında, ICRS skalasının tüm ölçütlerinde (yüzey, matriks, hücre dağılımı, hücre canlılığı, kartilaj mineralizasyonu) diğer gruplara göre anlamlı farklılık gösterdi. Ayrıca, ortalama canlı kondrosit sayısı ve yeni oluşan kıkırdak alanı bu grupta diğer tüm gruplara göre anlamlı derecede yüksek bulundu (p=0.0001).

Çıkarımlar: Mikrokırık veya periost flep tekniklerinin tek başına kullanımıyla karşılaştırıldığında, tamir kalitesinde daha belirgin artış meydana getiren kombine tekniğin kıkırdak defektlerinin tedavisinde daha etkin olduğu görünmektedir.

Anahtar sözcükler: Kıkırdak, eklem/yaralanma/transplantasyon; kondrosit; kondrogenez; periost/transplantasyon; tavşan; rejenerasyon; cerrahi flep; yara iyileşmesi/fizoloji.

Objectives: Microfracture and periosteal transplantation techniques were combined in order to enhance the quality of repair for the treatment of full-thickness cartilage defects.

Methods: In 40 mature New Zealand white rabbits, a full-thickness cartilage defect of 4 mm was induced on the weight-bearing surfaces of the medial condyles of the right femur. The rabbits were randomly divided into four groups equal in size. Control animals remained untreated following defect induction. Two groups were either treated with periosteal transplantation or the microfracture technique, while the fourth group underwent combination of the two techniques. All the animals were immobilized for two weeks postoperatively. At the end of 12 weeks, the animals were sacrificed and the specimens were removed for evaluation according to the criteria of the ICRS scale (International Cartilage Repair Society), and with respect to newly regenerated cartilage areas and the number of viable chondrocytes.

Results: Specimens treated with the combination of the two techniques exhibited significant differences from the other groups in all criteria of the ICRS scale (surface, matrix, cellular distribution, cell viability, and cartilage mineralization) except for subchondral bone criteria. In addition, the mean number of viable chondrocytes and newly regenerated cartilage areas were the highest in this group (p=0.0001).

Conclusion: Due to markedly improved quality of repair, the combination of the microfracture and periosteal flap techniques seems to be more effective than either of the techniques used alone in the treatment of cartilage defects.

Key words: Cartilage, articular/injuries/transplantation; chondrocytes; chondrogenesis; periosteum/transplantation; rabbits; regeneration; surgical flaps; wound healing/physiology.

Correspondence to: Dr. Taner Gunes. Gaziosmanpasa University, School of Medicine, Department of Orthopaedics and Traumatology, Tokat. Phone:+90 356 - 213 32 94 Fax: +90 356 - 213 31 79 e-mail: drtgunes@gmail.com

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The treatment of focal cartilage lesions presents problems which must be solved due to the functionality of cartilage tissue and its inability of regeneration. Although many treatment modalities have been used for regeneration, degenerative complications are always the issue.The goal of the treatment is to repair the defect by generating a healthy and durable hyaline cartilage which protects the joint against daily stresses and also maintains its biomechanical characteristics.

Inadequate regeneration of cartilage lesions is attributed to the avascular nature of the cartilage tissue, lower rate of mitotic activities and scarcity of chondrocytes.[1,2,3] In effect, a chondrocyte per se is metabolically very active. [3,4] However they are relatively fewer within tissues, and constitute only approximately 1 % of the cartilage tissue.[4] In addition, under normal circumstances chondrocytes of adult articular cartilage do not proliferate significantly.[5] Therefore for the regeneration process of the cartilage posttraumatically, as many chondrocytes as possible must be present on the lesion site.[6] The success of autologous chondrocyte transplantation especially in the repair of large defects is due to placement of large number of viable chondrocytes obtained with the cultivation of chondrocytes on the defective area, and also the enhancement of the number of chondrocytes by covering the defect with periosteum which contains chondrogenic precursor cells. Accordingly, this technique is called "double chondrogenic process".[6]

The goal of preclinical studies is basically involved with the reconstruction of the articular surface.[1,7,8] This goal is different from clinical studies intending to achieve painless functional joint. Besides, any animal model, though not applicable to directly to humans, can form the basis of trials to be performed in humans.[1,7] The key point is the selection of an animal model appropriate for the hypothesis to be searched and tested.[7] Since clinical problems related to cartilages usually do not involve subchondral bone, in animal models defects not penetrating subchondral bone have been used experimentally.[9] For rabbit models spontaneous healing of full thickness (4 mm) defects are not possible.[10]

Since recruitment of more cells into the defective area enhances the quality of repair, combination of microfracture and periosteal transplantation techniques originating from two different strategies was planned to be used in the treatment of cartilaginous defects of synovial joints, and repair tissue regenerated by combined technique was examined histopathologically.

Material and method

The study was performed with New Zealand white, 6- month mature rabbits weighing 3-4 kg. The rabbits were randomly assigned to 4 groups consisting of 10 rabbits each. A full thickness cartilage defect was generated in all rabbits. Control group (C) was left to spontaneous healing without applying any treatment. Periosteal transplantation was used for the treatment of rabbits in Group P. Microfracture technique was used for the rabbits in Group MF. For the experimental MF + P group, microfracture and periosteal flap techniques were combined. The area of repaired defect was immobilized for two weeks postoperatively in order to protect periosteal flaps from detachment. Any restriction except for 2 weeks of immobilization was not instituted, and the rabbits were fed with standard rabbit food, and water ad libitum. The rabbits were exposed to light and dark cycle for 12 hours alternatively, nurtured and treated in 60x40x40 cm cages. The study was realized in Gaziosmanpaşa University, Medical Faculty, Research Center for Experimental Animals after the approval of Ethics Committee (Gaziosmanpaşa Universıty, Dean of Medical Faculty, Ethics Committee of Medical –Surgical- Pharmacological Researches, Approval. No.: 03-GEKTIP-016).

Surgical technique

Ketamine (10 mg/kg i.m.) and xylazine (8 mg/kg i.m.) were used for anesthesia. Cephazoline sodium (20 mg/kg i.m.) was given for prophylaxis. Right lower extremity of every rabbit was shaved. After scrubbing and draping of the involved area, knee was explored through an anterior midline longitudinal incision. Following medial parapatellar arthrotomy, patella was dislocated laterally and the complete exposure of the knee was achieved. A full thickness cartilage defect was created under magnification on the weight bearing aspect of medial femoral condyle without traumatizing subchondral bony structures using a 4 mm dermal punch and a special curette. Subchondral bone was protected and bleeding from the base of the defect was avoided during the creation of a defect. An additional intervention was not performed for rabbits in the control group, and defect was left untreated for spontaneous healing.

A microfracture technique was applied from the periphery to the center of the defect, creating holes 0.5 mm in width and 1-2 mm in depth leaving intact tissue between the holes. Care was taken to bleed from every hole.

In Group P, the incision was extended distally, and a periosteal flap (10 x 5 mm) from anteromedial aspect of tibia was obtained using a sharp edged periosteal elevator as suggested in the literature.[11] The flap was divided into 2 pieces of 5 x 5 mm each. One piece was examined histologically for the presence of cambium layer. The edges of the other piece was rounded and placed inside the defect with its cambium layer facing to the defect. The edges of the specimen were fixed to the intact edges of the adjacent cartilage with intermittently placed separate sutures of 10/0 vicryl (Ethicon, San Angelo, TX, USA) under the magnification of surgical microscope. An average of 4 sutures were used for each flap.

In MF+P group, following microfracture technique used as in MF group, a periosteal transplantation was applied.

The stages of the surgical technique are shown in Figure 1.

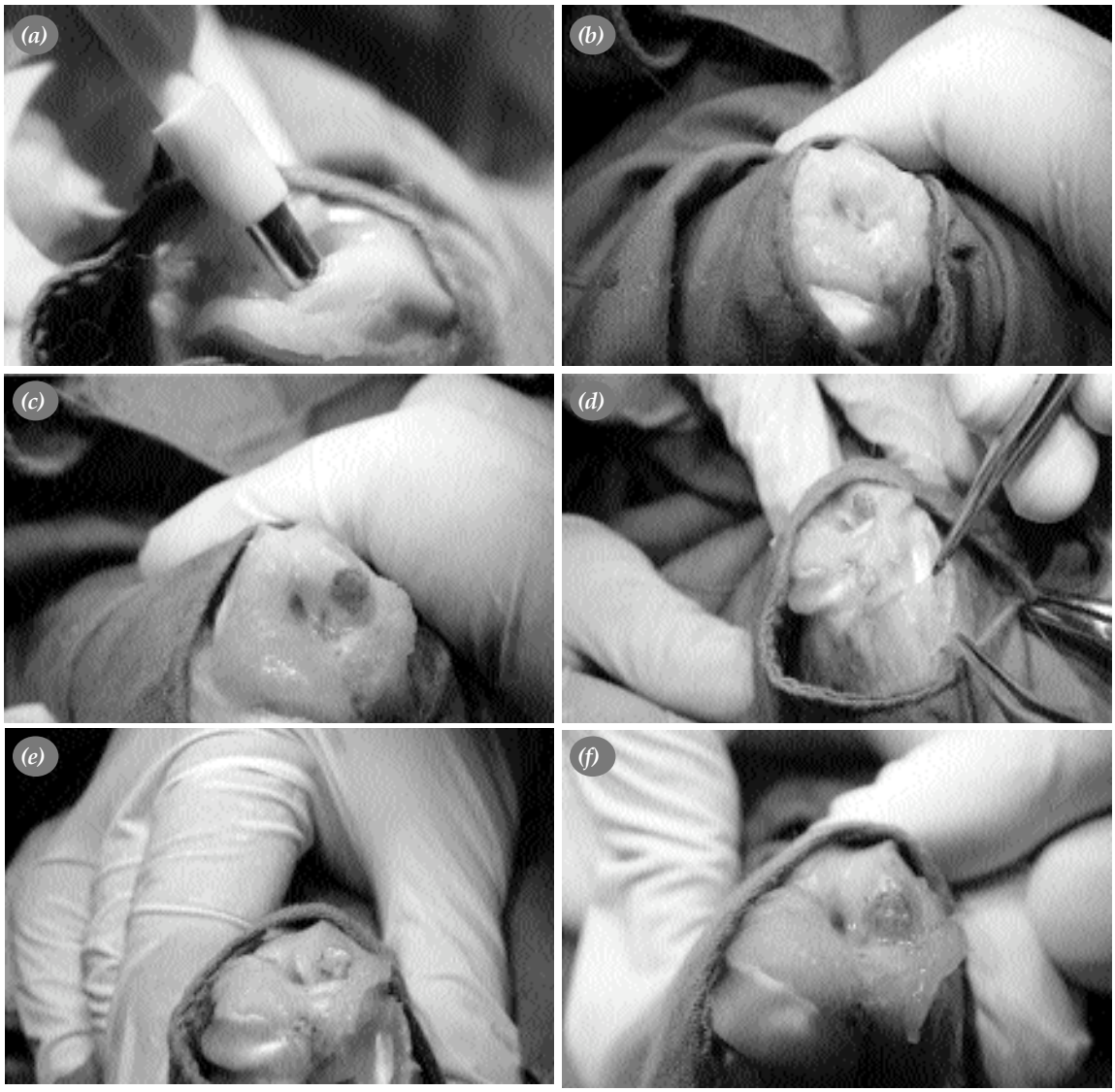

Figure 1. Stages of the surgical procedure. **(a)** Delineation of the borders of the defect with 4 mm dermal punch **(b)** The appearance of full thickness defect. **(c)** microfracture procedure. **(d)** harvesting the periosteum from anteromedial aspect of proximal tibia. **(e)** placement of periosteum on the defect. **(f)** the appearance of the flap after fixation to the peripheral cartilage tissue with intermittent sutures.

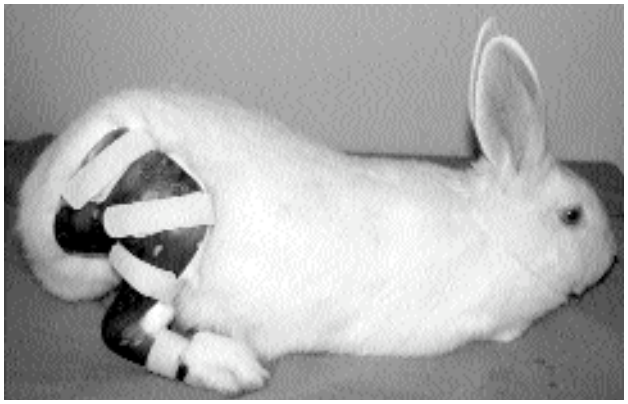

Figure 2. Brace used for postoperative immobilization

After the procedure, knees were irrigated with physiologic saline. Continuous sutures were used for the closure of the arthrotomy defect (4/0 vicryl, Ethicon, San Angelo, TX, USA) and skin (3/0 silk) incision. For the protection of the flap during the early postoperative period, knees were immobilized in the natural sitting position of the rabbit. To establish uniformity for the sake of comparison, 2 weeks of immobilization was applied also in other groups. For immobilization specially designed braces were used (Figure 2).

Postoperative care

For analgesia, acetaminophene was dissolved in 100 ml tap water to achieve a dose of 1-2 mg/kg. In addition, cephazoline sodium 20 mg/kg i.m. was used for 5 days. Daily wound care was realized with a brace. After 2 weeks, immobilization of rabbits was terminated. After this stage, any other restriction was not applied and rabbits were left in their nature.

Sacrification

At 12. weeks postoperatively, all rabbits were sacrificed using high doses of penthotal sodium. Any restriction in the range of motion of knees was not observed during delicate physical examination. After sacrification of the animals, distal femora and some part of distal tibias were resected avoiding any harm to the knee joint. Soft tissues in the resection material were dissected, in order to protect the structural integrity of knee joints. After the dissection of all soft tissues, knee joint capsule was opened respecting its anatomical integrity. Distal femur containing both condyles was detached.

Preparation for histological examination

Distal femur resection material was fixated in formaldehyde solution for 48 hours. After fixation distal femur material containing both condyles were kept in buffered rapid decalcification solution for 48 hours. Following decalcification procedure, distal femur were dissected on the frontal plane in-line with medial condyles keeping experimental cartilage defects in the center of the operation field. Both halves of the medial condyles including the defective areas were monitored for histological changes. During preparations for histological examination, after fixation in formaldehyde solution for 48 hours, dehydratation with alcohol, treatment with xylene, and infiltration with paraffine, sections were embedded in paraffine blocks. Five serial sections of 4 µm thickness taken from each half of medial condyles were stained with hematoxylene-eosin.

Table 1. ICRS (International Cartilage Repair Society) scale

Feature		Score
I	Surface	
	Smooth/continuous	3
	Discontinuities/irregularities	0
II	Matrix	
	Hyaline	3
	Mixture:Hyaline/fibrocartilage	2
	Fibrocartilage	1
	Fibrous tissue	0
III	Cell distribution	
	Columnar	3
	Mixed/columnar-clusters	2
	Clusters	1
	Individual cells/disorganized	0
IV	Cell population viability	
	Predominantly viable	3
	Partially viable	1
	<%10 Viable	0
V	Subchondral bone	
	Normal	3
	Increased remodelling	2
	Bone necrosis /granulation tissue	1
	Detached/fractured/callus at base	0
VI	Cartilage mineralization (calcified cartilage)	
	Normal	3
	Abnormal/inappropriate location	0

Histomorphometry

Cartilage regeneration was evaluated histologically on hematoxylene-eosin stained sections according to ICRS (International Cartilage Repair Society) Scale[12] (Table 1). Besides in the same sec-

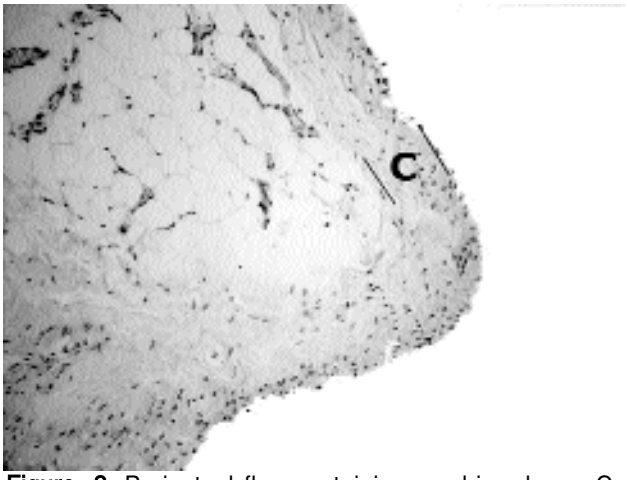

Figure 3. Periosteal flap containing cambium layer. C; cambium layer

tions, newly formed cartilage tissue was measured (mm2). For this calculation, 5 serial sections (overall 10 sequential sections) prepared from each half of the medial condyles which contained cartilage defect areas were examined with ocular micrometres, and newly formed areas of cartilage tissue were estimated. Newly formed areas of cartilage tissue in 10 sections were averaged. The resultant average value was accepted as the means of newly formed areas of cartilage tissue in each experimental animal. Also, viable chondrocytes in newly regenerated areas of cartilage tissue in sections stained with hematoxylene-eosin were counted. Chondrocytes were counted separately in 10 sequential sections, and then average number of chondrocytes in newly regenerated cartilage tissue was calculated. Chondrocytes were counted with a computer using a software program (ImageJ V1.33, National Institute of Health, USA). In Groups MF+P, and P where periosteal flaps were to be applied, periosteal specimens taken from the grafts were monitored histologically in order to demonstrate the presence of cambium layer, and to that effect sections in 4 µm thickness were dissected and stained with hematoxylene-eosin. Every specimen were evaluated as for the presence of cambium layer (Figure 3). Experimental animals without any evidence of cambium were excluded from histological and histomorphometric evaluations. Accordingly, one animal both in Group P, and MF+ P, were not included in the assessments.

Evaluations

All evaluations were performed by 2 separate independent pathologists unaware of the allottments of animals into groups.

Statistical evaluations

Kruskall-Wallis ANOVA test was used for the evaluation of parametres relevant to newly formed cartilage tissue area and the number of chondrocytes in all groups, while intergroup comparisons were performed with Mann-Whitney-U test (Bonferroni correction was used). For evaluations done according to ICRS scale chi-square test (likelihood ratio was used) was employed. P values less than 0.00833 were considered statistically significant.

Results

At the end of 12. weeks, septic arthritis developed in one rabbit, and 3 rabbits died of unknown causes in Group C. Also flaps detached in 2 rabbits, and periosteal flap of one rabbit didn't contain cambium layer in Group P. One rabbit became infected, and 2 rabbits died of unknown causes in MF group. In one rabbit flap detached, and cambium layer was not evident in periosteal flap specimens in MF+P group. Because of these unwanted complications 6 rabbits in Group C, 7 rabbits both in Group P and MF, and 8 animals in Group MF + P were included in evaluations. Histological findings of all groups are shown in Figure 4.

Cartilage areas

Mean areas of repaired and regenerated tissue formed within the defect were calculated (0.18+0.02 mm2 in C, 0.36+0.03 mm2 in MF,

Table 2. ICRS scale values of the groups

	Surface	Matrix	Cell Distribution	Cell Population	Subchondral Bone	Cartilage Mineralization
C	0+0.00	0.67+0.52	0.67+0.52	1+0.00	0.67+1.21	0+0.00
P	0.86+1.46	2+0.58	1.71+0.95	2.14+1.46	2.14+1.07	1.71+1.60
MF	0+0.00	1.86+0.69	1.43+0.79	2+1.29	0.86+1.07	1.29+1.60
MF+P	2.63+1.06	2.63+0.52	2.5+0.53	2.5+0.93	2+0.93	2.63+1.06
p value	0.0001	0.0001	0.001	0.001	0.056	0.003

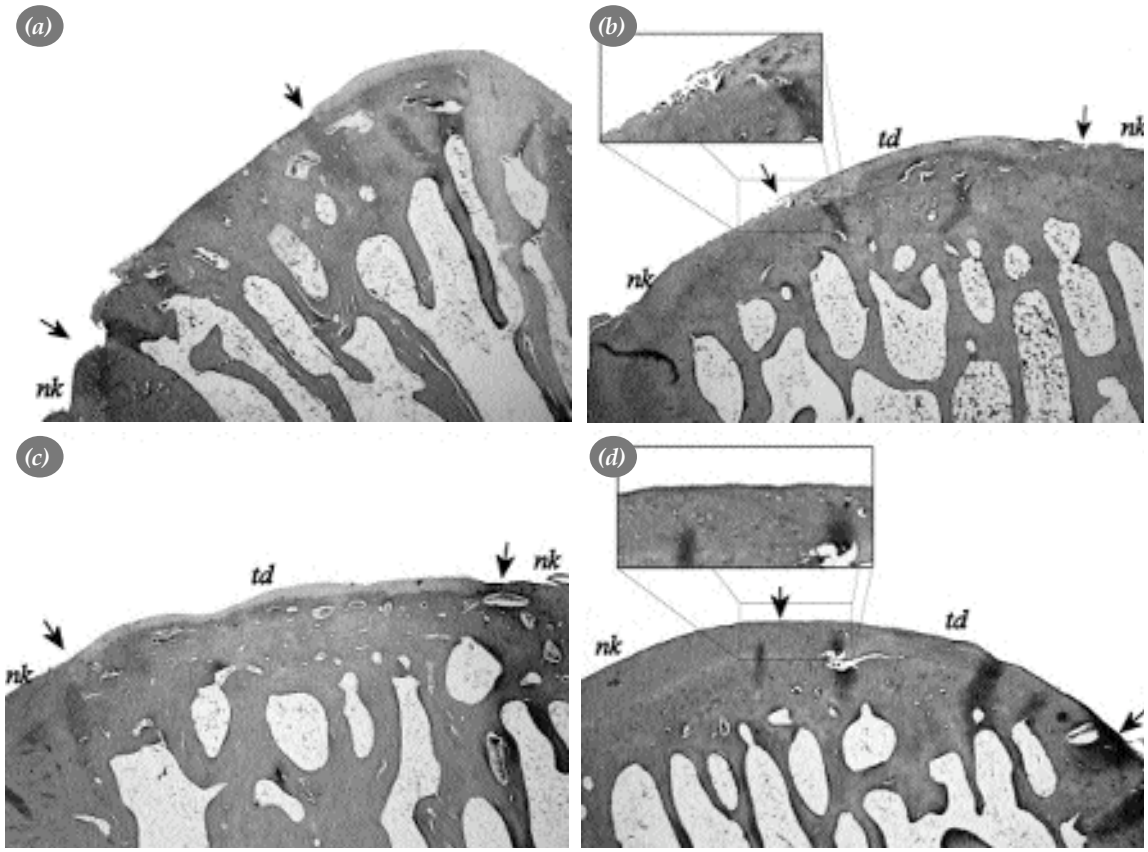

Figure 4. Histological sections obtained from experimental animals. **(a)** control group, **(b)** periosteal group, **(c)** microfracture group, **(d)** MF + P group. Apparently cartilage repair tissue didn't regenerate (a). In periosteal flap group defect area is covered almost completely with irregular cartilage repair tissue. **(b)** In microfracture group the cartilage repair tissue is relatively thinner and irregular when compared with the other repair groups. **(c)** Histological appearance of repair tissue in microfracture –periosteal flap group. The best results were obtained in this group where cartilage repair tissue with smooth surface has apparently attained the tissue depth of the normal cartilage tissue and integrated with it perfectly. **(d)** *arrows :* borders of repair tissue. *nk :* normal cartilage. td : repair tissue.

0.39+0.12 mm2 in P, and 0.64+0.03 mm2 in MF + P groups). Estimated areas in Groups MF and P didn't differ significantly(p=0.209), while this difference reached a statistically significant level in comparisons between MF +P, and the other groups (p=0.0001) .

Viable cell counts

Mean (± SD) numbers of viable chondrocytes in newly formed regenerated tissue in the defect were calculated to be 44.67+5.85, 102.86+20.33, 120.86+14.00, and 172.75+30.47 in Groups C, MF, P, and MF + P respectively. According to these estimates, viable cells in the repair tissue in treatment groups were higher than those found in the control group (C). Although the number of viable cells were higher in Group P when compared with Group MF, a significant difference was not found between these groups (p=0.128). In Group MF+P, mean number of viable cells were higher than those found in other groups(p=0,0001).

ICRS scale

In all groups, defects were evaluated according to ICRS scale criteria for surface, matrix, cellular distribution, cell population viability, subchondral bone and cartilage mineralization (calcified cartilage) in hematoxylene-eosin stained sections. ICRS scale values are shown in Table 2.A significant difference was found as for the scale's criteria for surface, matrix, cell distribution, cell population viability, and cartilage mineralization. However significant differences among groups as for the criteria concerning subchondral bone were not detected. The surface of repair tissue showed irregularities in

Group C and MF. Although surface of the repair tissue in Group P were better than Groups C and MF, the best group in this regard was Group MF + P (x2=20.761, p=0.0001). According to ICRS evaluation criteria for matrix, hyaline content of Group MF+ P were higher, and a significant difference existed between Group MF + P, and the other groups. (x2=31,054, p=0.0001). Cell distribution in Group MF+P was occasionally in the form of clusters or columns, whereas in other groups clusters and isolated patches were detected (x2=27.421, p=0.001). The proportion of cell population viability were higher in Group MF+P (x2=21.966, p=0.001). The number of specimens with nearly normal calcified cartilaginous mineralization in Group MF + P were higher than those found in other groups. (x2=13,666, p=0.003). All groups were found to be problematic with respect to subchondral bone criteria of the ICRS scale (x2=16,567, p=0,056).

Discussion

Complete regeneration of cartilage defects has not been reported yet.[1,13,14] Although various methods have been developed for the treatment of cartilage defect, a long-term solution is not available. The main problem of many techniques is insufficient biomechanical characteristics of newly formed repair or regenerated tissue. [15] Optimal treatment for cartilage defect must be cost-effective, reversible and above all should inhibit the development of osteoarthritis in the long run.[2]

Cartilage repair has two main objectives: 1. clinical resolution of pain, and recovery of articular functions, 2. inhibition of osteoarthritis and/or delay of its onset. [7,8,9] Another practical anticipation is the postponement of prosthetic replacement for a significantly longer time interval.[8] Many factors influence long-term success of cartilage repair. The degree of welding of the repair tissue to the defect area and long term maintenance of this integrity carry utmost importance. [9] Another important factor is the number of viable cells recruited to the defect area. All cartilage repair methods are concerned with the recruitement of chondrogenic cells into injured area. Repair which highly depends on mesenchymal cells, relies on the presence of these cells in the media, and as many cells as possible must be recruited to the defect area. [6]

The restoration of the articular surface and interventions for the repair of the defect are based mainly on two strategies [8,16] 1) enhancement of intrinsic capacity of the cartilage and subchondral bone to achieve improvement, 2) resurfacing of new articular facets through transplantation of chondogenic cells and chondrocytes which have potential for the regeneration of new cartilage tissue. Lavage and debridement,[16] subchondral drilling [2,17], abrasion,[2] microfracture[2,18,19] are techniques aimed at the enhancement of intrinsic capacity.[16] However periosteal transplantation,[16,17,20] perichondral arthroplasty,[16,17,21,22] autologous osteochondral transplantation,[1,15] and autologous chondrocyte transplantation [2,6,16,23] are also intended for chondral resurfacing.[16] Among these techniques, autologous chondrocyte transplantation differs from other methods in that this technique use two abovementioned different basic strategies in combination. Currently autologous chondrocyte transplantation is recommended especially for larger defects.[2] For the realization of this method, laboratories for tissue cultures must be established, standardized or work in collaboration with the other conventional laboratories. Besides, this technique requires two operative sessions. After the application of this technique, some authors observed 87 percent of newly formed repair tissue in the defect area [23], and 5 -year follow-ups yielded clinically successful outcomes at 90 % of the cases. [17,24] However in a randomized study after 2 years of follow-up, a significant difference in morphologic and histologic parametres between autologous chondrocyte transplantation and microfracture procedures was not found. Besides, a clinically significant correlation was not detected between histological findings and clinical outcomes. [25]

Microfracture method relies on "super clot" induced by recruitement of bone marrow elements in defect area, and the differentiation of mesenchymal stem cells of bone marrow origin to chondrocytes with resultant hyaline cartilage formation. [2,18,19] Microfracture method is a resurfacing procedure aimed at formation of a pluripotent media which induces tissue regeneration at defect area, and reinforces recovery potential of the body.[18] Although tissue formed after microfracture procedure is speculated to be a fibrocartilaginous tissue instead of hyaline cartilage, [26] Stedman et al. stated that tissue regenerated after microfracture procedure is a combination of hyalinous and fibrocartilaginous components, and its type 2 collagen content reaches up to 70 % a year after the operation.[19] Even a marked improvement was noted at first postoperative year , actual complete recovery was achieved 2-3 years postoperatively.[19] The longevity of the

regeneration is thought to depend on physiologic remodelling of the regenerated tissue, and additional procedures are contemplated for the shortening of this time interval. [9,19] We consider that combined microfracture and periost transplantation can decrease physiologic remodelling time of microfracture technique.

Chondrogenic ability of periosteum is acknowledged for a long time. [27] Mesenchymal precursor cells in the cambium layer of the periosteum are transformed into chondrocytes under the influence of local intraarticular factors which ensure chondrogenesis.[20,28] Studies have shown that chondrocytes in the repair tissue formed after periosteal transplantation were of periosteal origin.[28] In addition, periosteum contains bioactive factors effecting the formation of matrices and tissues which contain cellular components necessary for cartilage repair. [28] Nonetheless, chondrogenic potential of periosteum decreases with aging.[29] Flap transplantation induces cellular proliferation (postop. 1-10 days), cellular differentiation (postop. 7-28 days), and matrix formation (postop. 10-42. days).[28] In another study the depth of the regenerated tissue at 12. weeks of periosteal flap procedure was higher than that of the normal cartilage, and its integration with normal cartilage was satisfactory. At 24. weeks the thickness of the repair tissue was approximated normal values.[10] In this study tissue glues were used for the fixation of the flap. We didn't observe any evidence of thickening at 12. weeks after suture fixation of periosteal flaps. However some authors thought that periosteal transplantation per se does not contribute significantly to cartilage repair. [23] The largest series of periosteal transplantation belongs to Lorentzon et al.[30] According to these authors, periosteal transplantation per se is a satisfactory clinical method for the regeneration of the joint cartilage. One of the factors determining the quality of cartilage repair is the integration between the repair tissue and the surrounding healthy cartilage tissue. It was observed that the introduction of cultured chondrocytes into repair area induced the formation of repair tissue, but for the improved integration between repair tissue and healthy cartilage tissue periosteal transplantation was necessitated.[6] The authors attributed this to bioactive factors released from periosteum. Another factor contributed to improvement of repair quality is postprocedure rehabilitation with controlled passive movements. Studies have shown that dynamic compression induced by controlled passsive movements performed after microfracture,[18,31,32] and periosteal transplantation [16,20,28] procedures would regulate regenerative potentials of chondrocytes, and also improve the quality of resurfacing. However, some authors have suggested that clinically controlled passive manipulations after microfracture procedures and routine rehabilitation do not differ in clinical outcomes,[33]

In the English medical literature, only one study reported the usage of combined microfracture and periosteal transplantation.[34] Siebold et al. obtained satisfactory results clinically and radiologically in a limited number of patients with humeral head defects with this technique. However they didn't perform histological evaluations. Therefore the characteristics of the repair tissue achieved with this technique remained unexplained.

Among the limitations of this study, inability to perform controlled passive manipulations on rabbits during postoperative period, difficulty in suturing periosteal flap because of the intrinsic thinness of cartilage layer of rabbits (approximately 0.5 mm), [1, 8] detachment of periosteal flap despite immobilization during follow-up, and shorter follow-up period can be enumerated. In spite of these limitations, in our study combined technique provided wider repair tissue and recruitement of higher number of viable chondrocytes into the regeneration area when compared with other procedures. Besides, according to ICRS criteria of regenerated surface, matrix, cellular distribution, cell population viability and cartilage mineralization, combined technique provided better results. Under the light of all these information, combination of microfracture and periosteal transplantation technique has resulted in thicker and more qualified repair tissue compared with isolated usage of each technique per se. In addition, the results of this study suggest that periosteal transplantation combined with microfracture procedure might shorten the duration of above-mentioned physiologic remodelling process after microfracture process and might improve decreasing chondrogenic potential of periosteum with aging. The combined technique investigated in this study can be considered as a clinically alternative method in that it is a cost-effective approach performable in a single session.

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References

1. Breinan HA, Hsu HP, Spector M. Chondral defects in animal models: effects of selected repair procedures in canines. Clin Orthop Relat Res 2001;(391 Suppl):S219-30.
2. Sgaglione NA, Miniaci A, Gillogly SD, Carter TR. Update on advanced surgical techniques in the treatment of traumatic focal articular cartilage lesions in the knee. Arthroscopy 2002;18(2 Suppl 1):9-32.
3. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 2001;3:107-13.
4. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect 1998; 47:477-86.
5. Rosier RN, O'Keefe RJ. Autocrine regulation of articular cartilage. Instr Course Lect 1998;47:469-75.
6. Brittberg M. Autologous chondrocyte transplantation. Clin Orthop Relat Res 1999;(367 Suppl):S147-55.
7. O'Driscoll SW. Preclinical cartilage repair: current status and future perspectives. Clin Orthop Relat Res 2001;(391 Suppl):S397-401.
8. Reinholz GG, Lu L, Saris DB, Yaszemski MJ, O'Driscoll SW. Animal models for cartilage reconstruction. Biomaterials 2004;25:1511-21.
9. Frisbie DD, Oxford JT, Southwood L, Trotter GW, Rodkey WG, Steadman JR, et al. Early events in cartilage repair after subchondral bone microfracture. Clin Orthop Relat Res 2003;(407):215-27.
10. Carranza-Bencano A, Garcia-Paino L, Armas Padron JR, Cayuela Dominguez A. Neochondrogenesis in repair of full-thickness articular cartilage defects using free autogenous periosteal grafts in the rabbit. A follow-up in six months. Osteoarthritis Cartilage 2000;8:351-8.
11. O'Driscoll SW, Fitzsimmons JS. The importance of procedure specific training in harvesting periosteum for chondrogenesis. Clin Orthop Relat Res 2000;(380):269-78.
12. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander A, Hunziker E, et al. Histological assessment of cartilage repair: a report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). J Bone Joint Surg [Am] 2003;85 Suppl 2:45-57.
13. Jackson DW, Lalor PA, Aberman HM, Simon TM. Spontaneous repair of full-thickness defects of articular cartilage in a goat model. A preliminary study. J Bone Joint Surg [Am] 2001;83:53-64.
14. Prakash D, Learmonth D. Natural progression of osteochondral defect in the femoral condyle. Knee 2002;9:7-10.
15. Hangody L, Feczko P, Bartha L, Bodo G, Kish G. Mosaicplasty for the treatment of articular defects of the knee and ankle. Clin Orthop Relat Res 2001;(391 Suppl):S328-36.
16. O'Driscoll SW. The healing and regeneration of articular cartilage. J Bone Joint Surg [Am] 1998;80:1795-812.
17. Menche DS, Vangsness CT Jr, Pitman M, Gross AE, Peterson L. The treatment of isolated articular cartilage lesions in the young individual. Instr Course Lect 1998;47: 505-15.
18. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res 2001;(391 Suppl):S362-9.
19. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy 2003;19:477-84.
20. O'Driscoll SW. Articular cartilage regeneration using periosteum. Clin Orthop Relat Res 1999;(367 Suppl):S186-203.
21. Amiel D, Coutts RD, Abel M, Stewart W, Harwood F, Akeson WH. Rib perichondrial grafts for the repair of full-thickness articular-cartilage defects. A morphological and biochemical study in rabbits. J Bone Joint Surg [Am] 1985;67:911-20.
22. Bombaci H, Turkmen IM, Aker F, Bombaci E. The use of chondral rib in the treatment of articular cartilage defects. [Artricle in Turkish] Acta Orthop Traumatol Turc 2000; 34:403-11.
23. Brittberg M, Nilsson A, Lindahl A, Ohlsson C, Peterson L. Rabbit articular cartilage defects treated with autologous cultured chondrocytes. Clin Orthop Relat Res 1996;(326): 270-83.
24. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. J Bone Joint Surg [Am] 2003;85 Suppl 2:17-24.
25. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg [Am] 2004;86:455-64.
26. Robinson D, Nevo Z. Articular cartilage chondrocytes are more advantageous for generating hyaline-like cartilage than mesenchymal cells isolated from microfracture repairs. Cell Tissue Bank 2001;2:23-30.
27. O'Driscoll SW, Keeley FW, Salter RB. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg [Am] 1986;68:1017-35.
28. O'Driscoll SW, Fitzsimmons JS. The role of periosteum in cartilage repair. Clin Orthop Relat Res 2001;(391 Suppl): S190-207.
29. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. The chondrogenic potential of periosteum decreases with age. J Orthop Res 2001;19:95-103.
30. Lorentzon R, Alfredson H, Hildingsson C. Treatment of deep cartilage defects of the patella with periosteal transplantation. Knee Surg Sports Traumatol Arthrosc 1998; 6:202-8.
31. Salter RB, Simmonds DF, Malcolm BW, Rumble EJ, MacMichael D, Clements ND. The biological effect of continuous passive motion on the healing of full-thickness defects in articular cartilage. An experimental investigation in the rabbit. J Bone Joint Surg [Am] 1980;62:1232-51.
32. Kim HK, Moran ME, Salter RB. The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. J Bone Joint Surg [Am] 1991;73:1301-15.
33. Marder RA, Hopkins G Jr, Timmerman LA. Arthroscopic microfracture of chondral defects of the knee: a comparison of two postoperative treatments. Arthroscopy 2005;21:152-8.
34. Siebold R, Lichtenberg S, Habermeyer P. Combination of microfracture and periostal-flap for the treatment of focal full thickness articular cartilage lesions of the shoulder: a prospective study. Knee Surg Sports Traumatol Arthrosc 2003;11:183-9.