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The healing of full-thickness articular cartilage defects in rabbits: successful results with fibroblast growth factor

Tavşanlarda tam kat eklem kıkırdak kaybının iyileşmesi: Fibroblast büyüme faktörü ile başarılı sonuçlar

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Objectives: This experimental study aims to compare the effects of three techniques; free autogenous periosteal graft, demineralized bone matrix (DBM) and basic fibroblast growth factor (bFGF) combined with periosteal graft on the healing of full thickness joint cartilage defects in rabbits.

Materials and methods: This study used 87 adolescent 16 week-old New Zealand rabbits of both sexes, with an approximate weight of 2500-3750 g. The rabbits were randomly divided into four groups including a control group and three experimental groups. Cartilage defects were created in the posterior weight-bearing area of the medial femoral condyles of the rabbits. The surfaces of the osteochondral defects were covered with free autogenous periosteal graft, DBM and periosteal graft combined with bFGF in the experimental groups respectively. The rabbits were sacrificed at 4, 8 and 12 weeks postoperatively. Macroscopic and microscopic evaluations were performed.

Results: Periosteal grafts and DBM respond to the repair of cartilage defects in varying degrees. Although the macroscopic evaluation scores were higher in the bFGF group, there was no statistically significant difference between groups ($p>0.05$). The total scores on the histological grading scale were significantly higher in the bFGF group and control group than the other groups at 4th and 8th weeks ($p<0.05$). At the 12th week the total score was significantly higher in the bFGF group than the other three groups ($p<0.05$).

Conclusion: The application of bFGF promoted regeneration of articular cartilage and led to successful cartilaginous resurfacing of defects within 12 weeks. We suggest that bFGF when combined with periosteal grafts may have excellent repair capacity in the restoration of osteochondral defects.

Key words: Articular; cartilage; fibroblast growth factor 2.

Amaç: Bu deneysel çalışmada serbest otojen periosteal greft, demineralize kemik matriksi (DKM) ve temel fibroblast büyüme faktörü (bFGF) ile kombine edilmiş periosteal greftin kullanıldığı üç tekniğin tavşanlardaki tam kat eklem kıkırdak kaybının iyileşmesi üzerine etkileri karşılaştırıldı.

Gereç ve yöntemler: Bu çalışmada ağırlıkları yaklaşık 2500-3750 g olan 16 haftalık her iki cinsiyette 87 adet Yeni Zelanda tavşanı kullanıldı. Tavşanlar randomize olarak bir kontrol ve üç deney grubu olmak üzere dört gruba ayrıldı. Kıkırdak kaybı tavşanların medial femoral kondilitinin yük taşıyan posteriyor alanında oluşturuldu. Çalışma gruplarında osteokondral kayıpların yüzeylerine sırasıyla serbest otojen periosteal greft, DKM ve bFGF ile kombine periosteal greft uygulandı. Ameliyat sonrasında tavşanlar 4, 8 ve 12. haftalarda sakrifiye edildi. Makroskopik ve mikroskopik değerlendirmeler yapıldı.

Bulgular: Periosteal greft ve DKM'nin kıkırdak kaybının iyileşmesine yanıtı değişik düzeylerde idi. Makroskopik değerlendirme puanları bFGF grubunda daha yüksek olsa da, gruplar arasında istatistiksel olarak anlamlı bir fark yoktu ($p>0.05$). Histolojik derecelendirme skalasındaki toplam puanlar bFGF grubunda ve kontrol grubunda diğer gruplara göre 4. ve 8. haftalarda, anlamlı derecede yüksekti ($p<0.05$). On ikinci haftada, toplam puan bFGF grubunda diğer üç gruba oranla anlamlı derecede yüksekti ($p<0.05$).

Sonuç: bFGF uygulaması eklem kıkırdak yenilenmesini artırır ve 12 hafta içerisinde kaybın başarılı kıkırdak yüzeyle kaplanmasına yardım eder. Sonuç olarak, osteokondral kayıpların onarımında mükemmel bir onarım kapasitesi sağlamak için bFGF'nin periosteal greftle kombine edilerek uygulanmasını öneriyoruz.

Anahtar sözcükler: Eklem; kıkırdak; fibroblast büyüme faktörü 2.

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Full-thickness defects penetrating the subchondral bone undergo repair processes which result in the generation of either fibrous or fibrocartilaginous tissue or, to a very limited extent, hyaline cartilage. Bone marrow contains pluripotent mesenchymal progenitor cells, which can differentiate into multiple differentiated cell-types such as chondrocytes, osteocytes, adipocytes, and fibroblasts. These marrow-derived mesenchymal cells have an essential role in the repair of the full-thickness defects of articular cartilage.[1]

It is known that the sequence of events during chondrogenesis is regulated by various growth and differentiation factors such as basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-β), and members of the bone morphogenetic protein (BMP) family.[2-5] Recent studies reported that bone morphogenetic protein family members are expressed during cartilage repair, and BMP-2, BMP-4, TGF-β1, IGF-1 were measured in demineralized bone matrix (DBM).[6] Demineralized bone matrix has been used as another alternative treatment for cartilage repair in recent experimental studies.[7]

Among the growth factors, bFGF has attracted particular attention as the most potent known chondrocyte mitogen. Furthermore, it stimulates or stabilizes the synthesis of cartilaginous matrix by chondrocytes.[8]

It is known that periosteal transplant is one of the oldest procedures ever developed to repair cartilage defects and showed improved results in the long term.[9]

In this experimental study, we compared the histological effects of periosteal graft, DBM and exogenous bFGF combined with periosteal graft on the repair of full-thickness articular cartilage defects in mature rabbits.

MATERIALS AND METHODS

This study used 87 adolescent 16 week-old New Zealand rabbits of both sexes with an approximate weight of 2500-3750 g. The rabbits were randomly divided into four groups including a control group and three experimental groups. Each group again randomly divided into three subgroups according to the follow-up period of four, eight, and 12 weeks. Each subgroup in the experimental and the control group included eight and five rabbits, respectively.

Surgical technique

The right knee joint was approached by means of a medial parapatellar incision under sterile conditions. The patella was dislocated laterally to expose the articular surface. Full-thickness cylindrical defects (3.5 mm in diameter, 4 mm in depth) were created in the medial femoral condyle in the posterior weight-bearing area, with a hand drill equipped with a 3.5 mm. diameter drill-bit, as described previously.[10] All debris was removed and the joint cavity was carefully closed by precise suturing. After surgery, all animals were allowed to move freely in cage.

This was the only intervention in the control group (Group 1, 15 rabbits). In group 2 (24 rabbits), the defect was covered with periosteal-free grafts harvested from the medial aspect of the proximal portion of the tibia. The periosteal free graft was placed in the defect so that its cambium layer was facing up into the joint, and was secured to the base of the defect with fibrin glue. In group 3 (24 rabbits), the defects were covered with collagen sponge after the placement of 1300 mgr powdered DBM which was prepared from fresh intramembranous bones of New Zealand white rabbits, in the laboratory. In group 4 (24 rabbits), the defect was covered with periosteal-free grafts (as in group 2) and after the closure of the joint cavity, 5 μg recombinant human bFGF (Sigma®) was injected. The animals were sacrificed at four, eight and 12 weeks postoperatively by intravenous injection of 7.5% potassium chloride under isoflurane anesthesia.

Histological evaluation

The operated knee joint was excised, carefully cleaned of all neighbouring tissues, subjected to a macroscopic study and scored according to the system described by Carranza-Bencano et al.[11] The total score on the grading scale ranges from 0 points (no repair tissue) to 16 points (normal appearence). The specimens were fixed in 10% neutral formalin, decalcified, sectioned sagittally through the center of the defect and processed in paraffin, to obtain sections of 4μ for staining with hematoxylin-eosin.

The expression of collagen type I was detected with collagen I (500 μl) Quartett®. Collagen type II

TABLE I

Total scores of groups according to the macroscopic scoring system described by Carranza-Bencano et al.[11]

Group	Week 4	Week 8	Week 12	*p*
Group 1	10	9	12	>0.05
Group 2	11	10	13	>0.05
Group 3	11	12	10	>0.05
Group 4	12	14	14	>0.05

TABLE II

Total scores of groups according to the modified histological grading scale[2]

Group	Week 4	Week 8	Week 12	*p*
Group 1	20	10	6	<0.05
Group 2	29	23	9	<0.05
Group 3	25	19	7	<0.05
Group 4	22	8	3	<0.05

was evaluated with an antibody (Anti-collagen, type II Ab-2, MS-235-R7, Neomarkers®) raised against collagen II. The sections were incubated with a monoclonal antibody against anti-proliferating cell nuclear antigen (PCNA) (clone PC10; Dakopatts, Copenhagen, Denmark), diluted 1:400 with phosphate buffered saline, at room temperature for two hours. Ten sections were cut from the edge to the centre of the defect. The sections of each animal were examined independently by the investigator blinded to the group to which they belonged. Each preparation was scored on a histological scale, a variation of that described by Sellers et al.[2] The total score on the grading scale ranges from 0 points (normal cartilage) to 31 points (no repair tissue).

All statistical analyses were performed using the software SPSS 10.0 version for Windows (SPSS Inc, Chicago, IL, USA). A *p* value of ≤0.05 was considered statistically significant. Non-Parametric tests were used for statistic analysis of the results. The histological scores were compared with a Kruskall-Wallis one-way analysis of variance on ranks, and followed by Mann-Whitney U-test for all pairwise multiple-comparison to identify the differences between various treatment groups. Friedman test was used to compare the repeated measures.

RESULTS

Macroscopic study

Total mean scores of groups according to the macroscopic scoring system described by Carranza-Bencano et al.[11] are shown in table I. Although the scores were higher in group 4 (the defects treated with bFGF), there was no statistically significant difference between groups (p>0.05).

Histological findings

The scores of the histological evaluation are shown in table II (Figure 1a-d). The results of immunostaining can be found in table III (Figure 2a-d).

The total scores on the histological grading scale were significantly better in group 4 and in group 1 than the other groups at four and eight weeks (p<0.05). At 12 weeks the total score was significantly better in group 4 than the other three groups (p<0.05).

Immunostaining was seen as focal reaction in most of the sections. The evaluation of the immunostaining of type I and type II collagen revealed

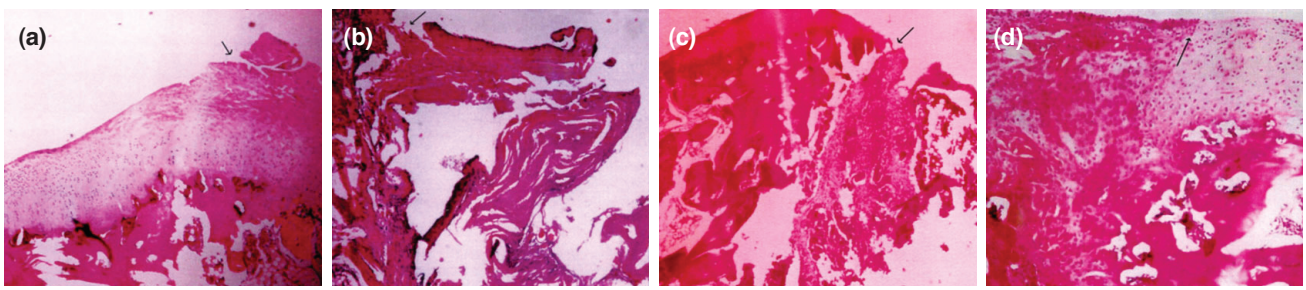

Figure 1. (a) In the group 1 at 12 weeks, filling of the defect with cartilaginous tissue, no chondrocyte clustering, but there is some surface irregularity (H-E x 200). **(b)** In group 2 predominantly fibrous tissue filling the defect at 12 weeks (H-E x 200). **(c)** In group 3, predomiantly cartilaginous tissue filling the defect at 12 weeks. The surface of the repair tissue is irregular (H-E x 200). **(d)** In group 4, completely cartilaginous tissue filling the defect at 12 weeks with a normal continuity with the remaining cartilage. The surface of the repair tissue is regular and the morphology of chondrocytes are similar to the normal cartilage (H-E x 200).

TABLE III

The results of the immunostaining in each group

Group	Week 4	Week 8	Week 12
Group 1			
Type I collagen	–	+	+
Type II collagen	–	–	–
PCNA	–	–	–
Group 2			
Type I collagen	–	+	–
Type II collagen	–	–	+
PCNA	–	–	–
Group 3			
Type I collagen	–	–	+
Type II collagen	–	+	–
PCNA	–	–	–
Group 4			
Type I collagen	+	+	–
Type II collagen	+	+	+
PCNA	–	–	–

PCNA: Anti-proliferating cell nuclear antigen.

that the intensity and distribution of immunostaining were significantly better in group 4 than the other groups ($p<0.05$). There was no difference between the groups in the immunostaining of PCNA ($p>0.05$).

Statistical analysis

There were no significant differences between the groups when analysing macroscopic appearance ($p>0.05$). Significant differences were found between the groups when we evaluated the histological scores ($p<0.05$).

DISCUSSION

Periosteal transplant is one of the oldest procedures to repair cartilage defects.[10] Previous studies,[10-12] have demonstrated that the capacity of periosteal-free grafts to produce hyaline cartilage enables them to be used to restore lesions of the joint surface. In a recent study by Atik et al.,[13] osteochondral multiple autograft transfer was performed either by arthrotomy or arthroscopy on 12 patients for the treatment of cartilage defects in the knee joint. The clinical results of this study were satisfactory and second-look arthroscopy of the five patients demonstrated a normal shiny appearance and color of the grafted area. It was reported that this is a promising surgical technique for the treatment of articular cartilage defects.

In a recent experimental study, the results of autologous chondrocyte transplantation (ACT) were compared with the results of periosteal grafting (PG). In the PG group, four weeks after the operation the lesion was only partially filled and did not significantly change after eight and 12 weeks. In most animals with PG, the repaired tissue was disrupted with small fissures and in all cases small fissures and cysts were noted throughout the entire period of observation. The tissue in the defect area was fibrous cartilage, usually much thinner than the surrounding tissue and integration of the repair tissue with the surrounding tissues was weak. It was concluded that ACT was superior to PG in the repair of damaged cartilage.[14] Our results were similar to those of PG group in this study. The cellular morphology included mostly spindle-shape (fibroblast-like) cells, that were decreased by time. The defect area showed clefts and fibrillations at all weeks. The surface of the defect area was irregular. The repair tissue was thinner than the surrounding tissue and showed a poor coaptation with the original cartilage.

The term BMP was used to describe the substance(s) in the DBM. The functions of BMPs have been evaluated in many in vitro studies. It is reported that BMPs may play an important role

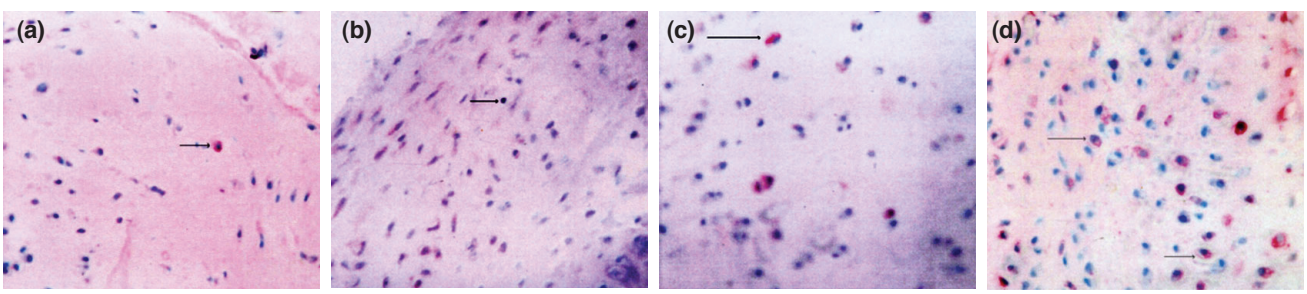

Figure 2. Type I collagen staining at 12 weeks (Immunoperoxidase x 400). **(a)** In group 1, **(c)** in group 3. Type II collagen staining at 12 weeks (Immunoperoxidase x 400). **(b)** In group 2, **(d)** in group 4.

in the development and growth of cartilage and bone, and also strongly promote the differentiation of chondroblasts and chondrocytes. RhBMP-7 induced in vivo proliferation of chondrocyte-like cells leading to partial healing of articular cartilage lesions[15,16] and rhBMP-2 accelerated the healing of full thickness osteochondral defects by inducing new subchondral bone and improving the histological appearance of overlying cartilage.[2] However, the function of BMPs and DBM in cartilage tissue has not been clarified.

In the DBM group of our study, 12 weeks after the operation most of the lesion was filled with an irregular repair tissue which included small voids. In most animals, the repair tissue showed decreased cellularity with a small number of round cells with the morphology of chondrocytes. The tissue in the defect area was usually thinner than the surrounding tissue and integration of the repair tissue with the surrounding tissues was not complete. These results were not better than those of the PG group.

It is known that growth factors are also expected to be useful tools to enhance osteochondral repair and among them, bFGF has aroused particular attention because it can stimulate the proliferation of chondrocytes, the synthesis of cartilaginous extracellular matrix and increased accumulation of type II collagen and proteoglycans.[17,18]

In a recent study by Mizuta et al.,[17] it was demonstrated that the successful cartilaginous resurfacing of full-thickness defects required the initiation and maintenance of expanding chondroprogenitor cell population prior to overt chondrogenesis in the defect cavities. This data suggests that bFGF-2 signaling plays a role in this process as a regulatory mechanism for a defect-size dependent regeneration of articular cartilage. A recent study[18] demonstrated that bFGF-2 administration for one day yielded the successful chondrogenic repair response to the same extent as administration for three days or two weeks. Histological analysis revealed that bFGF-2 administration for one day was sufficient to regenerate the epiphyseal structure, including resurfacing articular cartilage and the subchondral bone up to the original bone-articular junction.

In our study, the most successful results were found in the bFGF group. Our macroscopic and histological examination results demonstrated that the most successful cartilaginous resurfacing of full-thickness defects were found in the bFGF group with the best cellular morphology and the highest accumulation of type I and II collagen.

Our follow-up period was short and this experimental study did not give information about the durability of the repair tissue. Another limitation of our study is the lack of biomechanical tests. It is known that the development and maintaince of cartilage structure and mechanical characteristics are closely correlated to the effect of mechanical loading. The impact of load on cartilage structure and function is important especially in hyaline articular cartilage. Biomechanical tests are necessary to recognise the effects of mechanical loading on the histological structure and function of repair tissue.

The results obtained from our experimental study are encouraging. bFGF might be considered as a possible therapeutic alternative for patients affected by local arthrosis and areas of osteochondritis of the femoral condyle. Further clinical investigations are needed to clarify whether bFGF administration may prevent cartilage degeneration and/or promote cartilage regeneration and repair.

Declaration of conflicting interests

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