Original Article

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Comparison of Small-diameter-hole and Traditional Microfracture in Cartilage Repair and the Effect of Adding a Hyaluronic Acid-based Acellular Matrix Scaffold: An Animal Study

Kıkırdak Doku Onarımında Küçük Çaplı Delik Yönteminin Geleneksel Mikro Kırık Yöntemi ile Karşılaştırılması ve Hyalüronik Asit Temelli Hücresiz Matriks Skafold Eklemenin Etkisi: Bir Hayvan Çalışması

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ABSTRACT

Objective: Since, there is no standardized technique for the treatment of focal cartilage defects that can recreate original cartilage tissue; researchers continue to explore and evaluate various treatment modalities. This study compared post-operative healing of cartilage defects after treatment with small-diameter-hole microfracture (SDHM) technique with that of traditional microfracture technique. The effects of the hole density and augmentation with hyaluronic acid-based acellular matrix (HA-based AM) on cartilage healing were also investigated.

Methods: Articular cartilage defects measuring 5 mm in diameter and 3 mm in depth were created in each femoral trochlear groove of 21 New Zealand rabbits. Rabbits were assigned to seven groups comprising six knees each. The rabbits were sacrificed 12 weeks later, and the regenerated cartilage was harvested for histological evaluation using the Wakitani scoring system.

Results: All defects were filled with regenerated tissue macroscopically. Group I (14; range 10-14 points) had significantly higher Wakitani score than in groups VI (6; range 1-11 points) and

ÖZ

Amaç: Fokal kıkırdak defektlerinin tedavisinde hala orijinal kıkırdak dokusu sağlayan mükemmel bir yöntem yoktur, bu nedenle en iyi tedavi seçeneklerini bulmak için araştırmalar devam etmektedir. Bu çalışmada amaç, kıkırdak defektlerinde küçük çaplı delik (SDHM) ve geleneksel mikro kırık tedavilerinin iyileştirme kalitesini karşılaşmaktır. Bununla beraber delik yoğunluğunun ve defekti hyalüronik asit bazlı aselüler matriks (HA bazlı AM) ile desteklemenin kıkırdak iyileşmesi üzerindeki etkileri de incelenmiştir.

Yöntemler: Yirmi bir Yeni Zelanda tavşanının her iki femur trochlear oluğunda 5 mm çapında ve 3 mm derinliğinde artiküler kıkırdak defekti oluşturuldu. Her biri 6 dizden oluşan yedi grup oluşturuldu. Tavşanlar 12 hafta sonra sakrifiye edildi ve rejenere kıkırdak Wakitani skorlama sistemi kullanılarak histolojik değerlendirme için toplandı.

Bulgular: Tüm defektler rejenere doku ile makroskopik olarak dolduruldu. 1. Grup [14 (10-14) puan], VI [6 (1-11) puan] ve VII. [5 (3-10) puan] gruplara göre anlamlı derecede yüksek Wakitani

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VII (5; range 3-10 points) (p=0.043 and p=0.016, respectively). No significant differences were observed among the other groups. Augmentation with HA-based AM did not contribute to cartilage healing.

Conclusion: Improved cartilage healing was observed with increasing SDHM density than with traditional microfracture technique. SDHM combined with HA-based AM implantation did not improve the quality of the regenerated cartilage.

Keywords: Microfracture, cartilage repair, small-diameter-hole microfracture, hyaluronic-acid-based acellular matrix, cartilage healing

skoruna sahipti (p=0.043 ve p=0.016). Diğer gruplar arasında anlamlı bir fark gözlenmedi. Hyaluronik asit bazlı aselüler matriks ile destekleme, kıkırdak iyileşmesine katkıda bulunmadı.

Sonuç: SDHM yoğunluğunun artırılması, geleneksel mikro kırık ile karşılaştırıldığında kıkırdak iyileşmesini artırır. Hyalüronik asit bazlı aselüler matriks implantasyonu ile desteklenen SDHM'nin artırılması ise rejenere kıkırdak kalitesini artırmadı.

Anahtar Sözcükler: Mikrokırık, kıkırdak onarımı, küçük çap delikli mikrokırık, hyalüronik asit bazlı aselüler matriks, kıkırdak iyileşmesi

Introduction

Microfracture, a bone-marrow-stimulating technique introduced by, Steadman et al. (7) for the treatment of focal full-thickness, symptomatic cartilage defects, consists of multiple holes in the subchondral bone to enable pluripotent stem cells to migrate from the bone marrow into the defect and to promote formation of fibrocartilage repair tissue (1-7).

The amount of hyaline-like cartilage in the repair tissue depends on alterations in the subchondral bony architecture during microfracture, as compression of the cancellous bone around the holes prevents connection with the bone marrow, thus reducing the number of mesenchymal stem cells (MSCs) that can reach the defect (8,9). This disadvantage of microfracture led to the recent development of a technique called 'small-diameter-hole microfracture' (SDHM) (9-11). Some experimental studies showed that deep, small-diameter drill holes are more effective in promoting cartilage repair than large-diameter, shallow drill holes (10,11). The repair tissue after deep subchondral perforations contains less type I and more type II collagen, and the defect is better filled with hyaline-like cartilage (9-11). Despite promising results of SDHM in comparison with conventional microfracture, the role of hole density (how many SDHMs should be performed for a cartilage defect of a given size) has not been adequately investigated.

Another limitation of the marrow-stimulating techniques is related to the amount of defect filling. A thickness of 5 mm is necessary to fill a full-thickness articular cartilage defect in the knee and it is difficult to create sufficient thickness to fill in the defect without a scaffold. For this reason, several studies investigated the efficacy of MSCs transferred to a scaffold at the time of implantation to treat focal cartilage defect (12-15). The rationale of this treatment is that the scaffold triggers the proliferation and chondrogenic differentiation of MSCs. Moreover, the scaffold contributes to fill the defect.

An alternative to the engineered scaffold implantation is the autologous matrix-induced chondrogenesis (AMIC), which consists of implanting a cell-free scaffold over microfracture (16). Satisfactory results have been reported with this technique (17), albeit no evidence exists on the combination of matrix aided chondrogenesis and SDHMs (18).

The aim of the present study was twofold: to assess the efficacy of SDHMs with and without matrix augmentation in comparison with conventional microfracture for the treatment of focal full-thickness chondral defects of the knee, and to investigate the role of hole density on cartilage repair in SDHM technique. The hypotheses of the study were that SDHMs enhance cartilage repair with and without matrix augmentation, and that hole density positively correlates with cartilage repair.

Method

Twenty-one, mature, male New Zealand white rabbits with a mean weight of 3.4 kg (range: 3-4.2 kg) were used for the present study. All experiments were conducted in accordance with the guidelines of the local ethic committee for animal experimentation (approval number: 2014/140).

Surgical Technique

All of the surgical procedures were performed under general anaesthesia and by use of sterile conditions. A 2-cm longitudinal anterior skin incision was made over the right knee, and the joint was approached via a medial parapatellar arthrotomy. A cylindrical cartilage defect measuring 5 mm in diameter was created on the femoral trochlea (Figure 1A and 1B). Non-calcified and calcified layers of cartilage were removed from the defect area and care was taken to avoid damage to the subchondral bone.

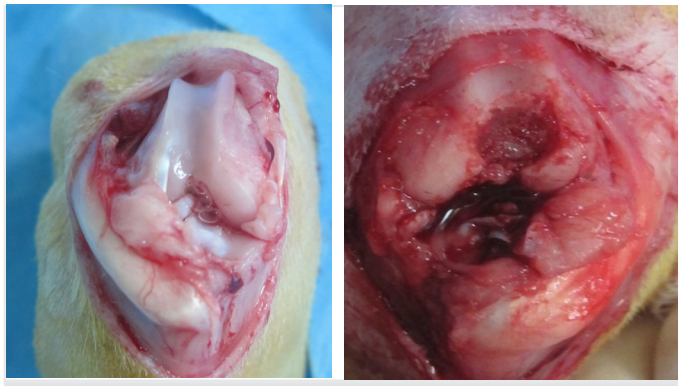

Figure 1. (A) Articular cartilage of the femoral condyles and trochlear groove of the rabbit. (B) A cylindrical cartilage defect measuring 5 mm in diameter and 3 mm in depth was created in the trochlear groove

Animals were divided into seven groups of six rabbits each, according to the treatment protocol. In group 1 (conventional microfracture or control group), three holes with 5 mm depth were created in the subchondral bone using a standard 1.2-mm arthroscopic awl. In groups 2 to 7, a custom-made device was used to create SDHMs into the defect, which measured 0.8 mm in diameter and 5 mm in depth. Four, five and six holes were created in groups 2, 4 and 6, respectively (Figure 2). Figure 3 illustrates the 6 holes of SDHM in the cartilage defect. In the remaining groups (3, 5 and 7 respectively), SDHMs were combined with a hyaluronic acid-based acellular matrix (HAAM) (Hyalofast; Anika Therapeutics, Bedford, MA, USA), which was placed over the defect after creating four, five and six holes in groups 3, 5, and 7, respectively (Figure 4). The joint was irrigated, haemostasis was controlled, the capsule was sutured with 2-0 Vicryl, and the skin was closed with 3-0 silk sutures.

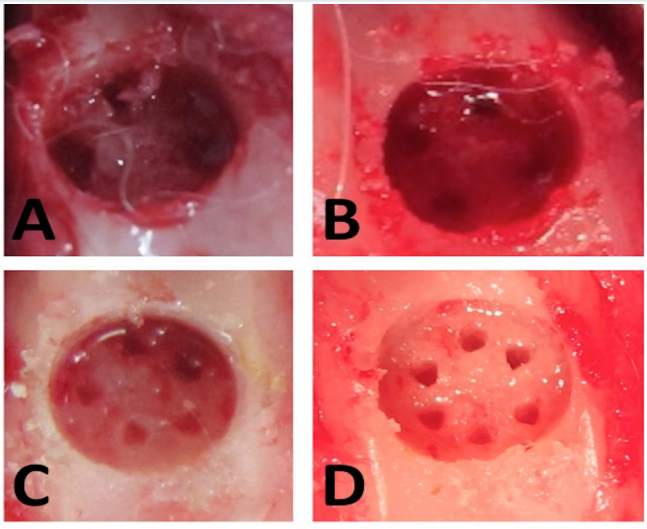

Figure 2. The defects were treated using (A) microfracture with three holes in group I, SDHM with (B) four holes in groups II and III, (C) five holes in groups IV and V, and (D) six holes in groups VI and VII

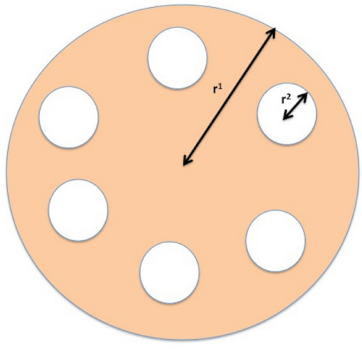

Figure 3. Six holes of SDHM are illustrated. r1 is the radius of the cartilage defect (2.5 mm) and r2 is the radius of the SDHM hole (0.4 mm)

SDHM: Small-diameter-hole microfracture

Postoperatively, the rabbits were returned to their cages without any immobilization of the operated limb and full weight-bearing was allowed as tolerated. General health monitored during recovery.

Outcome Measurements

The rabbits were sacrificed under general anaesthesia 12 weeks postoperatively. The distal femur was dissected and harvested for both macroscopic and histological evaluations.

Macroscopic appearance of the repair site was evaluated by three different investigators and rated according to the International Cartilage Repair Society (ICRS) evaluation score (19). This scoring system consists of a 12-point scale based on three categories (degree of defect repair, integration to border zone, and macroscopic appearance). The tissue samples were then fixed in 10% neutral buffered formalin for 72 hours, rinsed with water, and decalcified in decalcifying solution (OSTEOMOLL; Merck Millipore, Billerica, MA, USA). After decalcification, the samples were dehydrated gradually using increasing concentrations of alcohol (70%, 90%, 96%, and 100%) and cleared in xylene. Then, the samples were submerged in paraffin overnight at 60°C and embedded in paraffin blocks the next day. After the blocking process, 5-µm-thick sections perpendicular to the lesion surface were obtained from the samples and placed on slides, stained with haematoxylin and eosin (H&E) and toluidine blue for histological evaluation, and examined under a light microscope (Nikon Eclipse i5; Nikon, Tokyo, Japan).

Histological findings were scored using the scale described by Wakitani et al. (20) according to the intensity of basophilic staining caused by hematoxylin (20-22). Sections were graded according to cell morphology (maximum 4 points), matrix staining (maximum 3 points), surface regularity (maximum 3 points), cartilage thickness (maximum 2 points), and donor material integration into adjacent host cartilage (maximum 2

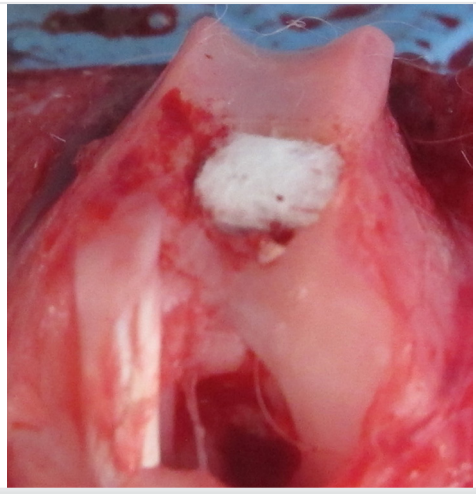

Figure 4. HA-based AM (Hyalofast, Anika Therapeutics, Bedford, MA, USA) was placed over the defect area after performing SDHM in groups III, V, and VII

SDHM: Small-diameter-hole microfracture

points). The maximum score was 14 points. A higher Wakitani score represents lower-quality repair tissue.

Three trained observers blinded to group allocation performed all macroscopic and histological evaluations.

Statistical Analysis

All the outcome measurements were expressed as mean values ± standard deviations. The data were analysed using statistical software SPSS 10.0 for Windows (IBM, Chicago, IL, USA). Groups were compared for histologic scores using the Kruskal–Wallis test. Post hoc Dunn test was used for multiple pairwise comparisons. Significance was considered for p values less than 0.05.

Results

There were no wound complications, infections, or deaths.

Gross evaluation showed that the defects were filled with the cartilage completely in group 6 and group 7, and partially in the other groups. The mean ICRS evaluation score of each group was showed in the table 1.

Histologic evaluation shows that matrix of hyaline cartilage is seen metachromasia and one of the best dyes for it is the toluidine blue. In addition, hematoxylin stains matrix of hyaline cartilage as basophilic. In our study, we scored the matrix staining in the Wakitani score (Table 2). The defect area was incompletely filled with fibrous tissue in group 1 and group 2, and completely filled with fibrocartilage tissue in group 3 and group 4. The defects were filled with repair tissue that contained hyaline cartilage in groups 5 to 7 (Figure 5). Although cartilaginous tissue was formed these groups, the basophilic matrix appeared in group 6 and group 7. The surface of the repaired tissue was more irregular in groups 1 to 5, and smoother in groups 6 and 7.

Overall histologic scoring assessment showed a significant difference between groups. Post hoc analysis showed that groups 6 and 7 had significantly better results than group 1 (p=0.043 and p=0.016, respectively). All other pairwise comparisons showed no significant differences.

Analysis of cell morphology subscore showed a significant difference between groups. Post hoc analysis showed that groups 6 and 7 had significantly better results than group 1 (p=0.041 and p=0.001, respectively).

Matrix staining subscore did not significantly differ among the groups.

Surface regularity subscore was significantly lower in groups 6 and 7 than in group 1 (p=0.029 and p=0.029, respectively).

Cartilage thickness and integration subscores did not significantly differ among the experimental groups.

Osteoarthritic changes were not observed in healthy cartilaginous tissue adjacent to the treatment area in all groups. The outer surface of the articular cartilage has normal appearance and has not wear or ridge and osteophyte. Vertical cracks, vascularization and inflammation have not been observed in the hyaline cartilage tissue. Chondrocytes, territorial and interterritorial matrix were normal in morphology. Histopathological findings were not found. Additionally, degeneration was not observed in the subchondral bone. Hyaline cartilage matrix was mostly made up of type II collagen. The metachromatic staining observed in our HE-stained sections showed that the amount of collagen was high and packed tightly. Metachromatic staining in the newly formed tissue matrix indicates that healing and cartilaginous tissue formation.

Table 1. Macroscopic evaluation of the samples according to the ICRS scoring system (point)

	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Min.	3	4	4	6	7	10	10
Max.	3	5	5	8	8	11	11
Mean	3	4.3	4.3	7	7.3	10.5	10.6

ICRS: International Cartilage Repair Society, Min.: Minimum, Max.: Maximum

Table 2. Histologic comparisons of the groups using Wakitani's score

	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	p
Morphology	4	3	3	2	2	2	1	0.001; VII vs I 0.041; VI vs I
Matrix	3	3	2	2	1	1	1	n.s
Surface	3	3	2	2	1.5	1	1	0.029; VII vs I 0.029; VI vs I
Thickness	2	1.5	1.5	1	1	1	1	n.s
Integration	2	1	1.5	1.5	1	1	1	n.s
Total	14	11.5	9.5	8	6.5	6	5	0.016; VII vs I 0.043; VI vs I

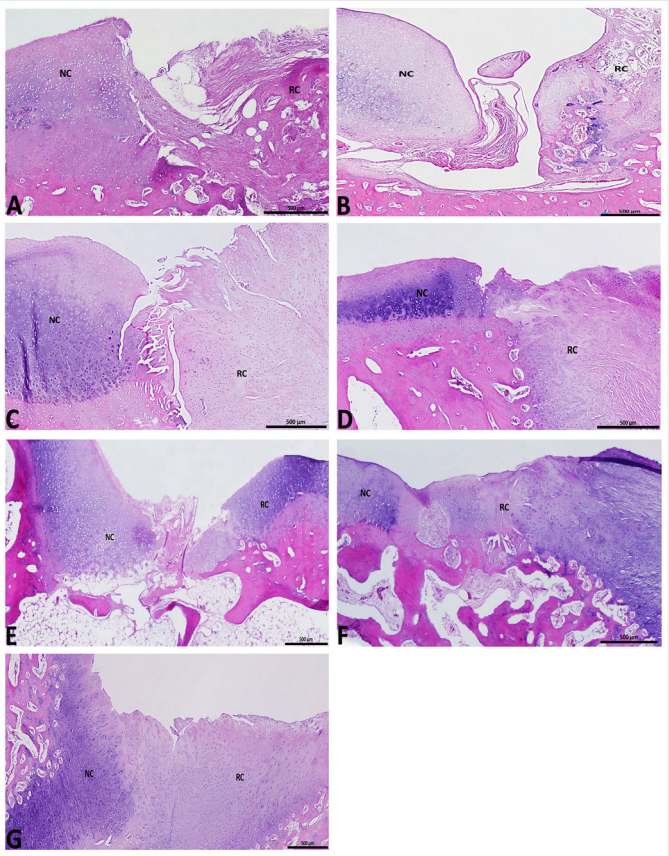

Figure 5. (A) Irregular surface, mostly non-cartilaginous tissue and non-metachromatic matrix staining in repaired cartilage (RC) tissue in Group 1. (B) Irregular surface, mostly non-cartilaginous tissue and significantly decreased matrix staining in Group 2. (C) Irregular surface, mostly fibro-cartilaginous tissue, significantly decreased matrix staining and integrated one edge between normal and repaired cartilage tissue in Group 3. (D) Moderate surface regularity, mostly fibro-cartilaginous tissue, significantly decreased matrix staining and integrated one edge between normal and repaired cartilage tissue in Group 4. (E) Regular surface, mostly hyaline cartilage, slightly decreased matrix staining and integrated one edge between normal and repaired cartilage tissue in Group 5. (F) Regular surface, mostly hyaline cartilage, metachromatic matrix staining and integrated both edges between normal and repaired cartilage tissue in Group 6. (G) Regular surface, hyaline cartilage, metachromatic matrix staining and integrated both edges between normal and repaired cartilage tissue in Group 7

NC: Normal cartilage tissue, RC: Repaired cartilage tissue, Staining: H&E, scale bar =500 µm

Discussion

The unique features of our method are as follows: 1) a SDHM with sufficient thickness for cartilage repair can be performed without a HA-based AM scaffold; and 2) increasing the number of SDHM applied to the defect area increases cartilage regeneration.

In recent years, several methods have been developed for repairing full-thickness cartilage defects. The MF technique performed arthroscopically as described by Steadman et al. (7) is a popular treatment method because it is easy applicable and cost-effective (23,24). Microfracture repairs the defective cartilage area by enabling the arrival of stromal cells. However, it results in shallow channels, wall compression, and increased trabecular thickness and density, as demonstrated by micro-computed tomography (microCT) and histology in several clinical and animal studies (25,26). Thus, it does not allow the regeneration of normal hyaline cartilage, perhaps because of the lack of sufficient MSCs or poor cellular differentiation (27-29). Compared with traditional microfracture, SDHM creates more holes with smaller diameters, which damage trabecular structures less. Therefore, more MSCs fill the defect (10,11,30,31).

Min et al. (30) compared the numbers of MSCs in 5-mm^2 cartilage defects in the femoral trochlear grooves of rabbits treated by microfracture with 3 (1.5 mm diameter) or 10 (0.8-mm diameter) holes, and found more MSCs in the latter group. They concluded that bone marrow stimulation technique affect the number of MSCs drained from the bone marrow, which may lead to increased cartilage healing. Eldracher et al. (10) performed microfracture using 1.8- or 1-mm awls and observed better osteochondral healing with the smaller awl. Orth et al. (11) created 4 × 8-mm cartilage defects on the femoral condyles and performed microfracture using 1- or 1.2-mm awls and found better histological cartilage healing in the small-awl group. Contrasting these studies, Marchand et al. (32) created 3.5x4.5-mm cartilage defects in rabbits and treated them using microfracture with two (0.9-mm in diameter) or three (0.5-mm in diameter) holes; they found no difference in cartilage healing.

In our study, the cartilage healing revealed by histological analyses of the 20-mm^2 defects did not differ significantly among groups with three (group I; the holes included 16.8% of the defect area), four (group II; 10% of the defect), or five (group IV; 12.5% of the defect) hole, whereas SDHM with six holes (groups VI and VII; 15% of the defect area) resulted in significantly higher scores compared with group I ($p=0.043$ and $p=0.016$, respectively). This suggests that SDHM involving a minimum of 15% of the defect area improves cartilage healing. Although microfracture involved a similar percentage of the defect (16.8%), we did not observe the same results with microfracture. We think that this difference depends on damage to the cancellous bone caused by the traditional awl. These results parallel the literature (10). Moreover, the cell morphology and cartilage surfaces scores were superior in groups VI and VII compared with group I.

Lim et al. (33) performed microfracture for the focal cartilage defects between 1 to 4 cm^2 and found good and excellent results and complete cartilage regeneration in 80% of the patients in second-look arthroscopy at the end of first year. To our knowledge, there is no a study investigating cartilage healing after SDHM, with ICRS evaluation system, using second-look arthroscopy. In the current study, we observed about normal cartilage regeneration in every sample in which six-holes SDHM was performed. In this group, the defects were completely filled

with hyaline-like cartilage and regular surfaces were seen. In conventional microfracture group, about 50% of the defects were filled with the bone and small cartilage islands.

Several studies have examined the effectiveness of HA-based scaffolds in cartilage healing (34-37). In our study, there was no difference between the HA-based acellular matrix groups and the SDHM-only groups (p=1.0 for group II vs. group III, group IV vs group V, and group VI vs group VII). Contrary to common belief and the literature, our study revealed that in the treatment of focal cartilage defects with SDHM, HA-based acellular matrix does not contribute to cartilage healing, although we still need more evidence regarding this issue. According to a hypothesis, the scaffold might block cells and factors derived from the synovium or cause high pressure in the chondral defect, resulting in prevention of cells and growth factors gushing out from the bone marrow, which leads to disadvantages for cartilage repair.

This study has some limitations. First, it would have been better if we had investigated the subchondral bone after SDHM and MF using microCT. Second, in addition to the histological analyses, the surface strengths of the repaired cartilages should be tested biomechanically. Third, the rabbits were 5 months old, and they reach skeletal maturity at their 7-8 months. It could be better if we used older rabbits in order to decrease the risk of spontaneous cartilage repair. Lack of immune-histo-chemical analysis for collagen type 1 or type 2, which was better to explore the hyaline cartilage nature of the regenerated cartilage, was another limitation. The last limitation is that weight bearing can not be restricted. Although microfracture has been applied to the trochlea, it may have affected as a result of increased pressure on the cartilage due to weight bearing.

Conclusion

SDMH with the optimum numbers of holes covering a minimum of 15% of the defect size can increase the quality of cartilage repair compared with the traditional microfracture (16.8% of the defect size) technique for defects of the same size in rabbits. HA-based AM implantation after microfracture does not improve the quality of the regenerated cartilage.

Ethics

Ethics Committee Approval: All experiments were conducted in accordance with the guidelines of the local ethics committee for animal experimentation (approval number: 2014/140).

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: G.U., F.Y. V.G., Concept: F.Y., V.U., V.G., Design: N.M.E., F.Y., M.E., Data Collection or Processing: V.U., O.E.T, G.U., Analysis or Interpretation: Y.G., G.U., F.Y., Literature Search: V.U., G.U., Writing: G.U., F.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg Am 1993;75:532-53.
2. Karthikeyan S, Roberts S, Griffin D. Microfracture for acetabular chondral defects in patients with femoroacetabular impingement: results at second-look arthroscopic surgery. Am J Sports Med 2012;40:2725-30.
3. Knutsen G, Drogset JO, Engebretsen L, Grontvedt T, Isaksen V, Ludvigsen TC, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg Am 2007;89:2105-12.
4. Saris D, Price A, Widuchowski W, Bertrand-Marchand M, Caron J, Drogset JO, et al. Matrix-Applied Characterized Autologous Cultured Chondrocytes Versus Microfracture: Two-Year Follow-up of a Prospective Randomized Trial. Am J Sports Med 2014;42:1384-94.
5. Kreuz PC, Steinwachs MR, Erggelet C, Krause SJ, Konrad G, Uhl M, et al. Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthritis Cartilage 2006;14:1119-25.
6. Bentley G, Biant LC, Carrington RW, Akmal M, Goldberg A, Williams AM, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. J Bone Joint Surg Br 2003;85:223-30.
7. 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.
8. Mithoefer K, Williams RJ, 3rd, Warren RF, Potter HG, Spock CR, Jones EC, et al. Chondral resurfacing of articular cartilage defects in the knee with the microfracture technique. Surgical technique. J Bone Joint Surg Am 2006;88 Suppl 1 Pt 2:294-304.
9. Chen H, Hoemann CD, Sun J, Chevrier A, McKee MD, Shive MS, et al. Depth of subchondral perforation influences the outcome of bone marrow stimulation cartilage repair. J Orthop Res 2011;29:1178-84.
10. Eldracher M, Orth P, Cucchiarini M, Pape D, Madry H. Small subchondral drill holes improve marrow stimulation of articular cartilage defects. Am J Sports Med 2014;42:2741-50.
11. Orth P, Duffner J, Zurakowski D, Cucchiarini M, Madry H. Small-Diameter Awls Improve Articular Cartilage Repair After Microfracture Treatment in a Translational Animal Model. Am J Sports Med 2016;44:209-19.
12. Kaneshiro N, Sato M, Ishihara M, Mitani G, Sakai H, Mochida J. Bioengineered chondrocyte sheets may be potentially useful for the treatment of partial thickness defects of articular cartilage. Biochem Biophys Res Commun 2006;349:723-31.
13. Ando W, Tateishi K, Hart DA, Katakai D, Tanaka Y, Nakata K, et al. Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. Biomaterials 2007;28:5462-70.

14. Cheuk YC, Wong MW, Lee KM, Fu SC. Use of allogeneic scaffold-free chondrocyte pellet in repair of osteochondral defect in a rabbit model. J Orthop Res 2011;29:1343-50.
15. Nakamura T, Sekiya I, Muneta T, Hatsushika D, Horie M, Tsuji K, et al. Arthroscopic, histological and MRI analyses of cartilage repair after a minimally invasive method of transplantation of allogeneic synovial mesenchymal stromal cells into cartilage defects in pigs. Cytotherapy 2012;14:327-38.
16. Kramer J, Bohrnsen F, Lindner U, Behrens P, Schlenke P, Rohwedel J. In vivo matrix-guided human mesenchymal stem cells. Cell Mol Life Sci 2006;63:616-26.
17. Volz M, Schaumburger J, Frick H, Grifka J, Anders S. A randomized controlled trial demonstrating sustained benefit of Autologous Matrix-Induced Chondrogenesis over microfracture at five years. Int Orthop 2017;41:797-804.
18. Benthien JP, Behrens P. Nanofractured autologous matrix induced chondrogenesis [NAMIC(c)]--Further development of collagen membrane aided chondrogenesis combined with subchondral needling: A technical note. Knee 2015;22:411-5.
19. van den Borne MP, Raijmakers NJ, Vanlauwe J, Victor J, de Jong SN, Bellemans J; International Cartilage Repair S. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. Osteoarthritis Cartilage 2007;15:1397-402.
20. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 1994;76:579-92.
21. Xia X, Li J, Xia B, Yang H, Zhang D, Zhou B, et al. Matrigel scaffold combined with Ad-hBMP7-transfected chondrocytes improves the repair of rabbit cartilage defect. Exp Ther Med 2017;13:542-50.
22. Dai Y, Gao Z, Ma L, Wang D, Gao C. Cell-Free HA-MA/PLGA Scaffolds with Radially Oriented Pores for In Situ Inductive Regeneration of Full Thickness Cartilage Defects. Macromol Biosci 2016;16:1632-42.
23. Bekkers JE, Inklaar M, Saris DB. Treatment selection in articular cartilage lesions of the knee: a systematic review. Am J Sports Med 2009;37 Suppl 1:148S-55S.
24. Schindler OS. Current concepts of articular cartilage repair. Acta Orthop Belg 2011;77:709-26.
25. Chen H, Sun J, Hoemann CD, Lascau-Coman V, Ouyang W, McKee MD, et al. Drilling and microfracture lead to different bone structure and necrosis during bone-marrow stimulation for cartilage repair. J Orthop Res 2009;27:1432-8.
26. Fortier LA, Cole BJ, McIlwraith CW. Science and animal models of marrow stimulation for cartilage repair. J Knee Surg 2012;25:3-8.
27. Dorotka R, Bindreiter U, Macfelda K, Windberger U, Nehrer S. Marrow stimulation and chondrocyte transplantation using a collagen matrix for cartilage repair. Osteoarthritis Cartilage 2005;13:655-64.
28. Henderson I, Lavigne P, Valenzuela H, Oakes B. Autologous chondrocyte implantation: superior biologic properties of hyaline cartilage repairs. Clin Orthop Relat Res 2007;455:253-61.
29. Otsuka Y, Mizuta H, Takagi K, Iyama K, Yoshitake Y, Nishikawa K, et al. Requirement of fibroblast growth factor signaling for regeneration of epiphyseal morphology in rabbit full-thickness defects of articular cartilage. Dev Growth Differ 1997;39:143-56.
30. Min BH, Choi WH, Lee YS, Park SR, Choi BH, Kim YJ, et al. Effect of different bone marrow stimulation techniques (BSTs) on MSCs mobilization. J Orthop Res 2013;31:1814-9.
31. Burr DB, Radin EL. Microfractures and microcracks in subchondral bone: are they relevant to osteoarthrosis? Rheum Dis Clin North Am 2003;29:675-85.
32. Marchand C, Chen G, Tran-Khanh N, Sun J, Chen H, Buschmann MD, et al. Microdrilled cartilage defects treated with thrombin-solidified chitosan/blood implant regenerate a more hyaline, stable, and structurally integrated osteochondral unit compared to drilled controls. Tissue Eng Part A 2012;18:508-19.
33. Lim HC, Bae JH, Song SH, Park YE, Kim SJ. Current treatments of isolated articular cartilage lesions of the knee achieve similar outcomes. Clin Orthop Relat Res 2012;470:2261-7.
34. Loken S, Jakobsen RB, Aroen A, Heir S, Shahdadfar A, Brinchmann JE, et al. Bone marrow mesenchymal stem cells in a hyaluronan scaffold for treatment of an osteochondral defect in a rabbit model. Knee Surg Sports Traumatol Arthrosc 2008;16:896-903.
35. Unterman SA, Gibson M, Lee JH, Crist J, Chansakul T, Yang EC, et al. Hyaluronic acid-binding scaffold for articular cartilage repair. Tissue Eng Part A 2012;18:2497-506.
36. Gobbi A, Scotti C, Karnatzikos G, Mudhigere A, Castro M, Peretti GM. One-step surgery with multipotent stem cells and Hyaluronan-based scaffold for the treatment of full-thickness chondral defects of the knee in patients older than 45 years. Knee Surg Sports Traumatol Arthrosc 2017;25:2494-501.
37. Liu Y, Shu XZ, Prestwich GD. Osteochondral defect repair with autologous bone marrow-derived mesenchymal stem cells in an injectable, in situ, cross-linked synthetic.