ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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THE EFFECTS OF HUMAN AMNIOTIC FLUID AND MEMBRANE ON CHONDRAL HEALING IN A RABBIT KNEE CARTILAGE DEFECT MODEL

İNSAN AMNİYOTİK SIVISI VE MEMBRANININ BİR TAVŞAN DİZİ KIKIRDAK DEFEKTİ MODELİNDE KONDRAL İYİLEŞME ÜZERİNE ETKİLERİ

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Öz

Amaç
Artiküler kıkırdağın kısıtlı intrensek iyileşmesi ve tamir kapasitesinin az olması nedeniyle çoğu tedavi metodları ile normal hyalen kıkırdak rejenerasyonu sağlanamamakta ve sonuç olarak osteoartroz gelişebilmektedir. Bu çalışmada insan amniyotik sıvısı ve membranının tavşanlardaki kondral defektlerdeki etkilerini incelemek amaçlanmıştır.

Gereç ve Yöntem
32 immatür albino Yeni Zelanda tavşanının 64 dizi bu çalışmaya dahil edildi. Tam kat kıkırdak defektleri tavşanların medial kondillerinin yük taşıma yüzeylerinde cerrahi olarak oluşturuldu. Tavşanlar randomize olarak dört gruba bölündü: ek tedavi verilmeyenler Grup 1, 0,3 ml insan amniyotik sıvısı (İAS) verilenler Grup 2, sadece insan amniyotik membranı (İAM) verilenler Grup 3, hem 0,3 ml İAS hem İAM verilenler Grup 4'ü oluşturdu. Kondiller histopatolojik olarak 4. ve 12. haftalarda modifiye O'Driscoll evreleme skalası ile değerlendirildi. Sonuçlar Mann-Whitney U ve ANOVA testleri ile istatistiksel olarak analiz edildi.

Bulgular
Gruplar arasında rejenere dokunun kalitesi açısından istatistiksel olarak anlamlı fark bulunmadı ($p>0.05$). Grupların ortalama sonuçları 12. haftada, 4. hafta sonuçlarından daha kötü olarak saptandı; buna rağmen bu farkın, sadece Grup 1 (sham grubu) ve Grup 4 açısından anlamlı olduğu görüldü (İAS+İAM) (sırasıyla, $p=0,007$ ve $p=0,014$).

Sonuç
Sadece İAS, sadece İAM ve her iki biyomateryalin kombine edildiği tedavi yöntemlerinin hiçbirinde kıkırdak defekt iyileşmesi daha iyi ve kaliteli olmadığı ve birbirlerine de bir üstünlüğü olmadığı görüldü. Bu çalışmada gösterilmiş olan 4. hafta sonuçlarının 12. hafta sonuçlarından daha iyi olmasının çalışmada kullanılan immatür tavşanlardaki intrensek tamir mekanizmasındaki erken rejenerasyon kapasitesinden kaynaklandığını düşünmekteyiz.

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Anahtar Kelimeler: Hyalen kıkırdak; Kıkırdak rejenerasyonu; Kondral defekt; İnsan amniyotik sıvısı; İnsan amniyotik membranı

Abstract

Objective
Due to the limited intrinsic healing and repair capacity of the articular cartilage, most treatment methods cannot achieve reliable regeneration of normal hyaline cartilage, resulting in early development of osteoarthritis. The purpose of this study was to determine the effects of human amniotic fluid and membrane on chondral defects.

Material and Methods
Sixty-four knees of 32 immature New Zealand rabbits were included in the study. Full thickness chondral defects were created in the weight-bearing surface of the medial condyles of the rabbits. The rabbits were divided randomly into four groups: no adjunct treatment was given in group 1, 0.3 ml human amniotic fluid (HAF) alone in group 2, human amniotic membrane (HAM) alone in group 3 and both of 0.3 ml HAF and HAM in group 4 was administered. The condyles were histopathologically evaluated at 4th and 12th week using the modified O'Driscoll Grading Scale.

Results
There were no significant differences in the quality of the regenerated tissue within and between groups ($p>0.05$). The mean results of groups at the 12th week were worse than results at the 4th week; however, the difference was statistically significant for only the sham group (group 1) and the combined therapy group (group 4) ($p=0.007$ and $p=0.014$, respectively).

Conclusion
HAF alone, HAM alone, and combined administration of both biomaterials neither affected chondral defect healing nor had any differences between each other. Nevertheless, we believe that some early regeneration due to an intrinsic repair mechanism is possible in immature rabbits as this study showed better results at 4th week than those at 12th week, although they are prone to degenerative processes in long-term follow-up. We suggest that a larger sample size in an experimental study would probably display a statistically significant difference when investigating effects of HAF, HAM, or both.

Keywords: Hyaline cartilage; Cartilage regeneration; Chondral defect; Human amniotic fluid; Human amniotic membrane

Introduction

Articular hyaline cartilage defects are among the most common injuries encountered in orthopedic practice. Treatment of cartilage problems has always been a challenge for orthopedic surgeons. Various conservative and surgical treatment modalities were attempted to treat hyaline cartilage lesions; however, depending on the limited intrinsic repair capacity of the articular cartilage, they still do not achieve reliable regeneration of normal hyaline cartilage, resulting in early development of osteoarthritis (1).

Hyaluronic acid was first introduced in the 1960s and is still widely used for the clinical treatment of osteoarthritis. Hyaluronic acid is claimed to moderate joint fluid viscosity, proliferate synovial cells, and contribute to joint proprioception (2). It prevents cartilage erosion, chondrocyte apoptosis and contributes to cartilage repair (3). HAF (obtained by amniocentesis especially in the 2nd trimester) contains high amounts of hyaluronic acid and hyaluronic acid-stimulating activator (4). Several studies have shown that HAF decreases scar tissue formation and enhances tissue healing by increasing endogenous and exogenous hyaluronic acid concentrations in the wound (5,6). HAF also include cartilage cell promoters such as growth factors (EGF, FGF, IGF etc.), fibronectin and laminin. Similarly, HAM is clinically used to treat intractable wounds and ulcers. Beneficial effects were also shown on nerve and tendon healing in experimental studies (7). Potential efficacy of HAM for cartilage defects were evaluated in some studies (8,9)

The aim of this study was to determine the effects of HAF injection, HAM wrapping, and combined application of both techniques on chondral defects and compare the advantages and disadvantages of these three application techniques.

Material and Methods

After the approval of Dokuz Eylül University, Faculty of Medicine, Animal Research Ethics Committee (date: 18.11.2004, number: 49) for animal experiments and the Dokuz Eylül University, Faculty of Medicine, Clinical and Laboratory Research Ethics Committee

(date: 30.11.2004, number: 158) for harvesting HAF and HAM, the study was carried out at Dokuz Eylül University, Faculty of Medicine, Multidisciplinary Experimental Animals Laboratory.

Human Amniotic Fluid Harvesting:
Sterile HAF was collected from amniocentesis of seronegative healthy pregnant women at between 16th and 24th weeks of pregnancy. The fluid was stored at -20 °C and was used within 1 week as described in the literature (10,11).

Human Amniotic Membrane Preparation
Sterile human amniotic membranes were harvested from placentas of seronegative parturients during cesarean sections. The amniotic membrane was meticulously dissected from the chorionic layer under sterile conditions. Then it was cleaned of blood by washing under sterile isotonic NaCl, and 10% gentamycin sulfate solution was added. It was kept at 4 °C and used within 4 hours as described in the literature (12).

Experiment Protocol
Sixty-four knees of 32 immature albino New Zealand rabbits weighing 1600-1800 g were included in the study. They were allowed to acclimatize to the environment, were kept under standard laboratory conditions (12 hours day/night illumination, 20-22 °C room temperature, and 50-60% humidity) for 1 week and were given food and water ad libitum. All rabbits were operated on bilaterally under general anesthesia by giving xylazine (5 mg/kg, im, Rompun, Bayer Türk Kimya San. Tic. Ltd. Şti., Gebze, Turkey) and ketamine (35 mg/kg, im, Ketalar, Eczacıbaşı İlaç San. ve Tic. A.Ş., Lüleburgaz, Turkey). The surgical field was shaved and cleaned with povidone iodine, an anterior longitudinal incision of 4 cm was applied to the skin, a medial parapatellar capsular approach was used to visualize the knee joint, and the patella was moved to the lateral side. Following maximum flexion of the knee joint, chondral defects of 3 mm in width and 7 mm in length were created with a scalpel at the weight-bearing surface of the medial femoral condyles of the rabbits. A previously prepared template with the sizes given above was used to determine the outer borders of the defect (Figure 1). As described in the literature, a No. 15 scalpel was used to create a full thickness chondral defect without damaging the subchondral bone (13).

The rabbits were randomly divided into four groups, each group containing 16 knees of 8 rabbits. Rabbits in each group were further divided into 2 subgroups: those euthanized and evaluated at 4 (early) and at 12 (midterm) weeks, respectively.

Figure 1
Template punch used to determine the outer borders of the cartilage defect (size: 3 mm × 7 mm)

Group 1 (sham control): The joint capsule was repaired and the defect was left untreated.

Group 2: HAF (0.3 mL) was injected into the knee joint following capsular repair.

Group 3: The defect was covered with HAM by suturing with 8/0 nylon suture and then the joint capsule was repaired.

Group 4: The defect was covered with HAM (Figure 2) and then the capsule was repaired. HAF (0.3 mL) was injected into the joint after capsular repair.

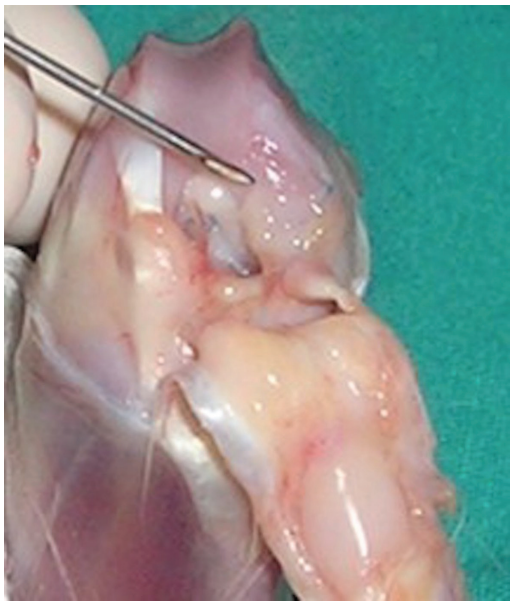

Figure 2
Amniotic membrane-wrapped femoral condyle defect shown with an injector needle

The joint capsule was repaired by 4/0 polyglactin suture and the skin was sutured with 4/0 polypropylene suture. No splint was applied and all rabbits were left free in the cage following the surgical procedure. All rabbits received cefazolin sodium at 50 mg/kg/day for two days for prophylaxis. Rabbits experiencing local or systemic infection were excluded from the study, and those dying in the early postoperative period were replaced with new rabbits. Neither HAF nor HAM caused any allergic or immunological reactions. All surgeries were performed by the same surgeon.

Histopathological Evaluation

Half of the rabbits in the groups were euthanized at the 4th week (early) and the rest were euthanized at the 12th week (midterm) by injection of a high dose of sodium thiopental (80-100 mg/kg, iv, Pental® Sodyum, İ.E. Ulagay İlaç Sanayi A.Ş, İstanbul, Turkey). Distal femoral condyles of the euthanized rabbits were stored in 10% neutral tamponized formalin solution in the pathology laboratory for 48 hours, then decalcified in 10% formic acid solution. Following 3 weeks of decalcification, the femoral condyles were embedded in paraffin blocks. Five sagittal cuts of 5-6 µm in slice thickness were taken from the medial femoral condyles, where the defect was created. Two of these slices were stained with hematoxylin-eosin and toluidine blue and evaluated for cell morphology, while the other two were stained with Safranin O and Sirius Red for matrix proteoglycans and collagen and the final slice was stained with Alcian blue (Figures 3a, 3b, 4a, and 4b). Some of the medial femoral condyles (n=36) were scored histopathologically under light microscope at the 4th week (early results) and the rest (n=28) were evaluated at the 12th week (midterm results) using the Modified O'Driscoll Grading Scale

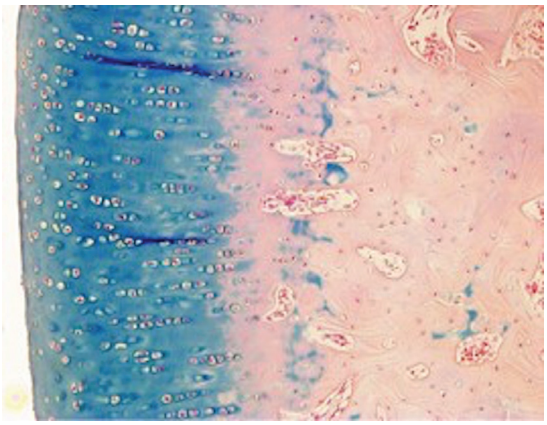

Figure 3a
Matrix staining of normal cartilage with Alcian blue at 20× magnification

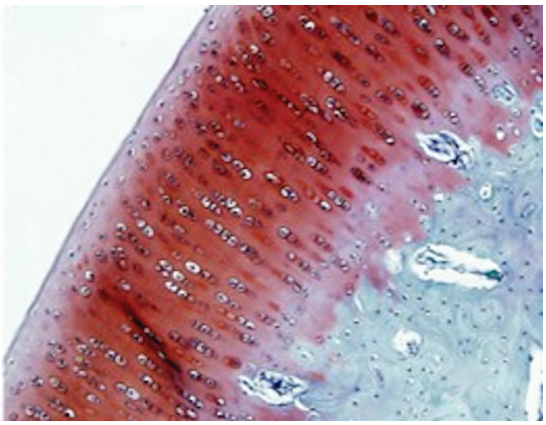

Figure 3b
Staining of proteoglycan of normal cartilage with Safranin O at 20× magnification

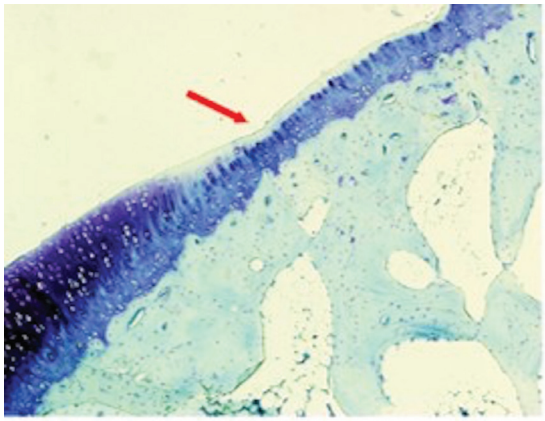

Figure 4a
Toluidine blue staining of the cartilage defect (red arrow) at 10× magnification demonstrating intact calcified cartilage layer

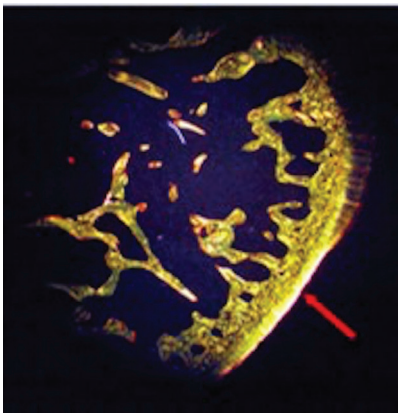

Figure 4b
Defect area (red arrow), stained with Sirius Red at 2× magnification under polarized light

(13). This grading scale scores samples between 0 to 16 in five categories including cell morphology, matrix staining, surface regularity, thickness of the cartilage, and bonding. A score of 16 points means perfect healing or normal articular cartilage (Table 1).

Statistical Analysis

Histopathological scores of all specimens were analyzed statistically with the nonparametric Mann-Whitney U test and the effectiveness and statistical differences of each treatment protocol were determined. Secondly, the three experimental treatment protocols, excluding the controls, were evaluated for early and midterm results with the ANOVA test. Data analysis was done with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Values of $p \leq 0.05$ were accepted as statistically significant.

Results

All of the 64 rabbits were histopathologically scored using the Modified O'Driscoll Grading Scale and scores of the experimental groups were processed in comparison with both the control group for every

Table 1 Modified O'Driscoll Grading Scale

Cell morphology	
Hyaline cartilage	4 points
Mostly hyaline cartilage	3 points
Hyaline and fibrocartilage	2 points
Mostly fibrocartilage	1 points
Mostly non-cartilage	0 points
Matrix staining	
Same as the normal area	4 points
Slightly reduced	3 points
Reduced	2 points
Significantly reduced	1 points
None	0 points
Surface regularity	
Smooth	2 points
Slightly irregular	1 points
Irregular	0 points
Thickness of the cartilage	
100% (300-400 μm)	4 points
75%	3 points
50%	2 points
25%	1 points
0%	0 points
Bonding	
Both edges integrated	2 points
One edge integrated	1 points
Both edges not integrated	0 points
Total	**16 Points**

Table 2 Comparison of 4th and 12th week mean scores

	4th week	12th week	P value
GROUP 1 (SHAM)	6.750± 1.035	4.625± 1.408	0.007*
GROUP 2 (HAF)	6.625± 2.066	4.625± 2.446	0.124
GROUP 3 (HAM)	7.100± 2.558	4.833± 1.329	0.069
GROUP 4 (HAF+HAM)	7.200± 2.201	4.000± 1.414	0.014*

treatment type and with the scores of same treatment at a different time point. First of all, results of the 4th week were compared with the 12th week. Results of the 12th week were worse than those of the 4th week; however, a statistically significant decrease was detected only in the sham group (group 1) and the group in which HAM was used with HAF (group 4) (p=0.007 and p=0.014, respectively). Comparisons of all results, standard deviations, and p-values are given in Table 2.

Secondly, all treatment groups were compared with the control group. In the comparison of results at the 4th week, the average score of the HAF treatment group (group 2) (6.625±2.066) was slightly lower than the average score of the sham group (group 1) (6.750±1.035), while the average scores of the HAM treatment group (group 3) (7.100±2.558) and the HAM+HAF treatment group (group 4) (7.200±2.201) were slightly higher. However, when the treatment groups were compared to the sham group, no statistically significant difference was seen (p>0.05).

In the comparison of results of treatment at the 12th week with the sham group, the average score of the HAF treatment group (group 2) (4.625±2.446) was similar to the average score of the sham group (group 1) (4.625±1.408), while the average score of the HAM treatment group (group 3) (4.883±1.329) was slightly higher. In contrast, the average score of the HAM+HAF group (group 4) (4.000±1.414) was slightly lower. None of these differences were statistically significant (p>0.05).

Then, treatment groups at 4th and 12th weeks were compared to each other. No statistically significant difference was detected (p>0.05).

As the final analysis, treatment groups at 4th and 12th weeks were compared by using ANOVA testing without the sham group. This yielded values of p=0.859 for the 4th week and p=0.730 for 12th week and these were statistically insignificant.

Discussion

The rabbit knee is similar to the human knee in terms of bone geometry, cartilage, and tendon structure. They show bone accretion and peak bone mass profiles similar to human (14). Many studies used rabbits before while investing effects of amniotic materials (11,15). Therefore, rabbits were preferred in this study.

The defect area, which was tried to be healed with intrinsic repair, degenerated with time (Figures 5a, 5b, 5c, 6a, and 6b) because the healed fibrocartilage tissue could not reflect accurate biomechanical properties of the hyaline cartilage (16). This study showed histologically better results at 4th week than those of 12th week. This was probably due to the fact that immature rabbits were used in this study as younger people are affected more by chondral defects. Wei et al. compared immature, adolescent and adult rabbits in terms of their healing capacity on a chondral defect model; they showed that the fastest and best healing was in immature rabbits (16). Also, the structural integrity was better in immatures than adults at 12th week and immature rabbits had significantly better bonding to adjacent cartilage than adolescent and adult rabbits at 6th week. However, many publications in the literature suggested the opposite result that this degeneration appeared at later weeks (17,18). O'Driscoll et al. found that chondrocyte numbers were maintained until the 12th week but decreased rapidly after the 18th week in a study of periosteal graft impacts on rabbit chondrogenesis (19). In the same study, mineral deposition and degeneration of chondrocytes were seen after the 12th week and degeneration of cartilage also increased with time.

HAF application, which was the first treatment applied in this study, can be considered as viscosupplementation. Recently, hyaluronic acid injections are widely used in the treatment of cartilage diseases and knee osteoarthritis. Solchaga et al. showed that spongious substances including hyaluronic acid were more effective compared to a control group considering hyaline cartilage production (17). Besides these effects, growth factors are also thought to have a positive impact on cartilage healing. It is reported that IGF-I, TGF-β, BMP-2, and EGF increase chondrocyte proliferation and stimulate type II collagen and proteoglycan synthesis (2,21,22). It has also been shown that IGF-I increases proliferative and metabolic activity (2,23). As per our hypothesis, the existence of several growth factors in amniotic fluid suggests that it may be effective on cartilage healing. These growth factors are NGF, IGF-I and -II, FGFs (acidic and basic FGF), and extracellular molecules like fibronectin and laminin. HAF is thought to decrease scar formation and increase the speed of tissue healing by increasing hyaluronic acid production exogenously and endogenously (5,6). It is also experimentally shown that HAF fosters peripheral nerve and tendon healing and helps tissue healing, decreases peritendinous adhesions, and increases cartilage tissue production on perichondrial flaps (10,11). These positive effects, and especially those on perichondrial flaps, encouraged us to pursue similar results for joint cartilage. In an animal study,

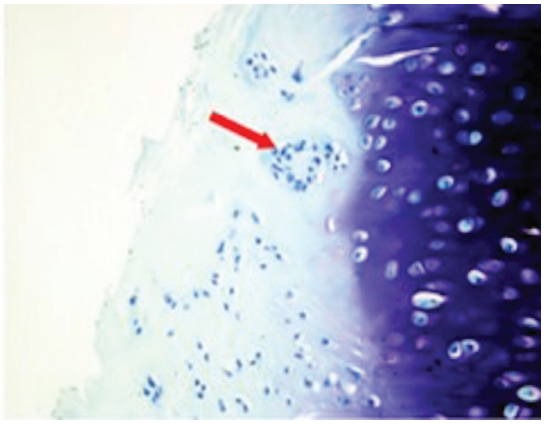

Figure 5a
Chondrocyte clusters (red arrow) in fibrocartilage area stained with toluidine Blue, indicating intrinsic repair, under 20× magnification

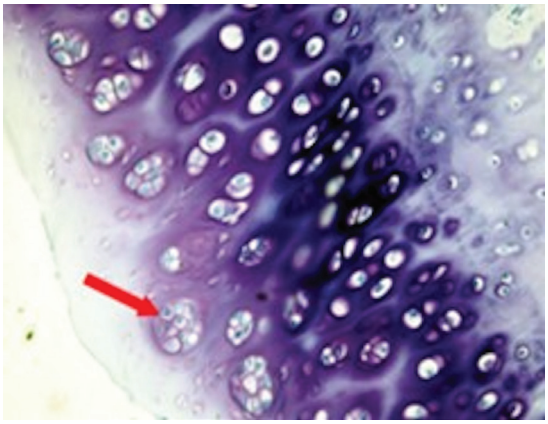

Figure 5b
Chondrocyte clusters (red arrow) in fibrocartilage area stained with toluidine Blue, indicating intrinsic repair, under 40× magnification

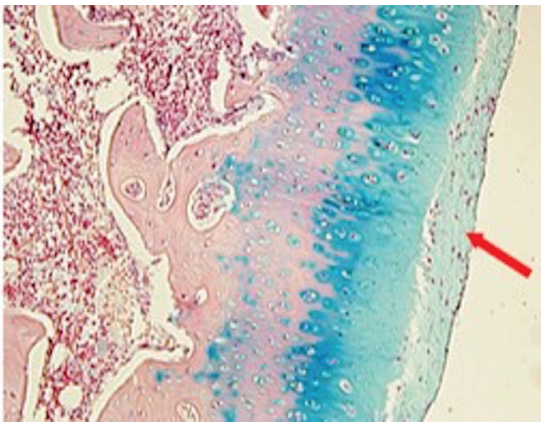

Figure 6a
Healing of chondral defect with fibrocartilage surface layer (red arrow), demonstrated as less staining with Alcian blue under 20× magnification

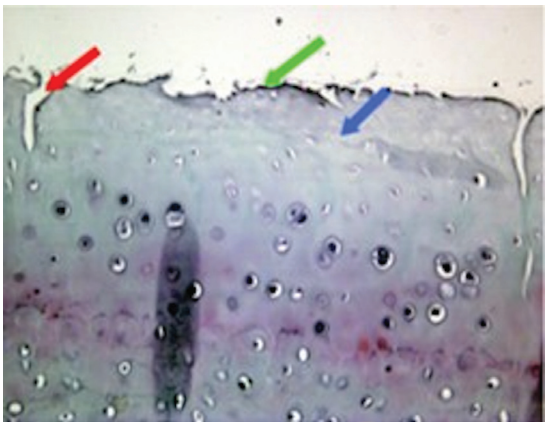

Figure 6b
Degenerated cartilage stained with Safranin O under 40× magnification showing cracks (red arrow), surface irregularity (green arrow), and focal chondrocyte necrosis (blue arrow), which all indicate degeneration

HAF was used to detect the effects on cartilage regeneration in rabbits. HAF was found to enhance neochondrogenesis from free perichondrial grafts. The rich content of hyaluronic acid and growth factors in HAF were the possible causes of this result (12). Vines et al. performed amniotic suspension allograft injections to treat Kellgren-Lawrence grade 3-4 knee osteoarthritis. Their study demonstrated the feasibility of intraarticular injection of amniotic suspension allografts for the treatment of knee osteoarthritis (24). Kavakli et al. administered HAF into the perichondrial bed of the costal cartilage of rabbits and found increased chondrogenesis in the perichondrial beds (25). You et al. had success in treatment of chondral defect by using human amnion derived mesenchymal stem cell sheets encapsulating cartilage particles (26). In our study, however, we did not obtain statistically significant results for the groups that underwent HAF treatment.

HAM is reported as a successful treatment modality for intractable wounds and ulcers (7). It is easy to produce and obtain. There are not many ethical issues that neither donor nor baby sees harm. It is osteoconductive, osteoinductive and also has antimicrobial, antiimmunogenic features ; thus it carries no risk when transplanted. It behaves like a scaffold and serves as a barrier to unpleasant conditions of joint hypothetically and can be combined with stem cells or grafts in order to increase its influence. The advantages of HAM have been experimentally shown in nerve and tendon healing (11,12). HAM enhances bone regeneration in large bone defects (27). HAM supported chondrocyte proliferation and regeneration of hyaline cartilage in vivo in a study (28). Zhang et al. investigated whether amniotic membrane-derived stem cells would be effective in a rabbit cartilage model and stated that the chondrocytes increased in damaged areas (29). Unfortunately in our study, we detected that both HAM alone application and HAM with HAF application had no statistically significant effect on joint cartilage healing.

After the comparison of these three treatments with each other and with the control group, no statistically significant values were seen. The most important reason for this was the similarity of the histologic points. Moreover, based only on these limited results, it should not be said that these agents are useless.

There are some limitations of this study. There had to be a control group to reveal whether the created cartilage defects could be standardized because creating standard cartilage defects with a scalpel in small animals is difficult. A small sample size was used in this study and we needed more rabbits to achieve statistically significant numbers. It was a histological study that biomechanical properties were not evaluated. Another probable reason why amnion derived treatments were not found useful in this study that cell populations in HAF and HAM show great diversity and variation among different donors and cultivation , therefore we do not have the knowledge of amount of ingredients of HAF and HAM in our study (30).

Conclusion

Application of HAF, HAM, and both of them together was ineffective for chondral healing with this number of animals. None of the treatments were superior to the others regarding these results. However, as the results were similar, the statistically insignificant results are not enough to lead us to say that these treatment modalities are useless. To show the effects of these agents, larger series are necessary in future studies.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Ethical Approvals

Dokuz Eylül University, Faculty of Medicine, Animal Research Ethics Committee (date: 18.11.2004, number: 49) and Dokuz Eylül University, Faculty of Medicine, Clinical and Laboratory Research Ethics Committee (date: 30.11.2004, number: 158) approved this study.

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants included in the study.

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References

1. 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. Arthrosc J Arthrosc Relat Surg 2003 May;19(5):477–84.
2. Brandt KD, Block JA, Michalski JP, Moreland LW, Caldwell JR, Lavin PT. Efficacy and safety of intraarticular sodium hyaluronate in knee osteoarthritis. ORTHOVISC Study Group. Clin Orthop Relat Res. 2001 Apr;(385):130–43.
3. Leighton R, Fitzpatrick J, Smith H, Crandall D, Flannery CR, Conrozier T. Systematic clinical evidence review of NASHA (Durolane hyaluronic acid) for the treatment of knee osteoarth-

ritis. Open Access Rheumatol Res Rev. 2018 May;10:43–54.
4. Dahl LB, Kimpton WG, Cahill RNP, Brown TJ, Fraser RE. The origin and fate of hyaluronan in amniotic fluid. J Dev Physiol. 1989 Oct;12(4):209–18.
5. Longaker MT, Adzick NS, Hall JL, Stair SE, Crombleholme TM, Duncan BW, Bradley SM, Harrison MR, Stern R. Studies in fetal wound healing, VII. Fetal wound healing may be modulated by hyaluronic acid stimulating activity in amniotic fluid. J Pediatr Surg. 1990 Apr;25(4):430–3.
6. Longaker MT, Scott Adzick N. The biology of fetal wound healing: A review. Plast Reconstr Surg. 1991 Apr;87(4):788–98.
7. Gruss JS, Jirsch DW. Human amniotic membrane: A versatile wound dressing. Can Med Assoc J. 1978 May 20;118(10):1237-46
8. Tabet SK, Conner DM, Guebert DA. The Use of Human Amniotic Membrane for Cartilage Repair: A Sheep Study. Stem Cell Discov. 2015;05(04):40–7.
9. Willett NJ, Thote T, Lin AS, Moran S, Raji Y, Sridaran S, Stevens HY, Guldberg RE. Intra-articular injection of micronized dehydrated human amnion/chorion membrane attenuates osteoarthritis development. Arthritis Res Ther. 2014;16(1):R47.
10. Özgenel GY, Filiz G. Effects of human amniotic fluid on peripheral nerve scarring and regeneration in rats. J Neurosurg. 2003 Feb;98(2):371–7.
11. Özgenel GY, Şamli B, Özcan M. Effects of human amniotic fluid on peritendinous adhesion formation and tendon healing after flexor tendon surgery in rabbits. J Hand Surg Am. 2001 Mar;26(2):332–9.
12. Özgenel GY, Filiz G. Combined Application of Human Amniotic Membrane Wrapping and Hyaluronic Acid Injection in Epineurectomized Rat Sciatic Nerve. J Reconstr Microsurg. 2004 Mar;20(2):153–7.
13. Grigolo B, Roseti L, Fiorini M, Fini M, Giavaresi G, Aldini NN, Giardino R, Facchini A. Transplantation of chondrocytes seeded on a hyaluronan derivative (Hyaff®-11) into cartilage defects in rabbits. Biomaterials. 2001 Sep;22(17):2417–24.
14. Norris S., Pettifor J., Gray D., Buffenstein R. Calcium metabolism and bone mass in female rabbits during skeletal maturation: effects of dietary calcium intake. Bone. 2001 Jul;29(1):62–9.
15. Karaçal N, Koşucu P, Çobanoğlu Ü, Kutlu N. Effect of human amniotic fluid on bone healing. J Surg Res. 2005;
16. Wei X, Gao J, Messner K. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. J Biomed Mater Res. 1997 Jan;34(1):63–72.
17. Solchaga LA, Yoo JU, Lundberg M, Dennis JE, Huibregtse BA, Goldberg VM, Caplan AI. Hyaluronan-based polymers in the treatment of osteochondral defects. J Orthop Res. 2000 Sep;18(5):773–80.
18. Tuncel M, Halici M, Canoz O, Yildirim Turk C, Oner M, Ozturk F, Kabak S. Role of insulin like growth factor-I in repair response in immature cartilage. Knee. 2005 Apr;12(2):113–9.
19. O'Driscoll SW, Fitzsimmons JS. The Role of Periosteum in Cartilage Repair. Clin Orthop Relat Res. 2001 Oct;391(391 SUPPL.):S190–207.
20. Elmalı N, Kaygusuz MA, Ozen S, Baysal O, Inan M, Karakaplan M, Şarlak O. The healing effect of intraarticular hyaluronic acid injection on osteoarthritic knee: a study on rabbit knees. Acta Orthop Traumatol Turc. 1999; 33: 211-215
21. Adams ME. An analysis of clinical studies of the use of cross-linked hyaluronan, hylan, in the treatment of osteoarthritis. J Rheumatol Suppl. 1993 Aug;39:16-8.
22. Wobig M, Bach G, Beks P, Dickhut A, Runzheimer J, Schwieger G, Vetter G, Balazs E. The role of elastoviscosity in the efficacy of viscosupplementation for osteoarthritis of the knee: A comparison of Hylan G-F 20 and a lower-molecular-weight hyaluronan. Clin Ther 1999 Sep;21(9):1549–62.
23. Fortier LA, Mohammed HO, Lust G, Nixon AJ. Insulin-like growth factor-I enhances cell-based repair of articular cartilage. J Bone Joint Surg Br. 2002 Mar;84(2):276-88.
24. Vines J, Aliprantis A, Gomoll A, Farr J. Cryopreserved Amniotic Suspension for the Treatment of Knee Osteoarthritis. J Knee Surg. 2015 Dec 18;29(06):443–50.
25. Kavakli K, Gurkok S, Caylak H, Genc O, Gamsizkan M, Yucel O, Karasahin E, Gozubuyuk A, Tasci C. Effects of human amniotic fluid on costal cartilage regeneration (an experimental study). Thorac Cardiovasc Surg. 2011 Dec;59(8):484-9.
26. You Q, Liu Z, Zhang J, Shen M, Li Y, Jin Y, Liu Y. Human Amniotic Mesenchymal Stem Cell Sheets Encapsulating Cartilage Particles Facilitate Repair of Rabbit Osteochondral Defects. Am J Sports Med. 2020 Mar 15;48(3):599–611.
27. Ghanmi S, Trigui M, Baya W, Ellouz Z, Elfeki A, Charfi S, Fricain JC, Keskes H. The periosteum-like effect of fresh human amniotic membrane on bone regeneration in a rabbit critical-sized defect model. Bone. 2018 May;110:392-404.
28. Cheng ZJ, So RP, Byung HC, Lee KY, Choong KK, Min BH. Human amniotic membrane as a delivery matrix for articular cartilage repair. Tissue Eng. 2007 Apr;13(4):693-702.
29. Zhang Z, Zeng L, Yang J, Guo L, Hou Q, Zhu F. Amniotic membrane-derived stem cells help repair osteochondral defect in a weight-bearing area in rabbits. Exp Ther Med. 2017 Jul;14(1):187–92.
30. Pipino C, Pierdomenico L, Di Tomo P, Di Giuseppe F, Cianci E, D'Alimonte I, Morabito C, Centurione L, Antonucci I, Mariggiò MA, Di Pietro R, Ciccarelli R, Marchisio M, Romano M, Angelucci S, Pandolfi A. Molecular and phenotypic characterization of human amniotic fluid-derived cells: A morphological and proteomic approach. Stem Cells Dev. 2015 Jun 15;24(12):1415–28.