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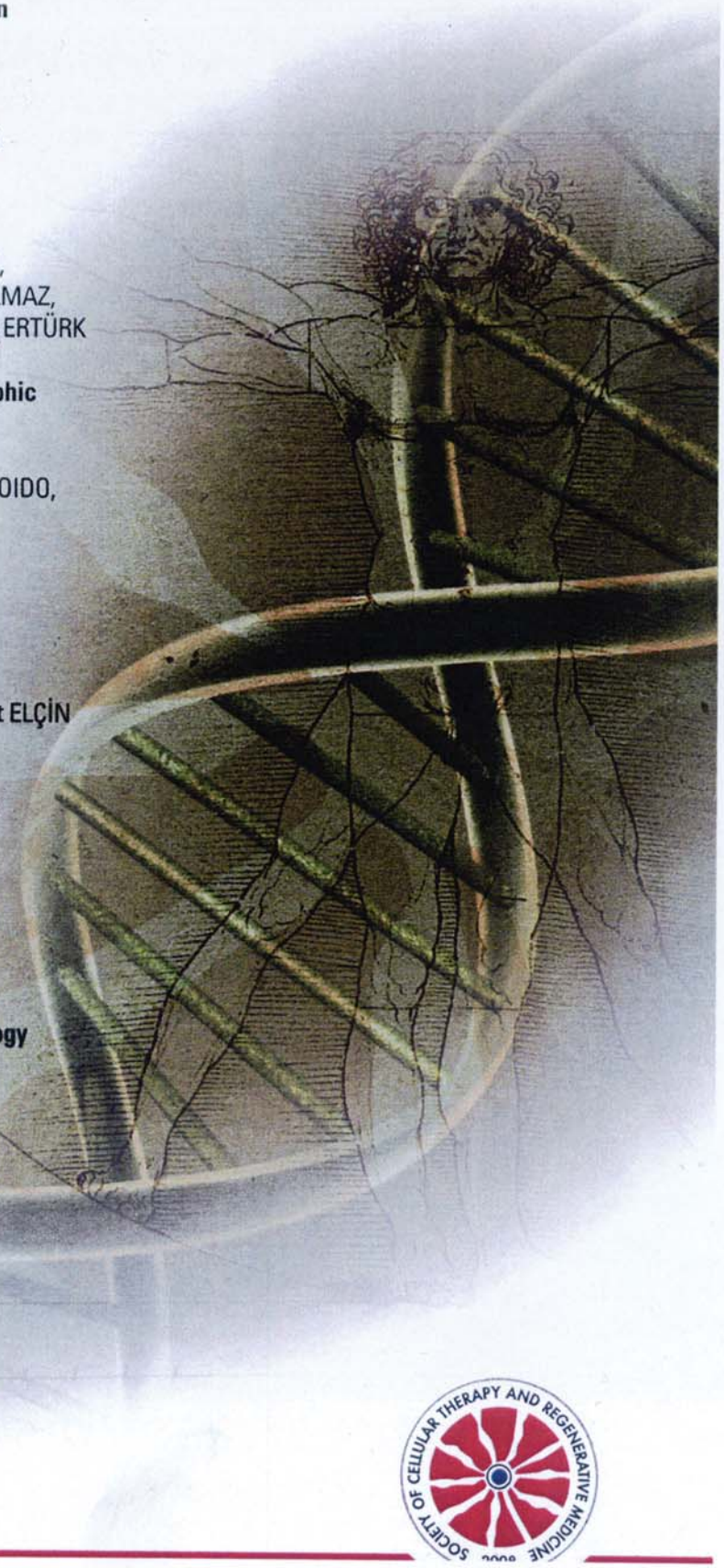

SOCIETY OF CELLULAR THERAPY AND REGENERATIVE MEDICINE

REVIEW

Recent Applications of Cellular Therapy in Orthopedic Surgery

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ABSTRACT Injured cartilage heals with inadequate scar tissue during its natural regeneration process. Treatment modalities before cellular therapies were focused on increasing the quality of the scar tissue rather than replacing it with the original one. Mechanical failure of the cartilage tissue causes arthritic changes in the long term. Arthritis is characterized by functional loss and pain and has a negative effect on the quality of life. Elderly patients are treated with total joint replacement, however, it remains the last option in younger patients with longer life expectancy due to the requirement of revision surgery in the long term. Therefore, prevention of joint arthritis in young patients is pivotal. The most important aspect of an ideal treatment method is to replace the missing tissue with another tissue that is original or similar as possible. As a product of interdisciplinary study, autologous chondrocyte implantation is one of the leading effective methods of cellular therapy in the musculoskeletal system. Autologous chondrocyte implantation is based on harvesting a small number of cartilage cells from the patient and replacing the missing tissue by transplanting the cells to the defective area after 3-4 weeks of in vitro propagation. In this review, autologous chondrocyte implantation treatment was discussed in the light of recent cellular therapy approach for chondrocyte injuries.

Key Words: Cartilage, chondrocyte, cell transplantation

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The major function of the cartilage tissue is to decrease the energy required for motion by reducing friction and to ensure painless range of motion.[1,2] The joint cartilage is a 2-4 mm, avascular, aneural and nonlymphatic structure, which results with delayed and inadequate healing when injured.[3-6] In addition, chondrocytes, which are the basic elements of the cartilage, have low mitogenic activity and very limited regeneration potential.[7] As a result, healing in joint injuries depends on the accumulation of fibrocartilaginous tissue, which has no histological and mechanical characteristic of the hyaline cartilage, in the defective area. Fibrocartilage that is biomechanically less endurable than cartilage cannot fulfill the pivotal functions of the cartilage such as absorption of shock and decreased friction, which are vital for long-term use. Depending on the location, size and burden area of the cartilage, biomechanical defects, loss of joint cartilage and functional loss predispose to osteoarthrosis characterized by pain.[7] Symptoms of osteoarthrosis decrease the activation

level and quality of life of patients, and eventually lead to joint replacement, which is not favored by clinicians due to the requirement of recurrent surgical interventions particularly in young patients. The treatment of progressive lesions due to the loss of joint cartilage is a major problem of the musculoskeletal surgery currently.

JOINT INJURIES AND CONVENTIONAL TREATMENT MODALITIES

Traumatic cartilage injuries are common among professional or amateur athletes and those who live an active life.[1,4,5] In relatively small (2-3 cm^2) cartilage defects, surgical interventions such as abrasion atrhroplasty, subchondral drilling, and microfracture are still considered current treatment options.[6,7] These surgical interventions provide a bridge between the defective area and the bone marrow and the mesenchymal stem cells recruited from the peripheral blood and bone marrow start the healing process. However, the fibrocartilaginous tissue produced by these methods does not possess the characteristics to fulfill the long-term expectations of a young and active patient.

Replacement of the hyaline joint cartilage with original or similar tissue is a widely investigated and discussed issue.[8-11] Transplantation of joint cartilage of low-burden areas to the defective area (mosaicplast) is one of the earliest interventions. However, this procedure has a major disadvantage such as the size of the treatable area being limited to the size of the donor area. Consequently, it may only be used in cartilage defects up to 4-6 cm^2. The inadequacy of the donor area in larger injuries and the problems that develop in the donor area are the major limitations of the procedure.[6,12] Joint replacement (restoration of cartilage surfaces using metal polyethylene) in large cartilage defects and in degenerative processes that develop in elderly patients is a serious treatment alternative. However, this procedure also has limitations such that, it may not fulfill the high functional expectancy of the young patients and may necessitate revision surgery in the long term. Thus, joint replacement remains the last treatment option in younger patients.

CELLULAR TREATMENT AND AUTOLOGOUS CHONDROCYTE IMPLANTATION IN CARTILAGE LOSS

Although the last century has witnessed great achievements in medicine, the developments in the field of cartilage injuries have been limited up to date. The pathophysiologic mechanisms and natural course of injuries have been investigated in detail. The results of these investigations suggested that healing was inadequate, moreover, healing would not be possible without intervention. Results of recent basic and clinical research on cellular treatment modalities for large (4-10 cm^2) cartilage defects are promising (Figures 1 A, 2 A, B).[3,8-11,13-15] In-vitro studies suggested that it was possible to enlarge the cartilage tissue by dedifferentiation and enhancement of cell numbers. Autologous chondrocyte implantation is a recent cellular treatment modality for the treatment of cartilage lesions involving large cartilage surfaces in young patients. This approach involves the harvesting of small numbers of cells from patients, in-vitro propagation and transplantation of cells to the defective area; the mean duration of this process is 3-6 weeks. This enables provision of adequate numbers of cells regarding the size of the defect and causes no problems in the donor area due to the low number of harvested cells. The "tissue engineering" approach described as the formation of functional tissues for implantation is an attractive alternative for the treatment of large chondral defects in the young and active population, despite its high cost.

Grande's work in 1982 provided a histological basis for autologous chondrocyte implantation defined as supporting with cultured chondrocytes of the periostal graft attached on the joint cartilage defect in rabbits.[14] Technically, the chondrocytes are enzymatically dissociated and forced to reversible dedifferentiation to be propagated as monolayers; thus 80% healing of defects with hyaline cartilage can be achieved. Lars Peterson et al. treated cartilage defects at the cellular level using this approach, which provided a basis for healing of joint surfaces lacking cartilage in humans.[3,8,9,11] Following the first autologous chondrocyte implantation in humans, favorable

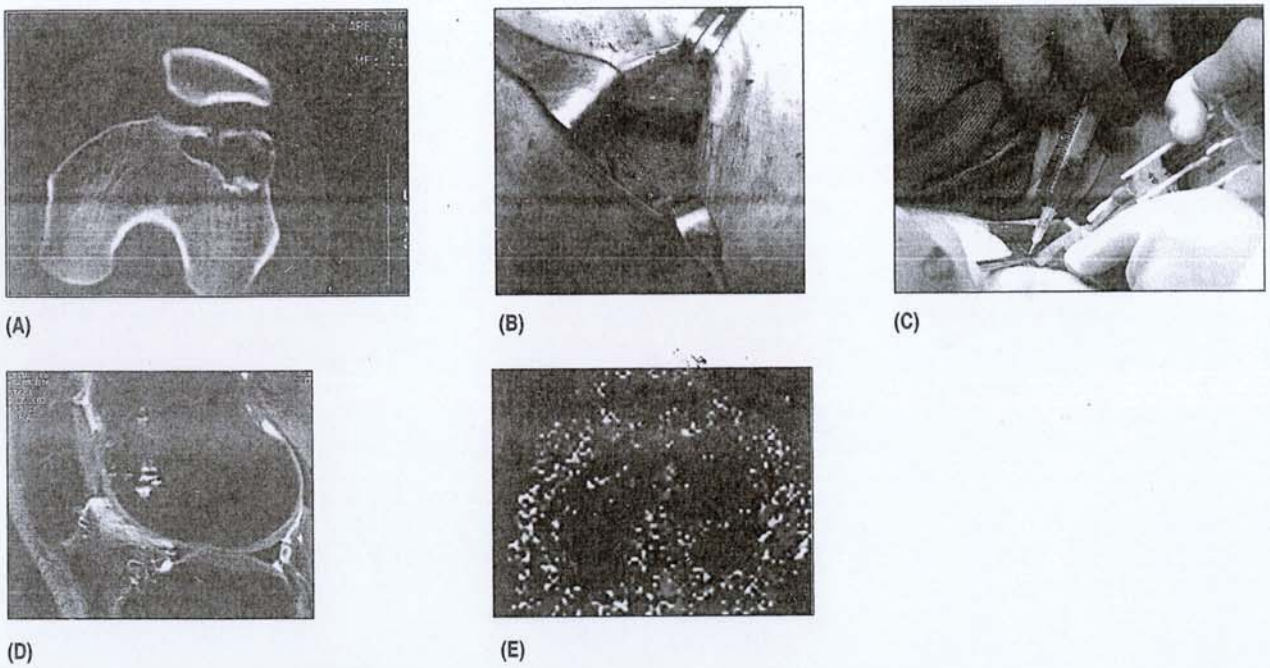

FIGURE 1: 38-years-old male (A): Large cartilage defect on the lateral femur chondyle in computed tomography scanning; (B) restoration of the defect with bone grafting in the first surgical session; (C) injection of autologous chondrocyte suspension to the defective area in the second session; (D) almost normal appearance of the cartilage defect in the magnetic resonance imaging in the fist year of surgical treatment; (E) activation signals in the defective area in perfusion magnetic resonance imaging after one year.

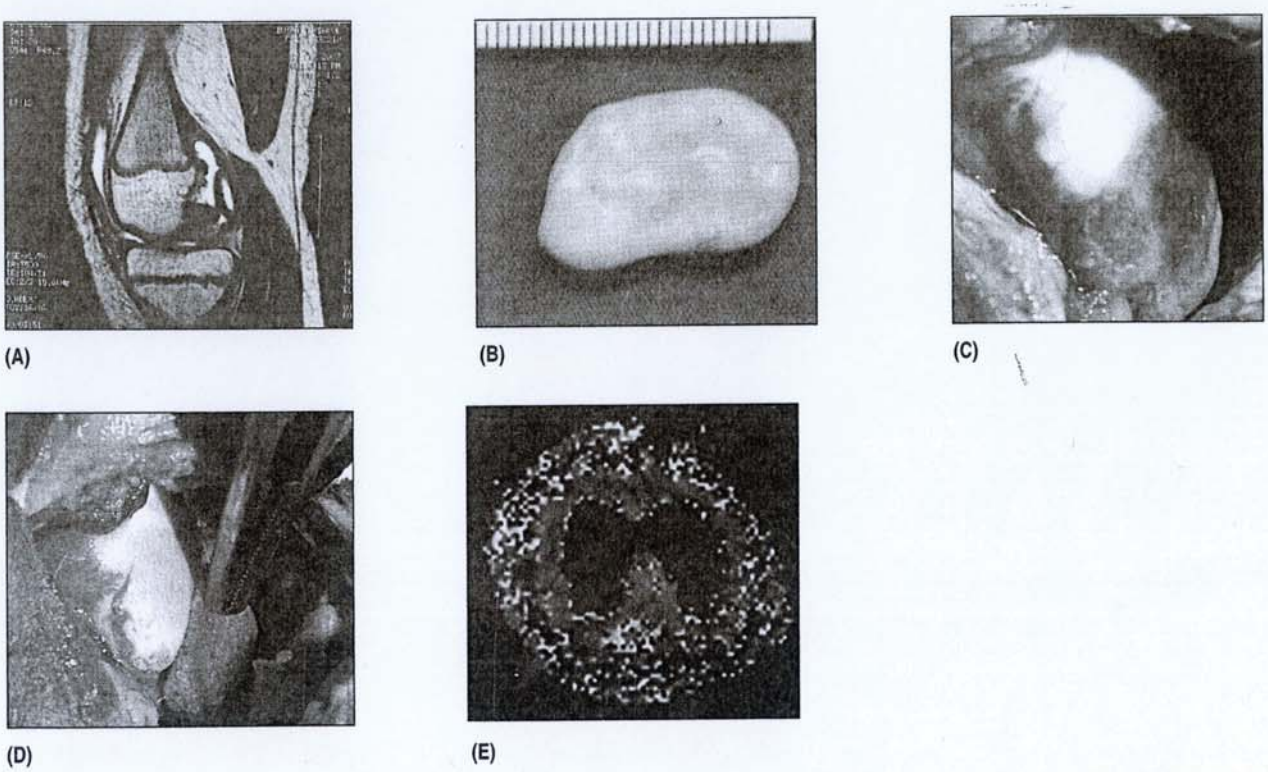

FIGURE 2: 13-years old, female; (A) The magnetic resonance image posterior to the joint of the traumatic cartilage piece that has detached from the lateral femur chondyle; (B) morphologic image; (C) transfer of chondrocytes, which were placed in a second generation polymer, to the defective area by surgical intervention; (D) image after fixation with fibrin cement; (E) perfusion magnetic resonance imaging at three months of autologous chondrocyte implantation.

results have been achieved during the follow-up of cases for more than ten years. This treatment modality is currently used for patients 15-55 years old without degenerative osteoarthritis and with symptomatic full layer cartilage defect. Reports indicated that increasing number of patients and long-term results would allow the development of novel joint surfaces.

Autologous chondrocyte implantation has shown progress since its first use with basic cellular propagation and reimplantation principles remained unchanged. The conventional approach in autologous chondrocyte implantation is to use cells harvested from the intact hyaline cartilage to bene-fit from the potential of mesenchymal cells to differentiate into chondrocytes. Following clinical diagnosis, chondral defects are confirmed by arth-roscopy and cartilage biopsy is obtained. In patients with cartilage loss accompanied by bone defect, a stable subchondral bed ready for use during implantation is prepared (Figure 1 B). A biopsy specimen, which includes 1-2 pieces of 4x10 mm cartilage, is obtained from the upper part of the medial femoral chondyle by curette in 98% of cases; this corresponds to approximately 200-300 mg of cartilage. The cartilage specimen is delivered to the laboratory within no more than six hours in transport medium. In the laboratory, after the specimen is fragmented and digested with various enzymes (collagenase, hyaluronidase, and DNAse), cellular expansion is initiated. Recently a new step of exposure to autologous serum has been added to the procedure. The tissue specimen is enzymatically digested, cultured for three weeks and is trypsinized after the number of cells increase 10-fold. This process yields an autologous chondrocyte suspension including hundred thousands of cells.[13,16,17]

Another issue investigated is increased production of cartilage components by stimulating cells mechanically during cell culture. Compressive and scissoring type forces were shown to increase collagen and proteoglycan synthesis and yield load capacities of various types. The structure of cartilage formed by tissue engineering revealed that it had a mechanical endurance of 30-50% of the normal cartilage after 6-8 weeks. These results suggested that mechanical stimulants had various effects on the development of chondrocytes and the production of extracellular matrix.

The developments in the field were recorded not only in the laboratory but also in surgical practice. In the early periods, chondrocytes propagated in liquid medium were injected under the periostal blanket formed by placing the periostal graft harvested from the medial tibia on the chondral defect and sealed by fibrin cement for impermeability (Figure 1 C). Control arthroscopic examination of first generation autologous chondrocyte implantation using the abovementioned technique revealed cartilage-like morphological and histological features and parallel to these observations, yielded good or excellent results in the functional evaluation (Figure 1 D, E).[3,10] The limitations of the technique such as non-homogenous distribution of cells in the liquid medium due to gravity, loss during injection, adhesion due to the use of periost tissue and hypertrophic changes stimulated research for second-generation chondrocyte implantation.

In the second-generation autologous chondrocyte implantation, chondrocytes harvested from the patients were cultured as monolayers or on three dimensional supporting structures and were transplanted to the defective area (Figure 2 C, D). Absorbable carriers developed by tissue engineering facilitated surgical intervention and allowed more homogenous distribution of cells. Chondrocytes to be implanted on the cartilage defect area are seeded on the polymer bed and thus, the negative effects of the periostal graft are avoided. Autologous chondrocytes are homogenously distributed within this spongy structure during implantation. The homogenous distribution of cells not only on the outer surface of the structure, but also in the inner pores, migration of the cells and the initiation of extracellular matrix production during the in-vitro cultivation period facilitate the development of a more effective cartilage tissue (Figure 2 E).[21-23]

Compared to the histological examination that is considered the gold standard to determine the implantation effectiveness of chondrocytes,

radiological imaging of the cartilage tissue using magnetic resonance imaging (MRI) is superior with its low cost and non-interventional nature.[24,25] This requires familiarity with the imaging characteristics of all components of cartilage that include mostly water, collagen, proteoglycans and 10% of cellular elements. In addition, certain molecules may be stained by contrast media. This will allow the identification of morphological characteristics as well as the biochemical composition of the cartilage tissue.[26] Gadalinium Enhanced MRI of Cartilage allows monitoring of surgical treatment of cartilage defects; this gives the opportunity of tracing the glycosaminoglycan concentration, which is associated with the biomechanical durability of the cartilage. Novel technologies such as diffusion MRI are also used in this field.[27]

OUR EXPERIENCE ON CELLULAR TREATMENT FOR CARTILAGE LOSS

Fourteen knee joints in thirteen patients were treated with autologous chondrocyte implantation in the Department of Orthopedics and Traumatology, Ankara University School of Medicine between 2002 and 2008. Chondrocyte implantation was performed by using cells cultivated in a foreign country in three cases; in the remaining cases, cell cultures propagated in the Ankara University laboratories were used. Enzymatically isolated 3-7x10^3 chondrocytes were grown as monolayers (2-4x10^5) in static cultures with chondrogenic DMEM medium including 10 ng/ml TGF-β, 50 μg/ml ascorbic acid, 10% serum and other supplements.[20,28] Cases were assessed with conventional methods such as T1 and T2 sequencing as well as Gadalinium Enhanced MRI of Cartilage; these assessments revealed that all cases had healed with almost normal joint cartilage surfaces during the sufficiently long (>12 months) follow-up period. All patients returned to their daily living activities without any complication.

CONCLUSION

In conclusion, the partnership of clinical sciences and tissue engineering is open to new developments. Loss of joint cartilage is still the center of interest for many researchers due to the limited healing potential of chondrocytes and the high incidence of osteoarthritis that decreases the quality of life. Previous methods used up to date for large defects have proved useless to obtain a stabile and smooth cartilage surface. Replacement of the hyaline joint cartilage with an identical or similar tissue has been a major research topic for many years. As the physiologic characteristics of cartilage cells and the response they give to injuries are better established, it will be easier to develop alternatives to these mechanisms.

Future research on cellular treatment for musculoskeletal surgery will focus on progenitor and stem cells derived from humans and animals. The key point in discovering a successful treatment modality is to clarify cartilage physiology and the pathophysiology of injuries or diseases; thus, multidisciplinary studies are pivotal. In this context, the cooperation of tissue engineering and orthopedic surgery has yielded favorable results for the treatment of cartilage injury.

REFERENCES

1. Mandelbaum BR, Seipel PR, Teurlings L. Articular cartilage lesions: Current concepts and results. In: Arendt EA, ed. Orthopedic Knowledge Update, Sports Medicine 2, AAOS, Illinois, 1999. p.19-28.
2. Kiviranta I., Jurvelin J. Biomechanical properties of articular cartilage- in vivo characterization ICRS 2000;3:14-15.
3. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochonritis dissecans of the knee with autologous chondrocyte transplantation (results at two to ten years). J Bone Joint Surg Incorp 2003;85-A,Suppl (2):17-24.
4. Binnet M S, Gürken İ, Karakaş A, Yılmaz C, Erekul S, Çetin C. Histopathologic assesment of healed osteochondral fractures. Arthroscopy 2001;17:278-85.
5. Binnet MS, Demirtaş M, Ateş Y, Mergen E. Traumatic articular cartilage injuries of the knee. Acta Orthop Traumatol Turc 1991;25: 326-9.
6. Kayaoğlu E, Binnet M S: Kıkırdak sorunlarında tedavi algoritması . Türkiye Klinikleri J Surg Med Sci 2 2006;2(39):51-4.
7. Shelbourne D, Jari S, Gray T. Outcome of untreated traumatic articular cartilage defects of the knee. J Bone Joint Surg 2003;85(A):8-16.
8. Brittberg M, Tallheden T, Sjögren-Jansson B, Lindahl A, Peterson L. Autologous chondrocytes used for articular cartilage repair: an update. Clin Orthop Relat Res 2001;(391 Suppl): S337-48.

9. Brittberg M, Nilsson A, Lindahl A, Ohlsson C, Peterson L. Rabbit articular cartilage defects treated with autologuos cultured chondrocytes. Clin Orthop Relat Res 1996;(326):270-83.
10. Minas T. Autologous chondrocyte imlantation for focal chondral defects of the Knee. Clin Orthop Relat Res 2001;(391 Suppl):S349-61.
11. Minas T, Peterson L. Advanced techniques in autologous chondrocyte transplantation. Clin Sports Med 1999;18(1):13-44, v-vi.
12. Oshima Y, Watanabe N, Matsuda K, Takenaka N, Kawata M, Takai S. Behaviour of graft and host cells in underlying subchondral bone after transplantation of osteochondral autograft. Microsc Res Tech 2002;58(1):19-24.
13. Freed LE, Vunjak-Novakovic G. Tissue Culture Bioreactors: Chondrogenesis as a model system. Principles of Tissue Engineering 1997. p.151-65.
14. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res 1989;7(2):208-18.
15. Robinson D, Ash H, Aviezer D, Agar G, Halperin N, Nevo Z. Autologous chondrocyte transplantation for reconstruction of isolated joint defects: The Assaf Harofeh experience. Isr Med Assoc J 2000;2(4):290-5.
16. Freed LE, Hollander AP, Martin I, Barry JR, Langer Ri, Vunjak-Novakovic G. Chondrogenesis in a cell-polymer-bioreactor system. Exp Cell Res 1998;240(1):58-65.
17. Obradovic B, Carrier RL, Vunjak-Novakovic G, Freed LE. Gas exchange is essential in bioreactor cultivation of tissue engineered cartilage. Biotechnol Bioeng 1999; 63(2):197-205.
18. Duda GN, Haisch A, Endres M, Gebert C. Mechanical Quality of tissue engineered cartilage: Results after 6-8 weeks in vivo. J Biomed Mater Res 2000;53(6):673-7.
19. Kiviranta I, Jurvelin J. Biomechanical properties of articular cartilage- in vivo characterization. ICRS 2000;3:14-15.
20. Emin N, Koç A, Durkut S, Elçin AE, Elçin YM. Engineering of rat articular cartilage on porous sponges: Effects of TGF-β1 and microgravity bioreactor culture. Artif Cell Blood Sub 2008, 36(2): 123-37.
21. Nehring D, Adamietz P, Meenen NM, Pörtner R. Perfusion cultures and modelling of oxygen uptake with three-dimensional chondrocyte pellets. Biotechnol Tech 1999;13(10): 701-6.
22. Rotter N, Aigner J, Naumann A, Hammer C, Sittinger M. Behaviour of tissue-engineered human cartilage after transplantation into nude mice. J Mater Sci Mater Med 1999; 10(10/11): 689-93.
23. Sittinger M, Reitzel D, Dauner M, Hierlemann H. Resorbable polyesters in cartilage engineering: Affinity and biocompatibility of polymer structures to chondrocytes et al. J Biomed Mater Res 1996;33(2):57-63.
24. Recht M, White LM, Winalski CS. MR imaging of cartilage repair procedures. Skeletal Radiol 2003;32(4):185-200.
25. Burkart A, Imhoff AB. Diagnostic imaging after autologous chondrocyte transplantation. Correlation of magnetic resonance tomography, histological and arthroscopic findings. Orthopade 2000;29(2):135-44.
26. Gillis A, Bashir A, McKeon B, Scheller A, Gray M, Burstein D. Magnetic resonance imaging of relative glycosaminoglycan distribution in patients with autologous chondrocyte transplants. Invest Radiol 2001;36(12): 743-8.
27. Mamisch TC, Menzel MI, Welsch GH, Bittersohl B, Salomonowitz E, Szomolanyi P, et al. Steady-state diffusion imaging for MR in-vivo evaluation of reparative cartilage after matrix-associated autologous chondrocyte transplantation at 3 tesla--preliminary results. Eur J Radiol 2008;65(1):72-9.
28. Elçin YM. Tissue Engineering, Stem Cells and Gene Therapies, Advances in Experimental Medicine and Biology Series: 534, New York and London: Kluwer Academic-Plenum Publishers; 2003. p.350.