Autologous Chondrocyte Transplantation for Reconstruction of Isolated Joint Defects: the Assaf Harofeh Experience

Dror Robinson MD PhD[1,3], Hana Ash PhD[2], David Aviezer PhD[2], Gabriel Agar MD[1], Nahum Halperin MD[1] and Zvi Nevo PhD[3]

[1]Department of Orthopedics, Assaf Harofeh Medical Center, Zerifin; [2]CTI Ltd., Kiriat Weizmann, Nes Ziona; and [3]Department of Clinical Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Israel

Key words: chondrocyte transplantation, joint cartilage, articular surface, bioengineering, cartilage repair

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Abstract

Background: Articular cartilage is incapable of undergoing self-repair since chondrocytes lose their mitotic ability as early as the first year of life. Defects in articular cartilage, especially in weight-bearing joints, will predictably deteriorate toward osteoarthritis. No method has been found to prevent this deterioration. Drilling of the subchondral bone can lead to fibrocartilage formation and temporary repair that slowly degrades. Animal experiments indicate that introducing proliferating chondrocytes such as cultured articular chondrocytes can reliably reconstruct joint defects.

Objectives: To describe our clinical experience in culturing and transplanting autologous chondrocytes.

Methods: Biopsies were obtained from 10 patients, aged 18–45, undergoing a routine arthroscopy in which a cartilage defect was identified with indications for cartilage transplantation. The biopsies were further processed to establish chondrocyte cultures. ACT was performed in 8 of the 10 patients because of persistent symptoms for at least 2 months post-arthroscopy. All patients (6 men and 2 women) had a grade IV cartilage defect in the medial or lateral femoral condyle, and three had a defect in the trochlear region as well. Biopsies were removed from the lateral rim of the superior aspect of the femur, and cells were cultured in a clean room. Following a 2 order of magnitude expansion, cells were implanted under a periosteal flap.

Results: The eight patients implanted with autologous cells were followed for 6 months to 5 years (average 1 year). Complaints of giving-way, effusion and joint locking resolved in all patients, and pain as assessed by the visual analogue score was reduced by an average of 50%. Follow-up magnetic resonance imaging studies in all patients revealed that the defects were filled with tissue having similar signal characteristics to cartilage.

Conclusions: Chondrocyte implantation is a procedure capable of restoring normal articular cartilage in cases with isolated joint defects. Pain can be predictably reduced, while joint locking and effusion are eliminated. The effect on osteoarthritis progression in humans has not yet been elucidated.

IMAJ 2000;2:290–295

Articular cartilage is incapable of undergoing self-repair. Adult cartilage itself does not contain undifferentiated stem cells, and the chondrocytes are all trapped in lacunae within a matrix that is inhibitory to cell proliferation [1]. Repair cells from bone marrow can only migrate into cartilage if the integrity of the subchondral bone plate is breached. In the latter situation, fibroblast-like precursors migrate from the bone marrow and form a fibrocartilaginous tissue, but this tissue possesses inferior biomechanical characteristics and tends to break down eventually [2].

Traumatic lesions of cartilage either do not undergo self-repair and stay empty or are filled initially with fibrocartilaginous tissue. Since these lesions often lead to the development of osteoarthritis [3], there is a need for reconstructing articular cartilage. Several methods are in clinical use today. Multiple drill holes through the subchondral bone plate or microfractures can lead to fibrocartilage formation [4]. Transplantation of allogeneic cartilage has been used, but since the cartilage is often only partially viable the long-term results are poor [5]. An exciting development is the transfer of osteochondral cylinders from non-weight-bearing areas into the weight-bearing areas of the same joint [6]. The integration of the cylinders with the surrounding cartilage has not yet been fully assessed, and neither are the long-term prospects of the cylinder technique known.

The possibility of transplanting isolated chondrocytes is intriguing. It is well known that isolated chondrocytes are able to regenerate a cartilage-like tissue following implantation. As cartilage lacks blood vessels and nerves, the newly formed tissue is remarkably similar to the original tissue. The cartilage above the tide mark remains uncalci-

ACT = autologous chondrocyte transplantation

fied, while the deeper regions undergo endochondral ossification. The biomechanical characteristics of the repair tissue are superior to those of the fibrocartilage formed following other cartilage repair techniques, and approximate those of hyaline cartilage [7]. The current study describes our experience with autologous chondrocyte transplantation.

Materials and Methods

Patients

All patients included in the study signed consent forms that were approved by the Institutional Committee for Human Studies and the Ministry of Health. Separate forms were signed for cartilage biopsy and for chondrocyte transplantation.

Inclusion criteria were: age range 18–45 years old; signing of an informed consent form; no clinical or serological evidence of inflammatory or generalized degenerative joint disease; outerbridge grade IV lesion (exposed subchondral bone) >2 cm^2 in area; no multicompartmental involvement of the joint; no significant ligament instability; no joint malalignment; extension to at least –10 degrees and flexion ≤140 degrees; and compliance with the postoperative physiotherapy protocol. The exclusion criteria included: prior joint infection, known malignancy, lack of significant clinical symptoms, and technical inability to perform ACT. All patients underwent radiographs, bone scanning and magnetic resonance imaging prior to the arthroscopic biopsy.

Arthroscopy

Arthroscopy was performed in patients with posttraumatic joint pain of at least 6 months. The procedure was carried out under general anesthesia and with a tourniquet to control bleeding. If a cartilaginous lesion meeting the inclusion criteria was identified, a cartilage biopsy was taken with a 3 mm hollow curette. Either one or two pieces of cartilage was removed either from the superolateral part of the trochlear cartilage at the cartilage-synovia rim or from the lateral part of the femoral notch. Biopsy size was approximately 3x3x5 mm. Multiple drilling to the bleeding subchondral bone using a 2 mm drill bit treated the cartilaginous defect. Loose fragments were removed and the defect edges trimmed. The postoperative protocol included partial weight bearing for 6 weeks, and physiotherapy comprising muscle strengthening and range of motion exercises beginning on the third postoperative day.

Evaluation of study group prior to transplantation [Table 1]

None of the patients had any systemic disorder or any indication of generalized arthritis. All patients had a clear history of trauma followed by pain and joint crepitations. All patients were treated by a physiotherapist for at least 2 months prior to the cartilage biopsy. An interval of at least 6 months elapsed from the trauma to the cartilage biopsy (range 6 months to 4 years, mean 1.5 years). Nine of the ten patients were engaged in litigation.

Table 1. Preoperative vs. postoperative evaluation in transplanted patients (n=8)

	Used in final evaluation	Pre-operative	Post-operative
How does your knee function?	*	3.5	2
1–4 scale; 4 is worst			
Patient's pain assessment		7.5	4
0–10 scale; 10 is worst.			
1–4 scale; 4 is worst	*	3.8	2.
(used in final evaluation)			
Symptoms (worst symptom determines score)	*	3.5	1.5
1–4 scale; 4 is worst			
Range of motion	*	1.2	1
Ligament examination	*	1	1
Compartmental findings (crepitus in involved compartment)		4	1
Harvest site pathology		Irrelevant	1.2
X-ray findings		1	1
Function test		4	2
Final evaluation		16.5	7.5

Clinical evaluation was based on the cartilage standard evaluation form/Knee issued by the International Cartilage Repair Society [8]. Knee radiographs were within normal limits in 8 of the 10 cases, while in the other 2 patients with a known osteochondral fracture the fragment had been previously removed. All 10 patients had increased bone scan uptake, indicating an osteochondral injury in the relevant area of the joint. MRI revealed an area of chondral damage surrounded by significant bone marrow edema in all patients. Increased amounts of joint fluid were noted in 8 of the 10 patients.

On average, two operative procedures (range 1 to 5) were performed on a transplanted joint prior to ACT. These included partial medial menisectomy in five patients, and removal of loose bodies and cartilage fragments in four. Chondral abrasion and drilling was performed in all patients at least once.

Pain score prior to surgery averaged 8.5 (range 7–10) on a Visual Analogue Scale numbered from 0 (no pain) to 10 (maximal pain). The functional status of all patients was 4 ("I am very restricted and I can do almost nothing with my joint without severe pain and disability") on a 1–4 grade score. Patient self-assessment of knee function prior to surgery was 3 on a 1–4 grade score as compared to the contralateral knee.

Arthroscopic findings

In seven patients a chondral defect, ranging in size from 2 to 8 cm^2, was located in the weight-bearing area of the medial femoral condyle [Figure 1]. In one of these patients a chondral defect measuring 2.3 cm^2 was also found in the patella; two patients had a trochlear defect of 10 cm^2 and one patient had a lateral femoral condyle defect measuring 12 cm^2. Fraying of the anterior cruciate ligament was observed in two patients, however the ligament fibers were continuous and the drawer test under anesthesia showed less than 5 mm. Partial medial menisectomy was performed in four patients with meniscal lesions. No synovitis was observed in any of the patients. Opposing joint surfaces were either normal or demonstrated grade I chondral changes.

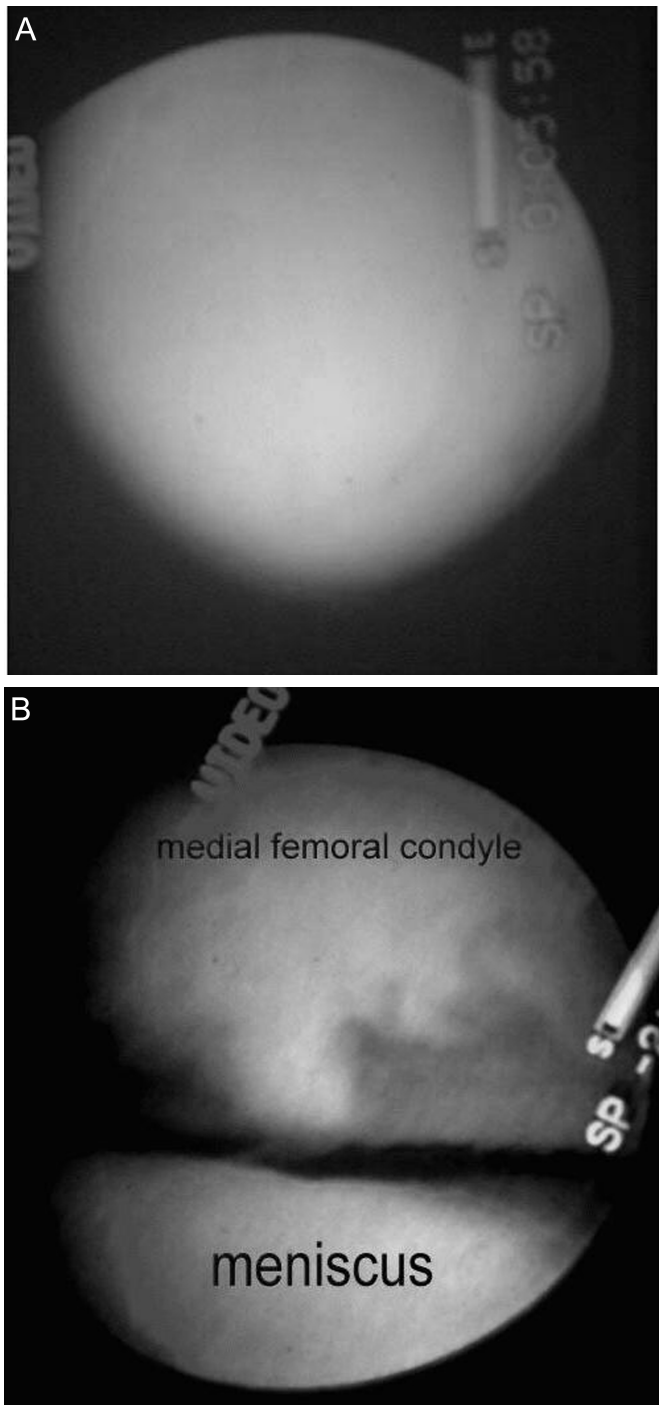

Figure 1. [A] Arthroscopic view of a grade IV defect of articular cartilage of the medial femoral condyle. **[B]** Arthroscopic view 4 months following transplantation demonstrating complete healing of the defect.

Cell culture procedures

All samples except for the first two were processed at CTI Ltd. (Kiriat Weizmann, Nes Ziona, Israel). The samples were processed in a dedicated tissue culture laboratory and were monitored repeatedly for sterility. Biopsies were treated under strictly sterile conditions in a clean room. The tissue pieces were dissected under x8 magnification and only hyaline-like cartilage was used. Explant cultures were established. Cells were cultured as monolayer in Dulbecco's minimal essential medium (Beit HaEmek Industries, Israel) to which 10% fetal calf serum (Life Technologies, Inc. Gibco/BRl, Frederick, MD, USA) was added. The serum was taken from herds believed to be free of prion disease.

Dedifferentiated chondrocytes were expanded by 2–3 orders of magnitude during 4–6 weeks of culture during which two subcultures were performed. Morphology of the cultures was monitored. When cells were grown under conditions known to induce cartilage formation *in vitro,* such as low oxygen and high density of the cells, cartilage nodules formed. Northern blotting of cultures demonstrated production of aggrecan, a proteoglycan specific for cartilage [Figure 2].

Histological, histochemical and immunohistochemical staining procedures

Cell cultures were assessed by immunohistochemistry performed according to methodology previously described. In brief, fibroblast growth factor receptor-3 antibodies prepared in rabbits were shown to selectively recognize FGFR3 and not type 1, 2 or 4 FGF receptors. The addition of purified soluble FGFR3 peptide obliterates staining by these antibodies, whereas similar FGF receptor type 1 or 2 peptides do not. Chondrocytes in culture tend to grow as a monolayer as long as the available growth area is adequate. As the culture matures, cell proliferation forces multilayering of the culture. When cells are grown for prolonged periods, especially under low oxygen conditions (8%), cartilaginous nodules are formed.

Cells in monolayer cultures were fixed in paraformaldehyde 4% in phosphate buffered saline 0.01 mol, pH 7.4. Culture dishes were stained with Meyer's hematoxylin and eosin, Masson's trichrome, safranin-O, and alcian blue (pH 1 and 2.5). All dishes used for immunohistochemical staining procedures with a peroxidase-antiperoxidase complex were pretreated with 3% H_2O_2 in methanol for

FGFR3 = fibroblast growth factor receptor-3

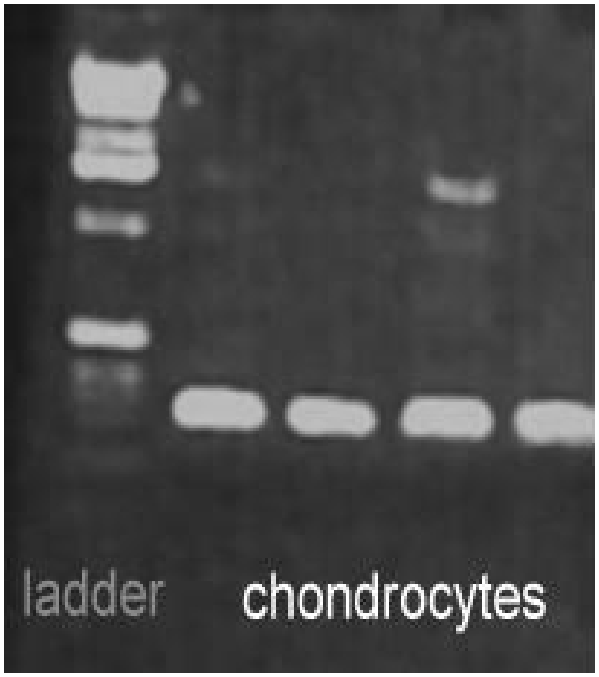

Figure 2. Photograph of a gel of polymerase chain reaction products of total cellular mRNA derived from isolated chondrocytes grown *in vitro* and induced to differentiate in micromass culture. The probe is for the core protein of aggrecan – the cartilage-specific proteoglycan. (UV illumination, ladder = DNA molecular weight calibration ladder, chondrocytes = cultures from 4 different patients).

removing the endogenous peroxidase. Normal swine serum abolished nonspecific background staining. Polyclonal self-prepared anti-FGFR3 antibodies and monoclonal anti-proliferating cell nuclear antigen (Dako, Glostrup, Denmark) were used as the primary antibodies. The dishes were then incubated with a secondary antibody (swine anti-rabbit, polyclonal, dilution 1:150; and rabbit anti-mouse, monoclonal, dilution 1:50, Dakopatts, Dako), and stained with peroxidase antiperoxidase rabbit complex (1:150) for the polyclonal and peroxidase antiperoxidase mouse complex (1:100) for the monoclonal. Diaminobenzidine was used as a substrate for both kinds of antibodies, yielding a brown color at sites of specific antigen presence. Negative antibody specificity controls were performed. Slides were stained either with the secondary antibody (swine anti-rabbit or rabbit anti-mouse, respectively) and peroxidase antiperoxidase complex, or peroxidase antiperoxidase complex alone.

Monolayer cultures of chondrocytes uniformly expressed FGFR3. Alcian blue and safranin-O are only expressed when the cultures are grown in high density as a multilayer culture. Proliferating cell nuclear antigen is uniformly expressed in the monolayer stage but not in the multilayer stage of the cultures.

Implantation procedure

Patients were considered for transplantation if symptoms of pain localized to the femoral condyles, accompanied by either locking or giving-way of severe crepitations, and had continued for at least 2 months post-arthroscopy. All patients were evaluated preoperatively by the International Cartilage Repair Society cartilage standard evaluation form/Knee.

The procedure was performed under general anesthesia, and tourniquet control was used. The joint was opened using a mini-arthrotomy incision located over the defect in five of eight patients. In the other three the presence of multiple lesions (1 case) or trochlea location (2 cases) necessitated formal anterior arthrotomy that included dislocation of the extensor mechanism laterally. The defect was thoroughly debrided. Application of 3% hydrogen peroxide allowed control of bleeding from the bone bed. The defect edges were made vertical to the joint surface and the defect was contoured into an oval shape. A periosteal flap oversized by 2 mm was removed from the proximal medial tibia and sutured to the defect using polyglycolic 6-0 sutures. Water tightness was achieved by spraying fibrin-based glue (Omrix, Nes Ziona, Israel), and $2x10^6$ cells/cm^2 defect were injected.

Postoperative protocol

Drainage of the joints was not used, thus obviating damage to the grafts. The limbs were bandaged in a bulky dressing for one day. On the second postoperative day continuous passive motion was instituted, which achieved 90 degrees of flexion on the fourth postoperative day. The patient was instructed not to bear weight for 6 weeks, but during the next 6 weeks partial weight bearing was begun. Full weight bearing commenced at 12 weeks post-surgery. During the first 12 weeks, physiotherapy was based on closed chain exercises, and isokinetic exercises were avoided. Patients returned to normal activities from 24 weeks onward.

Results

Study group

No postoperative complications were noted. Hospitalization following surgery averaged 5 days (range 4–7 days). All patients followed the postoperative protocol and evaluation was conducted 6 months post-transplantation. Functional status averaged 2 ("I can do almost everything that I want to do with my joint"). Patients assessed their implanted knee as 2 (70–90% of the contralateral knee). Pain intensity decreased to 3 (range 0–7) [Table 1].

Symptoms such as swelling during activity and giving way disappeared in seven of eight patients. Pain at activity level averaged 2 (nearly normal) and starting pain averaged 2 (nearly normal). Extension was full in all patients, and flexion reached 145 degrees in all. There was no joint crepitus. Dysesthesia due to damage to superficial nerves occurred in four patients following arthrotomy.

Post-transplantation surgery

One patient, who had undergone autologous cartilage grafting to the medial femoral condyle 4 months earlier, required repeat surgery due to the apparently unrelated appearance of an osteoblastoma in the lateral femoral condyle. Arthroscopy of the defect area demonstrated complete healing of a grade 4 defect [Figure 1].

Evaluation by MRI

In all patients repeat MRI scan was available 3 months after surgery. In addition to T1 weighted and T2 weighted images, fat-saturated proton-density fast-spin echo sequences were performed. MRI revealed a congruent joint surface in all patients [Figure 3], with the tissue appearing similar in signal to normal articular cartilage. No joint effusions were observed.

Discussion

ACT is a new evolving technique, thus its scope of success has not yet been fully defined [9]. The major theoretical advantage of this procedure might be its ability to generate hyaline-like cartilage. In general, cartilage defects in animals do not heal, or they heal by formation of fibrocartilage [2]. Other techniques generate either fibrocartilage alone as in microfractures or drilling, or a mixture of islands of articular cartilage surrounded by fibrocartilage as in osteochondral cylinder transplantation [10]. Our results presented here appear to indicate that articular cartilage surface congruity is restored following articular cartilage transplantation, as demonstrated by MRI. However, it should be remembered that clinical MRI today is limited in its capacity to define cartilage architecture, thus the tissue demonstrated on MRI may not be histologically typical articular cartilage.

It has been our policy not to recommend second-look arthroscopies and thus cartilage biopsies are not available. However, Hart [11], who performed cartilage biopsies in patients who underwent ACT, found typical articular hyaline cartilage in many cases. Furthermore, the same author concludes that synovitis of the joint ameliorated in all cases. This apparently indicates that the destructive process is halted by the chondrocyte transplantation. Further proof of the amelioration of the inflammatory process is the MRI appearance noted in our study. The lack of joint effusion supports Professor Hart's findings of an ameliorated arthritic process. It is of interest to note that in animals, even xenogeneic transplants, which are doomed to long-term rejection, prevent early osteoarthritis [12]. While only a few clinical reports on ACT have been published, there is quite an extensive literature on the procedure in experimental animals. Most authors, our group included, have reported that hyaline-like cartilage is formed by ACT in chicks, mice, guinea-pigs, rabbits and goats [2,7,13–16). About 20% of the implantations fail in most of these models, resulting in fibrocartilage. Recently, a failure of mesenchymal cell differentiation into cartilage rather than mis-differentiation into fibrous tissue was implicated as the reason for the failures [8]

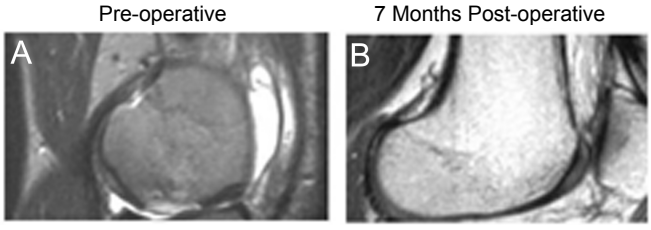

Figure 3. [A] MRI demonstrating osteochondral fracture of the medial femoral condyle, containing tissue fragments. **[B]** MRI at 5 months post-transplantation demonstrates filling of the defect with tissue, which is contoured to fit the condylar curvature. The tissue appears similar to hyaline cartilage in signal intensity.

The above-described results indicate that clinical improvement is anticipated following ACT, at least in the short term. Objective signs such as effusion, crepitations and local tenderness are expected to improve in the large majority of patients. Pain is decreased though not eliminated, and might disappear with time, as noted in the Global Genzyme Registry [17]. However, since these joints underwent multiple surgery, it is likely that some amount of pain will remain.

Further clinical and basic studies are necessary to define the role of chondrocyte transplantation as a method for repairing articular cartilage compared to other methods such as osteochondral grafting. Due to its lower cost the latter technique is appealing, although it lacks support of any long-term basic studies. The technique involves removing articular cartilage from one joint region and implanting it into another. There is no indication in the literature that such transplants integrate with their surroundings; in fact it is well known that cartilage matrix does not encourage adhesion. This is well demonstrated clinically by the common formation of discrete nodules in secondary synovial chondromatosis. However, mosaicplasty is currently an adequate procedure especially for smaller defects [6]. ACT is beset by the same problem of integrating with its surroundings. As the cells are implanted as mesenchymal cells, it is possible that they are able to integrate into the surrounding cartilage due to their mobility and secretion of a specific matrix [7], but the implants can dislodge in some cases [17].

A cost-effectiveness analysis in the United States demonstrated that ACT leads to a dramatic improvement in patients' quality of life, and the estimated cost per additional quality-adjusted life year is $6,791. The cost in Israel should be less due to cheaper production of the implants and reduced operating room and hospitalization costs. This technique might indeed provide the solution to alleviate joint symptoms caused by cartilage damage.

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Correspondence: Dr. D. Robinson, Dept. of Orthopedics, Assaf Harofe Medical Center, Zerifin 70300, Israel. Tel: (972-8) 977 9430; Fax: (972-8) 977 9434; email: robinsn@netvision.net.il.