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First Successful Autologous Chondrocyte Implantation in Pakistan

Tashfeen Ahmad, Masood Umer, Loay Lubbad, Riaz Hussain Lakdawala and Anwar Ali Siddiqui

ABSTRACT

Osteochondritis dissecans entails a hyaline cartilage defect of the articular surface causing pain and functional restriction in young adults, sometimes resulting in early degenerative arthritis. Conventional treatment methods such as abrasion chondroplasty and mosaicplasty have limitations in terms of quality of the resultant cartilage and donor site morbidity. A more recent technique, autologous chondrocyte implantation (ACI) results in hyaline cartilage formation and gives good long-term outcome, but requires a high-level cell culture facility and two surgical procedures. The patient was a young female with knee pain, intermittent locking and feeling of “joint mouse”. MRI scan and arthroscopy showed a 2x2 cm full thickness osteochondral defect in the medial femoral condyle. A free fragment of articular cartilage was found, which was extracted arthroscopically, and chondrocytes were cultured from it in the Juma laboratory. Subsequently, patient underwent surgery whereby the chondrocytes were injected under a periosteal patch sewn over the defect. Over six months, patient’s symptoms completely resolved and she returned to full function. A repeat arthroscopy after one year revealed complete filling of the previous defect with normal appearing cartilage indicating success of the procedure. This technology can be utilized for treating patients with a variety of conditions affecting hyaline cartilage of joints.

Key words: *Osteochondritis dissecans. Chondrocyte. Cell culture. Tissue engineering. Autologous chondrocyte implantation.*

INTRODUCTION

Articular cartilage degeneration with advancing age results in osteoarthritis, a leading cause of morbidity across the world.^{1,2} Defects in the cartilage may also occur in young age due to reasons such as injury and overuse, but quite often no underlying cause can be found. One such cartilage affliction is osteochondritis dissecans of the knee, which typically affects young adults and entails a full-thickness defect in the articular cartilage. At the outset, it causes significant symptoms and restriction of function, and later on, it may result in progressive cartilage degeneration and early onset osteoarthritis.^{3,4} Thus, adequate treatment is of utmost importance in such patients.⁵

We report a case of osteochondritis dissecans of the medial femoral condyle, where successful outcome was achieved by the use of autologous chondrocyte implantation (ACI).

CASE REPORT

A 28-year-old lady, housewife, presented with a six-month history of insidious onset pain in the left knee joint. This was associated with intermittent locking of the joint with flexion and extension, requiring jerky movements to “unlock” the knee. Moreover, she often

felt something moving inside her joint; typical of a “joint mouse”.

Physical examination revealed a mild effusion, crepitus and medial joint line tenderness. She had a positive McMurray’s test for the medial meniscus. Clinical impression was that of a torn meniscus or a loose body. Plain X-rays were unremarkable. MRI scan showed a full-thickness osteochondral defect at the anterior middle portion of the medial femoral condyle, a free-floating cartilage fragment and normal menisci and ligaments. An arthroscopy was performed whereby these findings were confirmed. A 2x2 cm full-thickness osteochondral defect (Outerbridge grade IV) was noted (Figure 1A,B). This defect was drilled down to bleeding subchondral bone with multiple passes of 20-gauge K-wire (Figure 1D,E). A free floating cartilage fragment was found (Figure 1C), extracted and placed in cell culture medium with full sterile precautions. The medium

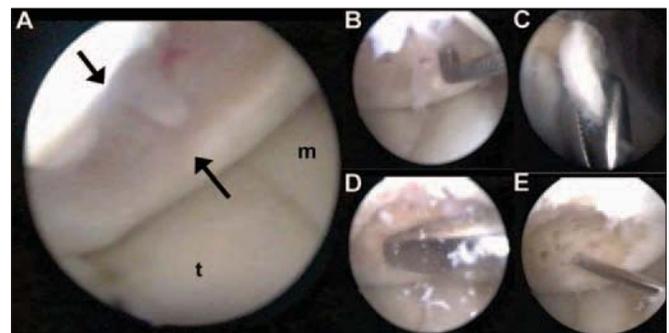


Figure 1 (A-E): Arthroscopic images showing (A) large defect (arrows) in the posterolateral aspect of the medial femoral condyle; (B) defect thickness confirmed by probing bone in the base of the defect; (C) extraction of free floating articular cartilage fragment; (D) abrasion of the defect and (E) drilling of defect with 20-gauge K-wire. m=medial meniscus, t=tibial articular

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comprised of DME/HAM F-12 1:1 mixture with 15 mM HEPES, supplemented with 10% Fetal bovine serum, 1.25 mg/ml Ascorbic acid, 50 µg/ml insulin, 50 µg/ml Transferrin, 5 µg/ml Selenium and 1 x antibiotics and antimycotic solution. All reagents were obtained from Sigma-Aldrich (St Louis, MO, USA).

The cartilage fragment was transported to the Aga Khan University basic science laboratory (Juma Research Laboratory). It was washed thoroughly with serum free medium to remove any traces of blood and transferred to a sterile plastic Petri dish to facilitate handling. With the help of scalpel blade the outer surface was gently abraded to reduce the chance of fibroblast contamination and then chopped into small pieces. Finally, the cartilage bits were transferred to 50 ml tube containing 0.125% collagenase IV enzyme in serum free medium and incubated overnight at 37°C with gentle rocking.

Next day, the digested cartilage material was filtered through sterile 100 micron cell strainer (Falcon, Franklin Lake, New Jersey, USA) to remove big undigested pieces. Filtered cells were centrifuged at 1000 rpm for 5 minutes and washed once with complete medium before they were seeded in 25 cm² tissue culture flasks (Falcon, Franklin Lake, New Jersey, USA). Flasks were incubated in a humidified chamber in 5% CO₂ atmosphere without any physical disturbance for 48 hours to aid attachment of cells. Thereafter, the medium was changed every 48 hours. Flasks were observed daily for any signs of contamination. Each flask was subcultured into two flasks of the same size when the cells reached 80% confluency. At the third passage, cells were harvested and stored in cell-freezing medium in liquid nitrogen.

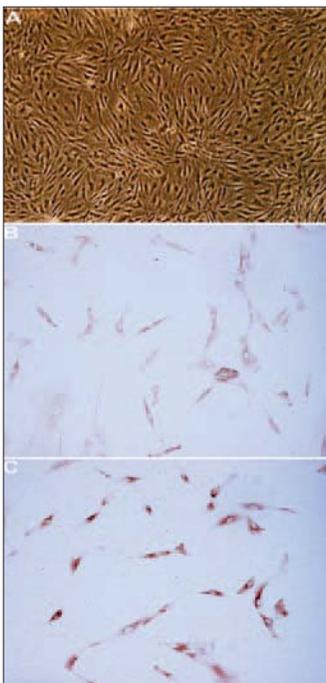


Figure 2 (A-C): Photomicrographs showing (A) chondrocyte-like cells in culture medium as observed under phase contrast microscopy; immunocytochemistry images showing cells positive for S-100 (B) and vimentin (C). Objective=20x.

Two weeks before surgery, the frozen cells were revived from liquid nitrogen and subcultured. Cells were also grown on glass cover slips for immuno-histochemistry staining with anti-vimentin and anti-S-100 antibodies (Abcam Inc., Cambridge, UK). Anti-vimentin antibody confirms the mesenchymal origin of cells while the anti-S-100

antibody differentiates chondrocyte cell type from fibroblasts as S-100 protein is expressed in chondrocytes and not fibroblasts. Cells were positive for both antigens, confirming the chondrocyte phenotype of the cells (Figure 2A-C).

At the day of the surgery, the cells were harvested from the tissue culture flasks and aliquots were sent to surgery room when the surgeon was ready for the transplantation. In the operating room, an arthroscopy was performed first, and it was noted that the cartilage defect persisted with presence of old organized clot in the base. An open arthrotomy was then performed and the defect debrided (Figure 3A). A periosteal patch was harvested from the mid-tibial shaft, and the outer surface was marked with a marking pen (Figure 3B). The flap was reversed such that the inner endosteal surface faced outside and sewn onto the defect margins using 6/0 vicryl sutures applied in a continuous running fashion, to achieve an airtight seal (Figure 3C). The margins were further sealed using BeriPlast™ fibrin glue (Figure 3D). The cultured autologous chondrocytes were injected under the periosteal patch using a 27 gauge needle. No leakage of the injected fluid was noted.

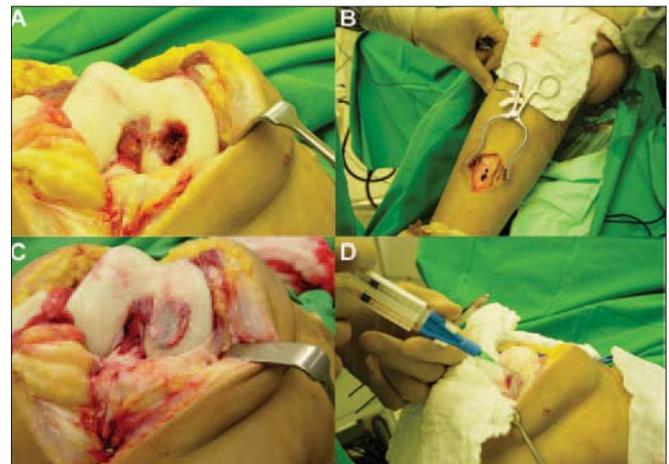


Figure 3 (A-D): (A) Osteochondral defect. (B) Periosteal patch harvested from tibia, outer surface of periosteal patch marked. (C) Patch reversed and sewn onto margins of defect with 6/0 vicryl sutures. (D) Margins sealed with fibrin glue and chondrocyte suspension injected underneath the defect.

Postoperatively, the patient was permitted active knee range of motion, but restricted from weight bearing for six weeks. The patient reported gradual improvement in pain and range of motion, such that she became completely asymptomatic after three months. Examination showed full, painless range of motion and ability to squat and get up with ease. A follow-up MRI done six months after the transplant confirmed filling of the defect. She also consented to a check arthroscopy, which was done one year after the transplant. The previous defect was completely filled with normal-

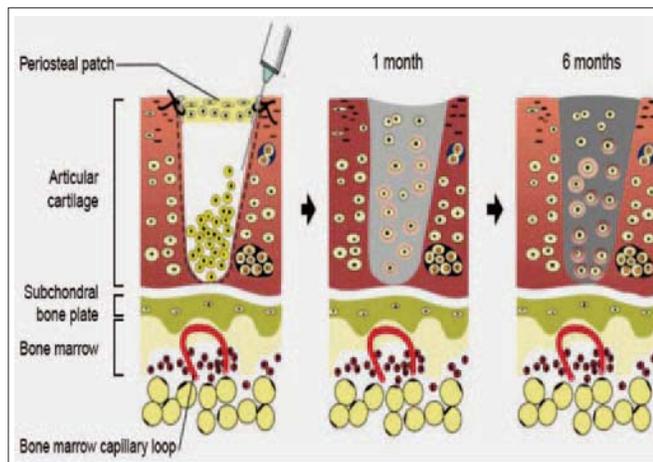


Figure 4: Drawings depicting hyaline cartilage regeneration following autologous chondrocyte implantation. The articular cartilage defect is debrided down to subchondral bone. A reversed periosteal patch with the “cambium” layer of osteoprogenitor cells facing down into the defect is carefully sutured onto the outer margins of defect (end to side) with interrupted 6/0 sutures and sealed with fibrin glue to ensure a water-tight chondral–periosteal compartment. Cultured chondrocytes are then injected carefully into the “contained” defect. These cells settle, adhere and proliferate, and also lay down new hyaline cartilage, resembling the native cartilage. Source: Oakes BW. Orthopaedic tissue engineering: from laboratory to the clinic. *MJA* 2004; 180: S35-S38. ©Copyright 2004. The Medical Journal of Australia - reproduced with permission.

looking cartilage.

DISCUSSION

This is the first reported case of successful ACI in Pakistan. Nearby countries where it has been started are India and Singapore.

The real challenge in the treatment of articular cartilage defects is the restoration of a smooth joint surface composed of hyaline cartilage. The natural history of untreated cartilage defects is usually replacement with fibrous tissue. With surgical therapy such as abrasion chondroplasty and drilling, fibrocartilage formation is induced. Both fibrous tissue and fibrocartilage have different surface characteristics and biomechanical properties as compared to the native hyaline cartilage. Thus, early good results are not maintained over a long-term.⁶ Another technique, mosaicplasty, has been claimed to result in formation of hyaline cartilage, but it entails extensive surgery on the normal cartilage and cannot be applied to large defects. Autografts comprising of osteochondral fragments have shown

good promise viz-a-viz restoration of the hyaline cartilage, but are associated with donor site morbidity, which increases with increasing size of the graft or defect. Allografts circumvent these limitations, but have their own limitations regarding availability and graft rejection by the recipient’s immune system. More recently, autologous chondrocyte implantation⁷ (Figure 4) has emerged as a promising tool. It utilizes patient’s own cartilage cells and potentially results in restoration of near-normal hyaline cartilage. It has minimal, if any, donor site morbidity and, therefore, can be used to fill large defects.^{8,9} However, it entails two procedures, one for harvesting the cartilage cells and second for implanting the cells and requires a basic science laboratory setup with cell-culture facility. Nevertheless, upto 90% good-excellent long-term results have been reported with this technique.

In Aga Khan University, with the establishment of the Juma Research Laboratory, a modern cell-culture facility has been setup. In the future, this technology can be utilized for treating patients with a variety of conditions affecting hyaline cartilage of joints

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