Autologous Chondrocytes Implantation with Three-Dimensional Collagen Scaffold

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Objective: *The authors report a patient with large traumatic knee cartilage defects treated with autologous chondrocytes implantation (ACI) in three-dimensional collagen scaffold.*
Material and Method: *A patient with grade 3-4 according to ICRS (International Cartilage Repair Society) Classification System was performed ACI with three-dimensional collagen scaffold. The two-stage procedure was performed. First, the cartilage was arthroscopic harvested. The chondrocytes were isolated in the laboratory. Second, the chondrocytes were re-implanted into the defects using three-dimensional collagen scaffold. The patients were clinically evaluated pre-operatively and post operatively and magnetic resonance imaging. The duration of follow-up was 12 months.*
Results: *There was no post operative complication. The clinical evaluations were excellent. The MRI showed the hyaline-like cartilage tissue formation at the defects.*
Conclusion: *The autologous chondrocytes implantation with three-dimensional collagen scaffold showed the excellent outcome. Long-term follow-up is required.*

Keywords: *Knee injury, Autologous chondrocytes implantation, Cartilage defects, Collagen scaffold*

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The articular cartilage is specific tissue that can endure the repetitive high loading in the knee joint. It consists of chondrocytes and extracellular matrices such as collagen and proteoglycans. The capacity of articular cartilage repair is limited because of the absence of blood supply, low mitotic activity, and immobility of articular chondrocytes[1,2]. The conventional enhancement procedures of intrinsic healing capacity of cartilage (abrasive chondroplasty, subchondral drilling, and microfracture) have been reported with the replacement of fibrocartilage and eventually following with pre-mature degeneration[3,4].

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The autologous chondrocyte implantation (ACI) has been developed using the expanded autologous chondrocytes to re-transplant into the cartilage defects[5,6]. The ACI consists of two procedures. First, the cartilage is arthroscopic harvested and the chondrocytes are isolated and cultured in the laboratory. Three to four weeks is needed to have the adequate number of cells. Second, the chondrocytes are re-implanted in to the encapsulated defects. Previous studies showed chondrocytes had the potential to provide the hyaline-like cartilage over the conventional procedures[7-9].

The disadvantages of first generation ACI which used chondrocytes in the suspended media are leakage of the chondrocytes from the periosteal flap and uneven distribution of chondrocytes. Many kinds of scaffolds have been successfully used to create the

three-dimensional suspension for chondrocytes. The three-dimensional cartilage-like tissue using collagen scaffold has been shown to maintain cartilage phenotype[10].

The present study reports the first ACI using three-dimensional collagen scaffold, and the clinical and magnetic resonance imaging (MRI) evaluation.

Material and Method

Patients

A 40 year-old-woman presented with left knee pain for 2 years after a motorcycle accident. At the time of injury, her knee was painful and swollen for weeks. The pain was constant while walking and aggravated when kneeling. The swelling occurred occasionally. There was no locking or giving way. The physical examination revealed an overweight stature with mild limping gait. The circumference of the left thigh was 1 cm smaller than the right thigh. The knee was swollen with no ballottement. The range of motion was full with no pain on passive motion. There was tenderness at the mid-medial joint line. The Mc Murray's test, stress test, and drawer test were negative. The plain x-ray of the knee was in normal limit.

She had performed knee arthroscopy on October 2007. The arthroscopic finding revealed small peripheral tear of medial meniscus, and grade 3-4 chondral lesion (Fig. 1) according to ICRS (International Cartilage Repair Society) Classification System[11]. The size of the lesion was 12x18 mm. The meniscus was repaired with outside-in technique. The cartilage was obtained and sent to the laboratory for chondrocytes isolation.

The patient consented for the ACI under the Ethics Committee regulation.

Cartilage harvest

Knee arthroscopy was performed. The slivers of cartilage (300-500 mg) were obtained from the minor load-bearing area on the upper medial femoral condyle of the injured knee. The cartilage samples were minced and transferred to the laboratory in the tubes containing DMEM (Gibco BRL) at ambient temperature.

Chondrocytes culture

The chondrocytes isolation was initiated not later than 6 hours after the operation. The cartilage was washed twice in Ham's F-12 medium (Gibco BRL, Paisley, Scotland) supplemented with gentamicin sulfate (50 μm/mL), amphotericin B (2 μm/mL), and

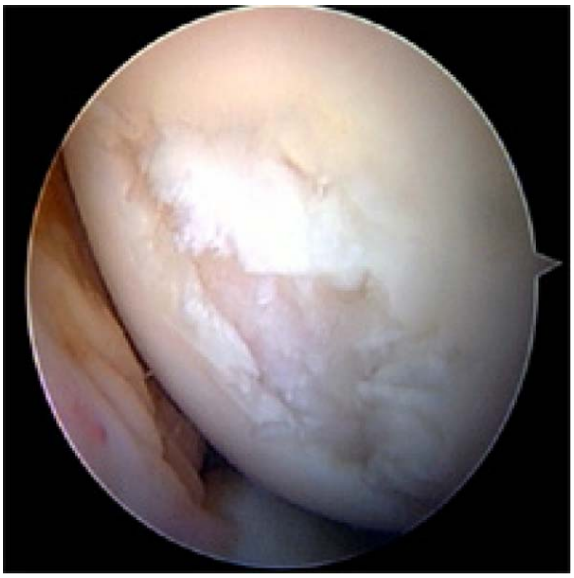

Fig. 1 The full-thickness chondral defect of medial femoral condyle

L-acorbic acid (50 μm/mL). The minced cartilage was digested 16-20 hours with clostridial collagenase (0.8 μm/mL, catalog no. C-9407, > 1200 IU/mg; Sigma, Free-hold, New Jersey) and deoxyribonuclease (0.1 μm/mL, catalog no. D-5025; Sigma). The isolated cells were resuspended in culture medium containing DMEM/F12 1:1 (Gibco BRL) with 10% serum and gentamicin sulfate (50 μm/mL), amphotericin B (2 μm/mL), L-acorbic acid (50 μm/mL), and L-glutamine (Gibco BRL). The chondrocytes were incubated in 5% Co_2, in air at 37°C[14]. After one week, the chondrocytes were trypsinized (trypsin-ethylene diaminetetra acetic acid 0.125%) and resuspended. The 3-4 weeks incubation was needed for adequate number of chondrocytes (Fig. 2). The chondrocytes were trypsinized and seeded in the 2.0 ml collagen scaffold. The quality-control procedures consisted of sterility testing and photographic recording of cell morphology. The chondrocytes seeding in collagen scaffold was transferred in cold sterile package[9,10].

Chondrocytes implantation

The knee arthrotomy was performed. The chondral lesion was debrided to the healthy cartilage. The subchondral bone plate must be carefully preserved. The collagen scaffold seeded with chondrocytes was sized and shaped. The collagen scaffold seeded with chondrocytes was fixed to the defect using fibrin glue. The periosteal graft was harvested from the proximal

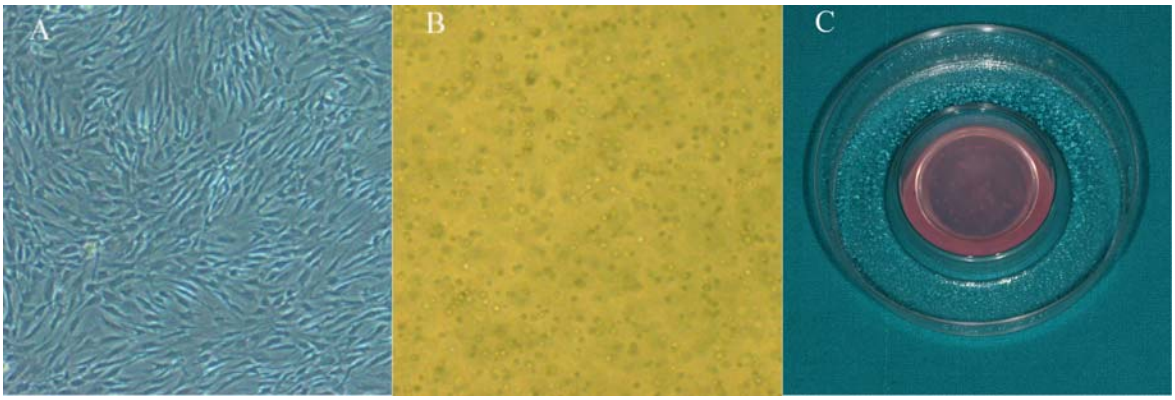

Fig. 2 Chondrocytes culture in monolayer (A), in collagen gel (B), and collagen gel (C) seeded with chondrocytes

tibia and sutured with interrupted sutures (Prolene 6-0) to the chondral defect facing the defect with the cambium layer. The fibrin glue was used to water-seal the pocket (Fig. 3). The wound was closed layer by layer and a compression dressing was applied.

Post-operative program

The pain control with intravenous analgesic and NSAIDs was performed. The isometric exercise was started immediately on the post operative day. The hinge brace at full extension and non-weight bearing were required for two weeks. The progressive weight bearing and active knee flexion were encouraged as tolerated after 2 weeks. The full weight bearing was started at 4-6 weeks. Running was restricted for 9 months.

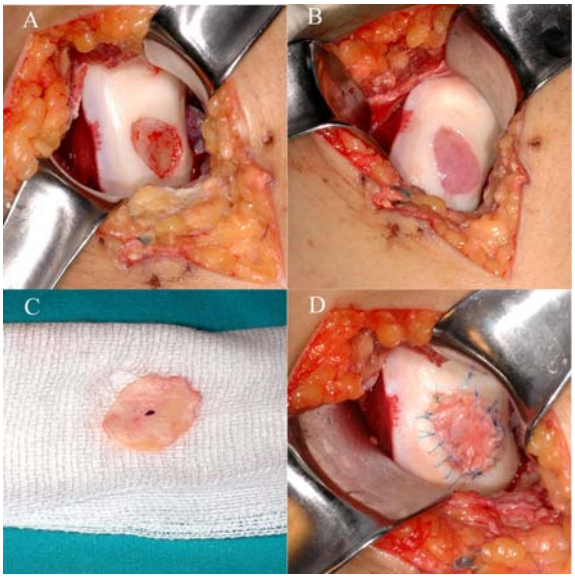

Fig. 3 ACI: debride lesion to healthy rim (A), fix collagen scaffold seeded with chondrocytes into the defect with fibrin glue (B), harvest periosteal graft from proximal tibia (C), suture periosteal graft over the lesion and seal with fibrin glue (D)

Clinical evaluation

The duration of follow-up was 12 months. The patients were clinically evaluated preoperatively and postoperatively with International Knee Documentation Committee Score (IKDC Score), Knee and Osteoarthritis Outcome Score (KOOS) including pain, symptoms, function in daily living (ADL), function in sports and recreation, knee-related quality of life[12]; and magnetic resonance imaging.

Results

The patients had no post operative complication. The International Knee Documentation Committee Score (IKDC Score) was 33 pre-operatively and 75 post operatively. The evaluation using KOOS showed excellent clinical improvement at duration of 12 months (Fig. 4).

The MRI (T1-2-weighted, fast-spin-echo image) at 6 months after ACI showed the meniscus repair with superficial cartilage-like repair tissue formation at the medial femoral condyle (Fig. 5). The hypertrophy of the periosteal graft was observed.

Discussion

The ACI using three-dimensional scaffold is world-wide accepted as the standard treatment for large full-thickness articular cartilage defects. The hyaline-like cartilage and long-term success have been

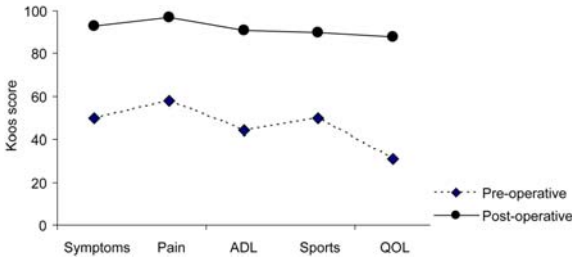

Fig. 4 The KOOS showed the excellent outcome after the ACI

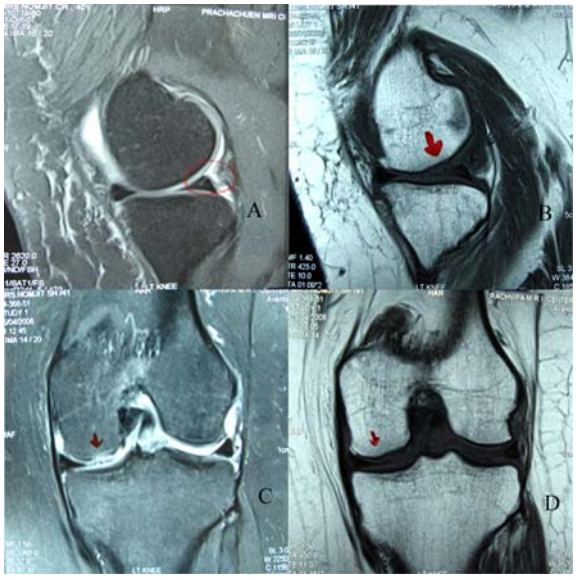

Fig. 5 The MRI at 6 months showed the meniscus repair (A), the defect filled with hyaline-like cartilage (B), with graft hypertrophy (C, D)

reported[8,13]. The presented patient had an excellent clinical result with good regeneration cartilage tissue at 1 year. Long-term follow is required. The authors found the periosteal hypertrophy from the MRI. The periosteal hypertrophy caused by the growth of viable periosteal cells, and few need for arthroscopic debridement[14]. Our patients had no symptoms at the time of follow-up. The collagen scaffold has been successful in clinical use[15]. The collagen scaffold can create the three-dimensional cartilage-like tissue with excellent chondrocytes distribution. The cartilage phenotypes such as collagen type II and proteoglycans production are reported with low antigenicity[10]. The superior biologic hyaline-cartilage and the long-term clinical results are expected. The autologous chondrocytes implantation (ACI) using three-dimensional collagen scaffold can be the proper treatment in the large full-thickness cartilage defect and the prevention of early degeneration.

Acknowledgement

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การรักษาการบาดเจ็บของกระดูกอ่อนข้อเข่าด้วยวิธีการปลูกถ่ายเซลล์กระดูกอ่อนที่เพาะเลี้ยงด้วย Three-Dimensional Collagen Scaffold

ชาญณรงค์ เกษมกิจวัฒนา, สุรพล เกษประยูร, กานดา ชัยภิญโญ, ชลวิช จันทร์ลลิต, โกสุม จันทร์ศิริ

วัตถุประสงค์*: เพื่อรายงานการรักษาการบาดเจ็บของกระดูกอ่อนด้วยวิธีการปลูกถ่ายเซลล์กระดูกอ่อน autologous chondrocytes implantation โดยใช้ three-dimensional collagen scaffold*

วัสดุและวิธีการ*: ผู้ป่วยมีการบาดเจ็บของกระดูกอ่อนขั้นรุนแรง ภายหลังได้รับอุบัติเหตุได้รับการผ่าตัดปลูกถ่ายเซลล์กระดูกอ่อน autologous chondrocytes implantation โดยใช้ collagen scaffold ขั้นตอนการรักษาผู้ป่วยจะได้รับการผ่าตัด 2 ครั้ง ครั้งแรกเป็นการผ่าตัดส่องกล้อง เพื่อตัดชิ้นเนื้อกระดูกอ่อนในบริเวณที่ไม่ได้ใช้งานนำไปเพาะเลี้ยงในห้องปฏิบัติการ ครั้งที่สอง ผู้ป่วยได้รับการปลูกถ่ายเซลล์กระดูกอ่อนของผู้ป่วยเองที่เพาะเลี้ยงโดย collagen scaffold กลับไปยังผิวข้อที่ได้บาดเจ็บ การติดตามผลการรักษาทางคลินิก และจาก MRI ที่ระยะเวลา 1 ปี*

ผลการศึกษา*: ผู้ป่วยมีอาการทางคลินิกดีขึ้นมากเทียบกับก่อนการรักษา จากผลการรักษาทางคลินิก การตรวจ MRI พบมีการทดแทนรอยบาดเจ็บที่ผิวข้อด้วย hyaline-like cartilage ไม่พบภาวะแทรกซ้อนภายหลังการรักษา*

สรุป*: การรักษาการบาดเจ็บของกระดูกอ่อนด้วยวิธีการปลูกถ่ายเซลล์กระดูกอ่อน autologous chondrocytes implantation โดยใช้ collagen scaffold ได้ผลเป็นที่น่าพอใจ อย่างไรก็ตามยังต้องการการศึกษา และติดตามผลระยะยาวต่อไป*