Autologous Chondrocytes Implantation for Traumatic Cartilage Defects of the Knee

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Objective: *To evaluate the results of autologous chondrocytes implantation in the patients with large traumatic cartilage defects of the knee.*
Material and Method: *Five patients (six knees) with grade 3-4 according to International Cartilage Repair Society Classification System were performed ACI between May 2006 and April 2007. 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. The patients were clinically evaluated preoperatively and postoperatively with Knee and Osteoarthritis Outcome Score (KOOS), magnetic resonance imaging, and arthroscopic assessment. The mean duration of follow-up was 19.8 $\pm$ 4.6 months.*
Results: *There was no postoperative complication. The clinical evaluation with Knee and Osteoarthritis Outcome Score (KOOS) showed significant improvement. The MRI showed the filling of regenerative cartilage tissue formation at the defects. The arthroscopic assessment showed the good defect fill, stiffness, and incorporation to the adjacent cartilage.*
Conclusion: *The autologous chondrocytes implantation showed the potential for the treatment of large cartilage defects. The excellent results allowed patients to return to normal activity level.*

Keywords: *Cartilage, Chondrocytes, Knee injuries, Transplantation, Autologous*

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The articular cartilage injury is a common finding in arthroscopic surgery. 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) had been reported. The full-thickness defects will be replaced with fibrocartilage and eventually following with pre-mature degenerative change[3-5]. The autologous chondrocyte implantation (ACI) had been developed using the expanded autologous chondrocytes to re-transplant into the cartilage defects[6,7]. 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. The previous study showed chondrocytes had the cellular plasticity and potential to provide the hyaline-like cartilage over the conventional procedures[8-11].

The purpose of the current study was to evaluate the results of ACI using clinical evaluation magnetic resonance imaging (MRI), and arthroscopy.

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Material and Method

Patients

Five patients (six knees) were performed ACI between May 2006 and April 2007, two lateral femoral condyle and four trochea lesions (Table 1). All patients had grade 3-4 according to ICRS (International Cartilage Repair Society) Classification System[12,13]. The mean duration of follow-up was 19.8 $\pm$ 4.6 months (range 16-27 months). The mal-alignment, ligament laxity, other pathology needed to be corrected before ACI. All consented for the ACI under the Ethics Committee regulation.

The ACI consists of a two-stage procedure. First, the cartilage was arthroscopic harvested. The chondrocytes were isolated and incubated in the laboratory. Second, chondrocytes were re-implanted into the defects.

Cartilage harvest

Knee arthroscopy was performed. The cartilage defect was examined. The slivers of cartilage (300-500 mg) were obtained from the minor load-bearing area on the upper lateral or 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 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, Freehold, 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% human 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. After one week, the chondrocytes were trypsinized (trypsin-ethylenediaminetetraacetic acid 0.125%) and resuspended. The 3-4 weeks incubation was needed for adequate number of chondrocytes (Fig. 1). The quality-control procedures consist of sterility testing and photographic recording of cell morphology. The transplanted chondrocytes were suspended in 1.0 ml of medium in cold sterile package[10] .

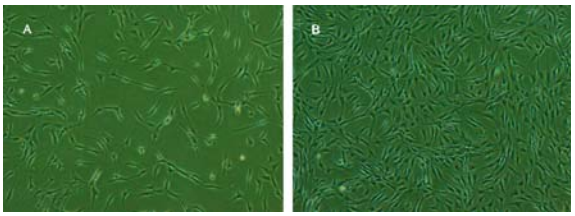

Fig. 1 Chondrocytes culture at 1 week (A) and 3 weeks (B)

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 periosteal graft was harvested from the anteromedial incision of proximal tibia. The periosteum graft was sutured with interrupted sutures (Prolene 6-0) to the chondral defect facing the defect with the cambium layer. The fibrin glue was used to make a

Table 1. Demographic data on the patients

Case	Gender, age	Side	Cartilage lesion		Operation
			Site	Size (cm^2)	
1	M, 15	R	Lateral condyle	2.4	Lateral meniscus repair, ACI
2	M, 42	L	Trochea	3.1	Tibial tuberosity advancement, ACI
		R	Trochea	2.0	Tibial tuberosity advancement, ACI
3	M, 39	L	Patella & trochea	2.0	ACL reconstruction, mosaicplasty of patella, ACI of trochea
4	M, 40	R	Trochea & lateral condyle	3.3	ACL reconstruction, mosaicplasty of trochea, ACI of lateral condyle
5	F, 42	R	Trochea	2.0	Tibial tuberosity advancement, ACI

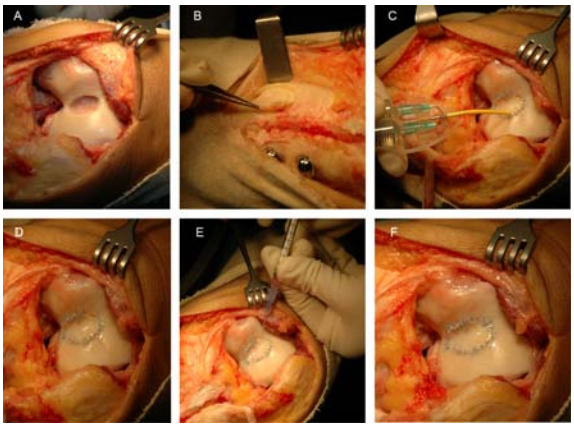

Fig. 2 The ACI procedures including debride lesion to healthy rim (A), harvest periosteal flap (B), suture periosteal graft over the lesion and seal with fibrin glue (C, D), inject chondrocytes into the pocket (E), and suture the opening hole and seal with fibrin glue (F)

water-seal pocket. The small upper edge was left opened for the chondrocytes injection. Chondrocytes in the suspended medium was injected in to the periosteum pocket and then closed with interrupted sutures. The fibrin glue was used to seal the pocket (Fig. 2). The wound was closed layer by layer and the compression dressing was applied.

Post-operative program

The pain control with intravenous analgesic and NSAIDs was performed. The isometric exercise was started immediately in 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 two weeks. Running was restricted for nine months

Clinical evaluation

The patients were clinically evaluated preoperatively and postoperatively with Knee and Osteoarthritis Outcome Score (KOOS) including symptoms, pain, function in daily living (ADL), function in sports and recreation, knee-related quality of life[14], magnetic resonance imaging, and arthroscopy. Non-parametric statistics included Wilcoxon signed Rank test was applied to test the difference between pre- and post operation with significance level at $p < 0.05$.

Results

All patients had no post-operative complication. The evaluation using KOOS showed clinical improvement at a duration of 19.8 $\pm$ 4.6 months (range 16-27 months) with statistical significance (Wilcoxon Signed Ranks test, $p < 0.05$) (Table 2, Fig. 3).

The T2W MRI (T2-weighted, fast-spin-echo image) at three months after ACI showed the filling of repair tissue with superficial cartilage-like tissue formation at the femoral trochea (Fig. 4A, arrow). The

Table 2. Clinical evaluation with KOOS (knee and osteoarthritis outcome score)

KOOS	Pre-operative	Post-operative	p-value
Symptom	59 $\pm$ 15	86 $\pm$ 9	0.042*
Pain	59 $\pm$ 21	80 $\pm$ 16	0.043*
ADL	67 $\pm$ 19	87 $\pm$ 11	0.043*
Sports & recreation	30 $\pm$ 14	67 $\pm$ 19	0.042*
Quality of life	38 $\pm$ 11	64 $\pm$ 9	0.042*

* Statistical significance, $p < 0.05$

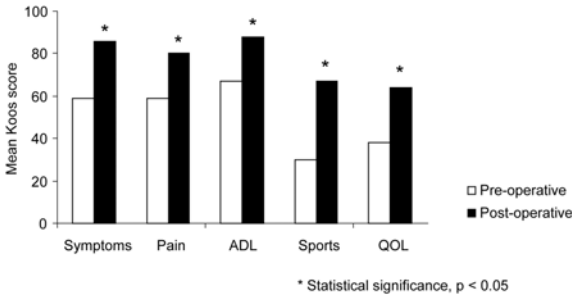

Fig. 3 The KOOS showed the functional outcome after the ACI

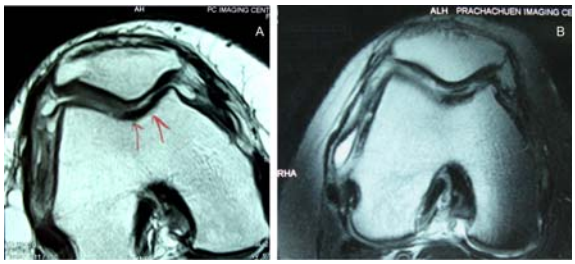

Fig. 4 The MRI at 3 months after ACI (A) showed good regenerative tissue formation within the defect, and 1 year (B) showed mild lamination

Fig. 5 The arthroscopic findings showed the excellent defect fill, incorporation, and stiffness at 2 years after ACI

hypertrophy of the periosteal graft was observed. The T2W MRI at one year after ACI showed good repair tissue formation with mild lamination of the superficial zone (Fig. 4B).

The arthroscopic assessment at two years after ACI showed 100% defect fill with the regenerative tissue. The probe indentation showed 80 % stiffness compared to the adjacent cartilage with well incorporation. There is a little periosteal graft hypertrophy (Fig. 5).

Discussion

The articular cartilage allows active articulation of the knee joint. The conventional treatments of large full-thickness chondral defects rarely achieved the hyaline-like cartilage[1]. The Autologous chondrocytes implantation (ACI) has been performed and has become the standard treatment for cartilage defects for over a decade in the Europe and United States[9]. The μ are advantages over the conventional techniques include abrasive chondroplasty, microfracture, and mosaicplasty. The superior hyaline-like cartilage over the conventional microfracture with long-term clinical success had been reported[15]. The hyaline-like cartilage after ACI is durable and can prevent early osteoarthritis in the patents with large cartilage defects of the knee. Currently, the conventional mosaicplasty can provide the hyaline-like cartilage after the treatment. However, the donor side morbidity such as painful scar at the donor side is the common complication. The size of the defects that are suitable for mosaicplasty is limited < 2 cm^2. The ACI showed clinical advantage over the conventional treatment in the large defects[2,10]. The proper patient selection is crucial; age < 45 years, no medical contraindication, unipolar lesion, and no malalignment and joint instability[10].

The presented patients have clinical improvement regarding the KOOS. The MRI becomes an increasingly important means of accessing articular cartilage and its repair. It correlated with the information obtained from clinical, arthroscopic and histologic evaluation[16]. The normal signal with the periosteal hypertrophy at the trochea lesions was found. The periosteal hypertrophy had been reported from the growing of viable periosteal cells. Some patients need the arthroscopic debridement of the hypertrophic tissue[17]. The presented patients have mild crepitation in flexion with no function deficit. None needed arthroscopic surgery at the time of follow-up. The arthroscopic assessment remains the gold standard for the postoperative evaluation. The repair is directly visualized, probed and a biopsy can be done to allow histomorphologic assessment. In the present study, the arthroscopic assessment showed the excellent defect fill and stiffness indentation compared to the adjacent cartilage. The core biopsy was not done in the present study due to the patient unwillingness.

However, the ACI has some limitations. First, the disadvantage of the chondrocytes in suspended medium that can leak. The good surgical techniques are required. Second, the uneven distribution of chondrocytes from the gravity causes the uneven chondrogenesis. The three-dimension culture in solid scaffold is the next generation for ACI. Third, the autologous chondrocytes have limited mitotic activity. The collagen type II and glycoaminoglycans contribute the major role to articular cartilage function. The production of collagen type II and glycoaminoglycans decrease when chondrocytes have been more multiplied. The defects were filled with poor hyaline-like cartilage. The better chondrocytes-expanded technique will be needed to restore the normal hyaline cartilage.

This present study showed the potential of autologous chondrocytes implantation for the treatment of large cartilage defects. The excellent clinical results, MRI, and arthroscopic finding will allow patients to return to normal activity level on a regular basis.

Acknowledgement

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References

1. Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. Cartilage injuries: a review of 31,516 knee arthroscopies. Arthroscopy 1997; 13: 456-60.
2. Shelbourne KD, Jari S, Gray T. Outcome of untreated traumatic articular cartilage defects of the knee: a natural history study. J Bone Joint Surg Am 2003; 85-A (Suppl 2): 8-16.
3. Mankin HJ. The response of articular cartilage to mechanical injury. J Bone Joint Surg Am 1982; 64: 460-6.
4. O'Driscoll SW. The healing and regeneration of articular cartilage. J Bone Joint Surg Am 1998; 80: 1795-812.
5. Nehrer S, Spector M, Minas T. Histologic analysis of tissue after failed cartilage repair procedures. Clin Orthop Relat Res 1999; 149-62.
6. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994; 331: 889-95.
7. Chanlalit C, Kasemkijwattanamd C, Varavit V. Autologous chondrocyte implantation for traumatic large cartilage defect. J Med Assoc Thai 2007; 90: 1435-42.
8. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander A, Hunziker E, et al. Histological assessment of cartilage repair: a report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). J Bone Joint Surg Am 2003; 85-A (Suppl 2): 45-57.
9. Micheli LJ, Browne JE, Erggelet C, Fu F, Mandelbaum B, Moseley JB, et al. Autologous chondrocyte implantation of the knee: multicenter experience and minimum 3-year follow-up. Clin J Sport Med 2001; 11: 223-8.
10. Peterson L, Minas T, Brittberg M, Lindahl A.Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. J Bone Joint Surg Am 2003; 85-A (Suppl 2): 17-24.
11. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg Am 2004; 86-A: 455-64.
12. Brittberg M, Peterson L. Introduction to an articular cartilage classification. ICRS Newsletter 1998; 1: 5-8.
13. ICRS Cartilage evaluation package [homepage on the Internet]. 2000 [cited 2002 Aug 6]. Available from: http://www.cartilage.org/files/contentmanagement/ICRS_evaluation.pdf
14. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)-development of a self-administered outcome measure. J Orthop Sports Phys Ther 1998; 28: 88-96.
15. Henderson I, Lavigne P, Valenzuela H, Oakes B. Autologous chondrocyte implantation: superior biologic properties of hyaline cartilage repairs. Clin Orthop Relat Res 2007; 455: 253-61.
16. Henderson I, Francisco R, Oakes B, Cameron J. Autologous chondrocyte implantation for treatment of focal chondral defects of the knee-a clinical, arthroscopic, MRI and histologic evaluation at 2 years. Knee 2005; 12: 209-16.
17. Kreuz PC, Steinwachs M, Erggelet C, Krause SJ, Ossendorf C, Maier D, et al. Classification of graft hypertrophy after autologous chondrocyte implantation of full-thickness chondral defects in the knee. Osteoarthritis Cartilage 2007; 15: 1339-47.

การรักษาการบาดเจ็บของกระดูกอ่อนข้อเข่าด้วยวิธีการปลูกถ่ายเซลล์กระดูกอ่อน

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

วัตถุประสงค์: *เพื่อศึกษาผลการรักษาการบาดเจ็บของกระดูกอ่อนด้วยวิธีการปลูกถ่ายเซลล์กระดูกอ่อน (autologous chondrocytes implantation)*

วัสดุและวิธีการ: *ผู้ป่วยที่มีการบาดเจ็บของกระดูกอ่อนขั้นรุนแรง (grade 3-4 International Cartilage Repair Society Classification System) จำนวน 6 ราย ได้รับการผ่าตัดปลูกถ่ายเซลล์กระดูกอ่อน (autologous chondrocytes implantation) ขั้นตอนการรักษาผู้ป่วยจะได้รับการผ่าตัด 2 ครั้ง ครั้งแรกเป็นการผ่าตัดส่องกล้องเพื่อตรวจพยาธิสภาพและตัดชิ้นเนื้อกระดูกอ่อนในบริเวณที่ไม่ได้ใช้งานนำไปเพาะเลี้ยงในห้องปฏิบัติการ ครั้งที่สองภายหลังการผ่าตัดครั้งแรกประมาณ 4 สัปดาห์ ผู้ป่วยได้รับการปลูกถ่ายเซลล์กระดูกอ่อนของผู้ป่วยเองกลับไปยังผิวข้อที่ได้บาดเจ็บ การติดตามผลการรักษาใช้ Knee and Osteoarthritis Outcome Score (KOOS) ซึ่งประกอบด้วยอาการ, ความเจ็บปวด, การใช้งานในชีวิตประจำวัน, การใช้งานเล่นกีฬา, และคุณภาพชีวิตภายหลังการรักษา; การติดตามผลจากเอกซเรย์คอมพิวเตอร์ (MRI); และการส่องกล้อง (arthroscopy) การติดตามผล รักษานานเฉลี่ย 19.8 $\pm$ 4.6 เดือน (ตั้งแต่ 16-27 เดือน)*

ผลการศึกษา: *ผู้ป่วยทุกรายมีอาการทางคลินิกดีขึ้นอย่างมีนัยสำคัญทางสถิติจาก Knee and Osteoarthritis Outcome Score (KOOS) การตรวจติดตามผล MRI และ การส่องกล้อง (arthroscopy) พบมีการทดแทนรอยบาดเจ็บที่ผิวข้อด้วย regenerative cartilage ไม่พบภาวะแทรกซ้อนตลอดผลการรักษา*

สรุป: *การติดตามผลการรักษาแสดงให้เห็นว่าการปลูกถ่ายเซลล์กระดูกอ่อน (autologous chondrocytes implantation) ในผู้ป่วยที่มีการบาดเจ็บของผิวข้อขั้นรุนแรง ได้ผลทางคลินิกเป็นที่น่าพอใจ การติดตามผลจาก MRI และการผ่าตัดส่องกล้องพบเนื้อเยื่อกระดูกอ่อนที่สร้างขึ้นใหม่ทดแทนการบาดเจ็บของผิวข้อเดิม*