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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. การนำผลงานวิจัยไปใช้ประโยชน์

ผลงานวิจัยได้มีการพัฒนา เชิงวิชาการ การเรียนการสอนและสร้างนักวิจัยรุ่นใหม่ที่สนใจศึกษาทำวิจัยการใช้ stem cell ทางโรคกระดูกและข้อเนื่องจากเป็นโครงการวิจัยแรกในประเทศไทยที่มีการนำเซลล์ต้นกำเนิดไปใช้กับการรักษาโรคข้ออย่างเป็นรูปธรรม และมีการเผยแพร่ผลงานเชิงสาธารณะ ในวงกว้างและสื่อสารมวลชนรวมถึงการประชุมวิชาการระดับชาติและนานาชาติทำให้เกิดความสนใจจากแพทย์นักวิจัย และประชาชนให้ได้รับข้อมูลที่เกี่ยวกับเทคโนโลยีและการพัฒนาการรักษาโรค ตลอดจนนำไปใช้ประโยชน์เชิงนโยบายในการกำหนดนโยบายมาตรการใหม่ที่เกี่ยวกับระเบียบข้อบังคับการรักษาโรคข้อในระดับองค์กรวิชาชีพ ซึ่งได้แก่ การทำ clinical guideline ของการรักษาโรคข้อเสื่อมของราชวิทยาลัยแพทย์ออร์โธปิดิกส์แห่งประเทศไทย ซึ่งแสดงถึงประโยชน์ของโครงการวิจัยอย่างกว้างขวาง

2. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

<u>นำเสนอผลงานวิจัย จำนวน 9 เรื่อง</u>

1. Channarong Kasemkijwattana, Suraphol Kesprayura, Suradej Hongeng , Cholawish Chanlalit, Kanda Chaipinyo, Ramida Watanapokasin, , Kosum Chansiri. The Co-implantation of Mesenchymal Stem Cell and Chondrocytes for Cartilage defects of the Knee. The Combined Meeting of The Royal College of Orthopaedic Surgeon of Thailand and the Meeting of Bone and Joint Decade (BJD) and Asian Federation of Sports Medicine (AFSM), Pattaya, Chonburi, October 18-22 2007: Award winner

2. Channarong Kasemkijwattana, Suradej Hongeng, Suraphol Kesprayura, Adisak Wongkajornsilp, Kanda Chaipinyo, Ramida Watanapokasin, Kosum Chansiri. Update in Tissue Banking and Tissue Engineering in Orthopedics: Cartilage Transplantation. The Annual Meeting of The Royal College of Orthopaedic Surgeons of Thailand, Pattaya, Chonburi, October 22-25 2008

3. Channarong Kasemkijwattana, Suradej Hongeng, Suraphol Kesprayura, Adisak Wongkajornsilp, Kanda Chaipinyo, Ramida Watanapokasin, Kosum Chansiri. Autologous BMSCs and Chondrocytes Implantation for Large Cartilage Defects. The Annual Meeting of The Royal College of Orthopaedic Surgeons of Thailand, Pattaya, Chonburi, October 22-25 2008

4. Channarong Kasemkijwattana, Suradej Hongeng, Adisak Wongkajornsilp, Suraphol Kesprayura, Kanda Chaipinyo, Cholawish Chanlalit, Kosum Chansiri. Application of Stem cells in Cartilage Treatment. The 4th WCRM Current Regenerative Medicine 2009, Centara Grand Hotel, Bangkok, July 4-7 2009

5. Channarong Kasemkijwattana, Suradej Hongeng, Suraphol Kesprayura, Adisak Wongkajornsilp, Cholawish Chanlalit, Kanda Chaipinyo, Kosum Chansiri. Autologous BMSCs and Chondrocytes Implantation for Large Cartilage Defects. The 4th WCRM Current Regenerative Medicine 2009, Centara Grand Hotel, Bangkok, July 4-7 2009

6. Channarong Kasemkijwattana, Suradej Hongeng, Adisak Wongkajornsilp, Suraphol Kesprayura, Kanda Chaipinyo, Cholawish Chanlalit, Kosum Chansiri. New Coming Technology for Orthopedic Surgery: Sport Medicine. The 34th Annual Meeting of the Royal College of Surgeon of Thailand 2009 , Pattaya, Chonburi, July 4-7 2009

7. Channarong Kasemkijwattana, Suradej Hongeng, Adisak Wongkajornsilp, Suraphol Kesprayura, Kanda Chaipinyo, Kosum Chansiri. Augmented autologous chondrocytes implantation with bone marrow mesenchymal stem cells The Sixth SICOT/SIROT Annual International Conference, a combined meeting with the Royal College of Orthopaedic Surgeons of Thailand (RCOST) 2009 , Pattaya, Chonburi, October 29 – 1 November 1 : Award winner

8. Augmented autologous chondrocytes implantation with bone marrow mesenchymal stem cells. The Singapore Orthopaedic Association Annual Conference November 18 – 21, 2009

9. Autologous chondrocytes implantation for ostechondral lesion of talus ในที่ประชุมวิชาการประจำปี 2553 ของราชวิทยาลัยแพทย์ออร์โธปิดิกส์แห่งประเทศไทย ในระหว่างวันที่ 22 – 24 ตุลาคม 2553 ณ โรงแรมรอยัลคลิฟ บีช รีสอร์ท พัทยา จังหวัดชลบุรี

3. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

<u>ตีพิมพ์เผยแพร่ผลงานวิจัย จำนวน 3 เรื่อง</u>

1. Kasemwattana C, Kespravura S, Chaipinyo K, Chanlalit C, Chansiri K. Autologous Chondrocytes Implantation for Traumatic Cartilage Defects of the Knee. J Med Assoc Thai 92(5):648-653, 2009

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ภาคผนวก

THE CO-IMPLANTATION OF MESENCHYMAL STEM CELLS AND CHONDROCYTES FOR CARTILAGE DEFECT OF THE KNEE

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Introduction:

The capacity of articular cartilage repair is limited because of low mitotic activity, the absence of blood and nerve supply, and immobility of articular chondrocytes. The cell-therapy (autologous chondrocytes implantation) was reported to provide superior biologic properties of hyaline-like cartilage over the conventional procedures. However, the chondrocytes have limited chondrogenesis. The recent study showed the potential of mesenchymal stem cells (MSCs) to differentiate to be chondrocytes and enhance chondrogenesis in the certain microenvironment. We aimed to study the feasibility to use both chondrocytes and MSCs for transplantation into the cartilage defect in term of the viability of the co-culture of both cells in monolayer and collagen scaffold.

Materials & Methods:

The slivers of cartilage were harvested from the upper minor load-bearing area of femoral condyle. The cartilage was minced and digested with trypsin and collagenase. The chondrocytes were isolated and cultured in the media (DMEM/F-12, serum, gentamicin sulfate, amphotericin B, L-ascorbic, and L-glutamine) at 37° C, 5% CO_2 in air. The bone marrow aspiration from the PSIS was suspended in the media and centrifuged to remove the red blood cell. The cell pellets were suspended in DMEM, serum, gentamicin sulfate, amphotericin B (Figure.1). The 8 dishes of $2x10^5$ cells of chondrocytes and MSCs were cultured in DMEM/F-12, serum, gentamicin sulfate, and amphotericin B, L-ascorbic, and L-glutamine at 37 ° C, 5% CO_2 in air. The 8 dishes of co-culture of $2x10^5$ cells of chondrocytes and MSCs were cultured in the same media. The morphology and number of the 3 groups were evaluated at 3 and 6 days. The co-culture of chondrocytes and MSCs were seeded in collagen scaffold. The viability and proliferation of chondrocytes and MSCs were observed.

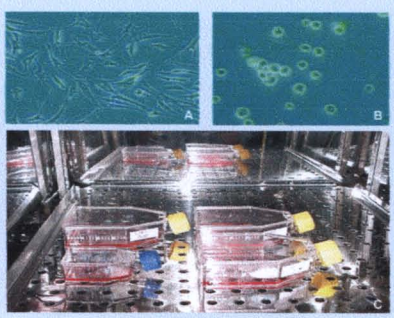


Figure.1 Chondrocytes (A), MSCs (B), culture chamber at 37° C, 5% CO_2 (C)

Results:

The morphology of the co-culture of chondrocytes and MSCs were excellent (Figure. 2). The number of co-culture cells at 3 and 6 days were $10.4 \pm 3.8 x10^5$, $53 \pm 4.9 x10^5$ cells. The number of isolated chondrocytes / MSCs at 3 and 6 days were $8.9 \pm 1.7 x10^5$ / $3.3 \pm 0.6 x10^5$ and $48 \pm 4.3 x10^5$/ $5.5 \pm 0.8 x10^5$ cells. There is no statistical significance in cell proliferation affected by the co-culture technique ($p>0.05$, Mann-Whitney U test). The co-culture of chondrocytes and MSCs were able to proliferate in collagen scaffold (Figure. 3).

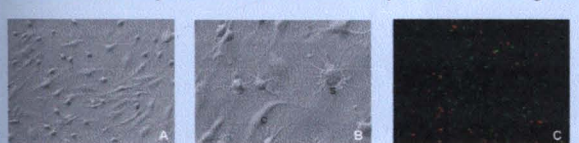


Figure. 2 Co-culture of chondrocytes & MSCs in monolayer (A), the morphology of chondrocytes (c) & MSCs (s) (B), labeled with green fluorescence bead for chondrocytes and red for MSCs (C)

Figure. 3 Co-culture of chondrocytes & MSCs in collagen scaffold at day 1(A), day 10 (B), and cell morphology (C)

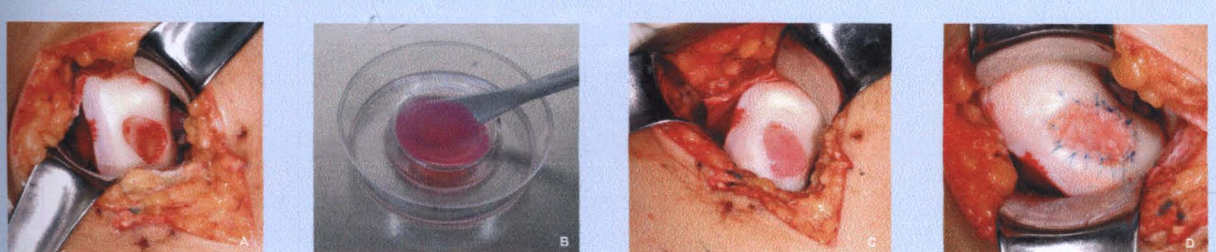


Figure. 4 The implantation of chondrocytes in collagen scaffold for cartilage defect (A), collagen scaffold seeded with chondrocytes (B), chondrocytes implantation (C), sealed with periosteal flap and fibrin glue (D)

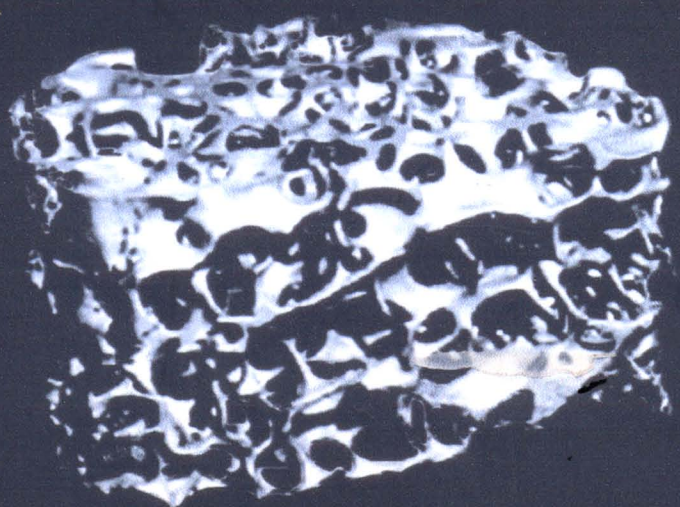
Conclusion:

The co-culture of chondrocytes & MSCs is feasible in both monolayer and collagen scaffold. The matrix synthesis in the co-culture environment is ongoing investigation. The co-implantation of autologous chondrocytes & MSCs in the collagen scaffold can be the alternative treatment for the large full-thickness chondral lesion.

Acknowledgements:

We'd like to thank Miss Aungkana Krajarng and Miss Peingjun Poyoi for technical supports. This study is granted by Srinakhrinwirot University and the Thailand Research Fund.

The 30th Annual Meeting of the Royal College of Orthopaedic Surgeons of Thailand



October 22-25, 2008
Pattaya, Thailand

Update in Tissue Banking and Tissue Engineering in Orthopedics Cartilage Transplantation

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The major challenge in the management of articular cartilage is the limited capacity of articular cartilage repair. The low mitotic activity, the absence of blood and nerve supply, and immobility of articular chondrocytes alter the natural history of cartilage defects to osteoarthritis. The biologic reconstruction of cartilage with engineering tissue in repair and restoration the articular cartilage become the alternative for the management of articular cartilage. The engineering tissues base on the transplantation of cells in combination with supporting matrices and biomolecules. The autologous chondrocytes implantation was the first successful tissue engineering in skeletal repair. The various biomolecules and matrices have been used in combination with chondrocytes implantation to enhance the cartilage repair. The mesenchymal stem cells have the potential as the cells and delivery of trophic factors to the repair cartilage and enhance chondrogenesis in the certain microenvironment. The combination of various engineering tissue procedures can be the future trend in the restoration of normal articular cartilage.

AUTOLOGOUS BMSCs AND CHONDROCYTES IMPLANTATION FOR LARGE CARTILAGE DEFECTS

Channarong Kasemkijwattana MD[1], Suradej Hongeng MD[2], Suraphol Kesprayura MD[3], Adisak Wongkajornsilp MD[4], Cholawish Chanbalit MD[1], Kanda Chaipinyo PhD[5], Ramida Watanapokasin PhD[5], Kosum Chansiri PhD[6]

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Introduction:

The autologous chondrocytes implantation is the convincing procedure to restore the normal hyaline cartilage. However, the capability of chondrocytes matrices synthesis is limited especially in the large cartilage defects. The autologous chondrocytes implantation resulted in the hyaline-like cartilage. The stem cells can be the progenitor cells and deliver trophic factors to the injured cartilage. The bone marrow mesenchymal stem cells (BMSCs) had been shown to differentiate to be chondrocytes which are capable of synthesis the cartilage matrices. Our previous study showed the cell synergy of BMSCs and chondrocytes in cell proliferation and differentiation (Figure.1). This study aimed to use the autologous BMSCs and chondrocytes implantation for the large cartilage defects of the knee.

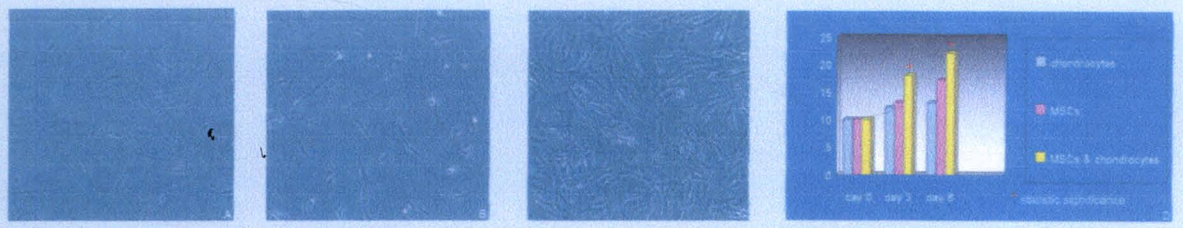

Figure.1 BMSCs (A), Chondrocytes (B), co-culture with better cell differentiation (C) and significant increase cells proliferation at 3, 6 days (D)

Materials & Methods:

The bone marrow aspiration was done from iliac crest. The cartilage slices were harvested arthroscopically. Both were transferred to the laboratory for BMSCs and chondrocytes isolation. The BMSCs and chondrocytes were co-cultured in the media (DMEM/F-12, serum, gentamicin sulfate, amphotericin B, L-ascorbic and L- glutamine) at 37° C, 5% CO_2 in air. The co-culture of BMSCs and chondrocytes were seeded into the collagen scaffold when the number of cells were adequate (Figure.2). The tissues seeded with BMSCs and chondrocytes were ready for implantation after the routine quality-control protocol. With the arthrotomy, the tissues seeded with BMSCs and chondrocytes were transplanted into the encapsulated periosteal graft in the knee joint. The defects were sealed with fibrin glue (Figure.3). The post-operative protocol was performed.

Figure.2 BMSCs and chondrocytes in collagen scaffold 20x (A), 4x (B), 1x (C)

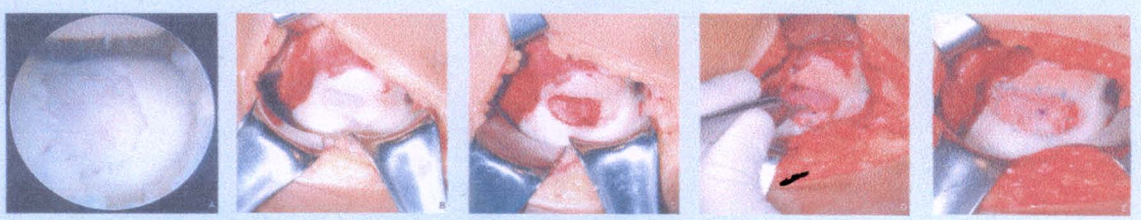

Figure.3 Chondral defect (A, B), after debridement (C), BMSCs and chondrocytes implantation (D), suture and seal with fibrin glue (E)

Result:

All patients have good immediate results without any complication. The clinical improvement after implantation for large defects was shown. The long-term follow-up will be required.

Conclusion:

The BMSCs have the potential as the progenitor cells and delivery of trophic factors to the repair cartilage and enhance chondrogenesis in the certain microenvironment. The autologous BMSCs and chondrocytes implantation can be the alternative to provide the hyaline cartilage in the large defects.

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Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, Ohgushi H, Wakitani S, Kurosaka M. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. Osteoarthritis and Cartilage 2007; 15(2): 226-231

Acknowledgement:

This study is granted by Thailand Research Fund.

The 4th World Congress on Regenerative Medicine

"Current Regenerative Medicine 2009"

March 12 - 14, 2009
at Bangkok Convention Center
and Centara Grand Hotel
at CentralWorld
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15.30-17.00	Session VI	Session VI	Session VI
	Room A: Invited Lectures	Room B: Oral Presentation	Room C: Invited Lectures
	Chair: Prof. Supachai Chaithiraphan Co-chair: Dr. Kriengkrai Hengrussamee, M.D.	Chair: Prof. Prasit Futrakul Co-chair: Dr. Kostas Papadopoulos	Chair: Prof. Amnuay Thitaphan Co-chair: Dr. Thanom Bunaprasert, M.D.
	Invited Lecture on "A Novel Autologous Progenitor Cell in Treatment of Ischemic Heart Disease" by Prof. Michael Belkin (Israel)	IM-04 Safety and Feasibility of Autologous Umbilical Cord Blood Stem Cell Intravenous Infusion In a Toddler with Spastic Diplegia/ Cerebral Palsy and Follow up Low Dose G-CSF i.m. Injections: A Case Report by Kostas Papadopoulos (Thailand)	Invited Lecture on "Regulation Roadmap Stem Cell and Tissue Engineering Product" by Dr. Thanom Bunaprasert, M.D. (Thailand)
	Invited Lecture on "Surgical Intramyocardial Implantation of Autologous Peripheral Blood Stem Cell for Cardiomyopathy: An Updated" by Dr. Permyos Ruengsakulrach, M.D. (Thailand)	BB-09 Injectable Smart Hydrogels with the Promising for Cartilage Tissue Engineering by Atchara Faikrua (Thailand)	Invited Lecture on "Application of Stem Cell in Cartilage Treatment" by Dr. Channarong Kasemkijwattana, M.D. (Thailand)

AUTOLOGOUS BMSCs AND CHONDROCYTES IMPLANTATION FOR LARGE CARTILAGE DEFECTS

Channarong Kasemkijwattana, MD[1], Suradej Hongeng, MD[2], Suraphol Kesprayura, MD[3], Adisak Wongkajornsilp M
Cholawish Chanlalit, MD[1], Kanda Chaipinyo, PhD[5], Kosum Chansiri, PhD[6]

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[2] Department of Pediatrics, Ramathibodi Hospital, Mahidol University, [3] Department of Orthopedics, Police General Hospital,
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[6] Department of Biochemistry, Faculty of Medicine, Srinakhrinwirot University.

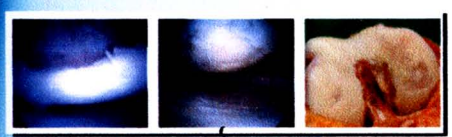


Figure.1 *Full-thickness chondral defect (A), heal with fibrocartilage (B), knee osteoarthritis (C)*

The osteoarthritis patients suffer from pain and disability. The primary cause of osteoarthritis is chondral defects. The healthy hyaline cartilage will be replaced by fibrocartilage following with the degenerative change after conventional treatments (Figure.1).

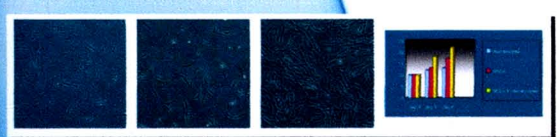


Figure.2 *BMSCs (A), Chondrocytes (B), co-culture with better cell differentiation (C) and significant increase cells proliferation at 3, 6 days (D)*

The tissue-engineering using autologous chondrocytes implantation is the standard procedure to restore the normal hyaline cartilage. However, the capability of matrices synthesis is limited. The more cells proliferate, the fewer matrixes synthesize. In the large cartilage defects, the autologous chondrocytes implantation resulted in the poor hyaline-like cartilage. The bone marrow mesenchymal stem cells (BMSCs) can be the progenitor cells and deliver trophic factors to the injured cartilage. The BMSCs had been shown to differentiate to be chondrocytes which are capable of synthesis the cartilage matrices. Our study showed the cell synergy of BMSCs and chondrocytes in cell proliferation and differentiation (Figure.2).

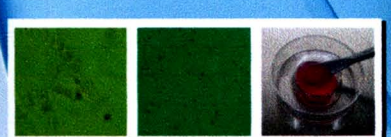


Figure.3 *BMSCs and chondrocytes in collagen scaffold 20x (A), 4x (B), 1x (C)*

The bone marrow aspiration was done from iliac crest. The cartilage slices were harvested arthroscopically. Both were transferred to the laboratory for BMSCs and chondrcytes isolation. The BMSCs and chondrcytes were co-cultured in the media. The co-culture of BMSCs and chondrcytes were seeded into the collagen scaffold when the number of cells was adequate (Figure.3).

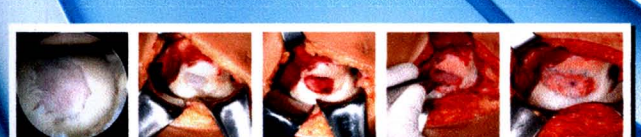


Figure.4 *Chondral defect (A, B), after debridement (C), BMSCs and chondrocytes implantation(D), suture and seal with fibrin glue (E)*

The tissues seeded with BMSCs and chondrcytes were ready for implantation after the routine quality-control protocol. With the arthrotomy, the tissues seeded with BMSCs and chondrcytes were transplanted into the en-capsulated periosteal graft in the knee joint. The defects were sealed with fibrin glue (Figure.4).

All patients have good immediate results without any complication. The clinical improvement after implantation for large defects was shown. The MRI showed the defects filled with the regenerative tissue. The long-term follow-up will be required.
The BMSCs have the potential as the progenitor cells and delivery of trophic factors to the repair cartilage and enhance chondrogenesis in the certain microenvironment. The autologous BMSCs and chondrocytes implantation can be the alternative to provide the hyaline cartilage in the large defects.

Acknowledgement : This study is granted by Thailand Research Fund.

Combined SICOT/RCOST 2009 Annual Meeting

29 October - 1 November 2009

Pattaya, Thailand

FINAL PROGRAMME

21901 **CK-MM (CREATINE KINASE MM ISOENZYME) LEVELS AS AN INDICATOR OF THE MAGNITUDE OF SKELETAL TRAUMA**
Raja VENKATARAMAN[1], Samuel CHITTRANJAN[2], R. SELVAKUMAR[2]
[1](United Kingdom), [2](India)

20969 **MAJOR REPLANTATION OF THE LOWER LIMB**
Saranatra WAIKAKUL (Thailand)

09:30-10:00 **Hip Arthroplasty** *(Hall A2)*

Plenary lecture

Introduction
Thanainit CHOTANAPHUTI (Thailand)

20220 **A DIRECT ANTERIOR APPROACH FOR HIP ARTHROPLASTY – TECHNIQUE, POTENTIAL, AND CRITICAL VALUATION**
Martin KRISMER (Austria)

10:00-10:30 *Coffee break/Poster discussion*

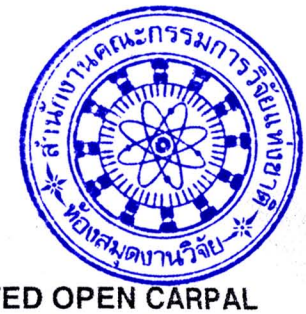
10:30-12:00 **Best Papers Session**

20058 **THE USE OF PLATELET GROWING FACTORS IN THE TREATMENT OF CHRONIC ACHILLEUS TENDONITIS**
Roberto PELUCCHI, Elisabetta DIOTTI, Maurizio LOVATO, Claudio MANZINI, Gianluca POZZI, Pasquale GIFUNI (Italy)

20718 **TOTAL HIP ARTHROPLASTY IN SICKLE CELL DISEASE**
Philippe HERNIGOU, Alexandre POIGNARD (France)

21018 **LATERAL APPROACH HAS AN ADVANTAGE IN TOTAL KNEE ARTHROPLASTY FOR VALGUS DEFORMED KNEE**
Hitoshi SEKIYA, Hisashi TAKADA, Kenzo TAKATOKU, Hideyuki SASANUMA (Japan)

21215 **REPAIR OF TEARS OF THE SUBSCAPULARIS: MINIMUM TWO YEAR FOLLOW-UP RESULTS OF 84 CASES**
István SZABÓ[1] Gilles WALCH[2], Pascal BOILEAU[2]
[1](Hungary), [2](France)



21274 **THE COMPARISON BETWEEN LIMITED OPEN CARPAL TUNNEL RELEASE USING DIRECT VISION AND TUNNELING TECHNIQUE AND TRADITIONAL OPEN CARPAL TUNNEL RELEASE – A RANDOMIZED CONTROLLED TRIAL STUDY**
Sorasak SUPPAPHOL, Preecha PITTAYAWUTWINIT, Patarawan WORATANARAT, Porntip CHATCHAIPUN (Thailand)

21520 **ARTHROSCOPIC AND MRI APPEARANCE AND RECONSTRUCTION OF THE ANTERIOR TALOFIBULAR LIGAMENT IN CASES OF APPARENT FUNCTIONAL ANKLE INSTABILITY**
Masato TAKAO, Ken INNAMI, Fumito KOMATSU, Takashi MATSUSHITA (Japan)

21700 **IN-VIVO KINEMATICS OF THE IVD ALLOGRAFT TRANSPLANTATION**
Stephen Ka Lok LAM, Dike RUAN, Yu DING, William LU, Keith D-K LUK (Hong Kong)

22333 **AUGMENTED AUTOLOGOUS CHONDROCYTES IMPLANTATION WITH BONE MARROW MESENCHYMAL STEM CELLS**
Channarong KASEMKIJWATTANA, Suradej HONGENG, Adisak WONGKAJORNSILP, Suraphol KESPRAYURA, Kanda CHAIPINYO, Kosum CHANSIRI (Thailand)

22461 **COMPUTER ASSISTED SURGERY: INTEREST ON TKR – PROSPECTIVE AND COMPARATIVE STUDY**
Ana TORRES, Eduardo SOLIS, Antonio MURCIA MAZON (Spain)

22493 **REPAIR ARTICULAR DEFECT WITH TISSUE-ENGINEERED CARTILAGE – A CLINICAL TRIAL ON HUMAN**
Hwa-Chang LIU, Chih-Hung CHANG (Taiwan)

12:00-13:30 *Lunch*

13:30-15:00 **Trauma: Hand & Forearm**

Moderators:
Horia-Bogdan ORBAN (Romania)
Piyawan CHATUPARISUTE (Thailand)

32nd Annual Scientific Meeting of SINGAPORE ORTHOPAEDIC ASSOCIATION

Paediatric Sports & Cartilage Regeneration

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Channarong Kasemkijwattana (Thailand)
Saw Khay Yong (Malaysia)

Local Faculty

Chang Haw Chong
Lim Jit Kheng
P.Thiagarajan

Symposium 1
Cartilage Repair Clinical Results

AUTOLOGOUS CHONDROCYTES IMPLANTATION AUGMENTED WITH STEM CELL

Channarong Kasemkijwattana, MD
Department of Orthopedics, Faculty of Medicine, HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakhrinwirot University

The autologous chondrocytes implantation is the convincing procedure to restore the normal hyaline cartilage. However, the capability of chondrocytes matrices synthesis is limited especially in the large cartilage defects. The autologous chondrocytes implantation resulted in the hyaline-like cartilage. The stem cells can be the progenitor cells and deliver trophic factors to the injured cartilage. The bone marrow mesenchymal stem cells (BMSCs) had been shown to differentiate to be chondrocytes which are capable of synthesis the cartilage matrices. Our study showed the cell synergy of BMSCs and chondrocytes in cell proliferation and differentiation. The stem cell technology was applied clinically to enhance the result of ACI in the large cartilage defects.

AUTOLOGOUS CHONDROCYTES IMPLANTATION FOR OSTEOCHONDRAL LESION OF TALUS

Chainarong Kasemkijwattana, MD[1], Visit Rungsinaporn, MD[1], Suraphol Kesprayura, MD[2], Suradej Hongeng, MD[3], Kanda Chaipinyo, PhD[4], Kosum Chansiri, PhD[5]

[1] Department of Orthopedics, Faculty of Medicine, HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakhrinwirot University, [2] Department of Orthopedics, Police General Hospital, [3] Department of Pediatrics, Ramathibodi Hospital, Mahidol University, [4] Faculty of Health Science, Srinakhrinwirot University, [5] Department of Biochemistry, Faculty of Medicine, Srinakhrinwirot University

Introduction: The osteochondral lesion of the talar dome is the common course of pain and disability. The contact surface of ankle articular cartilage is crucial regarding to the relatively small loading area. The conventional surgical treatments (debridement, drilling, and microfracture) are good for the small lesions. However, the large lesions will be replaced with fibrocartilage with poor clinical outcomes. The autologous chondrocytes implantation (ACI) is the accepted procedure to restore the hyaline cartilage in the knee joint. The extended indication to the chondral lesion of the talar dome was proven clinical benefit. In this study, we used ACI in the treatment of large cartilage lesion of the talar dome.

Materials & Methods: The symptomatic "unipolar" full-thickness chondral lesions were included. The two-stage procedure was required. First, the ankle arthroscopy was performed to assess the size of the chondral lesion and other pathology (Figure 1). The cartilage was arthroscopically harvested from the non-weight bearing part of ipsilateral knee and sent to the laboratory. The chondrocytes were isolated and cultured in the media (DMEM, serum, gentamicin sulfate, amphotericin B, L-ascorbic and L- glutamine) at 37°C, 5% CO_2 in air. With the adequate number of cells, the chondrocytes were seeded in the three-dimensional collagen scaffold for implantation (Figure 2).

Second, the malleolar osteotomy was performed to expose the talar dome. The lesions were debrided to the healthy rim. The periosteal graft was taken from the proximal tibia and sutured to the defects to form the en-capsulated pocket. The chondrocytes seeded in three-dimensional collagen scaffold were transplanted into the defects and sealed with fibrin glue. The malleolar reduction and internal fixation was done (Figure 3). The routine quality-control protocol was followed. The post-operative program including 2-4 weeks immobilization, following with the range of motion exercise. The partial weight bearing was advocated with full weight at 6-12 weeks.

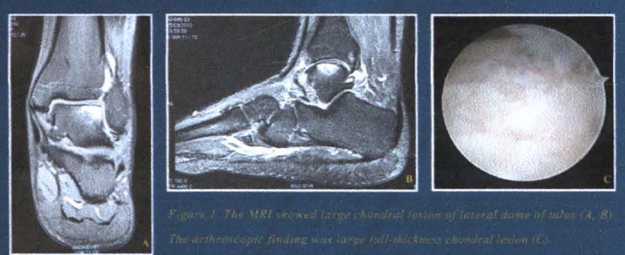

Figure 1. The MRI showed large chondral lesion of lateral dome of talus (A, B). The arthroscopic finding was large full-thickness chondral lesion (C).

Figure 2. The cartilage harvested from ipsilateral knee (A), the chondrocytes were isolated (B) and cultured in three-dimensional collagen scaffold (C, D).

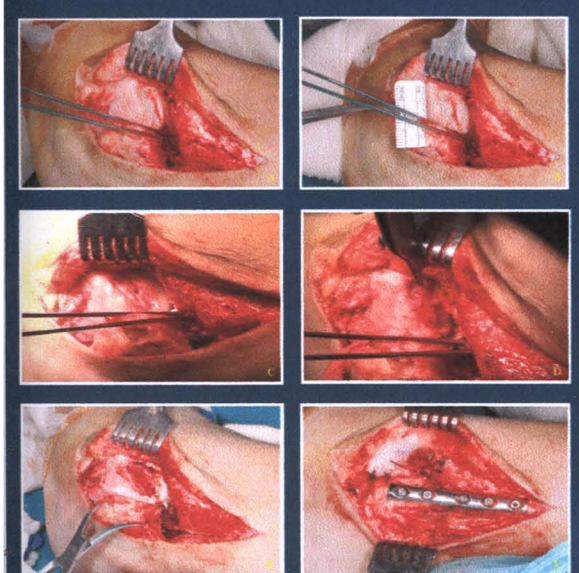

Figure 3. The lateral malleolus was osteotomized to expose the talar lesion (A, B). The periosteal flap was sutured to the lesion (C). The chondrocytes seeded in three-dimensional collagen scaffold were implanted and sealed with fibrin glue (D, E). The malleolus was fixed and the collateral ligaments were repaired (F).

Results: The patients showed the excellent early clinical results. There was no post-operative complication. The arthroscopic knee had the full recovery without any functional loss in a few weeks (Figure 4).

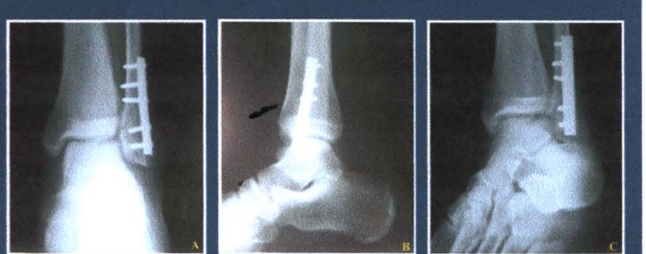

Figure 4. The x-ray showed good alignment of the ankle.

Conclusion: The ACI has the potential to treat the large chondral lesion of the talar dome. The promising results have been reported with long term follow-up. However, the technical demand and a period of rehabilitation program are required. The proper patient selection is the key success to restore the hyaline cartilage of the talus.

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Nam EK, Ferkel RD, Applegate GR. Autologous chondrocyte implantation of the ankle: 2- to 5-year follow-up. Am J Sports Med 2009; 37:274-284

Acknowledgement: This study was granted by Srinakharinwirot University and Thailand Research Fund.

Autologous Chondrocytes Implantation for Traumatic Cartilage Defects of the Knee

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Suraphol Kesprayura MD**, Kanda Chaipinyo PhD***,
Cholawish Chanlalit MD*, Kosum Chansiri PhD****

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**** *Department of Biochemistry, Faculty of Medicine, Srinakhrinwirot University, Bangkok, Thailand*

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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Full text. e-Journal: *http://www.mat.or.th/journal*

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 ± 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].

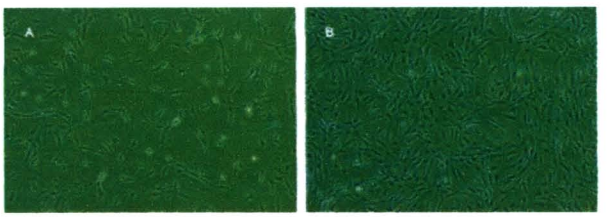

Fig. 1 Chondrocytes culture at 1 week (A) and 3 weeks (B)

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

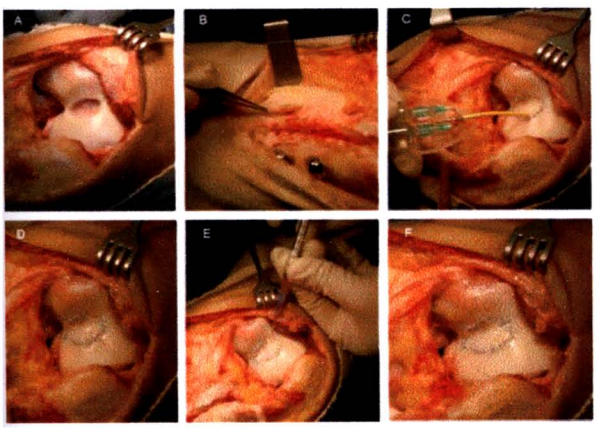

Fig. 2 The ACI procedures including debride lesion to healthy rim (A), harvest periosteal flap (B), suture periosteal graft over the lesion and seal with fibrin glue (C, D), inject chondrocytes into the pocket (E), and suture the opening hole and seal with fibrin glue (F)

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 ± 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 ± 15	86 ± 9	0.042*
Pain	59 ± 21	80 ± 16	0.043*
ADL	67 ± 19	87 ± 11	0.043*
Sports & recreation	30 ± 14	67 ± 19	0.042*
Quality of life	38 ± 11	64 ± 9	0.042*

* Statistical significance, $p < 0.05$

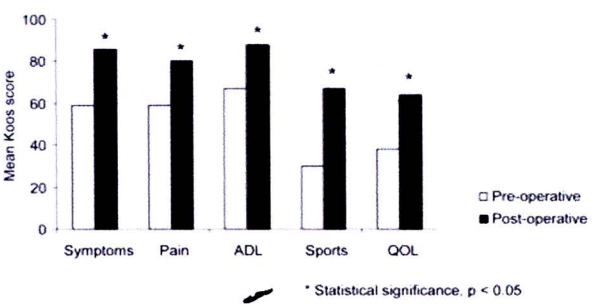

Fig. 3 The KOOS showed the functional outcome after the ACI

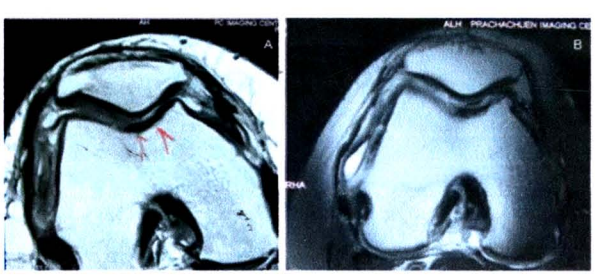

Fig. 4 The MRI at 3 months after ACI (A) showed good regenerative tissue formation within the defect, and 1 year (B) showed mild lamination

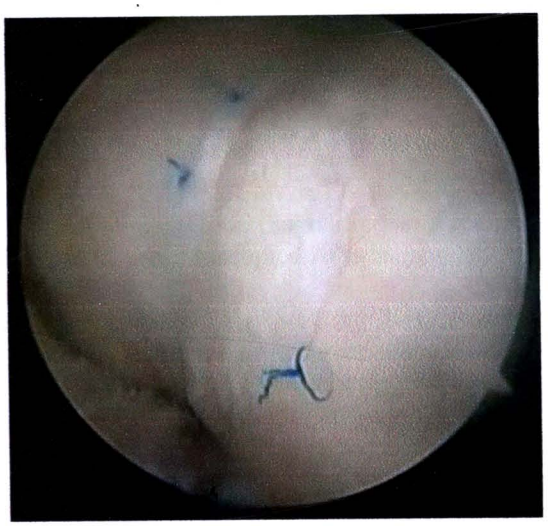

Fig. 5 The arthroscopic findings showed the excellent defect fill, incorporation, and stiffness at 2 years after ACI

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

This study has been granted by Srinakhrinwirot University.

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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 ± 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*

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

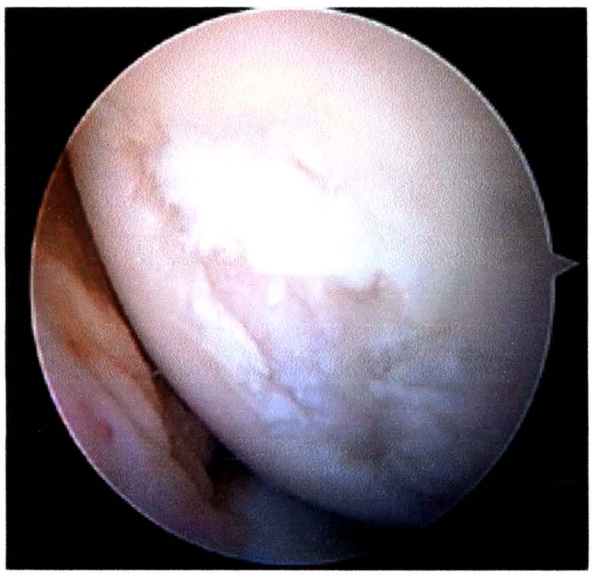

Fig. 1 The full-thickness chondral defect of medial femoral condyle

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

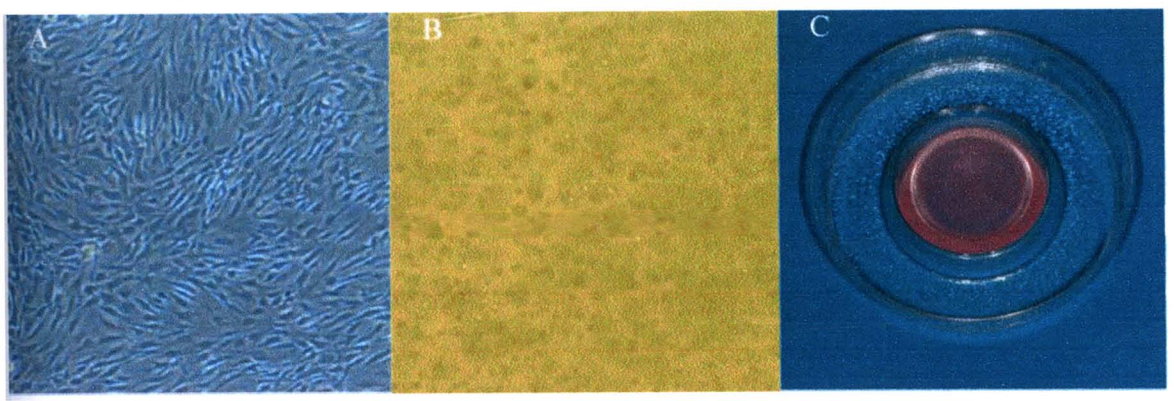

Fig. 2 Chondrocytes culture in monolayer (A), in collagen gel (B), and collagen gel (C) seeded with chondrocytes

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.

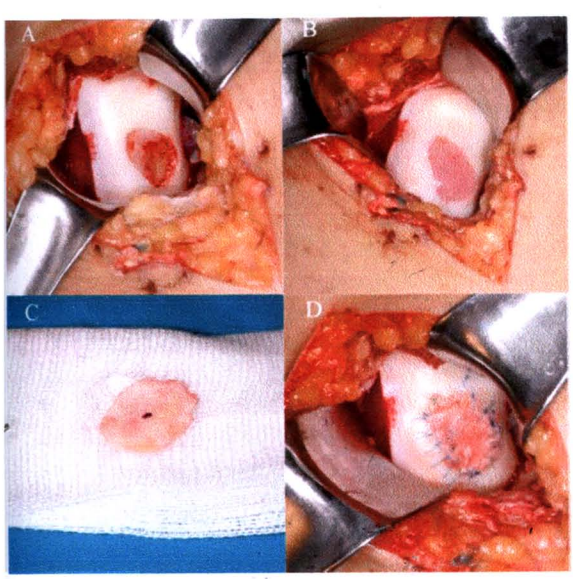

Fig. 3 ACI: debride lesion to healthy rim (A), fix collagen scaffold seeded with chondrocytes into the defect with fibrin glue (B), harvest periosteal graft from proximal tibia (C), suture periosteal graft over the lesion and seal with fibrin glue (D)

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

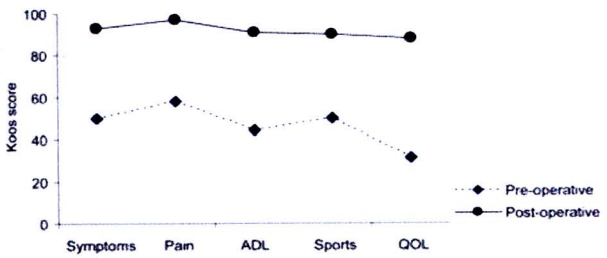

Fig. 4 The KOOS showed the excellent outcome after the ACI

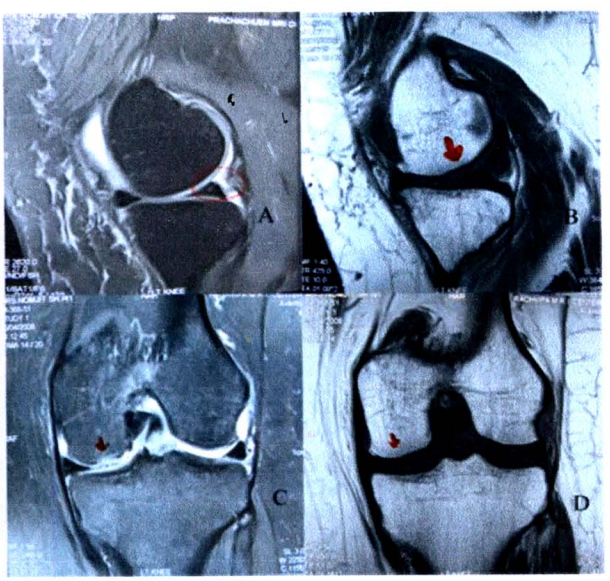

Fig. 5 The MRI at 6 months showed the meniscus repair (A), the defect filled with hyaline-like cartilage (B), with graft hypertrophy (C, D)

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-llke cartilage ไม่พบภาวะแทรกซ้อนภายหลังการรักษา*

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

Autologous Bone Marrow Mesenchymal Stem Cells Implantation for Cartilage Defects "Two Cases Report"

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Objective: *The authors reported the results of autologous bone marrow mesenchymal stem cells (BM-MSCs) implantation in two patients with large traumatic cartilage defects of the knee.*
Material and Method: *Two patients with grade 3-4 according to the International Cartilage Repair Society Classification System were performed autologous bone marrow mesenchymal stem cells (BM-MSCs) implantation on December 2007 and January 2008. The bone marrow aspiration was performed in the outpatient visit under local anesthesia and sent to the laboratory for BM-MSCs isolation and expansion. The BM-MSCs were re-implanted into the defects with the three-dimensional collagen scaffold. The patients were clinical evaluated preoperatively and postoperatively with Knee and Osteoarthritis Outcome Score (KOOS), International Knee Documentation Committee Score (IKDC Score) and arthroscopic examination. The duration of follow-up was 30-31 months.*
Results: *There was no postoperative complication. The clinical evaluation with Knee and Osteoarthritis Outcome Score (KOOS) and International Knee Documentation Committee Score (IKDC Score) showed significant improvement. The arthroscopic assessment showed the good defect fill, stiffness and incorporation to the adjacent cartilage.*
Conclusion: *The autologous bone marrow mesenchymal stem cells implantation showed the potential for the treatment of large cartilage defects. The one-stage procedure is the advantage over the conventional autologous chondrocytes implantation. The long-term follow-up with long last hyaline-like cartilage is required.*

Keywords: *Knee injury, Bone marrow mesenchymal stem cells, Cartilage defects*

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Articular cartilage consists of relatively few cells with low mitotic activity[1,2]. The poor self-repair of the cartilage will progress to osteoarthritis. The conventional procedures (abrasive chondroplasty, subchondral drilling, microfracture and mosaicplasty) are limited to the small defects[3,4]. The autologous chondrocytes implantation (ACI) had 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. The three to four weeks is needed to have the adequate number of cells.

Second, the chondrocytes are re-implanted into the defects[7-10]. The present study showed ACI had the potential to provide the hyaline-like cartilage over the conventional procedures[11,12]. However, the disadvantages of ACI are limitation in the number of chondrocytes and the requirement of two-stage procedure.

Bone marrow (BM) mesenchymal stem cells (MSCs) are multipotent, being capable of forming bone, cartilage and other connective tissue[13]. The BM-MSCs have high proliferation capacity and can be

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differentiated to chondrocytes[14,15]. The present study showed hyaline cartilage formation in the animal model[16-18] and in a clinical trial[19]. The BM-MSCs have the high potential to be the cell source for cartilage repair procedure with single-stage procedure.

The purpose of the current study was to evaluate the results of autologous BM-MSCs implantation using clinical evaluation and arthroscopic assessment.

Material and Method

Patients

Two patients were had autologous BM-MSCs implantation performed in December 2007 and January 2008; both had lateral femoral condyle lesions. The cartilage defects had grade 3-4 according to ICRS (International Cartilage Repair Society) Classification System[12,13]. The duration of follow-up was 30 and 31 months. The mal-alignment, ligament laxity, other pathology needed to be corrected before. All consented for the autologous BM-MSCs implantation under the Ethics Committee regulation.

The autologous BM-MSCs implantation consists of two-stage procedure. First, the BM was aspiration from anterior iliac crest under local anesthesia. The BM-MSCs were isolated and expanded in the laboratory. Second, the BM-MSCs were re-implanted into the cartilage defects.

BM-MSCs harvest and isolation

Heparinized bone marrow (BM) samples were obtained by aspiration from anterior iliac crest under local anesthesia and transferred to the laboratory at ambient temperature. Bone marrow mononuclear cells (BMMC) were separated by density gradient centrifugation with 1.073 g/ml Percoll solution (Sigma, MO, USA). Briefly, 10 ml of heparinized bone marrow cells were mixed in an equal volume of Dulbecco's Modified Eagle's Medium (DMEM) (BioWhittaker, USA) and centrifuged at 900 g for 10 min at room temperature. The washed cells were re-suspended in DMEM at density of 4 x 10^7 cells/ml and 5 ml aliquot was layered over 1.073 g/ml Percoll solution and centrifuged at 1,000 g for 30 min at room temperature. The interface mononuclear cells were collected and washed twice with DMEM. Total cell count and viability were evaluated by 0.2% Trypan blue exclusion. A total of 12 x 0^6 cells/ml of BMMC were cultured in DMEM complete medium supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, NY, USA) and 1% Penicillin-Streptomycin (Gibco BRL) at 37°C, 5% Co_2 in Co_2 incubator. On day 3 of cultivation, non-adherent cells were discarded and this process was repeated every 4 days. Upon 90% confluent, MSCs were trypsinized by 0.05% trypsin (Gibco BRL) and passaged for expansion (Fig. 1)[19]. The quality-control procedures consist of sterility testing and photographic recording of cell morphology. The 3-4 weeks incubation was needed to obtain an adequate number of MSCs for implantation.

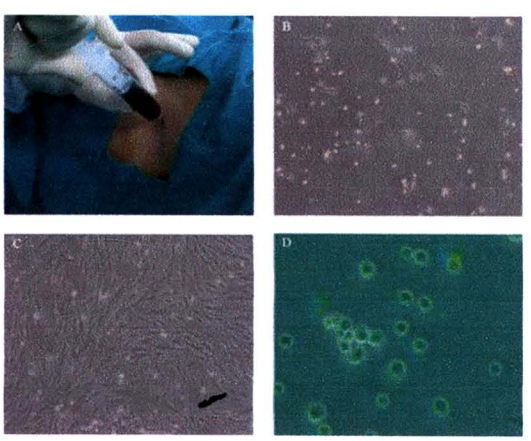

Fig. 1 Bone marrow aspiration (A), BM-MSCs isolation (B) and at 3 weeks (C), BM-MSCs in three-dimensional collagen scaffold (D)

Table 1. Demographic Data on the Patients

Case	Gender, Age	Cartilage lesion			Operation
		Side	Site	Size (cm^2)	
1	M, 24	L	Lateral condyle	2.5	double-bundle ACL reconstruction lateral meniscus repair, autologous BM-MSCs implantation
2	M, 25	L	Lateral condyle	2.2	ACL reconstruction, autologous BM-MSCs implantation

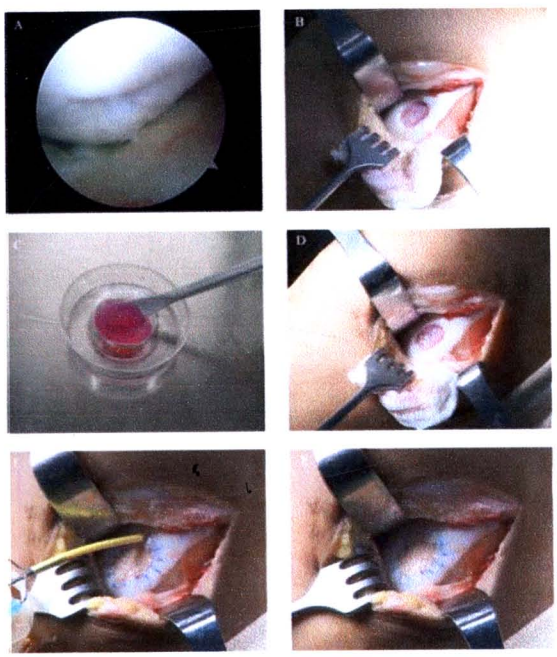

Fig. 2 Arthroscopic finding of the lesion (A), debride lesion to healthy rim (B), three-dimensional collagen scaffold seeded with BM-MSCs (C), fix into the defect with fibrin glue (D), suture periosteal graft over the lesion and seal with fibrin glue (E, F)

BM-MSCs implantation

The BM-MSCs were seeded in the atelocollagen (type I collagen, Koken) which is the three-dimensional collagen scaffolds. The grafts were sized and shaped according to the defects. The knee arthrotomy was performed. The chondral lesion was debrided to the healthy cartilage. The subchondral bone plate must be carefully preserved. The grafts were fixed to the defect using fibrin glue. The periosteal graft was harvested from the anteromedial incision of proximal tibia. The periosteum graft was sutured with interrupt suture (Prolene 6-0) to the chondral defect facing the defect with the cambium layer. The fibrin glue was used to make to water-seal pocket. The wound was closed layer by layer and the compression dressing was applied.

Post-operative program

The pain control with intravenous analgesic and NSAIDs were 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, active knee flexion and quadriceps/hamstrings strengthening exercises were encouraged as tolerate after 2 weeks. The patients had full range of motion at 3 months. The progressive weight training was encouraged. Running was restricted for 9 months.

Clinical evaluation

The duration of follow-up was 30-31 months. The patients were clinical 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[20]; and arthroscopic examination.The case reports were presented with preoperative and postoperative (at 30-31 months of follow-up) IKDC and KOOS respectively.

Results

The patients had no post-operative complication. The evaluation using the International Knee Documentation Committee Score (IKDC Score) and KOOS showed excellent clinical improvement at the time of 30-31 months follow-up (Table 2, 3).

The arthroscopic assessment showed the

Table 2. International Knee Documentation Committee Score (IKDC Score)

Case	Preoperative	Postoperative*
1	21	74
2	37	94

*At 30-31 months follow-up

Table 3. Knee and Osteoarthritis Outcome Score (KOOS)

Case		Preoperative	Postoperative*
1	Symptoms	39	89
	Pain	39	92
	ADL	30	62
	Sports	24	75
	QOL	31	75
2	Symptoms	36	100
	Pain	36	100
	ADL	32	100
	Sports	20	100
	QOL	31	94

*At 30-31 months follow-up
ADL = Function in daily living
QOL = Quality of life

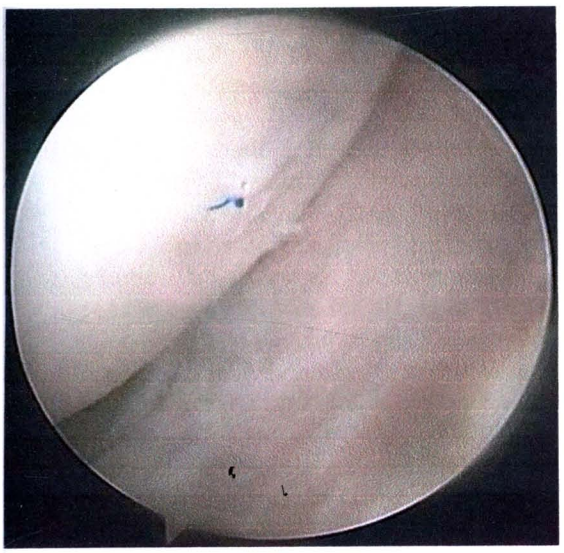

Fig. 3 The arthroscopic examination at 1 year after BM-MSCs implantation.

good defect fill, stiffness, and incorporation to the adjacent cartilage (Fig. 3).

Discussion

The ACI using expanded chondrocytes has been shown the promising results in the treatment of large cartilage defects over the conventional procedures[6,11]. However, the expanded chondrocytes have the limited chondrogenesis to achieve hyaline-cartilage from limited cell number for implantation[16]. The BM-MSCs are capable of self-renewal and differentiation to chondrocytes in the certain micro-environment[13]. The injured tissues induce the BM-MSCs to deliver trophic factors and differentiate to chondrocytes with "homing effect[14,15]". The BM-MSCs have shown the high potential as the cell sources for implantation with the same excellent clinical results compared to chondrocytes in vitro and *in vivo*[18,19,21]. The histology showed hyaline-like cartilage with abundant extracellular matrices[19]. The results were even better than chondrocytes in the older patient. The aging affects chondrogenesis of chondrocytes, but it seems to have less effect to BM-MSCs[21].

The present study showed the excellent clinical results and arthroscopic finding in the large defects at 30-31 months follow-up. The histologic study of the regenerative tissue could not be done regarding the patient's unwillingness. The other advantage of BM-MSCs is easy to obtain. The bone marrow aspiration can be done with the minimal invasive local anesthesia in the outpatient clinic. The patient satisfaction is high compared to the two-stage surgery of the conventional ACI.

However, the long term follow-up will be required. The BM-MSCs are multipotent, being capable of forming many connective tissues. The certain microenvironment which promote chondrogenesis differentiation and subsequent by maintain cartilaginous phenotype is required for further investigation. The biomaterial is another crucial factor for tissue-engineering. The BM-MSCs could be the alternative cell sources for cartilage regeneration procedure to provide superior biologic hyaline-cartilage in the large cartilage defects.

Potential conflict of interest

This study was granted by Thailand Research Fund.

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การปลูกถ่ายเซลล์ต้นกำเนิดจากไขกระดูกเพื่อรักษาการบาดเจ็บของกระดูกอ่อนข้อเข่า

ชาญณรงค์ เกษมกิจวัฒนา, สุรเดช หงส์อิง, สุรพล เกษประยูร, วิศิษฏ์ รังษิณาภรณ์, กานดา ชัยภิญโญ, โกสุม จันทร์ศิริ

วัตถุประสงค์: รายงานผลการรักษาการบาดเจ็บของกระดูกอ่อนด้วยวิธีการปลูกถ่ายเซลล์ต้นกำเนิด จากไขกระดูก (Autologous bone marrow mesenchymal stem cells implantation)

วัสดุและวิธีการ: ผู้ป่วยที่มีการบาดเจ็บของกระดูกอ่อนขั้นรุนแรง (grade 3-4 International Cartilage Repair Society Classification System) จำนวน 2 ราย ได้รับการผ่าตัดปลูกถ่ายเซลล์ต้นกำเนิดจากไขกระดูก (Autologous bone marrow mesenchymal stem cells implantation) ขั้นตอนแรกผู้ป่วยจะได้รับการเจาะดูดไขกระดูกจากกระดูกเชิงกราน (anterior iliac crest) ด้วยวิธีฉีดยาชาเฉพาะที่ (local anesthesia) นำไปแยกเซลล์ต้นกำเนิดจากไขกระดูกของผู้ป่วยเอง เพาะเลี้ยงใน three-dimensional collagen scaffold (atelocollagen type I collagen, Koken) ในห้องปฏิบัติการก่อนปลูกถ่ายเซลล์ต้นกำเนิดจากไขกระดูกใน three-dimensional collagen scaffold กลับไปยังผิวข้อที่ได้บาดเจ็บการติดตามผลการรักษาทางคลินิกโดยใช้ International Knee Documentation Committee Score (IKDC Score) และ Knee and Osteoarthritis Outcome Score (KOOS) ซึ่งประกอบด้วยอาการ, ความเจ็บปวด, การใช้งานในชีวิตประจำวัน, การใช้งานเล่นกีฬาคุณภาพชีวิตภายหลังการรักษาที่ระยะเวลา 30-31 เดือนร่วมกับการส่องกล้องตรวจ (arthroscopic assessment)

ผลการศึกษา: ผู้ป่วยมีอาการทางคลินิกดีขึ้นมากเทียบกับก่อนการรักษาจาก International Knee Documentation Committee Score (IKDC Score) และ Knee and Osteoarthritis Outcome Score (KOOS) การส่องกล้องตรวจพบเนื้อเยื่อกระดูกอ่อนใหม่ทดแทนรอยบาดเจ็บที่ผิวข้อ ไม่พบภาวะแทรกซ้อนตลอดผลการรักษา

สรุป: การติดตามผลการรักษาแสดงให้เห็นว่าการปลูกถ่ายเซลล์ต้นกำเนิดจากไขกระดูกในผู้ป่วยที่มีการบาดเจ็บของผิวข้อขั้นรุนแรง (autologous bone marrow mesenchymal stem cells implantation) ได้ผลเป็นที่น่าพอใจทั้งผลทางคลินิก และการติดตามผลจากการส่องกล้องตรวจ (arthroscopic assessment) นอกจากนั้นผู้ป่วยยังพึงพอใจที่ได้รับการผ่าตัดครั้งเดียวเทียบกับการผ่าตัดปลูกถ่ายเซลล์กระดูกอ่อน (autologous chondrocytes implantation) ซึ่งต้องได้รับการผ่าตัด 2 ครั้ง อย่างไรก็ตามยังต้องการติดตามผลการศึกษาระยะยาวต่อไป