

# Repair of the Complete Radial Tear of the Anterior Horn of the Medial Meniscus in Rabbits: A Comparison between Simple Pullout Repair and Pullout Repair with Human Bone Marrow Stem Cell Implantation

Jang-Hee Hong, MD, PhD<sup>1</sup>, Jong-Il Park, MD<sup>2</sup>, Kyung-Hee Kim, MD<sup>3</sup>, Young-Mo Kim, MD, PhD<sup>4</sup>, Yong-Bum Joo, MD<sup>4</sup> and Yoo-Sun Jeon, MD<sup>4</sup>

Departments of <sup>1</sup>Pharmacology, <sup>2</sup>Biochemistry, Chungnam National University School of Medicine; <sup>3</sup>Department of Pathology, Cancer Research Institute; <sup>4</sup>Department of Orthopedic Surgery, Chungnam National University School of Medicine, Daejeon, Korea

**Purpose:** To evaluate the degree of biological healing response that occurs between the anterior horn of the medial meniscus (MM) and the tibial plateau and investigate the biological healing response after injection of human bone marrow stem cells (hBMSCs) in a rabbit model.

**Materials and Methods:** Twenty-five rabbits with a mean body weight of 2.5 kg were chosen for this study. On the left knee, a complete radial tear was made at the anterior tibial attachment site of MM and after removal of tibial cartilage, pullout repair of the torn MM was performed on the tibial plateau. On the right knee, the same procedure was performed, and a scaffold (matrix gel) that contained human bone marrow stem cell was implanted between MM and the tibial plateau. A biopsy was performed at 2 (group 1), 4 (group 2), and 8 (group 3) weeks postoperatively. The authors compared the differences in the degree of biological healing of each group and investigated the degree of biologic healing after hBMSC implantation by comparing the left knee with the right knee.

**Results:** On the biopsy of 40 knees of 20 rabbits that survived after operation, all groups did not show the healing response between the undersurface of MM and the tibial plateau. There was no significant difference in terms of the pathological criteria such as fibroblasts and fibrochondrocytes etc., with and without hBMSC implantation.

**Conclusions:** There was no attachment between the repaired MM and the tibial plateau after complete radial tear on MM and the authors could not identify the effect of hBMSC.

**Key words:** Medial meniscus, Meniscal root ligament tear, Pullout repair, Human bone marrow stem cell, Biologic response.

Received April 27, 2011; Revised (1st) May 31, 2011; (2nd) July 13, 2011; Accepted July 18, 2011.

Correspondence to: Young-Mo Kim, MD.  
Department of Orthopedic Surgery, Chungnam National University School of Medicine, 33 Munwha-ro, Jung-gu, Daejeon 301-721, Korea.  
Tel: +82-42-280-7352, Fax: +82-42-252-7098  
Email: osdr69@cnu.ac.kr

Co-corresponding to: Kyung-Hee Kim, MD.  
Department of Pathology, Cancer Research Institute, Chungnam National University School of Medicine, 33 Munwha-ro, Jung-gu, Daejeon 301-721, Korea.  
Tel: +82-42-280-7195, Fax: +82-42-252-7098  
Email: phone330@cnuh.co.kr

\*This study was financially supported by research fund of Chungnam National University Hospital in 2009.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2011. THE KOREAN KNEE SOCIETY

www.jksrr.org

## Introduction

The meniscus is a fibrocartilaginous tissue composed of cartilage cells and a collagen fiber network of extracellular matrix. It is responsible for weight distribution, shock absorption, stability of the knee, joint lubrication, and proprioceptive sensation. A meniscal tear can lead to degenerative knee arthritis<sup>1)</sup>. The anterior and posterior horns of the medial meniscus (MM) are attached to the tibia by root ligaments whose tears and subsequent loss of meniscal function can lead to degenerative changes of the knee<sup>2,3)</sup>. Allaire et al.<sup>4)</sup> noted that root ligament tears resulted in changes in the tibiofemoral joint contact stress and biomechanics that could be comparable to total meniscectomy in their cadaver study and suggested the need for root ligament tear repairs to restore the joint biomechanics. Their study provides a rationale for arthroscopic repair of root ligament tear of the MM posterior horn<sup>5-9)</sup>. However, the outcomes of

arthroscopic repair of root ligament tear of the MM posterior horn that is common in Asians still remain controversial. We believe there are two prerequisites for successful healing after arthroscopic meniscus repair. First, the repaired posterior horn should be firmly attached to the tibia. Second, complete healing should be obtained between the undersurface of the meniscus and the tibial plateau. There have been some animal and cadaver studies regarding the pullout strength of the MM posterior horn<sup>10-12</sup>. In contrast, to our knowledge, the biological process of healing between the undersurface of the repaired meniscus and the tibial plateau has rarely been addressed<sup>13</sup>. In addition, the impact of stem cell injection on healing between the undersurface of the repaired meniscus and the tibial plateau appeared to have not been described in any study although mesenchymal stem cells were reported to differentiate into meniscal cells<sup>14</sup>. Studies on the root ligament tears of the MM posterior horn dealt with operative techniques providing only level IV scientific evidence<sup>4,15-17</sup>. In this study, we investigated the biological healing process after modified pullout repair of a complete radial tear made at the anterior tibial attachment site of MM in rabbits to assess whether the repaired meniscus was attached to the tibial plateau. In addition, the effect of human bone marrow stem cell (hBMSC) implantation in meniscal repair was also evaluated.

## Materials and Methods

This study was approved by our institutional review board for animal research. There were twenty-five New Zealand rabbits under the age of 1 year with a mean body weight of 2.5 kg (range, 2.4 to 2.6 kg) at the start of the study. However, 5 of them were lost to follow-up due to death and data from the remaining 20 subjects were used for analysis. A biopsy was randomly carried out at the 2<sup>nd</sup> postoperative week in 6 rabbits, 4<sup>th</sup> week in another 6, and 8<sup>th</sup> week in the remaining 8. The subjects were classified into 3 groups according to the time of biopsy: 6 in group I, another 6 in group II, and 8 in group III. All of the experiments were performed in an animal operating room at our institution.

### 1. Preparation of Human Bone Marrow Stem Cells (hBMSCs)

The hBMSCs were prepared using the same procedure described by Heo et al.<sup>18</sup>. Bone marrow was obtained from the hip bones of healthy donors. The mononuclear cells (MNCs) were isolated by centrifugation and cultured in Iscove's modified Dulbecco's medium (IMDM) with 20% heat inactivated bovine serum, 2 mM L-glutamine, penicillin (100 unit/mL), and streptomycin (100 µg/mL). After 7-10 days, the cells were trypsinized and

harvested in a new flask at 37°C under 5% CO<sub>2</sub> in air. The medium was replaced every 3-4 days. At 80% confluence, cells were removed with 0.025% Trypsin-EDTA and placed in fresh culture plates. Characterization of the cells was confirmed using a mesenchymal stem cell kit (Chemicon, Temecula, CA, USA). Differentiation into mesodermal lineage was confirmed using the method described by Yoo et al.<sup>19</sup>.

### 2. Anesthesia

Solid food was withdrawn for 6 hours before surgery and acepromazine (35 mg/kg) was administered intramuscularly. Ten minutes after the administration, Ketamin (50 mg/kg) was administered intramuscularly. The anesthesia was maintained with 2.5% isoflurane during surgery. Anesthesia was successfully induced and maintained in all cases. There was no intraoperative death.

### 3. Surgical Technique

Surgery was performed on both knees in all cases. On the left knee, the meniscal tear was repaired with sutures. On the right knee, a scaffold (matrix gel) containing human bone marrow stem cells was implanted between MM and the plateau before sutures.

#### 1) Left knee

A medial parapatellar longitudinal incision was made, subcutaneous soft tissue was dissected, and the patella was dislocated laterally. The joint capsule was opened and with the knee in flexion, the meniscus was exposed from the anterior

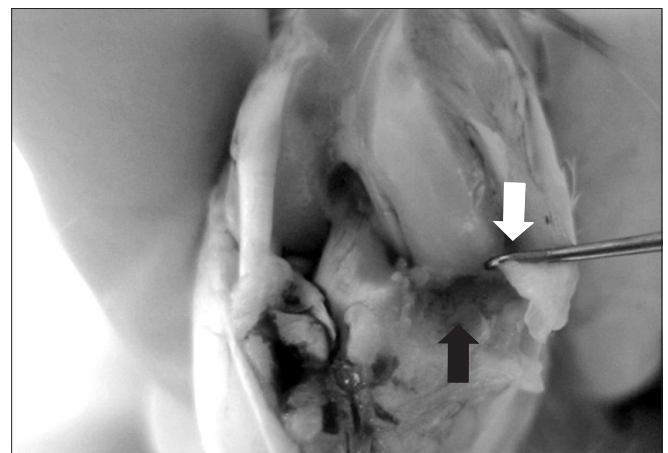


Fig. 1. The anterior horn of medial meniscus in a rabbit was cut from the tibial plateau (white arrow) and cartilage of the tibial plateau was removed by a curette (black arrow).

horn to the mid portion, not the posterior horn. The anterior intermeniscal ligament was cut at a site where the anterior cruciate ligament (ACL) is attached to MM using a #15 scalpel blade. The joint capsule and the MM anterior horn were medially translated to expose its attachment to the tibial plateau. Cartilage of the tibial plateau was removed with a curette until bleeding occurred (Fig. 1). A transtibial tunnel extending from a point 5mm medial to the proximal tibial tuberosity to immediately above the site of ACL attachment to the tibia was created using a 2mm-thick drill (Fig. 2A). A 3-0 Ethibond (Ethicon, Somerville, NJ, USA) (E1) was passed through the tunnel (Fig. 2B). The needle end of the suture (E1-1) was pulled out of the joint

between the MM and the joint capsule attachment site while applying pressure on the mid portion of MM (Fig. 2C). The E1-1 was introduced back into the joint through MM and the joint capsule attachment site at a point 2mm anterior to the suture exit, advanced medially from lateral to the site of ACL attachment to the tibia with tension to form an X-shaped suture over MM, pulled out of the joint immediately superior to the insertion site (Fig. 2D) for a pull out suture with the free end of E1 (E1-2) (Fig. 2E). The posteriorly protruded MM anterior horn was brought to its original place and fixed to the tibia by making a hole in the tibia using another 3-0 Ethibond (E2). In this way, MM was sutured with tension applied on the tibial plateau. After irrigation

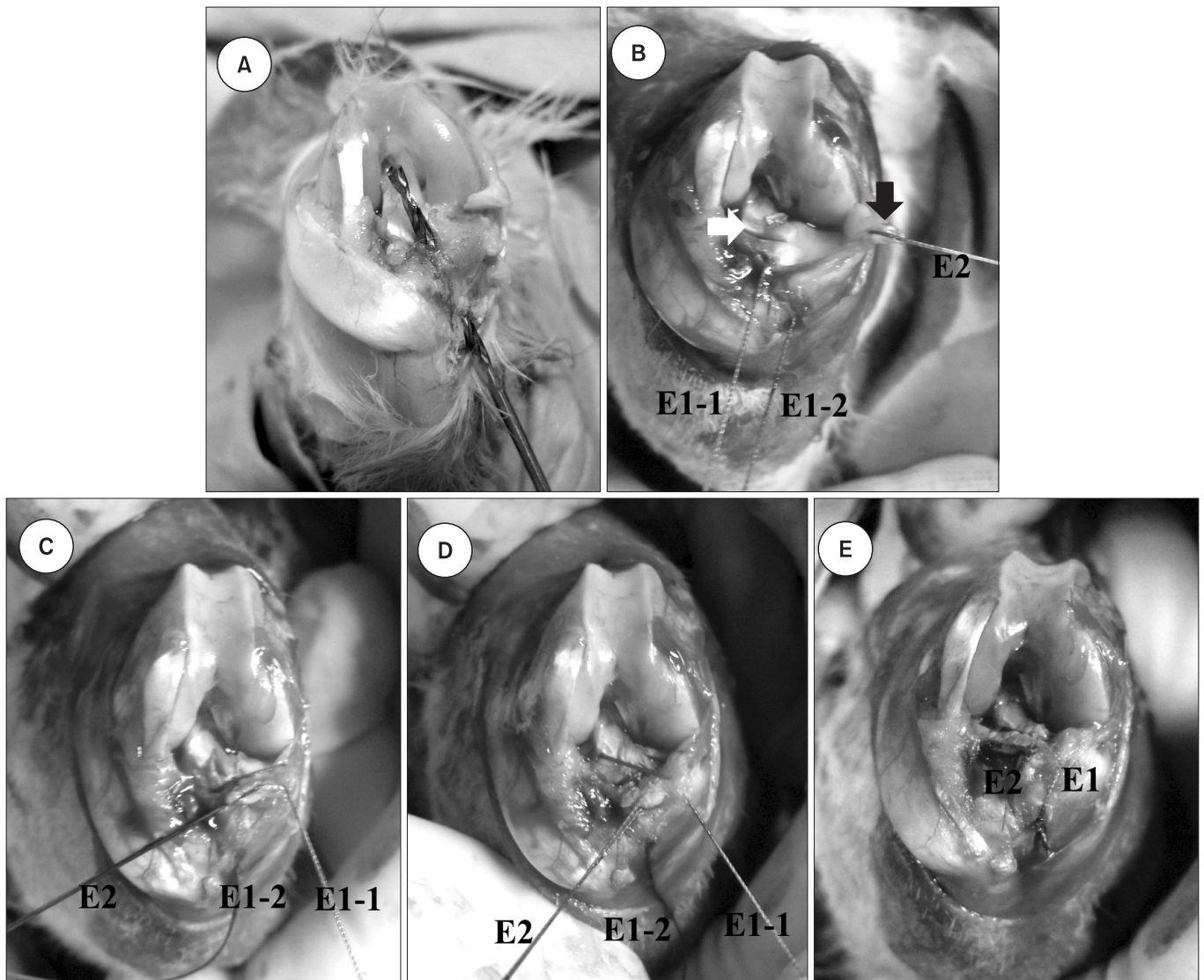


Fig. 2. The torn anterior horn of medial meniscus (MM) was reattached by a pullout suture technique. (A) A tibial tunnel was drilled for pullout suture. (B) Ethibond (E1) was inserted through the tibial tunnel and another Ethibond (E2) was sutured for anterior horn of MM (black arrow). Anterior cruciate ligament (white arrow). (C, D) MM was attached to tibial plateau by an X-shaped pullout suture and the root ligament of MM was reattached to its origin site. (E) The Ethibond (E1) was tied and anterior horn of MM was reattached to origin site (E2).

with saline water, interrupted suture was done with 4-0 Nylon for skin closure. The area created by the central and peripheral margins of MM, the anterior intermeniscal ligament attachment site to the MM anterior horn and the most posterior aspect of the exposed MM with the knee in full flexion was chosen as the surgical site.

## 2) Right knee

The joint was opened and the tibial cartilage was removed in the same way. One hundred  $\mu\text{g}$  ( $2 \times 10^6$  cells/mL) hBMSCs was mixed with the same amount of Matrigel at  $4^\circ\text{C}$ , to which 10  $\mu\text{L}$  cell suspension was pipetted (Gilson P20 pipette). The mixture was incubated at  $37^\circ\text{C}$  for 30 seconds and then injected between the MM and the removed cartilage. Suture closure was performed as was done on the left knee.

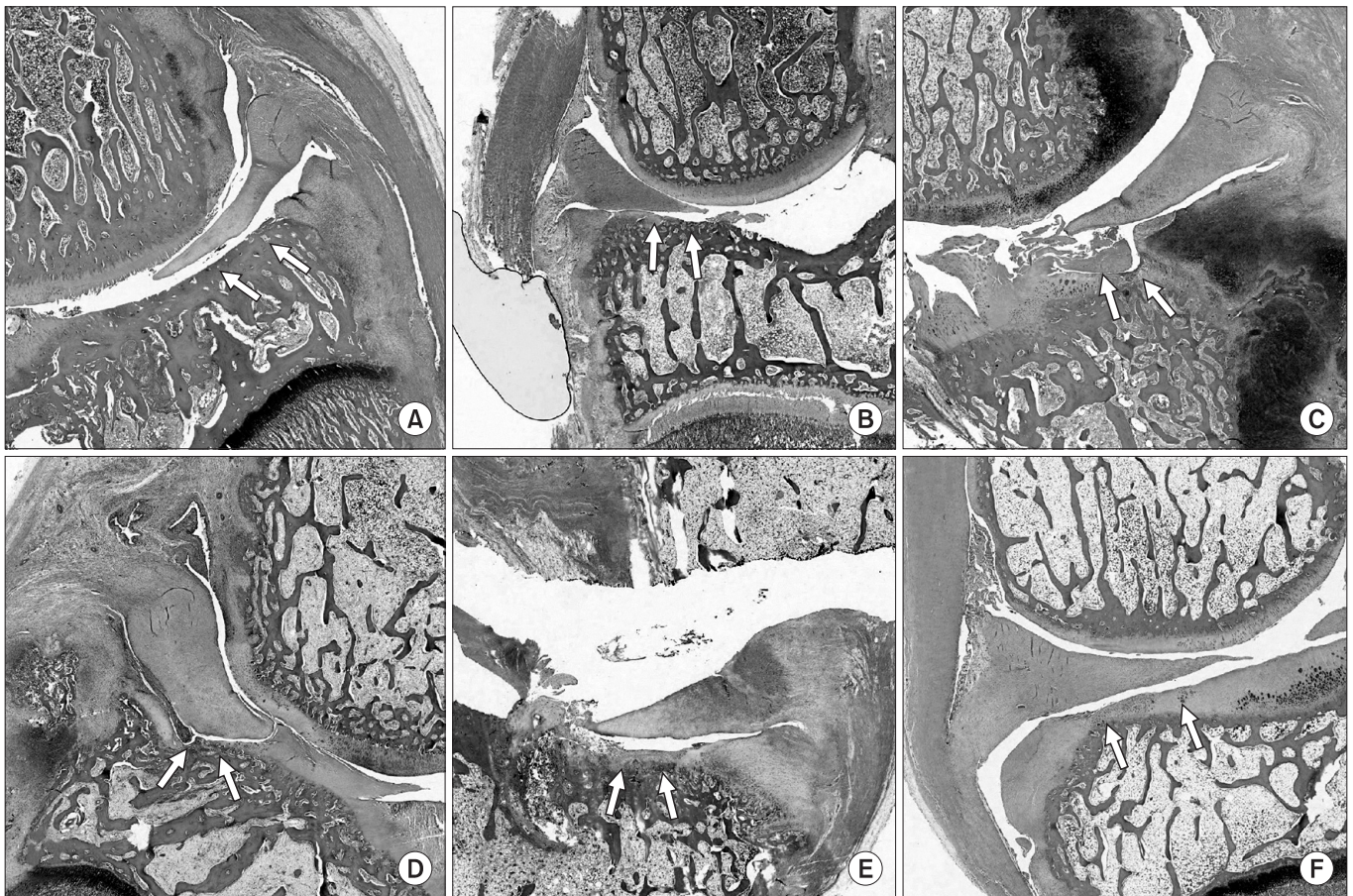
## 4. Postoperative Treatment

Proper wound dressing was performed and long leg cast

immobilization was applied. A biopsy was carried out at 2 weeks after surgery in group I, 4 weeks after surgery in group II, and 8 weeks after surgery in group III. During the biopsy, all biological events were recorded by a digital camera. The meniscus samples were examined with the naked eye by a histopathologist and were fixed with 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin. Inflammatory reaction and tissue response were evaluated with biopsy. Pathological criteria including inflammatory infiltration, connective tissue volume, macrophage, fibroblast, and fibrocartilage were used to assess the efficacy of hBMSC implantation.

## 5. Euthanasia

After sample collection, the rabbits were euthanized under anesthesia with an intravenous injection of a combination of embutramide (0.1 mg/kg), mebezone (0.1 mg/kg), and tetracaine (0.1 mg/kg).



**Fig. 3.** The pathologic features were observed using optical microscope. Attachment between the undersurface of medial meniscus and tibial plateau was not identified in all cases. There is some gap (black arrows) between the meniscus and tibial plateau in H&E stain ( $\times 40$  times). (A) Postoperative 2 weeks controlled group. (B) Postoperative 2 weeks stem cell inserted group. (C) Postoperative 4 weeks controlled group. (D) Postoperative 4 weeks stem cell inserted group. (E) Postoperative 8 weeks controlled group. (F) Postoperative 8 weeks stem cell inserted group.

**Results**

Two in group I and 3 in group II died after surgery. The cause of death was inflammation at the surgery site in 3 and food refusal in 2. The biopsy samples of the remaining 20 rabbits (40 knees) were observed under optical microscope. In all knees, a gap was observed between the undersurface of the MM anterior horn and the tibial plateau (Fig. 3), indicating that the undersurface of the MM anterior horn was not attached to the tibial plateau. Two cases of meniscus atrophy were observed in group II.

There were no histopathological differences regarding the use of hBMSCs because the gap between the undersurface of the MM anterior horn and the tibial plateau was observed in both the left knee and right knee in all cases. Attachment between the meniscus and the tibial plateau and distinctive inflammatory reaction were not observed in all cases (Table 1). The quantity of fibroblasts and fibrocartilage cells were similar in the left knee and the right knee, indicating no significant biological difference

with and without hBMSC injection (Fig. 3).

**Discussion**

The biomechanical properties of the meniscus have been addressed in many studies. In contrast, there is a paucity of studies in the literature on biological changes of the meniscus after root ligament repair<sup>13)</sup>, especially those involving hBMSC injection. Richmond and Sarno<sup>17)</sup> reported that the meniscus was reattached to the tibia after treatment of medial meniscal avulsion fractures via progression of the fibrocartilage calcification in the cancellous bone attached to the meniscus. However, considering that avulsion fracture treatments are about bone-to-bone healing, their study does not provide the basis for “bone-to-meniscus healing” after arthroscopic repair of the root ligament of the MM posterior horn. Therefore, we designed this study to investigate the healing process of a complete radial tear at the posterior tibial attachment site of MM in an animal model. Before study,

**Table 1.** Results in Stem Cell Inserted Group Versus Control Group

Group	Case number		Attachment between meniscus and tibial plateau	Inflammatory reaction
1	1	RSM/LCT	X/X	X/X
	2	RSM/LCT	X/X	X/X
	3	RSM/LCT	X/X	X/X
	4	RSM/LCT	X/X	X/X
	5	RSM/LCT	X/X	X/X
	6	RSM/LCT	X/X	X/X
2	7	RSM/LCT	X/X	X/X
	8	RSM/LCT	X/X	X/X
	9	RSM/LCT	X/X	X/X
	10	RSM/LCT	X/X	X/X
	11	RSM/LCT	X/X	X/X
	12	RSM/LCT	X/X	X/X
3	13	RSM/LCT	X/X	X/X
	14	RSM/LCT	X/X	X/X
	15	LSM/RCT	X/X	X/X
	16	RSM/LCT	X/X	X/X
	17	RSM/LCT	X/X	X/X
	18	RSM/LCT	X/X	X/X
	19	RSM/LCT	X/X	X/X
	20	RSM/LCT	X/X	X/X
Total	20	40		

RSM: stem cell inserted group of right knee, LCT: control group of left knee.

we performed preliminary examinations on the anatomical structures of the meniscus and the surrounding areas in rabbits and determined to use the MM anterior horn because it was easier to perform surgery on. When an intermeniscal ligament is injured, the MM anterior horn extrudes posteriorly in rabbits, which we assumed equivalent to meniscal extrusion in humans with root tears. We also assumed that the area immediately posterior to the intermeniscal ligament attachment site in rabbits was equivalent to the posterior tibial attachment site of MM in humans. There was no attachment between MM and the tibial plateau in all knees (Fig. 3). Unlike the successful MM anterior horn repair results in a rabbit model reported by Gao et al.<sup>13)</sup>, we could not observe reattachment of the MM anterior horn to the tibia after pullout repair. This was thought to be attributable to the fact that MM was sutured on the tibial plateau in our study. Unfortunately, it was impossible to remove the cartilage at the periphery of the tibia due to the small size of the knee joint of rabbits. We think that studies using animals with the knee joint similar in size with that of humans should be conducted to eliminate the possibility of errors caused by the knee joint size difference.

Stem cells are harvested from early stage embryos and can be grown in large quantities, differentiate into almost all types of cells in the body, and be transplanted without immune rejection to other patients and species<sup>14)</sup>. There have been many reports on the effects of the intraarticular injection of hBMSCs, but its function has yet to be established. According to Caplan and Dennis<sup>14)</sup>, mesenchymal stem cells (MSCs) appeared to enhance regeneration of the meniscus, although the MSCs observed in the meniscus were too few to account for the regeneration. In this study, we performed pullout repair alone on one knee and pullout repair with hBMSC injection on the other knee and compared the postoperative histological changes to evaluate the efficacy of hBMSCs. With the development of tissue engineering, absorbable polymer scaffolds have been developed, which enables intraarticular injections of growth factors and stem cells<sup>20,21)</sup>. However, we could not find statistically significant histopathological differences between the knees with stem cell injection and those without. We could not assess the impact of the intermeniscal ligament on the stability of the meniscus and identify whether the implanted stem cells were placed at the exact surgical site although we used a matrix gel scaffold to promote cell preservation. The limitations of this study include: 1) the knee joint of rabbits was too small to perform surgery at the exact site after proper tibial cartilage removal; 2) the surgery was performed on the MM anterior horn, not the posterior horn;

and 3) the follow-up period was not long enough for a long-term histological evaluation after meniscal attachment. Therefore, we think these limitations should be addressed in future prospective studies.

## Conclusions

There was no attachment between the MM anterior horn and the tibial plateau after complete radial tear repair using a pull out suture technique. In addition, we could not find statistically significant histopathological differences between the knees with hBMSC injection and those without.

## References

1. Arnoczky Sp, McDevitt CA. The meniscus: structure, function, repair and replacement. In: Buckwalter JA, Einhorn TA, Simon SR, eds. Orthopaedic basic science. 2nd ed. Rosemont: American Academy of Orthopaedic Surgeon; 2000. p531-45.
2. Gale DR, Chaisson CE, Totterman SM, Schwartz RK, Gale ME, Felson D. Meniscal subluxation: association with osteoarthritis and joint space narrowing. *Osteoarthritis Cartilage*. 1999;7:526-32.
3. Lerer DB, Umans HR, Hu MX, Jones MH. The role of meniscal root pathology and radial meniscal tear in medial meniscal extrusion. *Skeletal Radiol*. 2004;33:569-74.
4. Allaire R, Muriuki M, Gilbertson L, Harner CD. Bio-mechanical consequences of a tear of the posterior root of the medial meniscus. Similar to total meniscectomy. *J Bone Joint Surg Am*. 2008;90:1922-31.
5. Ahn JH, Wang JH, Yoo JC, Noh HK, Park JH. A pull out suture for transection of the posterior horn of the medial meniscus: using a posterior trans-septal portal. *Knee Surg Sports Traumatol Arthrosc*. 2007;15:1510-3.
6. Choi NH, Son KM, Victoroff BN. Arthroscopic all-inside repair for a tear of posterior root of the medial meniscus: a technical note. *Knee Surg Sports Traumatol Arthrosc*. 2008;16:891-3.
7. Kim YM, Rhee KJ, Lee JK, Hwang DS, Yang JY, Kim SJ. Arthroscopic pullout repair of a complete radial tear of the tibial attachment site of the medial meniscus posterior horn. *Arthroscopy*. 2006;22:795.e1-4.
8. Nicholas SJ, Golant A, Schachter AK, Lee SJ. A new surgical technique for arthroscopic repair of the meniscus root tear. *Knee Surg Sports Traumatol Arthrosc*. 2009;17:1433-6.

9. Ozkoc G, Circi E, Gonc U, Irgit K, Pourbagher A, Tandogan RN. Radial tears in the root of the posterior horn of the medial meniscus. *Knee Surg Sports Traumatol Arthrosc.* 2008;16:849-54.
10. Aros BC, Pedroza A, Vasileff WK, Litsky AS, Flanigan DC. Mechanical comparison of meniscal repair devices with mattress suture devices in vitro. *Knee Surg Sports Traumatol Arthrosc.* 2010;18:1594-8.
11. Kocabey Y, Taser O, Nyland J, Doral MN, Demirhan M, Caborn DN, Sarban S. Pullout strength of meniscal repair after cyclic loading: comparison of vertical, horizontal, and oblique suture techniques. *Knee Surg Sports Traumatol Arthrosc.* 2006;14:998-1003.
12. Kohn D, Siebert W. Meniscus suture techniques: a comparative biomechanical cadaver study. *Arthroscopy.* 1989;5:324-7.
13. Gao J, Wei X, Messner K. Healing of the anterior attachment of the rabbit meniscus to bone. *Clin Orthop Relat Res.* 1998;(348):246-58.
14. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem.* 2006;98:1076-84.
15. Griffith CJ, LaPrade RF, Fritts HM, Morgan PM. Posterior root avulsion fracture of the medial meniscus in an adolescent female patient with surgical reattachment. *Am J Sports Med.* 2008;36:789-92.
16. Marzo JM, Kumar BA. Primary repair of medial meniscal avulsions: 2 case studies. *Am J Sports Med.* 2007;35:1380-3.
17. Richmond JC, Sarno RC. Arthroscopic treatment of medial meniscal avulsion fractures. *Arthroscopy.* 1988;4:117-20.
18. Heo JY, Jing K, Song KS, Seo KS, Park JH, Kim JS, Jung YJ, Hur GM, Jo DY, Kweon GR, Yoon WH, Lim K, Hwang BD, Jeon BH, Park JI. Downregulation of APE1/Ref-1 is involved in the senescence of mesenchymal stem cells. *Stem Cells.* 2009;27:1455-62.
19. Yoo SW, Kim SS, Lee SY, Lee HS, Kim HS, Lee YD, Suh-Kim H. Mesenchymal stem cells promote proliferation of endogenous neural stem cells and survival of newborn cells in a rat stroke model. *Exp Mol Med.* 2008;40:387-97.
20. Peretti GM, Caruso EM, Randolph MA, Zaleske DJ. Meniscal repair using engineered tissue. *J Orthop Res.* 2001;19:278-85.
21. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with use of a collagen scaffold. Analysis of preliminary data. *J Bone Joint Surg Am.* 1997;79:1770-7.