The meniscus is semilunar fibrocartilage, and its injury causes dysfunction of the knee. We previously reported a high chondrogenic potential of synovial mesenchymal stem cells (MSCs). Here, we examined whether intra-articular injected synovial MSCs adhered to the defect of the meniscus, survived there, and differentiated into cartilage cells. MSCs were isolated from the synovium of GFP rats. Cylindrical defects were created in the menisci in wild rats, and GFP-positive synovial MSCs were injected into the knee. In the control group, 100 µl of PBS was injected into the contralateral knee. The menisci were analyzed after day 1, weeks 2, 4, 8, and 12. One day after injecting of 10^7 GFP-positive synovial MSCs, the meniscal defect was filled with the cells. The GFP-positive synovial MSCs expressed type II collagen, exhibited representative characteristics of chondrocytes by electron microscopy at 8 weeks, and could still be observed at 12 weeks. The histological score improved within 12 weeks but there were no statistical difference between the two groups at each period in this model. GFP mRNA expressions were not observed in distant organs at day 1. After intra-articular injection, synovial MSCs attached to the meniscal defect and differentiated into cartilage cells.

Key words: Mesenchymal stem cell, Synovium, Meniscus, Cartilage

Introduction

The meniscus is wedge-shaped semilunar fibrocartilage that lies between the weight bearing joint surfaces of the femur and the tibia. Meniscus has functions related to shock absorption, load transmission, and joint stability. Meniscus injury causes knee pain and dysfunction. For symptomatic meniscus injury, meniscectomy is often performed; however, it causes degeneration of articular cartilage. To preserve the meniscus, meniscal suture is performed; however, its indication is limited, and the outcome is not always satisfactory due to poor healing of the meniscus. A variety of methods are heretofore reported; however, they are not popular from the standpoint of effectiveness and invasion. Therefore, a novel treatment for meniscus injury is still needed.

The synovium plays a key role in meniscal healing. Seventy years ago, King observed the natural course of meniscal healing and found that synovium was induced to the site of meniscus injury and suggested
that the synovium contributes to meniscus repair. There are several reports describing effectiveness of synovial flap and free synovium to promote meniscal healing. These reports also indicated that synovium plays some roles during the natural course of meniscal healing; however, its reparative potential is fairly limited.

Mesenchymal stem cells (MSCs) are an attractive source for cell therapy because they are easily isolated and expanded. MSCs also have a multi-differentiation potential, which includes cartilage formation. We previously compared human MSCs derived from bone marrow, synovium, periosteum, adipose tissue, and skeletal muscle. We then demonstrated that synovial MSCs had the greatest expansion and chondrogenic differentiation ability. This indicates that synovial MSCs are a suitable cell source for the repair of cartilage including meniscus.

In this study, we made a hole in the meniscus of wild type Sprague-Dawley rats and injected GFP positive synovial MSCs into the joint. We examined whether injected cells adhered to the defect, survived there, and differentiated into meniscal cells. If such treatment is effective, injection of synovial MSCs may be a useful method to enhance repair of meniscus injury with little invasion in humans.

Materials and Methods

Synovial MSC preparation

All experiments were conducted in accordance with the institutional guidelines for the care and use of experimental animals. Thirteen-week-old male GFP rats, provided by Dr. Masaru Okabe of Osaka University, were anesthetized by an intra-peritoneal injection of sodium pentobarbital (25 to 30 mg/kg), and synovial tissues were harvested from knee joints. The tissues were minced, digested with type V collagenase (0.2%; Sigma, Lakewood, NJ, USA) for 3 hours at 37 °C, and passed through a 70-μm filter (Becton Dickinson, Franklin Lakes, NJ, USA). Nucleated cells from synovium were plated at 10^4 cells / 150 cm²-dish and cultured in complete medium (αMEM, Invitrogen, Carlsbad, CA, USA; 20% FBS, lot-selected for rapid growth of human MSCs, Invitrogen; 100 units/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B, and 2 mM L-glutamine, Invitrogen) for 14 days. Then, the cells were harvested after treatment with 0.25% trypsin and 0.02% EDTA, counted using a hemocytometer, and replated at 50 cells / cm². The cells were collected after 14 days, and frozen at -80 °C with Cryo 1 °C Freezing Container (Nalge Nunc International, Rochester, NY, USA) at 10^6 cells in 1 ml solution as Passage 1. The stocked cells were thawed in a water bath at 37 °C rapidly, plated in 150 cm²-dish, and harvested 3 to 4 x 10^6 cells after 5 days. Then, passage 2 cells were replated at 10^6 cells / cm² cultured for 14 days, and collected for further analyses and transplantation at Passage 3.11

Differentiation assay

For colony-forming assay, 100 cells were plated in 60-cm² dishes and cultured for 7 days. The dishes were then fixed with 4% paraformaldehyde and stained with 0.5% Crystal Violet in 4% paraformaldehyde for 5 minutes.

For adipogenesis, 100 cells were plated in 60-cm² dishes, cultured in complete medium for 7 days, and then the medium was switched to adipogenic medium that consisted of complete medium supplemented with 0.5 μM dexamethasone (Sigma-Aldrich, St. Louis, MO, USA) 0.5 mM isobutylmethylxanthine (Sigma-Aldrich), and 50 μM indomethacin (Sigma-Aldrich). After 4 days, the adipogenic cultures were stained with 0.3% Oil Red-O solution.12

For osteogenesis, 100 cells were plated in 60-cm² dishes, cultured in complete medium for 7 days, and then the medium was switched to calcification medium in the presence of 100 nM dexamethasone, 10 mM β-glycerophosphate and 50 μM ascorbic acid (Sigma-Aldrich). After additional 21 days, the dishes were stained with 0.5% Alizarin Red solution.13

For chondrogenesis, a pellet culture system was used. Approximately 8 x 10^5 cells were placed in a 15-ml polypropylene tube (Falcon, Bedford, MA, USA) and pelleted by centrifugation at 450 g for 10 minutes. The pellets were cultured for 21 days in chondrogenic media, which contained 500 ng/ml BMP-2 (Astellas Pharma Inc., Tokyo), in addition to high-glucose Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10 ng/ml TGF-β3 (R&D systems Minneapolis MN, USA), 10^-7 M dexamethasone, 50 μg/ml ascorbate-2-phosphate, 40 μg/ml proline, 100 μg/ml pyruvate, and 50 mg/ml ITS+TMPremix (Becton Dickinson). For histological analysis, the pellets were embedded in paraffin, cut into 5 μm sections, and stained with 1% Toluidine Blue.14

Surgical procedure for meniscal defect

Male Sprague-Dawley rats at 13-week-old (Sankyo Labo service Co, Tokyo) were used and anesthetized by the same methods mentioned above. A straight skin
incision was made on the anterior side of the knee, the anteromedial side of the joint capsule with the medial collateral ligament was cut horizontally at the level of the knee joint, and the anterior horn of the medial meniscus was dislocated anteriorly with a forceps. Then, a full-thickness cylindrical defect 1.0 mm in diameter was created in the anterior horn of the medial meniscus using a biopsy punch. Dislocated meniscus was replaced and the wound was closed in layers. The defects of the medial menisci were created in the both knees.

Knee injection of synovial MSCs

Just after the skin incision was closed, a 27-gauge needle was inserted at the center of the triangle formed by the medial side of patellar ligament, the medial femoral condyle, and the medial tibial condyle, toward the intercondylar space of the femur. Then, $10^5$, $10^6$ or $10^7$ GFP positive synovial MSCs in 100 µl PBS were injected into the right knee joint. For the control, the same volume of PBS was injected into the left knee joint. The knee joints were passively flexed and extended 10 times for dispersal of the cell suspension throughout the intra-articular space. The rats were allowed to walk freely in the cage.

Morphological evaluation

The rats were euthanized with a lethal dose of sodium pentobarbital, the whole medial meniscus was harvested and the meniscal defect was macroscopically observed under fluorescence. Then, the samples were fixed in 4% paraformaldehyde, decalcified with 0.5 M EDTA (pH 7.5) for 3 days at 4 °C, followed by gradient replacement with 20% sucrose for 24 hours at 4 °C. The meniscal samples were then rapidly frozen and cryosectioned in a cryostat (CM3000, Leica; Nussloch,! Germany) into 10 µm thick sections. Meniscal defects with injection of GFP positive cells (MSC group) and without injection of GFP positive cells (control group) were evaluated within unstained sections by using a fluorescent microscope system (IX 71, Olympus; Tokyo). Digital images of both groups were taken using the same exposure times (Penguin 600CL, Viewfinder 3.0, Pixera; Los Gatos, CA, USA). Also, sections were stained with Toluidine Blue.

Immunostaining

Sections were pretreated with 0.4 mg/ml proteinase K (DAKO, Carpinteria, CA, USA) in tris-HCl for 15 min at room temperature for optimal antigen retrieval. Residual enzymatic activity was removed by washing in PBS, and non-specific staining was blocked with PBS containing 10% normal horse serum for 20 min at room temperature. Primary anti-rat monoclonal antibody against human type II collagen (1:200 dilution with PBS containing 1% BSA; Daiichi Fine Chemical, Toyama) was applied to the sections, incubated at room temperature for 1 hour, and rinsed again with PBS. Immunostaining was detected by Vectastain ABC reagent (Vector Laboratories, Burlingame, CA, USA), followed by dianaminobenzidine staining. Counterstaining was performed with Mayer's hematoxylin.

Transmission electron microscopy

The centers of the repaired tissues in the meniscal defects at 8 weeks were selected in both the MSC and control groups, fixed with 2.5% glutaraldehyde in 0.1 M PBS for 2 hours, and washed overnight at 4 °C in the same buffer. The tissues were post-fixed with 1% OsO4 buffered with 0.1 M PBS for 2 hours, dehydrated in a graded series of ethanol, and embedded in Epon 812. Ultrathin sections at 90 nm were collected on copper grids, double-stained with uranyl acetate and lead citrate, and then examined with a transmission electron microscope (H-7100, Hitachi, Hitachinaka).

Histological grading score

Histological findings were quantified using a grading score for repaired meniscus (Table 1), which was modified based on repaired cartilage score. The score was assessed in a blinded manner by graders who were not informed of the group assignment.

Distribution of injected MSCs

Full thickness defect was created in the medial meniscus of the right knee in a wild-type rat, $10^7$ GFP synovial MSCs were injected into the right knee, and the rat was sacrificed at 24 hours. Synovium was harvested from the medial joint capsule, lateral joint capsule, and infrapatellar fat pad of the right knee and analyzed with fluorescence.

For RT-PCR, brain, lung, liver, kidney, and spleen were harvested and digested with 3 mg/ml collagenase for 3 hours. Total RNA was prepared by using the RNAqueous Kit (Ambion, Austin, TX, USA). To prepare a sufficient number of synovial cells from the right knee, after collagenase digestion of the synovium, the synovial cells were cultured for 14 days. GFP synovial MSCs were used as a positive control for GFP mRNA expression.

RNA was converted to cDNA and amplified by the Titan One Tube reverse transcription-polymerase
chain reaction (RT-PCR) System (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer’s recommendations. RT was performed for 30 min at 50 °C, followed by 2 min at 94 °C to inactivate the reverse transcriptase. PCR amplification of the resulting cDNAs was performed under the following conditions: 35 cycles of 94 °C for 30 s, 58 °C for 45 s, and 68 °C for 45 s. The reaction products were resolved by electrophoresis on a 1.5% agarose gel and visualized with ethidium bromide.

<table>
<thead>
<tr>
<th>PCR primers</th>
<th>Sequence</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP (forward)</td>
<td>5’-GACGTAACGGCCACAAGT -3’</td>
<td>(530 bp)</td>
</tr>
<tr>
<td>GFP (reverse)</td>
<td>5’-GGGTGTTCTGCTGGTAGTGG -3’</td>
<td></td>
</tr>
<tr>
<td>β-actin (forward)</td>
<td>5’-AGCCATGTACGTTACCCATCC-3’</td>
<td></td>
</tr>
<tr>
<td>β-actin (reverse)</td>
<td>5’-AGGAAGGAAGGCTGGGAAGAG -3’</td>
<td>(411 bp)</td>
</tr>
</tbody>
</table>

Statistical analysis

Statistical significance of the histological score difference was determined between the synovial MSC and control groups at each period and among periods in each group by two-way ANOVA using StatView-J 5.0 (SAS Institute, Inc, NY, USA). P values less than 0.05 were considered to be significant. If the differences between the synovial MSC and control groups at each period were not significant, statistical significance of the histological score difference was determined among periods in each group by one-way ANOVA.

Results

Characteristics of MSCs

Synovial cells in the GFP rats formed single cell derived-colonies when 100 cells were plated in 60 cm² dish and cultured for 14 days (Figure 1A). Most of the cells were spindle-shaped (Figure 1F). The cells differentiated into adipocytes (Figure 1B, G), osteoblasts (Figure 1C, H), and chondrocytes (Figure 1D, E, I) after induction. These results demonstrate that cells derived from the synovium of GFP rats exhibit the differentiation potential characteristic of MSCs.

Effect of number of synovial MSCs for knee injection

A full thickness defect 1 mm in diameter was created in the anterior horn of the medial meniscus of a wild type Sprague-Dawley rat (Figure 2). To examine the optimal number of synovial MSCs for knee injection to adhere to the meniscal defect in wild type Sprague-Dawley rats, 10⁵, 10⁶ or 10⁷ GFP positive synovial MSCs in 100 μl PBS were injected into the knee joint. Then 24 hours later, the meniscus was investigated under fluorescence. No green fluorescence was observed when 10⁵ cells were injected. Only very faint fluorescence was visible when 10⁶ cells were administered. GFP fluorescence was clearly visible when 10⁷ MSCs were injected (Figure 3). For further investigation, we focused on analyzing data obtained from injecting 10⁷ GFP positive synovial MSCs into the knee joint.

Features of meniscal defect after knee injection of synovial MSCs

Macroscopically, the meniscal defect was already covered with soft tissue at 2 weeks, and the covering tissue appeared to be thickened thereafter in both the synovial MSC and PBS control groups (Figure 4A). The difference in repaired meniscus appeared to be grossly invisible between the two groups. Green fluo-
rescence in the meniscal defect was still visible at 12 weeks in the synovial MSC treated group (Figure 4B). Histological observation at 2 weeks demonstrated that the meniscal defect was filled with fibrous tissue in both the synovial MSC and PBS control groups. At 4 weeks, cartilage matrix at the surface and fibrous tissue at the deep area of the meniscal defects increased in both groups (Figure 4C). At 8 weeks, the meniscal defect was filled with cartilage matrix in both groups. According to higher magnified observation, injected GFP positive cells still existed in the meniscal defect, and the cells produced type II collagen, though its expression was lower than that in native meniscus.

Electron microscopy of a representative cell showed several short processes with lacuna, characteristic of chondrocytes (Figure 4D). At 12 weeks, chondrocytes matured morphologically and gaps around the outside of the defect improved in both groups (Figure 4C). Histological findings were quantified using a grading score which was modified based on repaired cartilage score by Wakitani et al. There were no statistical differences of the score between the two groups at each period. The score increased within 12 weeks in both synovial MSC treated and control groups (Table 2). Distribution of injected synovial MSCs Twenty-four hours after 10⁷ GFP synovial MSCs were injected into the right knee, a large number of GFP cells were observed in synovial tissues (Figure 5A). To examine whether synovial MSCs mobilized to distant organs, GFP mRNA expressions in brain, lung, liver, kidney, and spleen were evaluated. RT-PCR showed GFP mRNA expression in the synovium, but not in these distant organs (Figure 5B).

Discussion
Here we demonstrated that synovial MSCs adhered to the injured site and differentiated into cartilage cells when synovial MSCs were injected into the knee joint. This is the first report of injection of synovial MSCs into the joint for intra-articular tissue injuries. Previously we compared rat MSCs derived from bone marrow, synovium, periosteum, adipose, and muscle. Each population of the cells was negative for CD11b (MAC-1), CD45 (leukocyte common antigen),
Fig. 2. Scheme for the experiment. (A) MSCs were isolated from synovium of GFP rats and expanded for the transplantation. Full thickness defects at 1 mm diameter were created in the anterior horn of the medial menisci in both knees of a wild-type rat. GFP synovial MSCs in 100 µl PBS were injected into the right knee, and 100 µl PBS was injected into the left knee. (B) Macroscopic feature of injured meniscus with 1 mm scale. (C) Normal and injured meniscus stained with Toluidine Blue (Bar = 1mm).

Fig. 3. Effect of number of synovial MSCs for knee injection. $10^5$, $10^6$ or $10^7$ GFP synovial MSCs in 100 µl PBS were injected into the right knee joint, and 24 hours later, the meniscus was investigated ($10^5$ and $10^6$ cells, n=2; $10^7$ cells, n=3). Representative macroscopic features of the meniscal defect under fluorescence are shown (Bar = 1mm).
Fig. 4D. Features of meniscal defect after knee injection of $10^7$ synovial MSCs. $10^7$ GFP synovial MSCs in 100 µl PBS were injected into the right knee, and only 100 µl PBS was injected into the left knee of a wild type rat. (A, B) Representative macroscopic features of the meniscal defect at 2, 4, 8, 12 weeks under light (A) and fluorescence (B) (Bar = 1 mm). (C) Representative histological features of the meniscal defect stained with Toluidine Blue (Bar = 1 mm). (D) Higher magnified histologies at 8 weeks, indicated in Figure 4C, stained with Toluidine Blue, under fluorescence, immunostained with type II collagen (Bar = 30 µm), and by transmission electron microscopy (Bar = 3 µm).

Fig. 5. Distribution of injected GFP synovial MSCs. A full thickness defect was created in the medial meniscus of right knee in a wild-type rat, $10^7$ GFP synovial MSCs were injected into the right knee, and the rat was sacrificed at 24 hours. (A) Synovium was harvested from the medial joint capsule (M), lateral joint capsule (L), and infrapatellar fat pad (I) of the right knee and analyzed with fluorescence (Bar = 5 mm). (B) RT-PCR analysis. GFP mRNA expressions in brain, lung, liver, kidney, spleen, and synovium of the right knee are shown. GFP synovial MSCs were used as a positive control /β-actin was used as an internal control.
and positive for CD90 (Thy1.1). The colony formation rate was the highest for synovium (1/100) and the lowest for bone marrow (4/10^5). After in vitro chondrogenic induction, the pellets derived from synovium and periosteum were larger and heavier than those from bone marrow, adipose, and muscle. For adipogenesis, the Oil Red-O positive colony rate in synovium and adipose was higher than that in muscle and bone marrow. These findings indicate that properties of MSCs depend on the source and that synovial MSCs are useful for a cartilage study in a rat model.

There have been some reports describing injection of bone marrow MSCs into the joint for meniscus injury. Murphy et al. removed medial meniscus entirely in a goat model, and 6 weeks later, 10^7 bone marrow MSCs were injected into the knee. The authors noted marked regeneration of the meniscus 6 months after the meniscectomy. However, type II collagen expression in regenerated meniscus seemed to be limited to the outer rim of the meniscus, which is known to retain high potential to heal due to an abundant blood supply. Caplan et al. suggested in their review paper that there were too few pre-labeled cells to account for the massive regeneration of the meniscus and inferred that the MSCs trophically enhanced the regeneration of the meniscus.

In two other papers, cartilage matrix was present around injected bone marrow MSCs in only a small portion of the injured meniscus, but the roles of injected MSCs were not fully demonstrated. In another report describing a tissue culture model, 10^6 cells/ml of GFP rat bone marrow MSCs in fibrin glue were transplanted into 1.2 mm circular defect of rat meniscus, and adherence and chondrogenesis of the MSCs were observed. Only a small portion of regenerated cartilage was shown in the meniscal defect at 12 weeks, but GFP cells were not detected at that time, and the origin of newly formed cartilage remained unknown. Unlike the previous reports, our study succeeded in demonstrating that injected cells adhered to the injured site and differentiated into chondrocytes based on their microenvironment. However, we cannot conclude that synovium MSCs are superior to bone marrow MSCs because experimental designs in the previous studies were not similar to ours; furthermore, we did not compare adherent and differentiation potential in synovium MSCs and bone marrow MSCs.

It is interesting to speculate as to why the cells adhered to the injured site and we consider 3 possible explanations. First, injured meniscus can produce some cytokines/chemokines, which recruit suspended cells in the knee joint to the meniscal defect. Ochi et al. reported that meniscal lesions expressed interleukin-1α, TGF-β, and PDGF in a rabbit model. These cytokines are known to have chemotactic effects. Second, blood clotting in the meniscal defect may cause injected MSCs to adhere to the meniscal defect. Indeed, we observed blood clots around meniscal defects 24 hours after the procedure (data not shown). According to Arnoczky et al., after a partial meniscectomy, a fibrin clot forms in the area of the removed tissue, which then acts as a scaffold for cellular ingrowth, though this does not always occur. Third, injected MSCs may fill in the meniscal defect physically. In this study, the number of synovial MSCs sufficient to adhere to the meniscal defect was 10^7 and the cells were suspended in 100μl PBS. This cell concentration is extremely high and the cell solution is very thick. Injected cells can occupy the whole space in the knee joint just after the injection, and the cells can dif-

### Table 2. Results of histological scoring system for repaired meniscus

<table>
<thead>
<tr>
<th>Cell morphology</th>
<th>Matrix staining surface</th>
<th>Matrix staining center</th>
<th>Surface regularity</th>
<th>Matrix integration density</th>
<th>Total Mean (18.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Full points)</td>
<td>(3.0)</td>
<td>(3.0)</td>
<td>(3.0)</td>
<td>(3.0)</td>
<td>(3.0)</td>
</tr>
<tr>
<td>2w</td>
<td>MSC (n=4)</td>
<td>0.3</td>
<td>0.0</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>4w</td>
<td>MSC (n=4)</td>
<td>0.8</td>
<td>1.3</td>
<td>1.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>1.8</td>
<td>1.5</td>
<td>2.0</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>8w</td>
<td>MSC (n=4)</td>
<td>3.0</td>
<td>2.8</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>3.0</td>
<td>2.8</td>
<td>3.0</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>12w</td>
<td>MSC (n=5)</td>
<td>2.2</td>
<td>2.4</td>
<td>2.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>1.2</td>
<td>1.6</td>
<td>1.6</td>
<td>2.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* p<0.05 versus total mean at 2w in the MSC or control group respectively.
fuse into the meniscal defect.

In our study, the matrix filled in the meniscal defect expressed type II collagen in the MSC group at 8 weeks. However, the level of type II collagen in the MSC group did not increase to the relevant level of the adjacent original meniscus. Even at 12 weeks, the structure of the matrix in the defect was different from that of normal rat meniscus, the center of which consists of trabecular bone. In our study, repaired tissue in the synovial MSC group at 12 weeks seemed to be still in the process of healing. Thereafter, the central part of repaired meniscus may have been replaced with bone. We previously transplanted synovial MSCs into the full thickness defect of the articular cartilage in rabbits. Rabbit MSCs differentiated into chondrocytes, then the border between the bone and cartilage was removed, and finally the thickness of regenerated cartilage reached a level similar to that of neighboring cartilage. A cell tracing system confirmed that transplanted MSCs still existed in the regenerated cartilage at 24 weeks.

Histological analysis demonstrated no statistical differences of the score between synovial MSC treated and control groups at each period. In this model, a one mm diameter defect in meniscus might have been too small to detect the effect of synovial MSCs. The defect in the control group was filled with cells and matrix. We propose 3 possible explanations for the origin of these cells. First, cells in the neighborhood of the meniscal defect contribute to the filling in the control group. The outside of the meniscal defect connects to vascularized trabecular bone. This blood supply provides a possible source of cells for filling the defect. Second, synovial membrane can be conducted to the site of meniscus injury and filled to the meniscal defect as previously reported. Third, synovial fluid may contain MSCs and these may mobilize to the meniscal defect. Jones et al. demonstrated MSCs derived from human synovial fluid which were obtained from swollen knee joints of patients suffering from rheumatoid arthritis and osteoarthritis.

When 10^7 synovial MSCs were injected into the knee joint, most cells adhered to synovium at day 1. Macroscopically, we did not observe hypertrophy of synovium (one of the features of synovitis) from day 1 through 12 weeks. However, synovitis will be one of our concerns for our own method. GFP mRNA expression was shown in the synovium, but not in distant organs by RT-PCR suggesting that the distribution of injected MSCs at 24 hours was localized to the site of injection; however, further investigation is required.

In this study, GFP cells were evaluated in unstained sections by fluorescent microscope, not by immunostaining with GFP antibody. To increase specificity of detection for GFP cells, the conditions were strictly controlled. We prepared two groups; the MSC group, in which GFP cells were injected, and the control group, in which GFP cells were not injected. The samples in both groups were processed concurrently, fixed, decalcified with EDTA for only 3 days, followed by gradient replacement with 20% sucrose for 24 hours at 4 °C, then rapidly frozen and cryosectioned. Digital images of both groups were taken with the same exposure times. Exposure times were regulated so that no auto-fluorescence could be observed in the meniscus of the control group or in the undisturbed meniscus of the MSC group.

Synovial cells are known to play crucial roles during the natural course of healing for injury of meniscus. Here, we demonstrated that injected cells adhered to the defect, survived there, and differentiated into cartilage cells.

Acknowledgements

We thank Kenichi Shinomiya, MD, PhD for supporting our studies; Yusuke Sakaguchi, MD, PhD for help with the MSC culture; Miyoko Ojima for expert help with histology; Izumi Nakagawa for excellent technical assistance.

This study is supported in part by grants from “the Japan Society for the Promotion of Science (19591752)” and “Center of Excellence Program for Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone in Tokyo Medical and Dental University” to TM, and “the Japan Society for the Promotion of Science (18591657)” to IS. Recombinant human BMP2 was distributed by Astellas Pharma Inc.

References

5. Jitsuiki J, Ochi M, Ikuta Y. Meniscal repair enhanced by an