Meniscus Regeneration by Syngeneic, Minor Mismatched, and Major Mismatched Transplantation of Synovial Mesenchymal Stem Cells in a Rat Model

Makiko Okuno,[1,2] Takeshi Muneta,[1] Hideyuki Koga,[1] Nobutake Ozeki,[1] Yusuke Nakagawa,[1] Kunikazu Tsuji,[3] Shinichi Yoshiya,[2] Ichiro Sekiya[4,5]

[1]Department of Joint Surgery and Sports Medicine, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Orthopaedic Surgery, Hyogo College of Medicine, Tokyo, Japan, [3]Department of Cartilage Regeneration, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan, [4]Department of Applied Regenerative Medicine, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan, [5]Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Received 13 September 2013; accepted 24 February 2014
Published online 18 March 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.22614

ABSTRACT: We compared the effect of syngeneic and allogeneic transplantation of synovial mesenchymal stem cells (MSCs) for meniscus regeneration in a rat model. Synovium was harvested from the knee joints of three strains of rats. The anterior half of the medial meniscus in both knees of F344 rats was removed and 5 million synovial MSCs derived from F344 (syngeneic transplantation), Lewis (minor mismatched transplantation), and ACI (major mismatched transplantation) were injected into the knee of the F344 rats. At 4 weeks, the area of the regenerated meniscus in the F344 group was significantly larger than that in the ACI group. Histological score was significantly better in the F344 and Lewis groups than in the ACI group at 8 weeks. DiI labeled cells could be observed in the knee joint in the F344 group, but were hardly detected in the ACI group at 1 week. The number of macrophages and CD8 T cells at synovium around the meniscus defect was significantly lower in the F344 group than in the ACI group at 1 week. Syngeneic and minor mismatched transplantation of synovial MSCs promoted meniscus regeneration better than major mismatched transplantation in a rat meniscectmized model. © 2014 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 32:928–936, 2014.

Keywords: mesenchymal stem cells; synovium; meniscus regeneration; syngeneic transplantation; allogeneic transplantation

The meniscus is a fibrocartilage whose functions are to increase surface contact area, to absorb mechanical loads, and to improve stability across the knee joint. Meniscal injury is one of the most common injuries of the knee joint, primarily due to sports injuries or degenerative conditions. For injured menisci, efforts have been made to preserve the meniscus as much as possible to prevent degenerative arthritis. For meniscal defects after meniscectomy or meniscal degeneration, transplantations of meniscal grafts or artificial menisci have been attempted; however, the invasiveness, durability and safety of the transplant remain controversial.[1] Therefore, a novel strategy for meniscus regeneration is required.

Mesenchymal stem cells (MSCs), especially those derived from synovium, are an attractive cell source for meniscus regeneration, because synovial MSCs have remarkable proliferation[2] and chondrogenic potential.[3–5] We previously reported that intraarticular injection of synovial MSCs promoted meniscal regeneration in a rat model.[6] It was a syngeneic transplantation model; therefore, possible immune reactions seem to be negligible.

MSCs have potent anti-inflammatory/immunosuppressive properties.[7] This suggests that allogeneic MSCs can be used therapeutically with equal efficacy to autologous MSCs without triggering the donor specific immune responses. However, this is still controversial, and the opposite results were also reported.[8–10] In regard to synovial MSCs, the influence of allogeneic transplantation has not been investigated at all. In this study, we compared the effect of syngeneic and allogeneic transplantation of synovial MSCs for meniscus regeneration in a rat model. For allogeneic transplantations, major antigen mismatched and minor antigen mismatched MSCs were also prepared.

MATERIALS AND METHODS

Animals

This study was approved by the Animal Experimentation Committee of Tokyo Medical and Dental University. Wild-type male Lewis rats (Charles River, Kanagawa, Japan), F344 rats (Charles River) and ACI rats (Saitama Experimental Animals Supply, Saitama, Japan) at the age of 10–14 weeks were used for the experiments.[11–13]

Preparation of Synovial MSCs

Synovium was obtained from male F344, Lewis, and ACI rats. The synovial membranes of bilateral knee joints were excised, minced, and digested for 3 h at 37°C with type V collagenase (0.2%; Sigma-Aldrich, St. Louis, MO), and passed through a 45-μm filter (Becton Dickinson, Franklin Lakes, NJ). Synovial cells were cultured in αMEM (Invitrogen, Carlsbad, CA) containing 10% FBS (Invitrogen), 100 U/ml penicillin (Invitrogen), 100 μg/ml streptomycin (Invitrogen), and amphotericin B (Invitrogen) for 14 days. Then the cells were harvested and frozen at −80°C as passage 0. The stocked cells were thawed in a water bath at 37°C plated in a 150 cm^2 dish, and harvested after 5 days. Then the cells were replated in 150 cm^2 dishes and harvested in confluent for transplantation.

Grant sponsor: The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.
Correspondence to: Ichiro Sekiya (T: +81-3-5803-4017; F: +81-3-5803-4020; E-mail: sekiya.arm@tmd.ac.jp)

In Vitro Differentiation Assay

For in vitro chondrogenesis, 2.5×10^5 cells were placed in a 15 ml polypropylene tube (BD Falcon, Franklin Lakes, NJ) and pelleted by centrifugation at 450*g* for 10 min. The pellets were cultured for 21 days in chondrogenic media, which contained 1,000 ng/ml BMP-7 (Stryker Biotech, Hopkinton, MA), in addition to high-glucose Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10 ng/ml transforming growth factor-β3 (TGF-β3) (R&D Systems, Minneapolis, MN), 10^{-7} M dexamethasone, 50 μg/ml ascorbate-2-phosphate, 40 μg/ml proline, 100 μg/ml pyruvate, and 50 mg/ml ITS + TMPremix (BD Falcon).[14]

For adipogenesis, the cells were cultured in the adipogenic medium that consisted of a complete medium supplemented with 0.5 μM dexamethasone, 0.5 mM isobutylmethylxanthine (Sigma-Aldrich), and 50 μM indomethacin (Wako, Osaka, Japan). After 14 days, the adipogenic cultures were stained with 0.3% Oil Red-O solution.[15,16]

For calcification, the cells were cultured in the calcification medium in the presence of 100 nM dexamethasone, 10 mM β-glycerophosphate (Wako), and 50 μM ascorbic acid. After 3 weeks, the dishes were stained with 0.5% alizarin red solution.[5]

Meniscectomy and MSC Injection

F344 rats at 10–14 weeks of age were used for recipients. Under anesthesia by isoflurane inhalation and intraperitoneal injection of tribromoethanol, bilateral knee joints were exposed with a straight incision on the anterior side of the knee. After the patellar tendon was dislocated laterally and the anterior insertional ligament of the medial meniscus was cut, the anterior half of the medial meniscus was resected at the level of the medial collateral ligament. The posterior part of the medial meniscus was left remained, and the wound was closed in layers.[17,18] Immediately after the skin incision was closed, a 27-gauge needle was inserted at the center of the triangle formed by the medial side of the patellar ligament, the medial femoral condyle, and the medial tibial condyle, toward the intercondylar space of the femur. Then 5×10^6 synovial MSCs of F344, Lewis, and ACI rats in 50 μl PBS were injected into the right knee joint. For the control, the same volume of PBS was injected into the left knee joint.

Synovial MSCs derived from the Lewis rat are a minor antigen mismatch to the F344 rat, which means their histocompatibility antigens differ only partly. Synovial MSCs derived from the ACI rat are a major antigen mismatch to the F344 rat, which means their histocompatibility antigens differ greatly (Fig. 1).[11–13]

Quantification for Size of Regenerated Menisci

The removed medial menisci were photographed, and their sizes were quantified with Image J software (version 1.43, National Institutes of Health, Bethesda, MD).

Histology

Regenerated meniscus tissue was fixed in 4% paraformaldehyde for 7 days, decalcified in 20% ethylenediaminetetraacetic acid (EDTA) solution for 10 days, then embedded in paraffin wax. The specimens were sectioned in the sagittal plane at 5 μm and stained with safranin-o and fast green, and hematoxylin and eosin. Histological sections were visualized using an Olympus BX 53 microscope (Olympus, Tokyo, Japan). The regenerated meniscus was evaluated using the

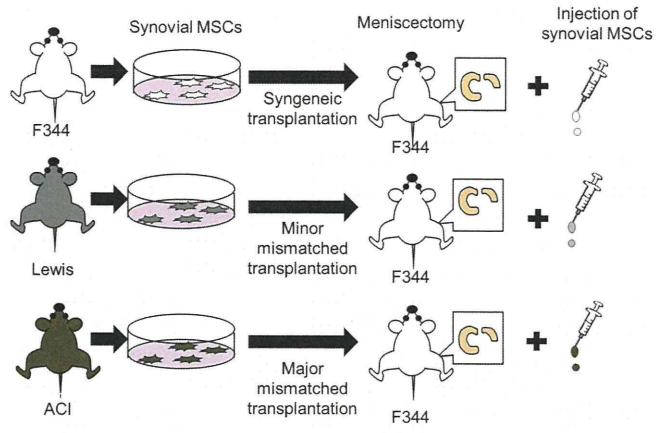

Figure 1. Scheme for this study. Synovium was harvested from the knee joints of three strains of rats: F344, Lewis, and ACI. The anterior half of medial meniscus in both knees of F344 rats was removed and 5 million synovial MSCs of three strains were injected into the right knee of F344 rats. F344 MSC transplantation is regarded as a syngeneic model, Lewis MSC transplantation as a minor mismatched model, and ACI MSC transplantation as a major mismatched model.

Table 1. Modified Pauli's Meniscus Score

I. Morphology
0 Sharp like normal meniscus
1 Moderately sharp
2 Slightly sharp
3 Dull
II. Surface indentation
0 Smooth
1 Slight fibrillation or slightly undulating
2 Moderate fibrillation or markedly undulating
3 Sever fibrillation or disruption
III. Cellularity of Chondrocyte
0 Normal
1 Moderate number of chondrocytes
2 Small number of chondrocytes
3 No chondrocyte detected
IV. Collagen organization/alignment and fiber organization
0 Collagen fibers organized, homogenous eosinophilic staining of collagen ground substance
1 Collagen fibers organized, diffuse foci of hyaline or mucinous degeneration
2 Collagen fibers unorganized, confluent foci or bonds of hyaline or mucinous degeneration, fraying
3 Collagen fibers unorganized, fibrocartilaginous separation (edema, cystic formation), severe fraying and tears
V. Matrix staining (Safranin-O, Fast green)
0 Same as normal, well stained around chondrocytes of inner side of the meniscus
1 Moderate
2 Slightly stained
3 Not stained at all
VI. Collagen type II staining
0 Same as normal, well stained around chondrocytes of inner side of the meniscus
1 Moderate
2 Slightly stained
3 Not stained at all

modified Pauli's score, 0–15 points, with 0 representing normal and 15 representing the worst (Table 1).[18,19]

DiI Labeling

On the day of implantation, 5×10^6 cells were resuspended in α-MEM at 1×10^6 cells/ml, then the fluorescent lipophilic tracer 1,1′-dioctadecyl-3,3,3′,3′-teteramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR) was added at 5 μl/ml in αMEM. After incubation for 20 min at 37°C with 5% humidified CO_2, the cells were centrifuged at 450*g* for 5 min, washed twice with PBS, then resuspended in PBS for the transplantation.[20] DiI labeled MSCs were transplanted into F344 rats at 8–10 weeks of age. Macroscopically, DiI labeled MSCs on the tibial side of the knee joint were evaluated at 1 week.

Immunohistochemistry

Monoclonal antibodies for rabbit anti-type I collagen (1:200, Abcam, Cambridge, England), human anti-type II collagen (1:200, Daiichi Fine Chemical, Toyama, Japan),[18] mouse anti-rat ED1 antibody[21] (1:400, Abcam) as a macrophage marker, and mouse anti-rat CD8 antibody (1:200 Abcam) as a cytotoxic T lymphocyte marker were used. Paraffin-embedded sections were deparaffinized in xylene, rehydrated in graded alcohol, and washed in PBS. All subsequent incubations were performed in a humidified chamber. For type II collagen, the section was pretreated with peroxidase K (Dako, Glostrup, Denmark) in Tris HCl buffer for 15 min at room temperature for optimal antigen retrieval. For ED1 and CD8, the sections were immersed in sodium citrate buffer (Dako), put in 4% paraformaldehyde for 15 min, put in hot water of 95°C for 20 min, then endogenous peroxidases were quenched using 0.3% hydrogen peroxidase in methanol for 15 min. Primary antibodies for type II collagen were applied to sections and incubated at room temperature for 1 h. Primary antibodies for type I collagen, ED1 and CD8 were applied to sections and kept overnight at 4°C.

After extensive washes with PBS, the sections were incubated in the biotinylated goat anti rabbit IgG for type I collagen, in the biotinylated horse anti-mouse IgG for type II collagen, CD8, and ED1 for 30 min at room temperature. Immunostaining was detected with Vectastain ABC regent (Vector, Burlingame, CA) followed by diaminobenzidine staining. The sections were counterstained with hematoxylin.

ED1 and CD8 Expressed Cells Were Evaluated 1 Week After Transplantation

Synovial MSCs (5×10^6) of F344 and ACI rats in 50 μl PBS were injected into the knee joint of F344. After 1 week, the removed whole knees were fixed in 4% paraformaldehyde for 7 days, decalcified in 20% ethylenediaminetetraacetic acid (EDTA) solution for 10 days, then embedded in paraffin wax. The specimens were sectioned in the sagittal plane at 5 μm and stained with ED1 and CD8. The number of ED1 and CD8 positive cells in synovium was counted in high power fields at 400 times using an Olympus BX 53 microscope (Olympus).

Statistical Analysis

The StatView 5.0 program (SAS Institute, Cary, NC) was used and the Kruskal–Wallis test was performed for analyses. p Values <0.05 were considered to be statistically significant. For quantification of ED1 and CD8 cell number, the Mann–Whitney's U-test was performed.

RESULTS

Synovial MSCs From Three Strains of Rats

The colony-forming cells derived from synovium of three strains of rats, F344, Lewis, and ACI, were spindle-shaped (Fig. 2). The colony-forming cells differ-

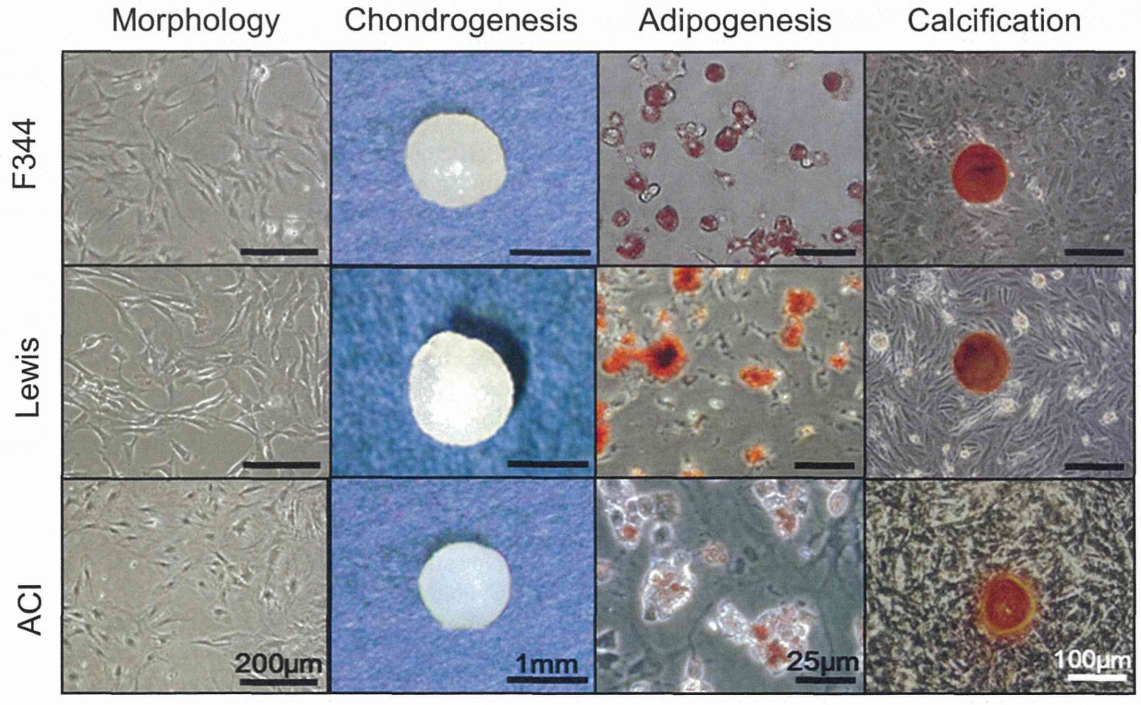

Figure 2. Morphology and multipotentiality of synovial MSCs of three strains of rats. For chondrogenesis, cartilage pellets are shown. For adipogenesis, the cells were stained with oil red-o. For calcification, the cells were stained with alizarin red.

entiated into chondrocytes, adipocytes, and calcified when cultured in the differentiation medium. These findings indicate that colony-forming cells derived from synovium had characteristics of MSCs in three strains of rats.

Macroscopic Analyses for Regenerated Meniscus

At 2 weeks, the area of the regenerated meniscus in each transplantation group appeared larger than that in the control group (Fig. 3A), but there were no significant differences among the transplantation groups (Fig. 3B). At 4 weeks, the area of the regenerated meniscus increased in both the transplantation groups and the control group (Fig. 3A). Quantification analyses demonstrated that the area of the regenerated meniscus in the F344 group was significantly larger than that in the ACI group (Fig. 3B). At 8 weeks, the area of the regenerated meniscus further increased in both the transplantation groups and the control group (Fig. 3A). There were no significant differences among three transplantation groups (Fig. 3B).

Histological Analyses for Regenerated Meniscus

At 2 weeks, the matrix of the regenerated meniscus was better stained with safranin-o in the F344 group than in the Lewis and ACI groups (Fig. 4). At 4 weeks, matrix staining increased and lacuna formation was observed in the F344 group. Matrix staining slightly increased in the Lewis group, while matrix staining remained unchanged in the ACI group. At 8 weeks, collagen fiber became better organized in the F344 group. Matrix stainability increased in the Lewis group but still remained unchanged in the ACI group.

Stainability of type II collagen was already observed at 2 weeks, and increased at 4 weeks and 8 weeks in the F344 and Lewis groups; however, it was hardly detected in the ACI groups until 8 weeks (Fig. 5A). Stainability of type I collagen was detected at the

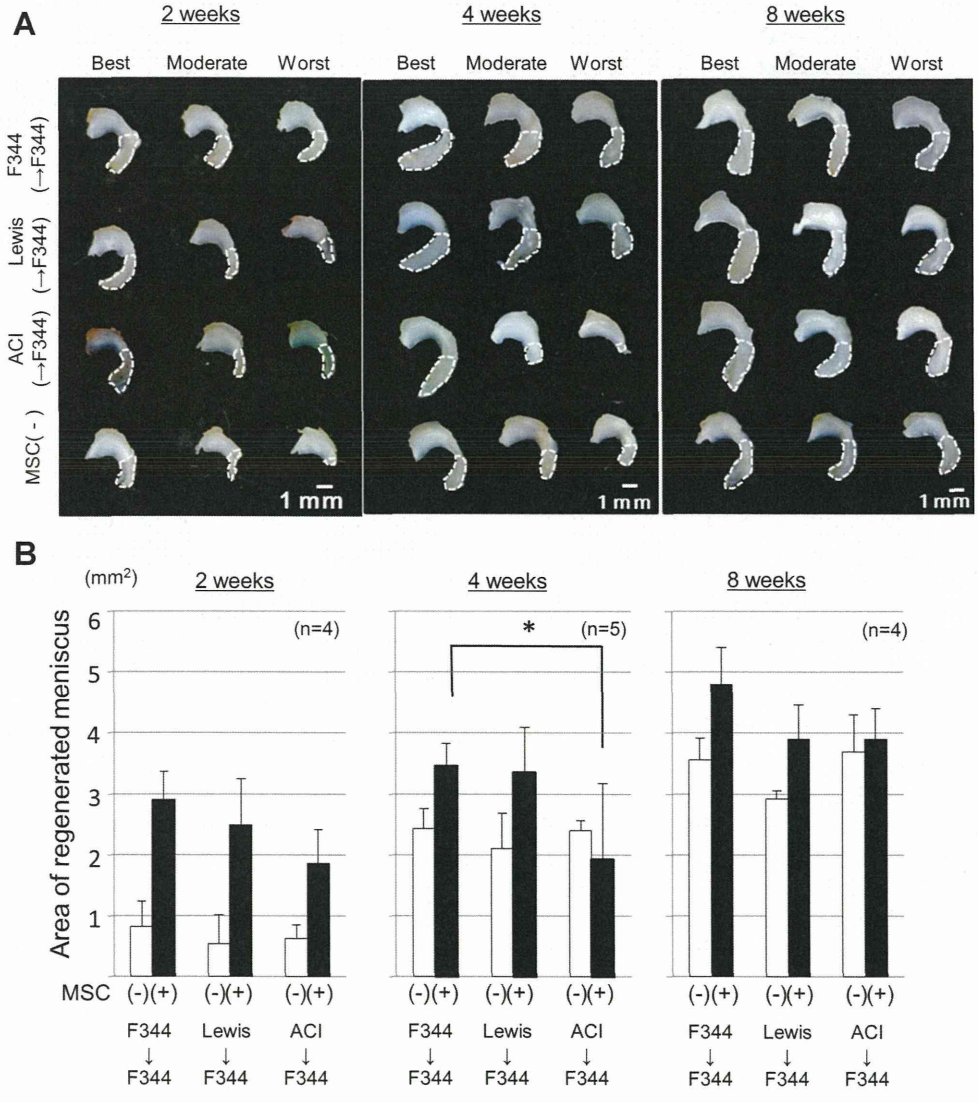

Figure 3. Macroscopic analysis of regenerated menisci at 2 weeks. (A) Whole pictures for medial menisci. Regenerated meniscus is surrounded with dot line. (B) Quantification for area of regenerated medial meniscus. Average values with standard deviation are shown ($p < 0.05$ by Kruskal–Wallis test).

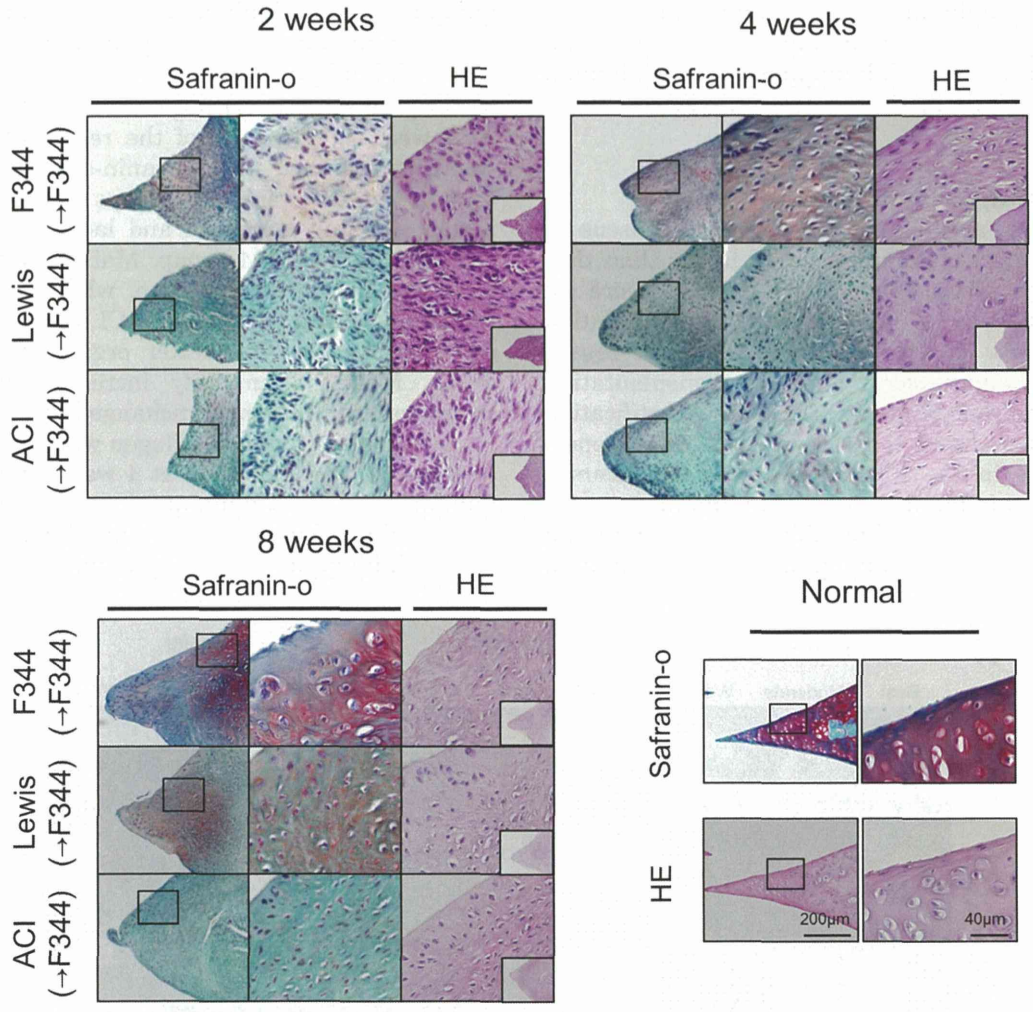

Figure 4. Histological analyses for regenerated and normal menisci. Representative sections stained with safranin-o and hematoxilin and eosin (HE) are shown.

surface of the regenerated meniscus in the F344 group at 8 weeks (Fig. 5A).

The modified Pauli's score (Table 1)[18] was significantly better in the F344 group than in the ACI group at 4 weeks and significantly better in the F344 and Lewis groups than in the ACI group at 8 weeks (Fig. 5B).

Immunological Analyses

One week after DiI labeled MSCs were transplanted into the meniscectmized knee, DiI labeled cells were observed at the tibial side of the knee joint in the F344 group. Contrastingly, DiI labeled cells were hardly detected in the ACI group (Fig. 6). The number of ED1 positive macrophages and CD8 T cells at synovium around the meniscus defect was significantly higher in the ACI group than in the F344 group respectively (Fig. 7).

DISCUSSION

In this study, synovium was obtained from F344, Lewis, and ACI rats for MSC preparation. Then, synovial MSCs derived from those three strains of rats were transplanted into F344 rats. The major histocompatibility complex (MHC) class I genes are comprised of A, Pa, F, B, D, and E loci (Fig. 8).[11–13] The F344 and the Lewis of their major loci in MHC are the same, and those of their MHC-linked loci are different. This means their histocompatibility antigens differ only partly, and it was referred to as minor mismatched transplantation in this study. The F344 and the ACI of their class I major antigen in MHC are different, which means their histocompatibility antigens differ greatly. Here, it was referred to as major mismatched transplantation.

We compared the effect of synovial MSCs on meniscus regeneration by syngeneic, minor mismatched, and major mismatched transplantation in a rat model. The size of the regenerated meniscus in the syngeneic transplantation group was significantly larger than that in the major mismatched group at 4 weeks. The histological score in the syngeneic and minor mismatched transplantation groups was significantly better than that in the major mismatched group at

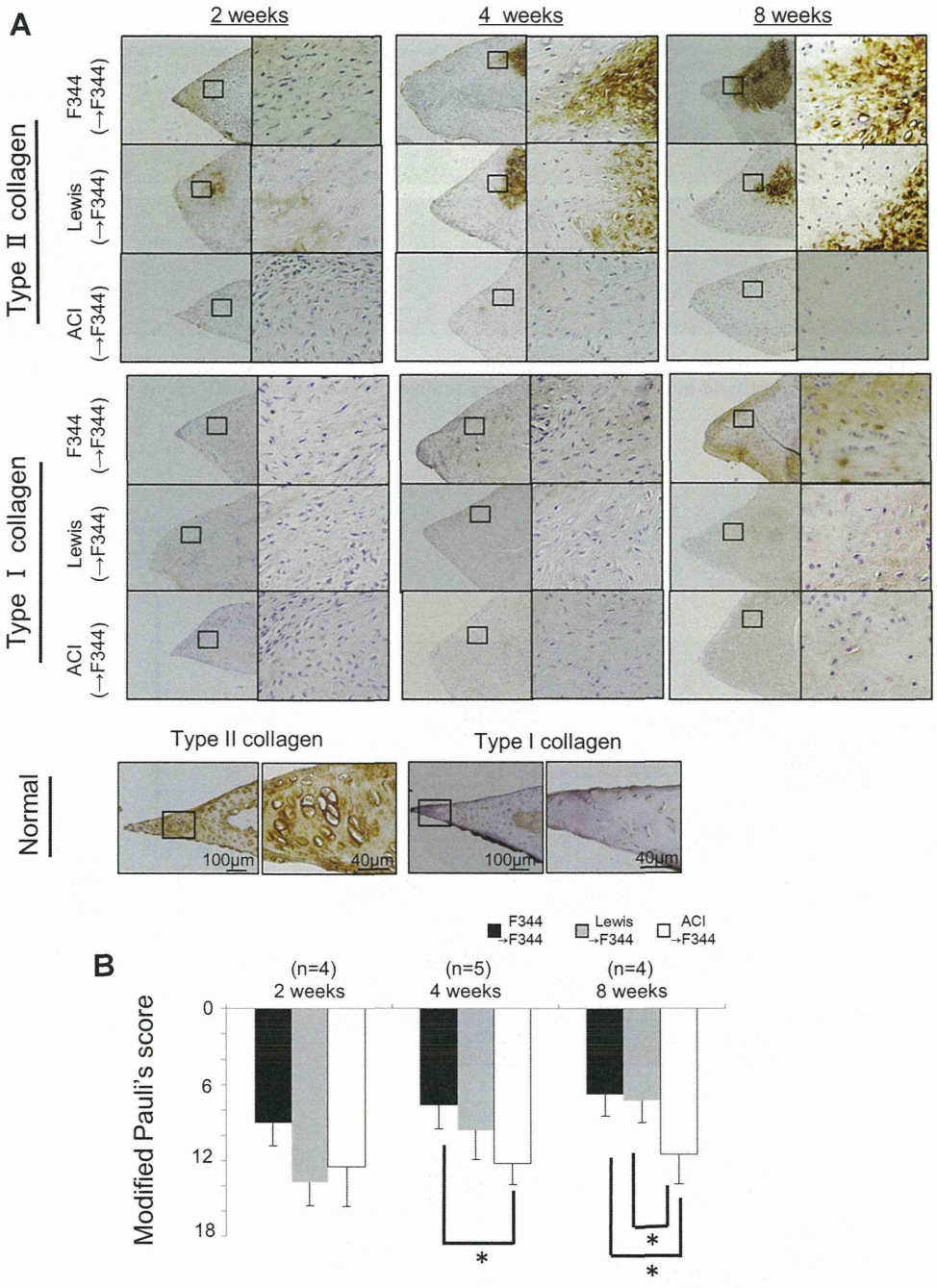

Figure 5. Regenerated meniscus immunostained with type II and type I collagen. (A) Representative sections. (B) Quantification for histology by modified Pauli's score system. Average values with standard deviation are shown ($p < 0.05$ by Kruskal–Wallis test).

8 weeks. The minor mismatched transplantation had a similar effect but the major mismatched transplantation had an inferior effect to the syngeneic transplantation on the meniscus regeneration.

Although the regenerated meniscus in the F344 group was significantly larger than that in the ACI group at 4 weeks, no significant difference was found at 8 weeks. In this study, we used a model in which a spontaneous repair took place. According to our previous study using a similar rat model, the size of the regenerated meniscus was significantly larger in the knee transplanted with syngeneic synovial MSCs than in the knee without transplantation at 2, 4, and 8 weeks, but the effect of syngeneic synovial MSC transplantation was not significant at 12 weeks.[6] In our current study, the size of regenerated meniscus was similar between the transplanted knee and the control knee in the ACI group (Fig. 3B). Spontaneous healing potential would mask the effect of transplantation of synovial MSCs at 8 weeks.

Histologically, the regenerated meniscus was significantly better in the F344 and Lewis groups than in

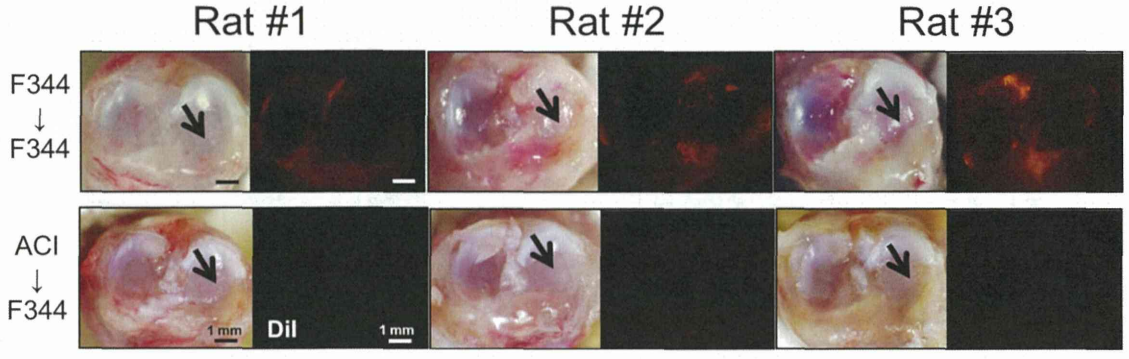

Figure 6. Macroscopic images for DiI labeled MSCs. Tibial side of the knee joint 1 week after DiI labeled MSC transplantation is shown for biright field and fluorescent field in three knees. Meniscectomized area is shown with arrow.

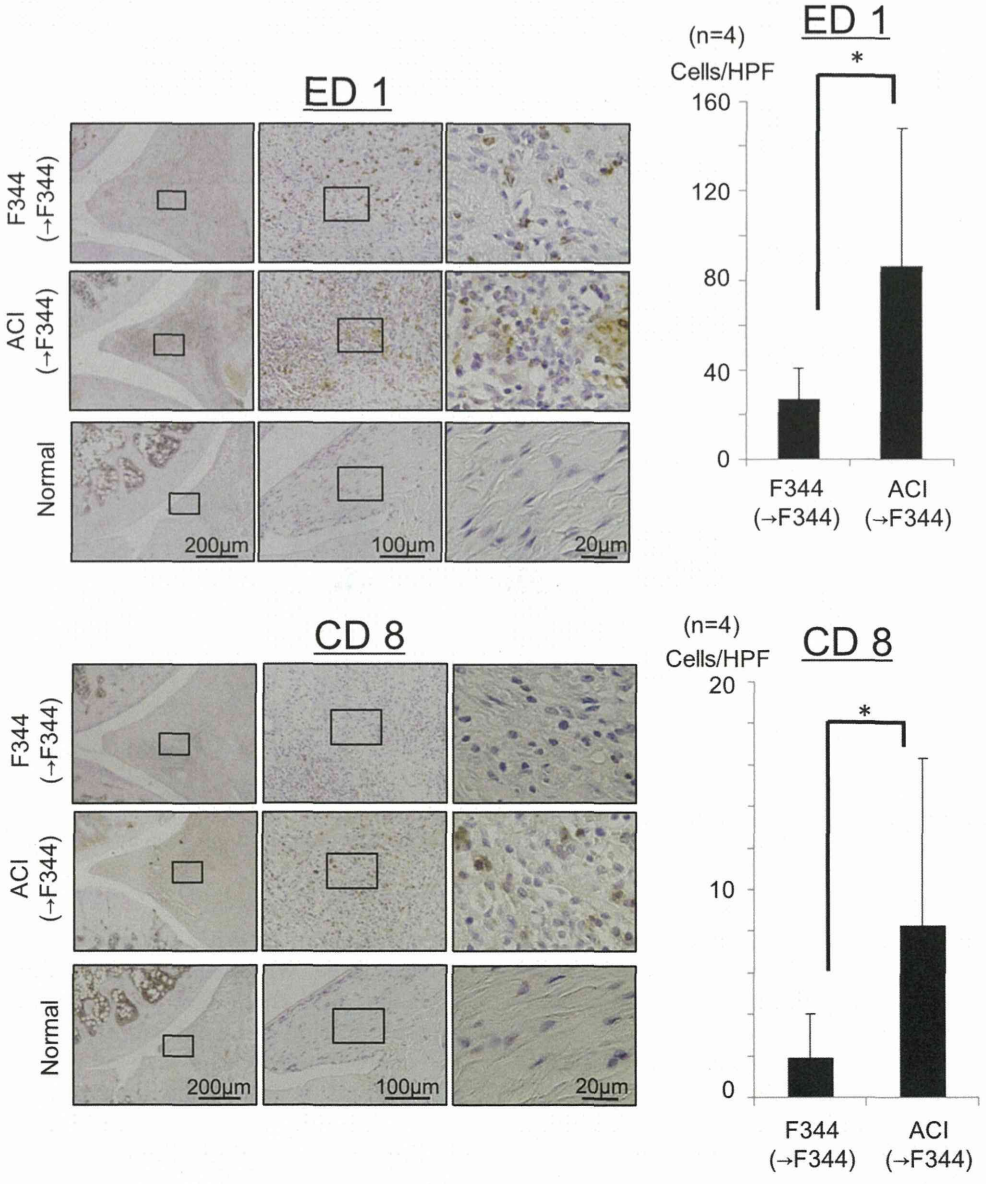

Figure 7. Synovium immunostained with ED1 and CD8. Synovium around the meniscus defect was immunostained 1 week after MSC transplantation. For the normal knee, the same area is shown. Quantitation for ED1 and CD8 positive cell number per a high power field (HPF) is also shown ($n = 4$; $p < 0.05$ by the Mann–Whitney's U-test).

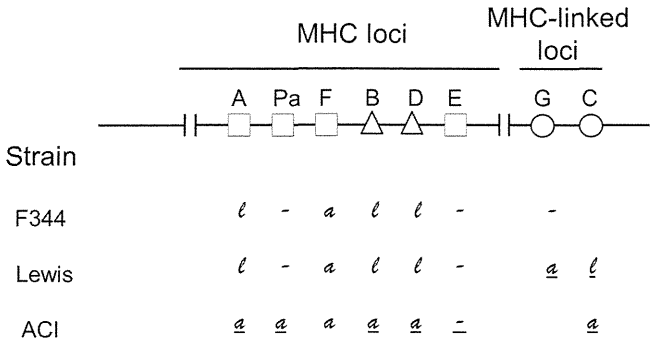

Figure 8. Major histocompatibility complex (MHC) and MHC-linked loci of F344, Lewis, and ACI rats. The square denotes the class I major transplantation antigens; the traiangle, class II antigens; and the circle, the class I medial transplantation antigens. Alleles in Lewis and ACI rats different from those in F344 rats are underlined.[11–13]

the ACI group at 8 weeks (Fig. 5B). However, the regenerated meniscus in the F344 and Lewis groups was different from the normal meniscus from the viewpoints of contour, cellularity, matrix staining, and type II collagen immunostaining. It will take more time for the meniscus to mature by transplantation of synovial MSCs. According to our previous study using a similar rat model, the meniscus further matured 12 weeks after transplantation of syngeneic synovial MSCs.[6]

One week after DiI labeled F344 MSCs were transplanted into the meniscectmized F344 rat knee, DiI labeled cells were observed in the knee joint. On the other hand, DiI labeled cells were hardly detected 1 week after ACI synovial MSCs were transplanted into F344 rat. The number of macrophages and CD8 T cells at synovium around the meniscus defect increased 1 week after ACI synovial MSCs were transplanted into F344 rat. CD8 T cells are known as cytotoxic T lymphocytes. These suggest that ACI synovial MSCs transplanted into the knee joint decreased rapidly through an immunological response.

To create a defect model of the medial meniscus, we removed only the anterior half of the medial meniscus. Though total meniscus resection models may be more popular, they seem to be highly invasive and complicated. In our current model, the medial collateral ligament could be preserved, and we could complete this model with low invasiveness, with relative ease, and with high reproducibility. Though our model mimics limited pathological conditions of meniscal lesions, it was useful to investigate the effectiveness of an unestablished treatment for meniscal regeneration.[6,17,22–24]

The current study is the first report in which comparisons were made between syngeneic and allogeneic transplantation of MSCs for meniscal regeneration. There are some studies in which the effect of autologous (or syngeneic) and allogeneic transplantation of MSCs for bone formation has been investigated in rat models. Kotobuki et al. reported that MSCs derived from Lewis bone marrow and MSCs derived from ACI bone marrow seeded on ceramic scaffolds were rapidly destroyed after those were implanted subcutaneously into F344 rats, though syngeneic MSCs produced bone matrix, in a rat ectopic bone formation model.[10,18] Lopez et al. reported that MSCs derived from Fisher adipose tissue and MSCs derived from ACI adipose tissue promoted posterolateral spinal fusion after those were implanted into Fisher rats, but inflammatory cell infiltration was significantly higher when allogeneic MSCs were transplanted.[10] These suggest that allogeneic MSCs cannot be always used therapeutically with equal efficacy to autologous MSCs without triggering the donor specific immune responses.

In a clinical situation for meniscal regeneration by synovial MSC therapy, autologous transplantation would be theoretically safer than allogeneic transplantation. However, for cost and efficiency, allogeneic transplantation will be attractive and this model is often referred to as "off-the-shelf" stem cell therapy. Our study indicates that if allogeneic synovial MSCs are used, their histocompatibility antigens should be closer to those of the recipient.

In conclusion, syngeneic and minor mismatched transplantation of synovial MSCs promoted meniscus regeneration better than major mismatched transplantation in a meniscectmized rat model. Major mismatched transplantation of MSCs might be refused by immunity.

ACKNOWLEDGEMENTS

This study was supported by "the Project for Realization of Regenerative Medicine" by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. We thank Miyoko Ojima for her help with histology and Izumi Nakagawa for managing our laboratory. Recombinant human BMP-7 was distributed by Stryker Biotech.

REFERENCES

1. Englund M, Roemer FW, Hayashi D, et al. 2012. Meniscus pathology, osteoarthritis and the treatment controversy. Nat Rev Rheumatol 8:412–419.
2. Nimura A, Muneta T, Koga H, et al. 2008. Increased proliferation of human synovial mesenchymal stem cells with autologous human serum: comparisons with bone marrow mesenchymal stem cells and with fetal bovine serum. Arthritis Rheum 58:501–510.
3. Sakaguchi Y, Sekiya I, Yagishita K, et al. 2005. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 52:2521–2529.
4. Koga H, Muneta T, Nagase T, et al. 2008. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res 333:207–215.
5. Yoshimura H, Muneta T, Nimura A, et al. 2007. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell Tissue Res 327:449–462.
6. Horie M, Sekiya I, Muneta T, et al. 2009. Intra-articular injected synovial stem cells differentiate into meniscal cells

directly and promote meniscal regeneration without mobilization to distant organs in rat massive meniscal defect. Stem Cells 27:878–887.
7. Griffin MD, Ryan AE, Alagesan S, et al. 2013. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells: what have we learned so far? Immunol Cell Biol 91: 40–51.
8. Kotobuki N, Katsube Y, Katou Y, et al. 2008. In vivo survival and osteogenic differentiation of allogeneic rat bone marrow mesenchymal stem cells (MSCs). Cell Transplant 17:705–712.
9. Coathup MJ, Kalia P, Konan S, et al. 2013. A comparison of allogeneic and autologous mesenchymal stromal cells and osteoprogenitor cells in augmenting bone formation around massive bone tumor prostheses. J Biomed Mater Res A 101:2210–2218.
10. Lopez MJ, McIntosh KR, Spencer ND, et al. 2009. Acceleration of spinal fusion using syngeneic and allogeneic adult adipose derived stem cells in a rat model. J Orthop Res 27:366–373.
11. Gill TJ III, Kunz HW, Misra DN, et al. 1987. The major histocompatibility complex of the rat. Transplantation 43: 773–785.
12. Gill TJ III, Smith GJ, Wissler RW, et al. 1989. The rat as an experimental animal. Science 245:269–276.
13. Melhem MF, Kunz HW, Gill TJ III. 1993. A major histocompatibility complex-linked locus in the rat critically influences resistance to diethylnitrosamine carcinogenesis. Proc Natl Acad Sci USA 90:1967–1971.
14. Sekiya I, Vuoristo JT, Larson BL, et al. 2002. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. Proc Natl Acad Sci USA 99:4397–4402.
15. Sekiya I, Larson BL, Smith JR, et al. 2002. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells 20:530–541.
16. Sekiya I, Larson BL, Vuoristo JT, et al. 2004. Adipogenic differentiation of human adult stem cells from bone marrow stroma (MSCs). J Bone Miner Res 19:256–264.
17. Katagiri H, Muneta T, Tsuji K, et al. 2013. Transplantation of aggregates of synovial mesenchymal stem cells regenerates meniscus more effectively in a rat massive meniscal defect. Biochem Biophys Res Commun 435: 603–609.
18. Ozeki N, Muneta T, Koga H, et al. 2013. Transplantation of Achilles tendon treated with bone morphogenetic protein 7 promotes meniscus regeneration in a rat model of massive meniscal defect. Arthritis Rheum 65: 2876–2886.
19. Pauli C, Whiteside R, Heras FL, et al. 2012. Comparison of cartilage histopathology assessment systems on human knee joints at all stages of osteoarthritis development. Osteoarthritis Cartilage 20:476–485.
20. Koga H, Muneta T, Ju YJ, et al. 2007. Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. Stem Cells 25:689–696.
21. Iyoda M, Shibata T, Kawaguchi M, et al. 2009. Preventive and therapeutic effects of imatinib in Wistar-Kyoto rats with anti-glomerular basement membrane glomerulonephritis. Kidney Int 75:1060–1070.
22. Horie M, Driscoll MD, Sampson HW, et al. 2012. Implantation of allogenic synovial stem cells promotes meniscal regeneration in a rabbit meniscal defect model. J Bone Joint Surg Am 94:701–712.
23. Horie M, Choi H, Lee RH, et al. 2012. Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. Osteoarthritis Cartilage 20:1197–1207.
24. Hatsushika D, Muneta T, Horie M, et al. 2013. Intraarticular injection of synovial stem cells promotes meniscal regeneration in a rabbit massive meniscal defect model. J Orthop Res 31:1354–1359.

Osteoarthritis and Cartilage xxx (2015) 1–11

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Synovial mesenchymal stem cells promote healing after meniscal repair in microminipigs[1]

Y. Nakagawa †, T. Muneta †, S. Kondo †, M. Mizuno ‡, K. Takakuda §, S. Ichinose ||, T. Tabuchi ¶, H. Koga †, K. Tsuji †, I. Sekiya ‡ *

† *Department of Joint Surgery and Sports Medicine, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan*
‡ *Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan*
§ *Department of Biodesign, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan*
|| *Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan*
¶ *Yaesu Clinic, Tokyo, Japan*

ARTICLE INFO

Article history:
Received 23 September 2014
Accepted 5 February 2015

Keywords:
Meniscus repair
Meniscus injury
Mesenchymal stem cells
Synovium
Pig

SUMMARY

Objective: The induction of synovial tissue to the meniscal lesion is crucial for meniscal healing. Synovial Mesenchymal stem cells (MSCs) are an attractive cell source because of their high proliferative and chondrogenic potentials. We examined whether transplantation of synovial MSCs promoted healing after meniscal repair of extended longitudinal tear of avascular area in a microminipig model.

Design: Longitudinal tear lesion was made in medial menisci and sutured in both knees, and then a synovial MSC suspension was administered for 10 min only in unilateral knee. The sutured meniscus was evaluated morphologically and biomechanically at 2, 4, and 12 weeks. The behavior of transplanted MSCs was also examined.

Results: The meniscal healing at 12 weeks was significantly better in the MSC group than in the control group; macroscopically, histologically and by T1rho mapping analysis. Transmission electron microscopic analysis demonstrated that the meniscus lesion was occupied by dense collagen fibrils only in the MSC group. Biomechanical analysis revealed that the tensile strength to failure of the meniscus higher in the MSC group than in the control group in each microminipig. Synovial tissue covered better along the superficial layer from the outer zone into the lesion of the meniscus in the MSC group at 2 and 4 weeks in each microminipig. Synovial MSCs labeled with ferucarbotran were detected in the meniscus lesion and adjacent synovium by MRI at 2 weeks.

Conclusion: Transplantation of synovial MSCs promoted healing after meniscal repair with induction of synovium into the longitudinal tear in the avascular zone of meniscus in pigs.

Introduction

Meniscus injuries are a common cause of knee dysfunction, and surgical treatment for meniscus injury is one of the most frequent orthopedic operative procedures[1]. Since a meniscectomy increases the risk of osteoarthritis[2,3], a meniscal suture repair is recommended as a first choice surgery for meniscus tear. However, meniscal suture repair is generally limited only to the vascular area and even for the vascular area, failure rate after suture repair is about 30%[4,5]. New procedures to widen the indication for meniscal suture repair, to improve outcomes, are required.

The induction of synovial tissue to the meniscal lesion will be crucial for meniscal healing. Indeed, King reported approximately

[1] This study was supported by the Highway Program for Realization of Regenerative Medicine from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan Science and Technology Agency (JST) and a grant-in-aid for Research on Regenerative Medicine for Clinical Application from the Ministry of Health, Labor and Welfare (MHLW).

* Address correspondence and reprint requests to: I. Sekiya, Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. Tel: 81-3-5803-4017; Fax: 81-3-5803-0170.

E-mail addresses: ynakagawa.orj@tmd.ac.jp (Y. Nakagawa), muneta.orj@tmd.ac.jp (T. Muneta), kondo.orj@tmd.ac.jp (S. Kondo), mizuno.arm@tmd.ac.jp (M. Mizuno), takakuda.mech@tmd.ac.jp (K. Takakuda), ichinose.bioa@tmd.ac.jp (S. Ichinose), t.tabuchi@adic.or.jp (T. Tabuchi), koga.orj@tmd.ac.jp (H. Koga), tsuji.orj@tmd.ac.jp (K. Tsuji), sekiya.arm@tmd.ac.jp (I. Sekiya).

http://dx.doi.org/10.1016/j.joca.2015.02.008