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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*

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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.
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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

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* 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).

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80 years ago that meniscus lesions did not heal unless they communicated with the synovium and capsule[6]. Unfortunately, the reparative potential of synovium for meniscus lesion is limited, and therefore, spontaneous repair of the injured meniscus in the avascular zone cannot be usually expected. To promote this process, several methods are attempted such as supplementation with synovial flaps[7], synovial grafts[8] and induction of synovium by rasping[9]. However, these techniques are not common practice at present due to the difficulty in performing the procedures and lack of efficacious outcomes. Novel applications using synovium are expected to be developed to improve the outcome of meniscus repair.

Mesenchymal stem cells (MSCs), which can be isolated from synovium[10], are attractive for meniscus repair because synovial MSCs have a high proliferative and chondrogenic potential[11–14]. Intraarticular injection of synovial MSCs promoted meniscus regeneration after the anterior half of the medial meniscus was resected in rats[15], rabbits[16], and pigs[17]. MSCs in the synovial fluid of the knee increased in number after meniscus injury[18] and characteristics of MSCs in synovial fluid were close to synovial MSCs[19,20], indicating a physiological role of synovial MSCs during meniscal healing in the natural course. In this study, we examined whether transplantation of synovial MSCs promoted healing after meniscal repair in a pig model that was closer to human clinical pathology than the anterior half meniscal defect model we previously examined. This method broaden its indication for meniscal suture repair with promising outcomes.

Materials and methods

Animals

All experiments were conducted in accordance with the institutional guidelines for the care and use of experimental animals at Tokyo Medical and Dental University. Twelve-month-old, skeletally mature microminipigs[21] (Fujimicra Corp, Shizuoka, Japan) were used. The average weight was 13 kg. Synovial MSCs from three microminipigs were prepared and their colony forming abilities were examined. Synovial MSCs with highest colony forming ability were chosen as donor MSCs. One transgenic microminipig ubiquitously expressing green fluorescent protein (GFP) was also used to prepare GFP+ synovial MSCs for cell tracking. This GFP+ microminipig was developed by hybridizing GFP transgenic Jinhua pigs[22] and wild type microminipigs. Seventeen microminipigs were used as recipients but two were excluded because of postoperative complications; death just after surgery and wound infection in the control side at 2 weeks. Therefore, fifteen microminipigs were analyzed; four microminipigs were evaluated at 2 weeks after transplantation, four microminipigs at 4 weeks, five microminipigs at 12 weeks, and two microminipigs for GFP observation.

Cell isolation and culture

Synovium harvested through an arthrotomy of the knee was digested with 3 mg/ml Collagenase D (Roche Diagnostics, Mannheim, Germany) for 3 h, and cultured at a clonal density in complete culture medium (CCM): αMEM (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum for 14 days[23].

Characterization experiments

To characterize cells derived from the synovium of microminipigs as MSCs, we performed colony forming unit assays, *in vitro* differentiation assays, and examined surface markers, as described previously[23–28].

Surgical procedures

Under general anesthesia, both knees were operated on at the same time, in each animal. One knee was assigned to the MSC group and the other to the control group. Each knee was approached through a medial parapatellar arthrotomy. To expose a whole medial meniscus, the medial collateral ligament was cut and the knee was maximally flexed and externally rotated. A full-thickness longitudinal tear was created in the junction between the internal third and middle third of the meniscus in the avascular area from the anterior to the posterior portion with use of a 1.0 mm diameter biopsy punch (Kai Corp, Tokyo, Japan). The tear was repaired by mattress suture using 4-0 nylon (Bear Medical Corp, Chiba, Japan). For transplantation of synovial MSCs, with the tibial joint surface facing upward, 2×10^7 synovial MSCs at passage two suspended in 100 μl of glucose-added, acetic acid maintenance infusion solution (Veen-3G inj, Kowa Corp, Tokyo, Japan) were placed directly into the meniscal lesion with use of a 23-gauge needle. Knees were then held stationary for 10 min. In the control knees, the same volume of solution without cells was placed as a control, and the knees were once again held stationary for 10 min. The medial collateral ligament, capsule, and skin were closed in layers with non-absorbable sutures. The microminipigs were allowed to move freely in their cages without any fixation method.

Macroscopic observation

The medial menisci were photographed using an Olympus MVX10 stereoscopic microscope (Olympus Corp, Tokyo, Japan). The macroscopic features were scored on a modified scoring system based on that developed by Ruiz-Iban *et al*[29]. The lesion was divided into anterior, middle, and posterior parts. The score was calculated by summing up the scores from 0 to 3 of each of the three parts. Consequently, the final scores were between 0 and 9 points (Supplemental Table 1).

Histological analysis

Sections at 5 μm were stained with toluidine blue, picrosirius red (Picrosirius red Stain Kit, Polysciences, Inc., Warrington, PA), or hematoxylin & eosin (HE). Histological pictures were taken by light microscopy (Olympus IX71). The histology was scored on a modified scoring system based on that developed by Zellner *et al*[30]. The repair was graded by summing up the scores from 0 to 3 of 6 individual subgroups. Consequently, the final scores were between 0 points (no repair) and maximal 18 points (complete reconstitution of the meniscus) (Supplemental Table 2). To quantify the induction of synovial tissue to the lesion, an original score was used (Supplemental Table 3). These scores were evaluated by two independent observers in a blinded manner. Picrosirius red stained sections were observed with a polarizing microscope (Olympus BX51). To evaluate picrosirius red positive areas in the lesion, bright diffracted light areas were measured using Image J software (National Institutes of Health, Bethesda, MD)[31].

Immunohistochemistry

Type I and II collagen was visualized using diaminobenzidine staining. For type II collagen, a rabbit polyclonal antibody (ab34172, Abcam) and for type I collagen, a mouse monoclonal antibody (ab90395, Abcam, Cambridge, UK) was used[17]. For Ki67, a rabbit polyclonal antibody (NB500-170, Novus Biologicals, Littleton, CO), and species-specific AlexaFluor-555 conjugated secondary antibody were used.

Transmission electron microscopy

The specimens were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer for 2 h. The samples were washed with 0.1M phosphate buffer, post-fixed in 1% OsO4 buffered with 0.1M phosphate buffer for 2 h, 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, Tokyo, Japan)[32].

MRI for T1rho mapping

After sacrifice, the knee joints were removed and examined by MRI 3.0 T imager (Achieva; Philips Medical Systems, Andover, MA). The knee joint was set up in the coils for the wrist MRI (Phillips Medical System). The sagittal T1rho mapping was analyzed with Ziostation2 software (Ziosoft, Inc., Tokyo, Japan) for the T1rho mapping. The region of interest was set manually. Parameters are listed in Supplemental Table 4.

Biomechanical analysis

After removal of suture threads, the meniscus was divided into three parts; an anterior, middle and posterior part. The anterior and posterior parts were used for the biomechanical analysis, and the middle part was used for histological analysis. The specimen was set on a mechanical testing frame (Autograph, Shimadzu Corp, Kyoto, Japan). One edge of the meniscus was held tightly with a vice grip (SC-3, Imada Corp, Aichi, Japan) and the other edge was held with a small chuck (PGC, Imada Corp). The specimen was then split across the repair tissue with a defined crosshead speed of 1 mm/min. The force needed to separate the meniscus in the area of the repair was recorded. Then, the cross-sectional area of the repaired tissue was measured using a stereoscopic microscope (Olympus MVX10), and the tensile strength at failure was calculated. The average value for failure strength of the anterior and posterior parts was calculated. Intact meniscus was also examined.

Cell tracking for GFP+ MSCs

To examine whether transplanted cells adhered to the meniscus lesion, GFP+ microminipig synovial MSCs were transplanted into two knee joints of two microminipigs. After 1×10^7 GFP+ MSCs at passage three were transplanted and the skin was closed, the knee was flexed and extended 100 times, and then the meniscus with tibial plateau was photographed using a stereoscopic microscope (Olympus MVX10).

Cell tracking for synovial MSCs labeled with ferucarbotran by MRI

For this assay only, synovial MSCs at passage two were cultured with ferucarbotran (Resovist, Bayer Schering Pharma, Leverkusen, Germany) which is a superparamagnetic iron oxide, at a final concentration of 100 μg/mL for 1 day. Two weeks after transplantation, the knee joints were examined by MRI 3.0 T imager (Achieva). Parameters are listed in Supplemental Table 4. Only these two pigs received ferucarbotran MSCs.

Statistical methods

Comparisons between the MSC group and the control group were analyzed using the Wilcoxon's signed rank test. *P* value was calculated, except cases in which sample number was less than five. *P* value of <0.05 was considered statistically significant.

Additional methods

Detailed methods for characterization of cells and immunohistochemistry are described in Supplemental Methods.

Results

Synovial cells from a microminipig had characteristics of MSCs

Cells derived from the synovium of microminipigs were colony-forming (Fig. 1A). These cells differentiated into chondrocytes, adipocytes and mineralized tissue (Fig. 1A). Flow cytometric analysis demonstrated that the majority of these cells expressed CD90, CD44, and CD105, and were negative for CD11b, and CD45 (Fig. 1B).

Transplanted synovial MSCs promoted healing after meniscal repair

For this procedure, a longitudinal tear was created in the avascular area, then a suspension containing synovial MSCs was placed on the sutured meniscus (Fig. 1C). Menisci were graded on the progress of repair in three parts (anterior, middle, and posterior) by macroscopic examination (Fig. 2A). At 2 weeks, the MSC group had undergone partial repair of at least two parts and partial repair of three parts in one case (Fig. 2B). In the control group, menisci were partially repaired in only one part, with the exception of one case in which no healing was observed at all. In the MSC group at 4 weeks, complete healing at two or three parts was observed in three cases, with at least partial repair in all parts in all cases. In the control group, menisci repair was much less robust in all cases, with no healing at all for two parts observed in two cases. In the MSC group at 12 weeks, complete healing at three parts was observed in the best case, and partial healing at three parts was observed in the worst case. In the control group, no healing in at least one part was still observed in two cases. Macro scores for meniscus healing (Supplemental Table 1) were higher in the MSC group than in the control group in each microminipig at 2, 4 and 12 weeks (Fig. 2B).

Histologically, in the MSC group at 2 weeks, the lesion was partially connected in all cases (Fig. 3A). The lesions were filled with a large number of polygonal, spindle shape cells, or acellular matrix. In the control group, the lesion was partially connected in only one case. In the MSCs group at 4 weeks, the lesion appeared to be connected more tightly as in the representative case. In the control group, the lesion was still connected partially in all cases. At 12 weeks, in the MSC group, the lesion appeared to be fully connected and/or healed completely even in the worst case. The lesion also became closer to the adjacent normal meniscus in terms of metachromasia and cell morphology. In the control group, the lesion was still connected partially in all cases. Histological scores for meniscus healing (Supplemental Table 2) were higher in the MSC group than in the control group in each microminipig at 2, 4 and 12 weeks (Fig. 3B). At 12 weeks, the MSC group expressed more type II collagen, both in punctuate small foci and dispersed throughout. Type I Collagen was diffusely present in both MSC and control groups (Fig. 3C). To examine collagen fiber volume and organization in the lesion, sections were stained with picrosirius red and observed under polarized microscopy (Fig. 3D). Though the picrosirius red positive area in the lesion increased with time in both groups, the picrosirius red positive area ratio in the lesion was higher in the MSC group than in the control group in each microminipig at 2, 4, and 12 weeks (Fig. 3E).

Transmission electron microscopic analysis demonstrated that the lesion in the MSC group at 12 weeks was occupied by dense collagen fibrils with smaller diameter than those observed in an intact meniscus (Fig. 3F). Contrarily, in the control group, organized collagen fibrils were not observed in the lesion.

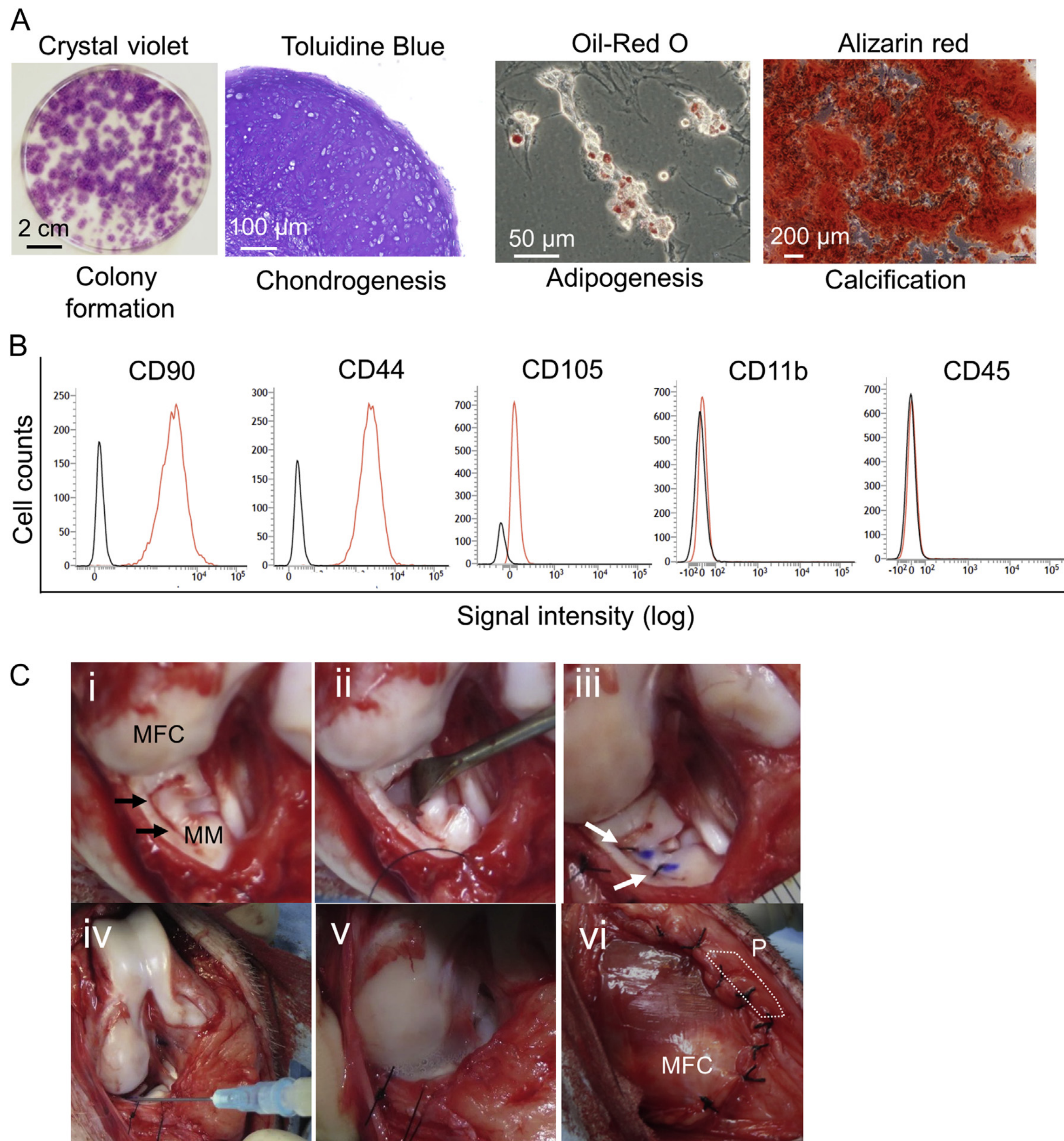

Fig. 1. Characteristics of cells from microminipig synovium and experimental set up. (A) Representative images for colony formation and multilineage differentiation of synovial MSCs. (B) Epitope profile. (C) Procedure. (i) After exposing the medial meniscus, a longitudinal tear (indicated with black arrows) was created from the anterior to posterior segment using a skin biopsy punch with a diameter of 1 mm (ii) Instability of the meniscus was confirmed. (iii) Meniscus was sutured with nylon thread (indicated with white arrows). (iv) Synovial MSC suspension was placed on the sutured meniscus. (v) The sutured meniscus was immersed in a synovial MSC suspension for 10 min (vi) The anterior and medial capsule was closed. MM, medial meniscus; MFC, medial femoral condyle; P, patella.

Images from T1rho mapping showed that T1rho values at the inner area of the lesion in the control group at 12 weeks were higher than in the MSC group (Fig. 3G). T1rho values in the MSC group were significantly lower than in the control group, showing T1rho values in the MSC group were closer to that of the intact meniscus than in the control group (Fig. 3H).

Synovial MSCs promoted induction and formation of synovial tissue in the lesion

We also evaluated the induction of synovial tissue into the meniscus lesion. In the MSC group, synovial tissue already covered the lesion along the superficial layer of the meniscus from the outer zone down into the lesion in the inner zone at 2 weeks, covered an even wider area around the lesion at 4 weeks, and increased coverage even further at 12 weeks (Fig. 4A). Contrarily, in the control group, synovial tissue did not reach the lesion at 2 and 4 weeks, until finally reaching at 12 weeks. Synovial coverage scores for meniscus healing (Supplemental Table 3) were higher in the MSC group than in the control group in each microminipig at 2 and 4 weeks (Fig. 4B). In the MSC group, the lesion was still covered with synovium but the synovial cell layer decreased at 12 weeks.

To analyze proliferation of the cells in the synovial tissue that were induced into the meniscal lesion, the expression of Ki-67, a proliferation marker, was examined. At 4 weeks, there were many Ki-67 positive cells in the MSC group, but only a few in the control group (Fig. 4C).

Transmission electron microscopic observation demonstrated a great number of spindle-shaped or polygonal cells around the lesion at the superficial layer in the MSC group at 4 weeks. This

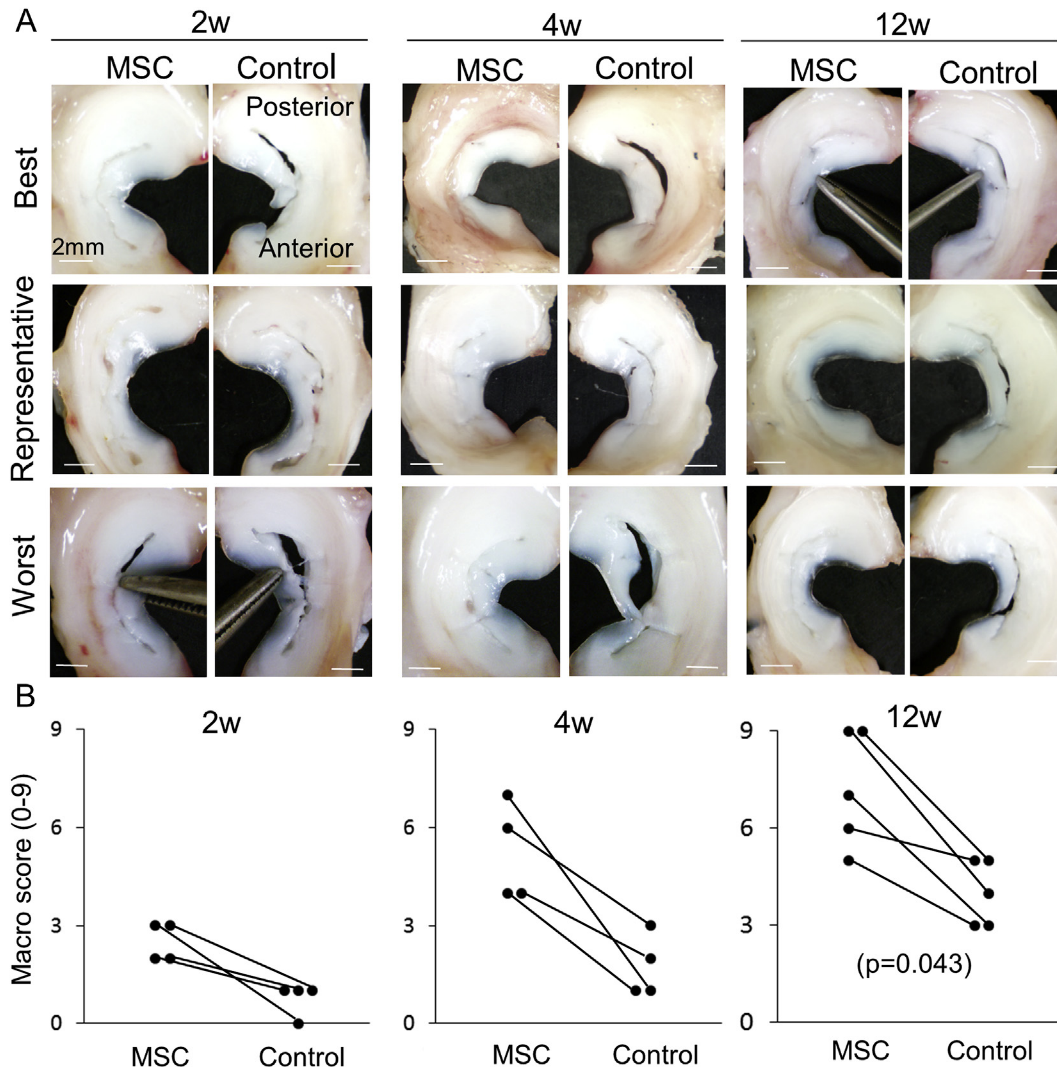

Fig. 2. Macroscopic analysis. (A) Macroscopic features. To remove individual variability, the menisci in the MSC and control groups of the same pig are shown in the same row. For "Best", "Representative", and "Worst", each case in the MSC group was selected. In some pictures, the forceps were used to pull at the inner side of the meniscus in order to display the lesion. (B) Quantification of macroscopic findings evaluated by modified Miguel's scoring system[29] (Supplemental Table 1). *P* value was calculated by Wilcoxon's signed rank test except cases in which sample number was less than five.

image was similar to that observed in an intact synovium (Fig. 4D). Contrarily, only a few cells were observed around the lesion in the control group.

Synovial MSCs increased tensile strength at failure of the meniscus

For biomechanical analysis (Fig. 5A), the average tensile strength at failure was 10.5 N/mm^2 in the MSC group, 4.6 N/mm^2 in the control group, and 20.8 N/mm^2 in the intact meniscus. The failure occurred where the lesion was first made in all cases. The tensile strength at failure of the meniscus was higher in the MSC group than in the control group in each microminipig (Fig. 5B).

Transplanted synovial MSCs located around the lesion and adjacent synovium

GFP+ cells were observed in the lesion and synovium around the lesion 10 min after synovial MSC suspension was added to the lesion. This finding was observed in each of the two microminipigs tested (Fig. 6A). Most synovial MSCs took up particles of ferucarbotran 24 h after incubation (Fig. 6B). Ferucarbotran can be visualized as scattered black dots in MRI. Two weeks after the transplantation of synovial MSCs, particles of ferucarbotran were observed around the lesions and anterior synovium (Fig. 6C).

Discussion

We were able to demonstrate that both macro and histological scores for meniscus healing were higher in the MSC group than in the control group in a longitudinal meniscal repair model in each microminipig. T1rho values in the MSC group were closer to that of intact meniscus than in the control group. The tensile strength at failure of the meniscus was higher in the MSC group than in the control group in each microminipig at 12 weeks. Transplantation of synovial MSCs was able to promote healing of repaired meniscus.

MRI provides a useful tool to examine healing after meniscal repair. In this study, we employed T1rho mapping to evaluate the quality of the repaired meniscus. In articular cartilage, T1rho

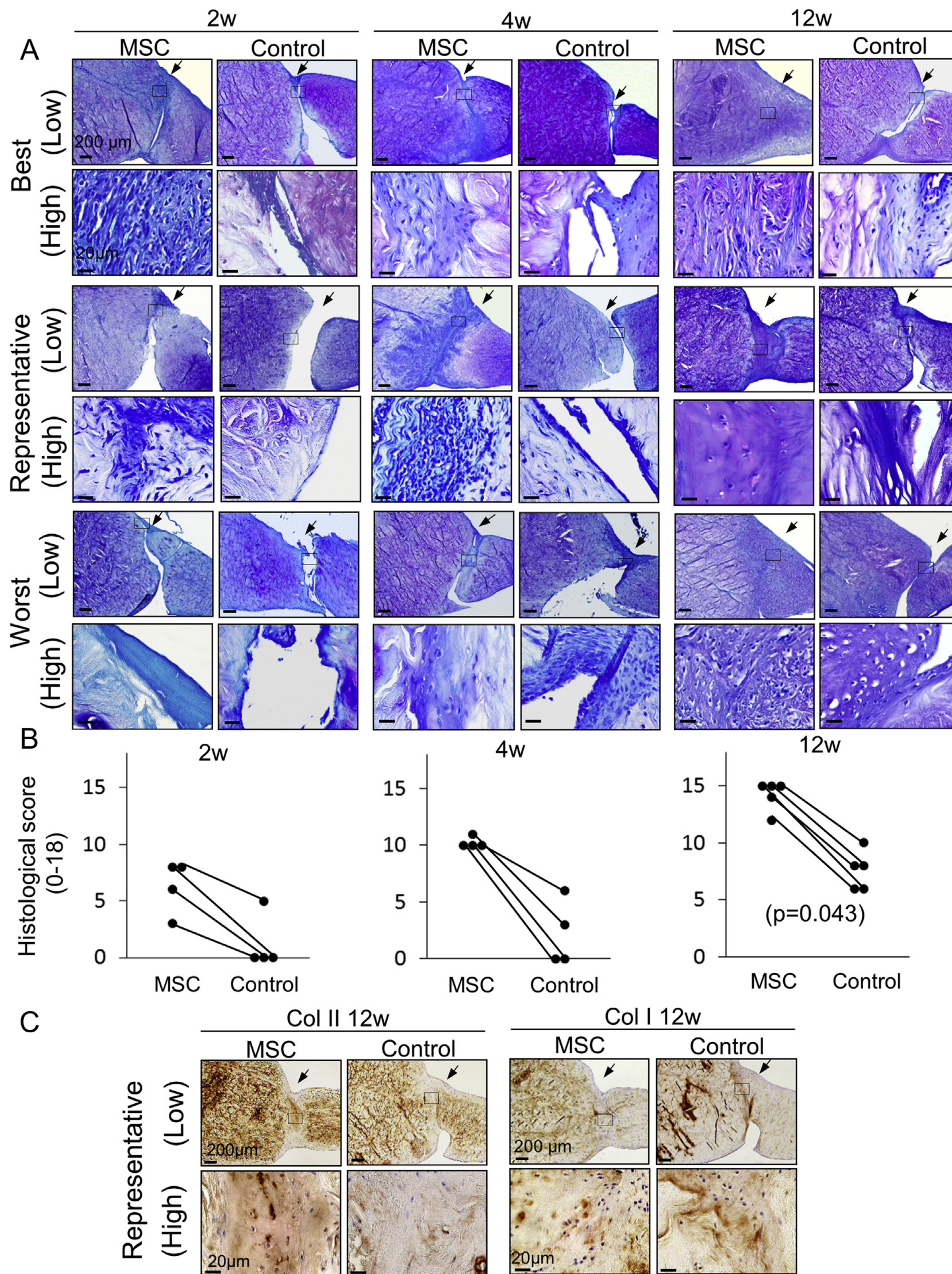

Fig. 3. Histological analysis. (A) Histological sections stained with toluidine blue for the meniscal lesions. Lesions are indicated with black arrows. For "Best", "Representative", and "Worst", each case in the MSC group was selected. (B) Quantification of histology evaluated by modified Zeller's scoring system[30] (Supplemental Table 2). *P* value was calculated by Wilcoxon's signed rank test. (C) Histological sections immunostained with type II and type I collagen. The lesions are indicated with arrows. (D) Histological sections stained with picrosirius red under polarized microscope. The lesions are indicated with arrows. Borders between the lesion and the normal area are indicated with dotted line. (E) Quantification of picrosirius red positive area ratio in the lesion. *P* value was calculated by Wilcoxon's signed rank test. (F) Transmission electron microscopic images of collagen fiber architecture in the lesions both in the MSC group and the control group at 4 weeks, and the same area in an intact meniscus. (G) Representative T1rho mapping images with color bar. Lesions are indicated with white arrows. (H) T1rho values of injured medial meniscus and intact meniscus. *P* value was calculated by Wilcoxon's signed rank test.

mapping has been shown to evaluate glycosaminoglycan (GAG) concentration[33].

Transplanted allogeneic MSCs could provide two mechanisms for meniscal healing. Firstly, MSCs have the differentiation potential to go to meniscal cells. According to our previous study, LacZ+ synovial MSCs could be observed in the regenerated meniscus 12 weeks after transplantation in a rat model[15]. Secondly, MSCs produce a wide variety of cytokines and other trophic support factors. Our species specific microarray analyses showed that synovial MSCs increased the gene expression of hundreds of genes including

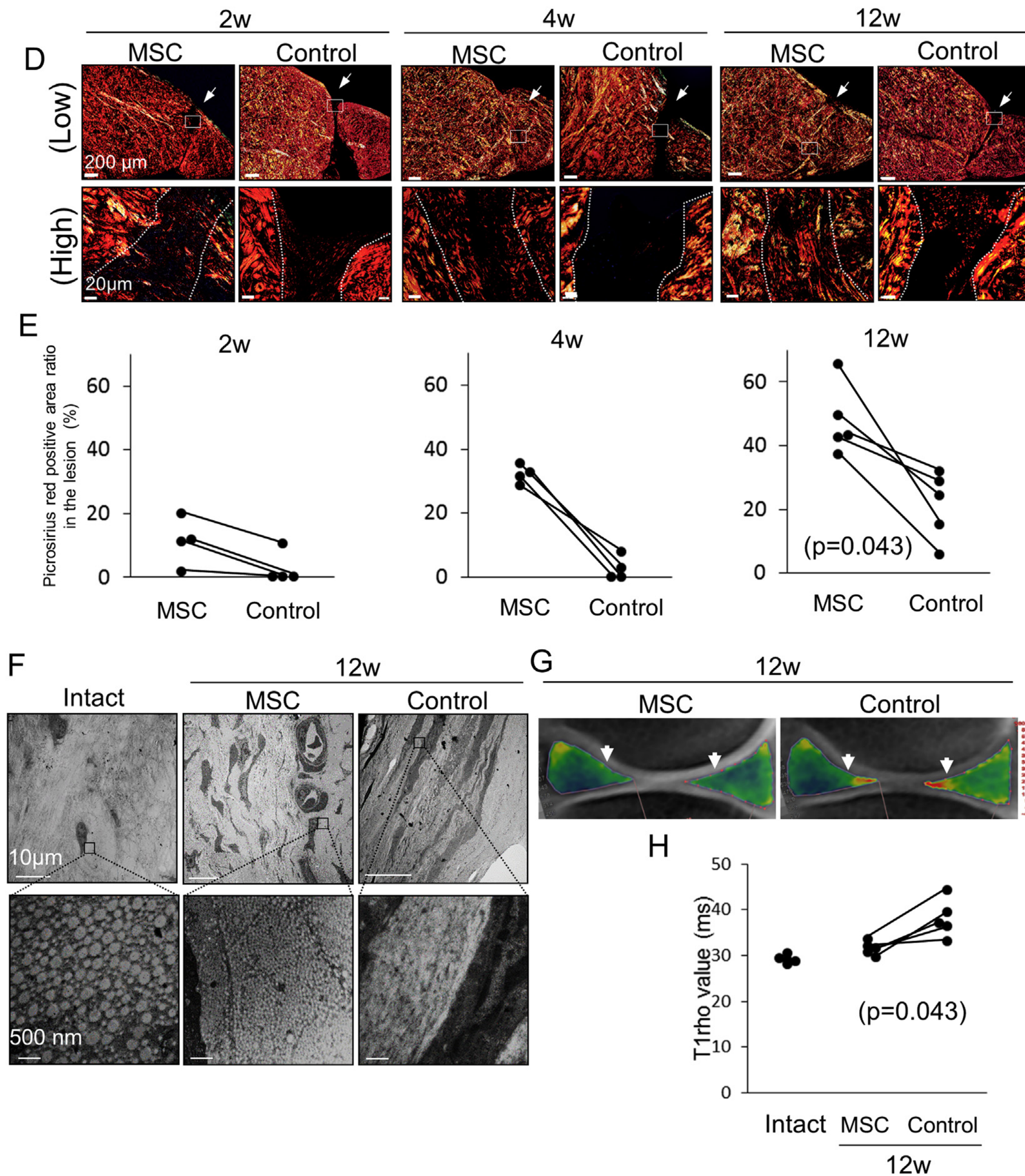

Fig. 3. (*continued*).

PRG4, BMP2, and TSG-6 after transplantation into the knee in a rat osteoarthritis model (in submission). The current study revealed that one of the important cytokine effects caused by synovial MSCs was the induction of synovial tissue to the meniscal lesion or the formation of synovial tissue in the meniscal lesion. A number of papers have provided support for the importance of this induction of synovial tissue to the meniscal lesion for meniscal healing[7,8].

Synovium induced into the meniscal lesion had a large number of Ki67 positive, proliferating cells at 4 weeks. The lesion was still covered with synovium but the synovial cell layer was decreased at 12 weeks possibly because the initial proliferative phase transitioned to the remodeling phase in the induced synovium.

Biomechanical testing is an important evaluation analytic for functional meniscal repair. There are several methods that have been employed to various experimental models[30,34–36]. We divided the meniscus into three parts, the anterior and posterior parts were used for biomechanical testing, and the average values for the tensile strength at failure was calculated. This method is similar to reports by Pabbruwe *et al*[36]. The remaining middle part was used for histological analysis. With this method, we could perform both biomechanical and histological analyses on the same meniscus in order to reduce the additional animals used in the experiment.

In this study, we transplanted 20 million cells per meniscus. We previously reported that intraarticular injections of 50 million allogeneic synovial MSCs promoted meniscus regeneration in a massive meniscal defect model using minipigs[17]. The averaged body weight of microminipigs used in this experiment was approximately 13 kg and that of minipigs used in our previous report was approximately 32 kg. We scaled the dosage of cells with body weight (40%) between the two strains.

To track the synovial MSCs, we used GFP+ synovial MSCs, which were observed in the lesion and synovium around the lesion 10 min after the synovial MSC suspension was immersed on the lesion. To track synovial MSCs by MRI, we used synovial MSCs labeled with

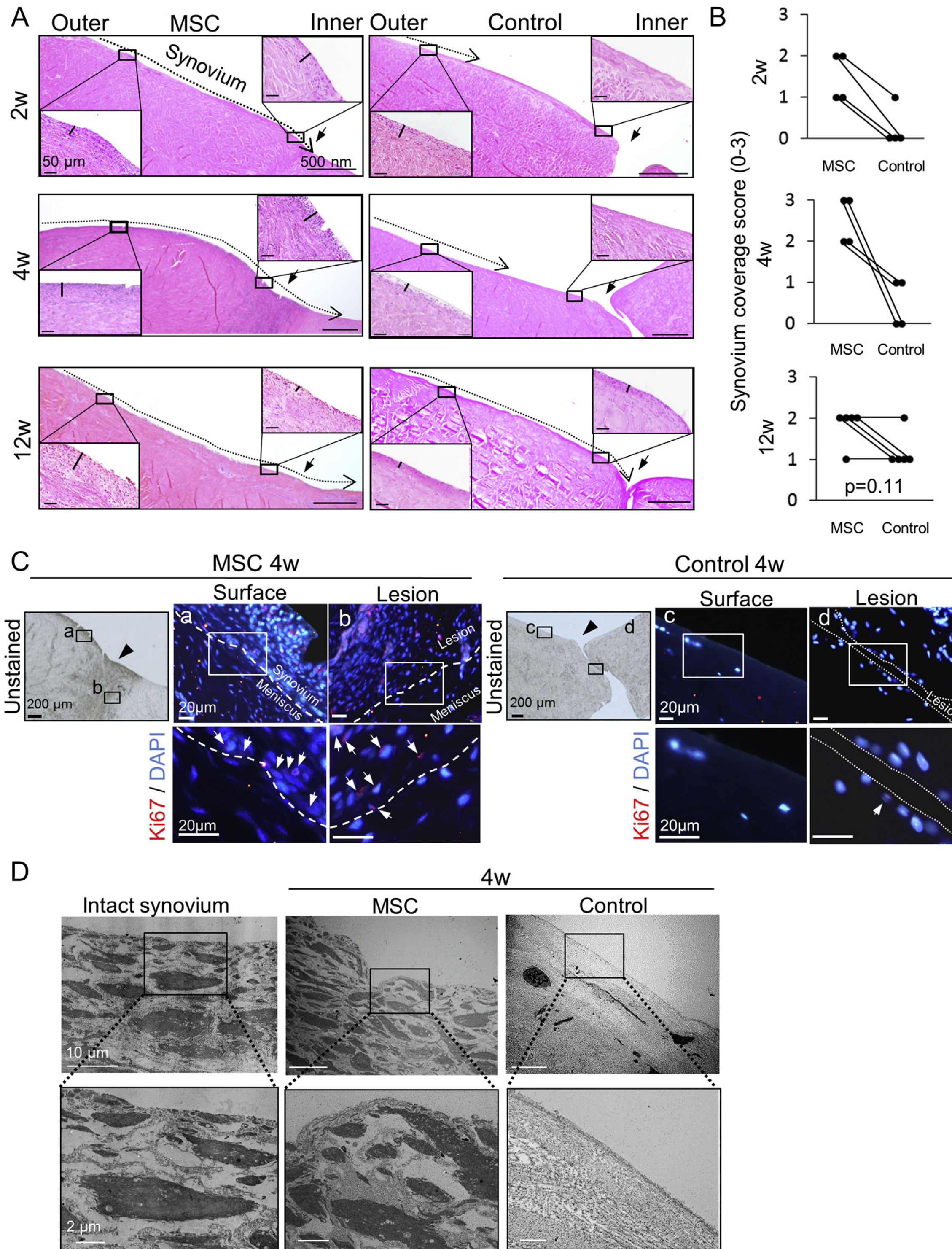

Fig. 4. Analysis for induction and formation of synovial tissue from the outer zone to the lesion of the meniscus. (A) Histological sections stained with hematoxylin and eosin for the femoral side of the meniscal lesions. Continuous synovial tissues from outer zone going to lesion in the inner zone are indicated with dotted arrows. Lesions are indicated with black arrows. In the insets, synovial tissues covering meniscus are indicated with black bars. (B) Quantification of histology for synovial coverage (Supplemental Table 3). *P* value was calculated by Wilcoxon's signed rank test. (C) Histological sections stained with Ki67. The lesions are indicated with a black arrow head. Ki67 positive cells are indicated with white arrows. (D) Transmission electron microscopic images of the surface in the lesion both in the MSC group and the control group at 4 weeks, and synovium in an intact meniscus.

particles of ferucarbotran[37]. Particles of ferucarbotran were observed around the lesions and anterior synovium 2 weeks after transplantation. We previously reported that more than 60% of synovial MSCs suspended in phosphate buffered saline (PBS) placed upon an osteochondral defect adhered to the defect in 10 min[38] and ultimately promoted cartilage regeneration[23] and the addition of magnesium in the cell suspension enhanced this adherence through the activation of integrins[39]. In this study we suspended

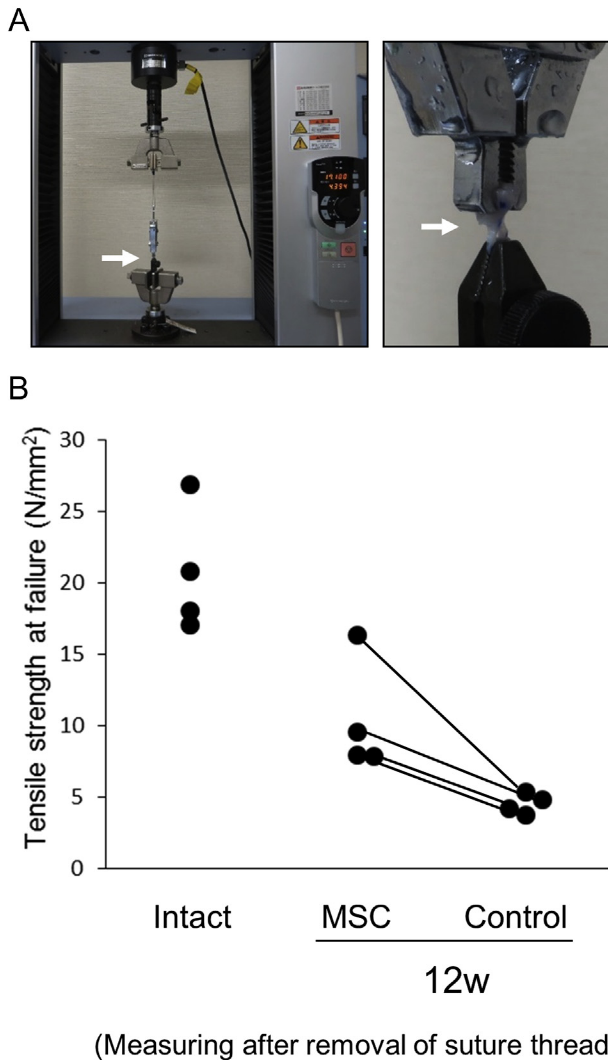

Fig. 5. Biomechanical analysis (A) Mechanical testing frame and vice grip. Meniscus is indicated with an arrow. (B) Tensile strength at failure for injured meniscus and intact meniscus.

synovial MSCs in Veen 3G-inj containing 2.5 mM magnesium. For the GFP+ synovial MSC tracking experiments, we used 10 million MSCs instead of the 20 million MSCs transplanted in the other experiments. These cells were readily detected without the need to increase the dose to 20 million MSCs, as was the case in assays for efficacy. These data confirmed the ability of MSCs to adhere to the meniscus lesion.

In this study, allogeneic synovial MSCs were used. Though we did not use immunosuppressive drugs to reduce the risk of infection, we did not observe obvious signs of immunoreaction in any of the recipient pigs. When we injected allogeneic synovial MSCs into the knee after the anterior half of the medial meniscus was resected in rabbits[16], and pigs[17], obvious immunoreactions were similarly absent. These data suggest that the transplantation of allogeneic synovial MSCs into the knee is a safe procedure. In human clinical practice, allogeneic synovial MSCs have not yet been employed, though allogeneic bone marrow MSCs have been used. Vangsness *et al.* reported that intraarticular injections of allogenic bone marrow MSCs to patients after partial meniscectomy did not cause serious adverse events including immunological reaction in a clinical study[40].

In terms of clinical implications, we propose three limitations to the current study. First, we transplanted synovial MSCs immediately after we produced a meniscus lesion. In a clinical situation, patients requiring cell therapy will have a degenerated meniscus that will have been injured for what could be a long time after their initial meniscus injury event. Ruiz-Iban *et al.* reported that the effect of MSCs on meniscal lesion was different between acute and chronic lesions[29]. Investigations into whether synovial MSCs are also effective in the repair of chronic lesions will be the subject of our future work. Second, we did not follow the healing outcomes after meniscal repair more than 12 weeks. Though transplantation of synovial MSCs significantly improved the healing of the meniscus at 12 weeks, the repaired meniscus appeared to be less than fully mature both morphologically and biomechanically. Third, the number of animals sampled in each experiment was relatively low.

Despite these limitations, the present study provides evidence that synovial MSCs have the possibility to enhance healing of meniscal repair. Therefore, the combination of synovial MSC cell therapy and surgical suture repair can increase the indication for clinical repair of meniscus injuries. This may contribute to increasing the chance of saving a patient's meniscus, preventing the development of osteoarthritis in patients with meniscus injuries.

In conclusion, the transplantation of synovial MSCs promoted healing of meniscal repair with the induction of synovium into the longitudinal tear in the avascular zone in the microminipig meniscus.

Author contributions

Yusuke Nakagawa: Conception and design, collection of data, analysis and interpretation of the data, and manuscript writing. Takeshi Muneta: Conception and design, interpretation of the data, and administrative support. Shimpei Kondo: Collection of data, and analysis of data. Mitsuru Mizuno: Analysis of data. Kazuo Takakuda: Technical support for biomechanical experiment. Shizuko Ichinose: Collection of data for electron microscopy. Hideyuki Koga: Interpretation of data. Kunikazu Tsuji: Technical support. Ichiro Sekiya: Conception and design, financial support, manuscript writing, and final approval of manuscript.

Conflict of interest

No conflict of interest for any of the authors.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.02.008.

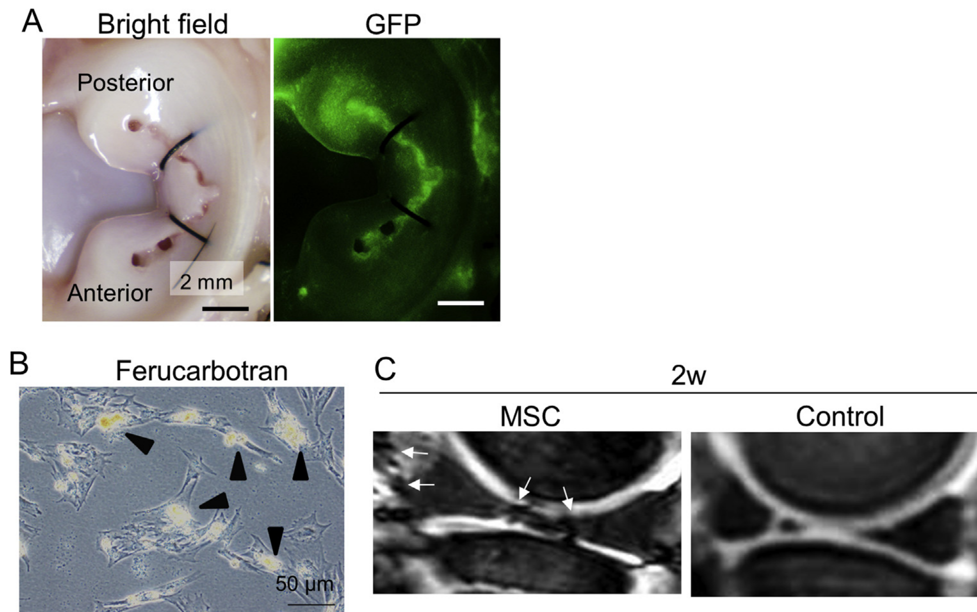

Fig. 6. Distribution of transplanted MSCs. (A) Macroscopic features. The sutured meniscus was immersed with synovial MSC suspension for 10 min, the knee was flexed and extended 100 times, and then the meniscus with tibial plateau was photographed. (B) Synovial MSCs taking up particles of ferucarbotran indicated with arrow heads. (C) Ferucarbotran are visualized as scattered black dots in MRI. Synovial MSCs labeled with ferucarbotran are indicated with arrows.

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