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Repetitive allogeneic intraarticular injections of synovial mesenchymal stem cells promote meniscus regeneration in a porcine massive meniscus defect model

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SUMMARY

Objective: A new strategy is required in order to regenerate a meniscus for extensive defects. Synovial mesenchymal stem cells (MSCs) are an attractive cell source for meniscus regeneration due to their high proliferation and chondrogenic potential. We examined the effect of repetitive intraarticular injections of synovial MSCs on meniscus regeneration in a massive meniscal defect of pigs. We followed up the efficacy using MRI evaluation in addition to macroscopic and histological observations.

Design: Two weeks before the injection of synovial MSCs, the anterior half of the medial menisci was resected in both knees of pigs. Fifty million allogeneic synovial MSCs were injected into the right knee at 0, 2, and 4 weeks and followed up by sequential MRI. The regenerated meniscus, adjacent articular cartilage, and subchondral bone were evaluated by MRI at 2, 4, 8, 12 and 16 weeks. They were also evaluated macroscopically and histologically at 16 weeks ($n = 7$).

Results: The resected meniscus regenerated significantly better in the MSC group than in the control group based on histological and MRI analyses. Macroscopically, the meniscal defect already appeared to be filled with synovial tissue at 2 weeks. Articular cartilage and subchondral bone at the medial femoral condyle were also significantly more preserved in the MSC group based on MRI, macroscopic, and histological analyses.

Conclusions: Intraarticular injections of allogeneic synovial MSCs appeared to promote meniscus regeneration and provide protection at the medial femoral articular cartilage in a porcine massive meniscal defect model.

Introduction

The meniscus is a wedge-shaped fibrocartilaginous structure and plays important roles in load distribution, shock-absorption, and knee joint stability. It has a poor healing potential due to its largely avascular nature, and loss of meniscal function leads to accelerated osteoarthritis[1]. For a massive meniscal defect in a clinical situation, the meniscal allograft is indicated, but it is comparatively invasive, and selecting the proper size of allograft is difficult[2,3]. A novel strategy for meniscus regeneration remains necessary.

It was already reported more than 70 years ago that meniscus lesions did not heal unless they communicated with the synovium and capsule[4]. However, the reparative potential of synovium for meniscus lesion is limited and therefore spontaneous repair of the injured meniscus cannot be expected in many cases. Synovial mesenchymal stem cells (MSCs) are an attractive cell source for articular cartilage and meniscus regeneration. In comparison with other tissue-derived MSCs, synovial MSCs have a high chondrogenic potential[5,6] and remarkable expansion ability with autologous human serum[7].

We previously reported that intraarticular injection of synovial MSCs promoted meniscus regeneration after the anterior half of the

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medial meniscus (MM) was resected in rats[8] and rabbits[9]. We hypothesized that this method would also be useful in bigger animals that are more closely related to humans. The purpose of this study was to examine the effect of repetitive intraarticular injections of allogeneic synovial MSCs on the regeneration of the meniscus in a massive meniscal defect model of pigs. We followed up on meniscus regeneration sequentially using MRI, and examined the results macroscopically and histologically at the endpoint. The articular cartilage and subchondral bone were also sequentially evaluated to examine the function of the regenerated meniscus. This study showed the effect of synovial MSCs on meniscus regeneration, and it may lead to the development of a new treatment for meniscus regeneration in clinical situations.

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 and Jichi Medical University. Thirteen-month-old, skeletally mature Mexican hairless pigs (National Livestock Breeding Center, Ibaraki, Japan) were used. The average weight of these pigs was 32 kg. All pigs were bred under specific pathogen-free conditions and had free access during the study period to food and water in a post-operative care cage (400 cm wide, 1210 cm long and 1090 cm high). To remove donor variation, only one pig was used as a donor for transplantation of allogeneic synovial MSCs, and 10 pigs were used as recipients.

Cell isolation and culture

Synovial tissue was taken together with the underlying connective tissue from the suprapatellar pouch, which overlays the non-cartilaginous areas of the femur through an arthrotomy of the knee. Synovial tissue with the underlying connective tissue was digested in 3 mg/mL collagenase D solution (Roche Diagnostics, Mannheim, Germany) in α-minimal essential medium (α-MEM; Invitrogen, Carlsbad, CA) at 37°C for 3 h, filtered through a 70-μm nylon filter (BD Biosciences, Franklin Lakes, NJ). The nucleated cells were plated in 150-cm^2 culture dishes (Nunc, Rochester, NY) at 5×10^5 cells/dish in complete medium (α-MEM supplemented with 10% fetal bovine serum (FBS), 100 U/L penicillin, 100 μg/mL streptomycin and 250 ng/mL amphotericin B [all from Invitrogen]) in 150-cm^2 culture dishes (Nunc), then cultured for 14 days at 37°C, 5% CO_2 with saturated humidity. The medium was changed to remove non-adherent cells every 4–5 days. The adherent cells were harvested with 0.25% trypsin-EDTA (Invitrogen) and cryopreserved as passage 0. Aliquots of 2×10^6 cells in 2 mL of stock medium [α-MEM supplemented with 10% FBS and 5% dimethylsulfoxide (Wako, Osaka, Japan)] were frozen slowly in a Cryo 1°C freezing container (Nunc) and cryopreserved at −80°C. To expand the cells, a frozen vial of the cells was thawed, plated in 60-cm^2 culture dishes at 1×10^6 cells/dish, and cultured for 4 days as passage 1. Then the cells were re-plated in 150-cm^2 culture dishes at 5×10^5 cells/dish and cultured for 14 days as passage 2. Passage 2–4 cells were used for further studies. The number of total cell doublings was 12 at passage 0, 18 at passage 1, 25 at passage 2, 30 at passage 3, and 37 at passage 4.

Colony-forming unit assay

Five hundred cells were plated in 60-cm^2 dishes, cultured in complete medium for 14 days, and stained with 0.5% crystal violet in methanol for 5 min to observe cell colonies.

In vitro differentiation assay

For chondrogenesis, 2.5×10^5 cells were collected at the bottom of a 15-ml polypropylene tube (BD Biosciences) by centrifugation at 450 × g for 10 min. The pellets were cultured in chondrogenesis medium consisting of high-glucose Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 1 μg/mL bone morphogenetic protein (BMP)-7 (provided from Stryker Biotech, Hopkinton, MA), 10 ng/mL transforming growth factor (TGF)-β3 (R&D Systems, Minneapolis, MN), 100 nM dexamethasone (Sigma–Aldrich, St Louis, MO), 50 μg/mL ascorbate-2-phosphate, 40 μg/mL proline, 100 μg/mL pyruvate and 1:100 diluted ITS + Premix (6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 ng/mL selenious acid, 1.25 mg/mL bovine serum albumin and 5.35 mg/mL linoleic acid; BD Biosciences). For microscopy, the pellets were embedded in paraffin and cut into 5 μm sections. They were stained with safranin-o fast green, and immunostained with type II collagen[10–12].

For adipogenesis, cells were cultured for 21 days in adipogenic medium consisting of complete medium supplemented with 100 nM dexamethasone, 0.5 mM isobutyl-methylxanthine (Sigma–Aldrich) and 50 μM indomethacin (Wako). The adipogenic cultures were fixed in 4% paraformaldehyde, then stained with fresh oil red-o solution[13].

For calcification, cells were cultured for 21 days in calcification medium consisting of complete medium supplemented with 1 nM dexamethasone, 20 mM β-glycerol phosphate (Wako) and 50 μg/mL ascorbate-2-phosphate (Sigma–Aldrich). The cells were fixed in 4% paraformaldehyde, then stained with 0.5% alizarin red solution[14].

Meniscectomy

Two weeks before the injection of synovial MSCs, a straight incision was made on the medial side of the knee, the anteromedial side of the joint capsule was cut, and the anterior horn of the MM was exposed under general anesthesia. Then the meniscus was dislocated anteriorly with a forceps, cut vertically at the level of the medial collateral ligament, and the anterior half of MM was excised in both knees.

Intraarticular injection of synovial MSCs

5×10^7 synovial MSCs were suspended in 1 mL of PBS and injected into the right knee three times at 2-week intervals (the first injection day was determined as day 0). The same volume of PBS was injected into the left knee as the control. For the injection, a 23-gauge needle was inserted at the center of the triangle formed by the medial side of the patellar ligament, the medial femoral condyle (MFC), and the medial tibial plateau, toward the intercondylar space of the femur. Immediately after the injection, the medial side of the injected knee was kept down for 10 min so that the injected synovial MSCs could be attached around the defect of the meniscus[15]. After waking up from anesthesia, the pigs were allowed to walk freely without fixation, and without the administration of immunosuppressive drugs to reduce the risk of infection.

Macroscopic examination and histological analysis

After 16 weeks, seven pigs were sacrificed by overdose intravenous injection of KCl under adequately deep general anesthesia. Samples such as MFCs were examined macroscopically for color, integrity, and smoothness, and evaluated using the International Cartilage Repair Society (ICRS) macroscopic score[16]. Specimens of