



Ultrasound-Guided Harvesting of Synovium for Regenerative Medicine of Cartilage and Meniscus Using Synovial Mesenchymal Stem Cells

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Abstract: Mesenchymal stem cell (MSC) therapy for cartilage or meniscus pathologies, including osteoarthritis, requires the easy and safe collection of MSC source materials. Synovial MSCs are attractive cell sources for joint pathology because of their high proliferative and chondrogenic potential in vitro and in vivo. We developed an ultrasound-guided harvesting procedure for synovium for the regenerative medicine of cartilage and meniscus. A ~1-cm skin incision is made at the proximal side of the patellae, and a forceps is inserted under ultrasound guidance of the suprapatellar pouch to grasp the synovium. Here, several synovium samples were retrieved and transported sterilely for culture at the cell-processing facility. After a 14-day culture of the nucleated cells, crystal violet confirmed colony formation. Cell growth was enough for MSC therapy of joint pathology ($0.89 \pm 0.06 \times 10^6$ cells/dish). No adverse events occurred during synovium harvesting. A key advantage of this procedure is its minimal invasiveness, as synovium is harvested from a 1-cm skin incision in the knee joint. A disadvantage is the possible risk of hemostasis, as arresting bleeding at the synovial harvest site is difficult, even though the suprapatellar pouch contains no major vessels.

Mesenchymal stem cell (MSC) therapy has become a widely applied clinical procedure for the treatment of cartilage or meniscus pathologies, including osteoarthritis (OA).^{1,2} One of the most attractive cell sources is synovial MSCs, which have high proliferative and chondrogenic potential in vitro and in vivo.³⁻⁵ The use of synovial MSCs for joint pathology is reasonable because articular chondrocytes and synovial lining cells

share a similar origin from Gdf-5-expressing progenitor cells,⁶ and the gene profiles of MSCs derived from cartilage, meniscus, and synovium are more similar than those derived from adipose tissue or bone.⁷ Clinical trials have confirmed that transplantation of synovial MSCs onto a cartilage defect improves the magnetic resonance imaging score and Lysholm scores.⁸ In addition, the transplantation of synovial MSCs onto a repaired meniscus with degenerative injury improves the clinical evaluation scores at the 2-year follow-up.¹

The emerging global interest in MSC therapy amplifies the importance of obtaining MSC source materials easily and safely. From this point of view, adipose tissue has become one of the most popular cell sources for regenerative medicine for knee OA⁹ because, even though the cellular origin of adipose tissue and cartilage or meniscus is quite different, adipose tissue can be readily harvested in an outpatient setting with the use of local anesthesia. By contrast, harvesting of synovium requires arthroscopy in an operating room under local or lumbar spinal anesthesia.^{1,8} The ability to harvest synovium more easily in the outpatient clinic would increase the availability of synovial MSC therapy in patients with knee OA¹⁰ or meniscus pathology.¹¹

The diagnosis of joint disease or trauma in the orthopaedic field is greatly aided by ultrasound

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evaluations, so ultrasonography is increasingly used to assess degenerative and traumatic tendon disorders in the upper and lower limbs, ankle instability, gluteal tendon tears, and meniscal cysts.¹² Ultrasound-guided treatments, such as injections for shoulder, knee, ankle, and hips or surgical interventions for hematoma evacuation, have been reported.¹³⁻¹⁶ Here, we describe a procedure for ultrasound-guided harvesting of synovium for regenerative medicine of the cartilage and meniscus.

Indication

This technique is indicated for treatment of pathologies of the knee joint that require synovial MSC therapy. This procedure is contraindicated in patients with a hemorrhagic predisposition or who are taking anticoagulants because of the difficulty to control bleeding.

Patients

This procedure was performed with the approval of the institutional review board of Tokyo Medical and Dental University (research protocol identification number: M2019-279). We performed ultrasound-guided harvest of synovium in 3 patients just before total knee arthroplasty (TKA) under general anesthesia. During TKA surgery, the synovium within the suprapatellar pouch is routinely removed; therefore, for this study, we performed this procedure in patients undergoing TKA. In future, this procedure would be conducted in patients who are indicated for regenerative medicine in the outpatient clinic.

Surgical Technique (With Video Illustration)

The patient is positioned supine on a standard operating table. An appropriate portal is made by inserting a 23-G spinal needle under the guidance of ultrasound, and local anesthesia of (10 ml 0.5% xylocaine with a 200,000-fold dilution of epinephrine) is administered subcutaneously. A 20-mL volume of the same anesthetic is then injected into the knee joint with an 18-G needle. A skin incision approximately 1 cm in length is made to insert the forceps at the proximal side of the patellae to access the suprapatellar pouch. Note that the scalpel must penetrate the capsule and into the knee joint. An ultrasound probe (11-MHz linear probe, SONIMAGE MX1; Konica Minolta, Inc. Tokyo, Japan) covered with a sterile sleeve is set horizontally on the skin of the suprapatellar pouch (Fig 1A). The forceps, with a round upper jaw and a flat lower jaw (Keisei Medical Industrial, Co., Ltd. Tokyo, Japan; Fig 1B), is inserted through the skin incision under ultrasound guidance and the synovium is grasped to remove a sample (Fig 1 C and D, Table 1). This procedure is performed several times. After completion of the harvest (Fig 1E), the synovium samples are transported

sterilely for culture at the cell-culture processing facility.

Manipulation of Synovial MSCs After Harvest

The synovium is digested with 3 mg/mL collagenase (Sigma-Aldrich Japan, Tokyo, Japan) at 37°C for 3 hours and then filtered through a 70-μm cell strainer (Greiner Bio-One GmbH, Frickenhausen, Germany). The nucleated cells are plated in 150-cm² culture dishes (Nalge Nunc International, Rochester, NY); the complete medium includes minimal essential medium-α supplemented with 10% fetal bovine serum, 100 U/L penicillin, 100 mg/mL streptomycin, and 250 ng/mL amphotericin B (all from Thermo Fisher Scientific, Waltham, MA). The disseminated cells are cultured at 37°C, in 5% CO₂ and saturated humidity. After 14 days, the cultured cells are harvested with 0.25% trypsin–ethylenediaminetetraacetic acid (Thermo Fisher Scientific), and the numbers of cells are counted. For colony assays, 100 cells are plated in 55-cm² culture dishes and cultured in complete medium for 14 days. The dishes are stained with 0.5% crystal violet (Fujifilm Wako, Osaka, Japan) in 10% formalin for 5 minutes and washed twice with distilled water.

Proliferation Potential of Synovial MSCs

After the 14 days of culture (Fig 1F), staining with crystal violet confirmed colony formation by the synovial cells (Fig 1F). The colonies consisted of spindle-shaped cells (Fig 1G). No adverse events occurred during the harvest of synovium in any of the cases.

Discussion

We have introduced a technique for ultrasound-guided harvesting of synovium for regenerative medicine. This technique provides a safe and easy harvest of synovium in the outpatient clinic using local anesthesia and will facilitate the widespread use of synovial MSC therapy for treatment of meniscus or cartilage disorders, including OA. The tips of this procedure are to keep the ultrasound probe still on the suprapatellar pouch, to make the skin incision with a spinal needle, to penetrate the capsule with the scalpel into the knee joint, and to grasp the synovium securely with forceps under ultrasound guidance (Table 1). The clear detection of the head of the forceps in the joint makes this procedure safe and easy. We used the forceps with a round upper jaw and a flat lower jaw, as this made grasping the synovium quite easy.

The pitfalls of this procedure are as follows: the forceps must be inserted at an appropriate depth in the joint, and sterile conditions must be maintained throughout the procedure (Table 1). If the head of the forceps is opened within the muscles, this creates a risk of injury to the muscles. In patients with obesity, care should be taken to distinguish between the subcutaneous adipose tissue and

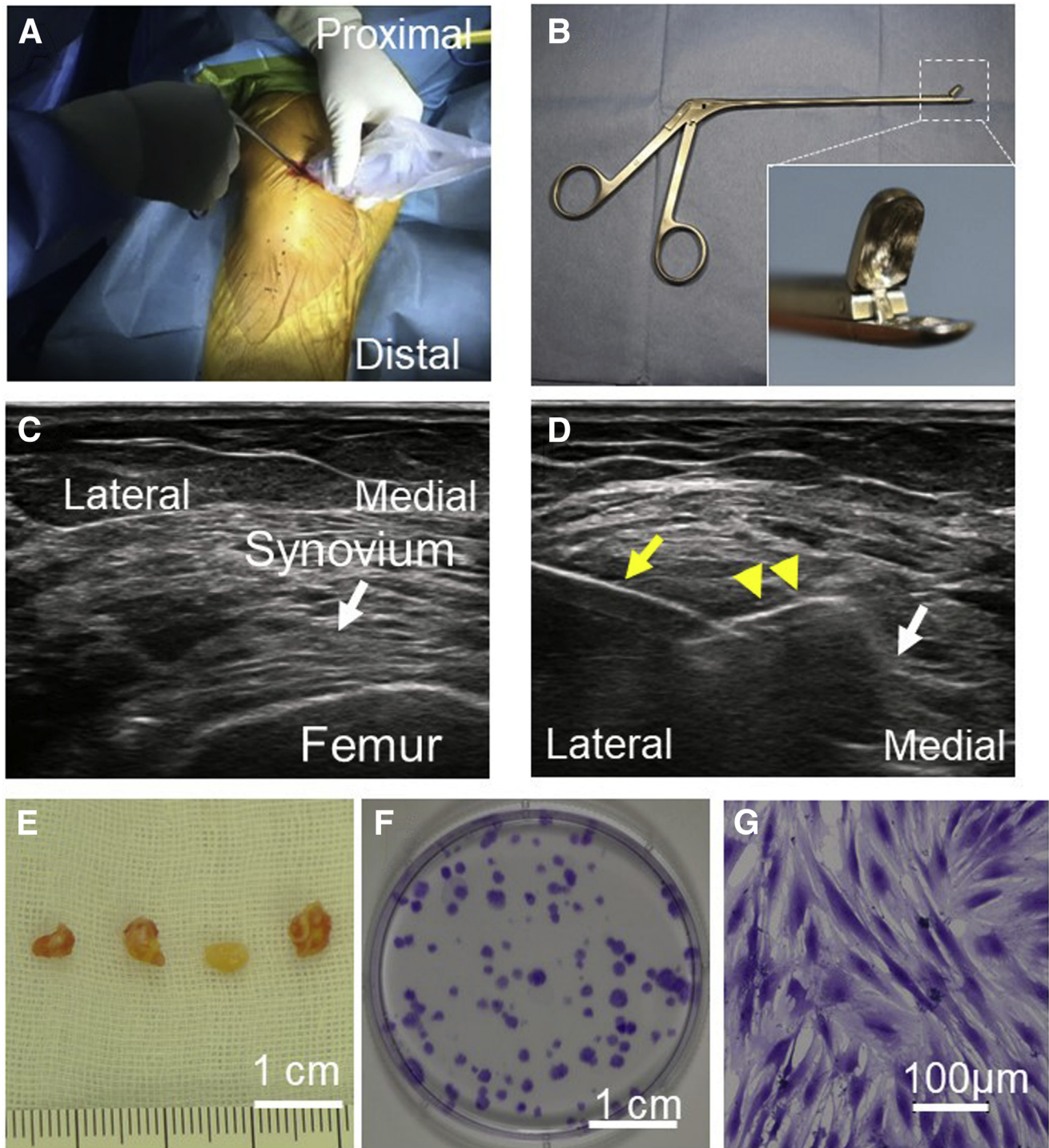


Fig 1. A case of ultrasound-guided harvest of synovium. (A) Collection from the right knee. The ultrasound probe was set on the skin of the suprapatellar pouch to obtain the short axis of the image. The forceps were inserted from lateral side of the suprapatellar pouch. (B) Forceps used for harvest. (C) Ultrasound image of the suprapatellar pouch. Arrow indicates the synovium. (D) Ultrasound image taken while harvesting the synovium. Yellow arrow indicates the forceps inserted into the suprapatellar pouch and yellow arrowhead indicates the head of the forceps. White arrow indicates the synovium. (E) The pieces of synovium harvested under ultrasound. (F) Culture dish stained with crystal violet. Synovial cells were cultured for 14 days. (G) Morphology of the synovial mesenchymal stem cells.

synovium because both tissues appear similar on ultrasound images. In addition, because the synovium is cultured in the cell-processing facility and cultured cells

are transplanted into the joint, sterile conditions should be maintained as much as possible throughout the harvest of synovium.

Table 1. Tips and Pitfalls

Tips	Pitfalls
<ul style="list-style-type: none"> Keep the ultrasound probe still on the suprapatellar pouch and make the skin incision with a spinal needle Penetrate the capsule with the scalpel into the knee joint Grasp the synovium correctly with forceps under ultrasound guidance 	<ul style="list-style-type: none"> Ensure that the forceps is inserted at an appropriate depth in the joint Maintain sterile conditions throughout this procedure

The most critical advantage of this procedure is that synovium can be harvested with a single skin incision about 1-cm long, so this is a minimally invasive harvest of synovium from the knee joint (Table 2). Harvesting of synovium with local anesthesia is possible in any clinics or hospitals equipped with ultrasonography. In addition, the ultrasound-assisted procedure is safe because the head of the forceps can be visualized, even though no serious vessels or nerves exist in the suprapatellar pouch. After cell culture, the numbers of cells collected in this study were sufficient for the regenerative medicine used in our previous clinical study upon transfer and culture in the appropriate dishes.^{1,8}

The harvested synovium is transported to the outsourced cell-processing facility inside a sterile box. After expansion of the synovial MSCs for several weeks, they can be transported back again to clinics or hospitals for injection. Building and maintaining a cell-processing facility is substantially expensive¹⁷; therefore, the ability to outsource the synovial MSC expansion step is highly practical for dozens of patients with joint pathology, and this procedure will advance regenerative medicine in this field.

The main disadvantage of this procedure is the possible risk of hemostasis (Table 2). When using arthroscopy, one option for coagulating a bleeding source is the use of a radiofrequency device; however, stopping bleeding at the synovial harvest site is difficult using this technique, even though no major vessels

Table 2. Advantages and Limitations of This Technique

Advantages	Limitations
<ul style="list-style-type: none"> It is possible to conduct this procedure in the outpatient clinic in any hospital or clinic that has an ultrasound device Minimally invasive procedure, with a single skin incision approximately 1-cm long Safe procedure with visualization of forceps and synovium under ultrasound guidance 	<ul style="list-style-type: none"> Difficulty to control bleeding; therefore, the procedure is not recommended for patients with a hemorrhagic predisposition or taking anticoagulants

are distributed in the suprapatellar pouch. Local anesthesia with epinephrine can reduce bleeding, and compression should be applied against unpredictable bleeding. In addition, this procedure should be contraindicated in patients with known complications of hemorrhagic disease or who are taking anticoagulation medications.

The synovial MSCs described in this paper had the ability to adhere to plastic dishes, form colonies, and differentiate into chondrocytes, adipocytes, and calcification in vitro. They also had a specific surface marker pattern as previously reported.^{3,18,19} These characteristics meet the definition of the criteria for MSCs by the International Society for Cell Therapy.²⁰ Many clinical cases using autologous MSCs derived from mesenchymal tissues, such as synovium, bone marrow, and adipose tissue, have been reported, but no serious complications, such as tumor formation, have been reported.²¹ Clinical cases of autologous synovial MSCs for cartilage damage, meniscus damage, and OA are still insufficient to determine their efficacy. Large-scale prospective comparative studies are needed to overcome this limitation.

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