

Preparation of Synovial Mesenchymal Stem Cells from a Rat Knee Joint

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[Abstract] Mesenchymal stem cells (MSCs), first described in human bone marrow, are emerging as promising cell-based therapeutics for a wide range of diseases (Caplan and Correa, 2011). MSCs have been isolated from various organs in the body, and synovial MSCs were first reported by De Bari *et al.* (2001). We previously reported that synovial MSCs have superior proliferation and chondrogenic potentials as compared to bone marrow-, muscle-, and adipose- derived MSCs in humans (Sakaguchi *et al.*, 2005) and rats (Yoshimura *et al.*, 2007). In addition, administration of synovial MSCs for osteochondral defect promoted cartilage regeneration in a rabbit (Koga *et al.*, 2008) and a pig model (Nakamura *et al.*, 2012). In 2008, we started a clinical trial in human and obtained satisfactory results of symptoms and regenerated cartilage by Magnetic Resonance Imaging (Sekiya, *et al.*, 2015). We have also engaged in multiple research lines using synovial MSCs for meniscus regeneration in rats (Horie *et al.*, 2009; Horie *et al.*, 2012; Katagiri *et al.*, 2013; Okuno *et al.*, 2014; Ozeki *et al.*, 2015). In this article, we demonstrated how to harvest the synovium including infrapatellar fat pad from a rat knee joint, and to describe the technique of isolation and culture of rat synovial MSCs.

Materials and Reagents

1. Culture dish (culture area: 56.7 cm², diameter: 100 mm) (Thermo Fisher Scientific, catalog number: 150350)
2. Cell strainer (70 µm) (VWR International, Greiner Bio-One GmbH, catalog number: 89508-344)
3. Clip (Mitsuya, catalog number: GM-590) cut by pliers in a half size (Figure 1B)
4. Styrene foam (RIKAKEN) (Figure 1C)
5. 50 ml Falcon tube (Corning, catalog number: 352070)
6. 8-12 weeks old Lewis rat (Charles River Laboratories International)
7. Collagenase V (Wako Pure Chemical Industries, catalog number: 038-17851)
8. α-minimal essential medium (α-MEM) (Thermo Fisher Scientific, catalog number: 12561-056)
9. Fetal bovine serum (FBS) (Thermo Fisher Scientific, catalog number: 12483-020)
10. Streptomycin, Penicillin, Amphotericin B (Antibiotic-Antimycotic, 100x) (Thermo Fisher)

Scientific, Gibco™, catalog number: 15240-062)

11. Phosphate buffer saline (PBS) (Thermo Fisher Scientific, Gibco™, catalog number: 14190-235)
12. α -MEM containing 10% FBS with antibiotics (see Recipes)
13. 3 mg/ml Collagenase solution (for synovium from both knee joints) (see Recipes)

Equipment

1. 37 degree, 5% CO₂ forced-air incubator (ASTEC, catalog number: SCA-165DS)
2. Centrifuge machine (KUBOTA Corporation, model: Model 8730)
3. Scalpel holder (Natsume, catalog number: No.3 D-11) with blade (Natsume, catalog number: No.11 D-13), scissors (Natsume, catalog number: B-12), and tweezers (Natsume, catalog number: A-6) (Figure 1A)

Procedure

A. Preparation of the surgery (10 min)

1. Sacrifice a rat by delivering CO₂ in a vinyl bag.
2. Remove the hair around the knee joints, and sterile clean the legs with 70% ethanol (Figure 1D).
3. Put the rat on the styrene foam, and cover the sterile sheet on the rat, keeping the right leg out of the hole. Stabilize the ankle with clip on the styrene foam (Figure 1E).

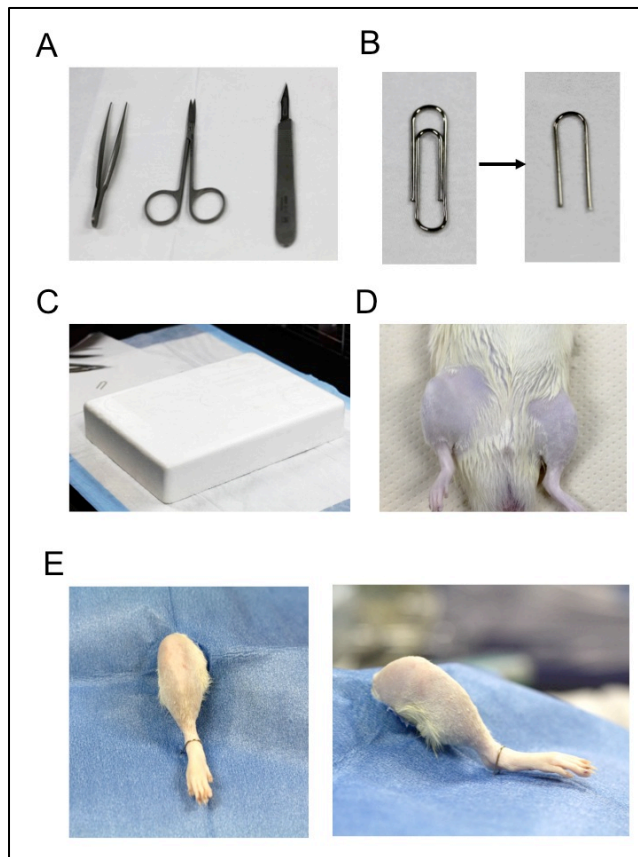


Figure 1. Preparation of the surgery. A. Surgical instruments; B. Clip for stabilization of legs; C. Styrene form; D. Removal of the hair; E. Settings of the legs.

- B. Harvesting the synovium from the knee joint (Video 1) (10 min for both knee joints)
1. Expose the patellar tendon with straight skin incision.
 2. Cut the patellar tendon transversely, and peel the tendon upward and downward to expose the infrapatellar fat pad.
 3. Separate the infrapatellar fat pad from the femur and tibia.
 4. Put the synovium into PBS in a Falcon tube.

Video 1. Harvesting the synovium including infrapatellar fat pad from the right knee



C. Isolation and culture of synovial MSCs

1. Mince the synovium with a scalpel into 2-3 mm pieces.
2. Put the minced synovium in collagenase solution.
3. Place the tube in the incubator for 2-3 h. Shake it 2-3 times per hour.
4. Filter the digested solution through a cell strainer.
5. Centrifuge the tube at 580 x *g* for 5 min.
6. Remove the supernatant, and plate the cells on the 56.7 cm² dish (about 1,000 cells/cm²).
7. Change the medium twice a week and culture for 2 weeks with α -MEM containing 10% FBS with antibiotics.

Representative data

We can usually harvest 1×10^4 cells from one synovium (Figure 2). After 2 weeks, we can obtain 5×10^5 - 1×10^6 MSCs from one dish. The cells are heterogenous, and we usually use these cells of passage 2-4 in the experiments. We reported these data in our previous reports (Yoshimura *et al.*, 2007).

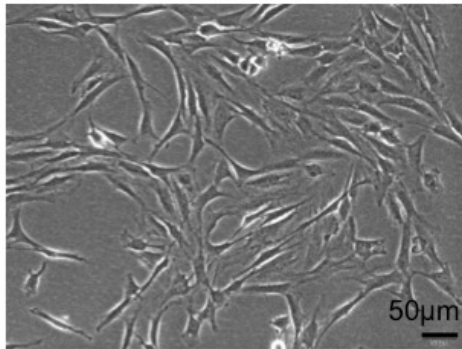


Figure 2. Representative morphology of synovial MSCs (passage 1, day 7). Scale bar = 50 μ m

Recipes

1. α -MEM containing 10% FBS with antibiotics
 α -MEM 445 ml
FBS 50 ml
100x Antibiotic-Antimycotic 5 ml
2. Collagenase solution 3 mg/ml (for synovium from both knee joints)
 α -MEM 3 ml
Collagenase V 9 mg

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