European Review for Medical and Pharmacological Sciences 2020; 24: 8273-8280

Ankle synovium-derived mesenchymal stem cells for the treatment of osteochondral lesion of the talus: a novel cell harvesting technique and clinical applications

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Abstract. – OBJECTIVE: To describe a novel technique for harvesting mesenchymal stem cells (MSCs) from ankle synovium and demonstrate their multipotency for chondrogenesis and osteogenesis in the treatment of a large osteochondral lesion of the talus (OLT).

PATIENTS AND METHODS: MSCs were harvested under local anesthesia from a biopsy of the ankle synovium of a patient with OLT and cultured for 4 weeks. In accordance with the International Society for Cellular Therapy (ISCT) criteria, synovial-derived MSCs were analyzed for cell surface markers using flow cytometry and grown in differentiation induction medium to demonstrate multilineage differentiation potentials *in vitro*. The patient received an injection of synovial-derived MSCs in a collagen matrix with a fibrin sealant to augment surgery for the lesion.

RESULTS: Harvesting of ankle synovium yielded a culture of 30 million spindle-shaped stem cells in 4 weeks. Cell surface marker expression of the MSCs met the ISCT standards. After 21 days, cultured cells could be differentiated into adipocytes, osteocytes and chondrocytes. The patient recovered uneventfully from surgery and showed satisfactory improvements in pain and ankle motion.

CONCLUSIONS: Ankle synovium is a potential source of MSCs for the treatment of OLT. We developed a novel harvesting technique that was simple, convenient and had no complications.

Key Words:
Mesenchymal stem cells, Synovial-derived, Ankle, Osteochondral lesion, Talus.

Introduction

Human synovial-derived mesenchymal stem cells (MSCs) have been described in several reports[1,2]. De Bari et al[2] were the first to isolate MSCs from human synovial membranes (SMs), demonstrating that these cells have the ability to proliferate widely in culture, and that the *in vitro* multilineage differentiation potential can be preserved[2]. The knee joint is an available source of MSCs[3]. To address the potential for variation in MSCs derived from different sources, the International Society for Cellular Therapy (ISCT, 2006) provided a set of minimal criteria to define cells as multipotent MSCs composed of three features[4]. First, the cells must be plastic-adherent when maintained under standard conditions. Second, they must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules. Third, the MSCs must be able to differentiate into osteoblasts, adipocytes and chondrocytes *in vitro*. These criteria have been applied widely to identify SM-derived MSCs in subsequent studies. However, little is known about the potential for intra-articular synovium from the ankle as a source of MSCs. In the present study we describe a technique for harvesting MSCs from the ankle synovium and demonstrate that these synovial-derived MSCs are multipotent MSCs that can undergo chondrogenesis and osteogenesis for the treatment of osteochondral lesion of the talus (OLT).

Patients and Methods

Radiographic (Figure 1) and magnetic resonance imaging (Figure 2) data were collected from a 32-year-old male patient with a large osteochondral lesion at the posteromedial aspect of the left talus with a varus deformity of the distal tibia. His symptoms had not improved following the previous conservative treatment, leading to his decision to undergo surgery. We planned the following treatments: administration of synovial-derived MSCs associated with a collagen matrix and fibrin

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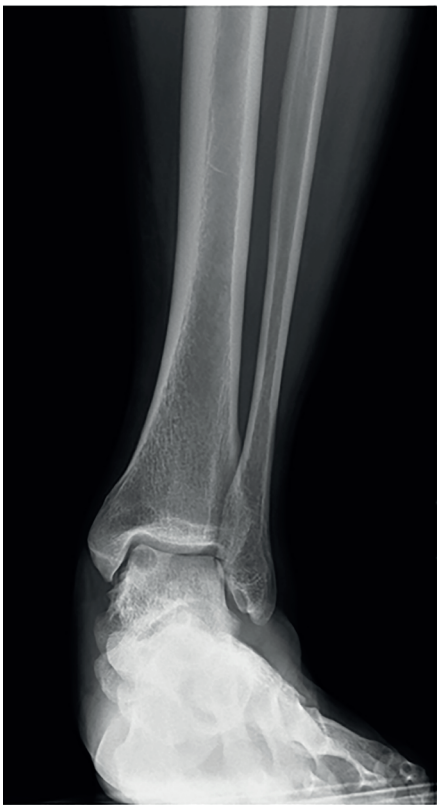

Figure 1. Anteroposterior radiograph of the left ankle showing the varus alignment of the ankle with a large osteochondral lesion of the talus on the medial side.

glue and covered by autologous platelet rich plasma (PRP) fibrin; autologous bone grafting at the OLT site; and supramalleolar osteotomy. The first stage required the patient to undergo harvesting of the ankle synovium for MSCs. The harvesting was performed with the patient ambulatory, using local anesthesia and core needle biopsy devices under fluoroscopic control (Figure 3). The patient gave informed consent and the study was approved by the Ethics Committee of the author's institute.

Within 4 hours of collection, the ankle synovium was transported to the laboratory and processed under sterile cell culture facilities. First, the synovium was washed with phosphate-buffered saline (PBS; Life Technologies, Carlsbad, CA, USA) supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Millipore, Billerica, MA, USA). The sample was then minced into small pieces, followed by dissociation with 0.2% collagenase (EMD Millipore, Billerica, MA, USA) for 3 hours, as described previously[5]. After filtering through a 70-µm nylon filter, digested cells were recovered by centrifugation and cultured in an alpha MEM medium (Hyclone, South Logan, UT, USA) containing 10% human serum (Sigma-Aldrich, St. Louis, MO, USA), 10 ng/mL basic fibroblast growth factor (Life Technologies, Carlsbad, CA, USA), 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 100 U/mL penicillin and 100 µg/mL streptomycin.

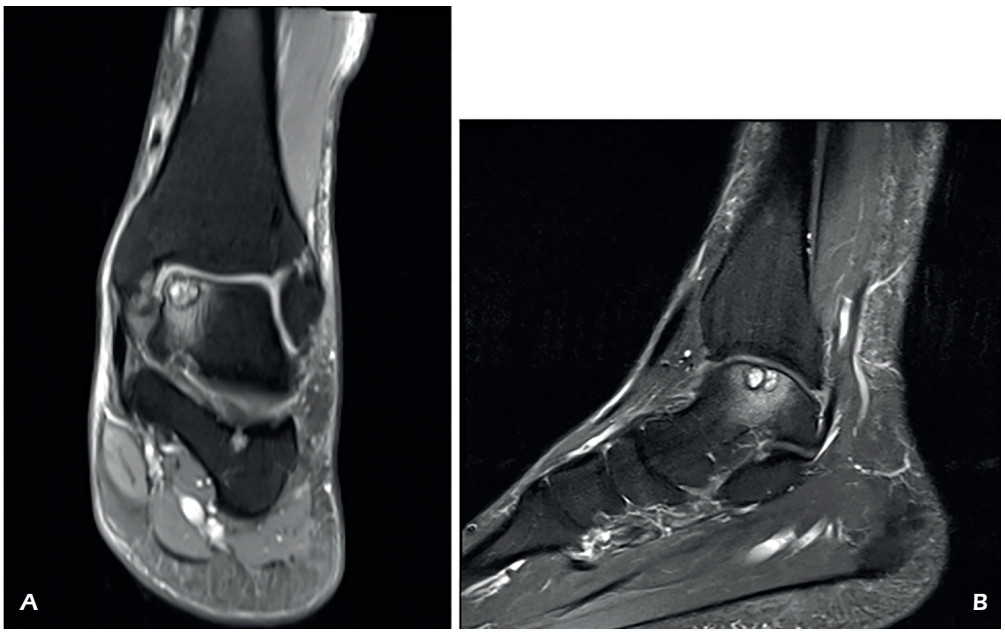

Figure 2. Magnetic resonance images demonstrating the large osteochondral lesion of the talus on the medial side with surrounding bone edema (**A**, coronal view; **B**, sagittal view).

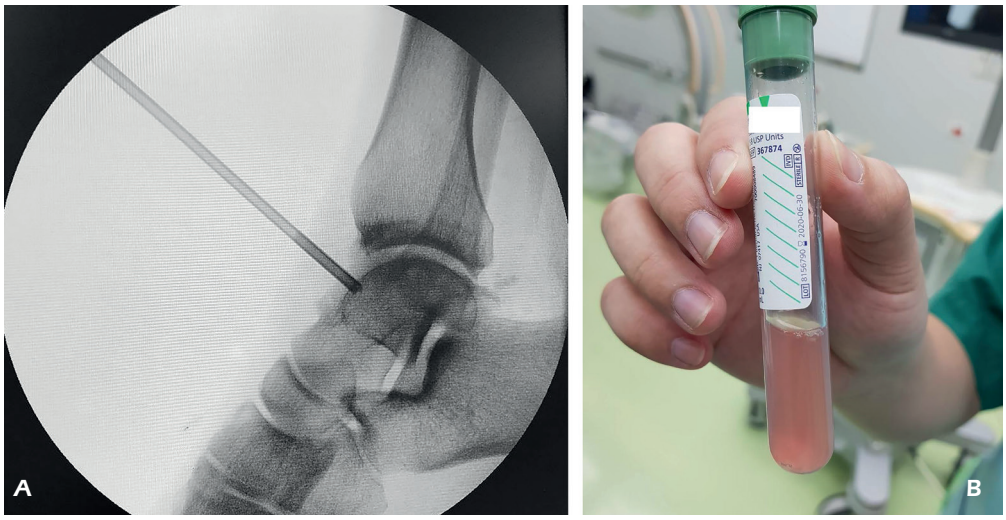

Figure 3. The core needle biopsy under fluoroscopic control (**A**) and biopsy content (**B**).

Cultures of synovial-derived MSCs were maintained at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed every 3-4 days. Cells were subcultured using TrypLE™ Select enzyme (Life Technologies, Carlsbad, CA, USA) when they reached about 90% confluence. During the cell culture period, morphology of the expanded cells was routinely observed under an inverted microscope. The number and viability of the cultivated cells were determined using a trypan blue (Life Technologies, Carlsbad, CA, USA) exclusion assay. Cells were counted using Countess Automated Cell Counter (Life Technologies, Carlsbad, CA, USA). Before proceeding with subsequent experiments, the sterility of the cultivated cells was determined by bacterial culture and endotoxin testing using a limulus amebocyte lysate assay (Lonza, Walkersville, MD, USA).

At passage 4, synovial-derived MSCs were characterized by flow cytometry analysis using a standard panel of MSC markers as follows: CD45-FITC, CD73-APC, CD90-ECD and CD105-PE (all from BioLegend, San Diego, CA, USA). Cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA) and data were analyzed with CytExpert software (Beckman Coulter). At least 10,000 cell events were acquired. Cells stained with appropriate isotype controls served as negative controls.

To assess multilineage differentiation potentials, cultivated cells were induced to differentiate into chondrogenic, osteogenic and adipogenic lineages using the appropriate StemPro medium for each type (all from Life Technologies, Carlsbad, CA, USA). At passage 4, cells were seeded in a 35-mm tissue culture dish. After reaching 50-70% confluency, the medium was replaced with induction medium (as described earlier), which was changed twice a week. After 21 days of differentiation, the culture was fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) then stained with the following lineage-specific dyes (all from Sigma-Aldrich, St. Louis, MO, USA): Alcian Blue 8GX for proteoglycan and glycosaminoglycan formation; Alizarin Red S for mineralization of bone matrix; and Oil Red O for lipid droplet deposition. Morphology of the stained cells was observed under an inverted microscope.

Following the verification of synovial-derived MSC multipotency, the patient underwent surgery. After completing the supramalleolar osteotomy (Figure 4), OLT debridement, subchondral bone curettage and drilling, we performed autologous bone grafting at the OLT. The source of bone graft was the distal tibia at the osteotomy site. Collagen I/III matrix (Chondro-Gide, Geistlich, Wollhusen, Switzerland) was cut to roughly the size of the lesion, placed over the lesion, and then trimmed for a more precise covering. The lesion area was 12 × 6.5 mm and the lesion depth was 8 mm. The synovial-derived MSC solution was injected into the collagen matrix (Figure 5), and the two were allowed to merge for 3 minutes, then fixed to the lesion with human fibrin sealant (Fibringluraas, Shanghai RAAS, Shanghai, China). The stability of the matrix fixation was tested by dorsiflexion and plantarflexion movements of the ankle several times, with fixation deemed

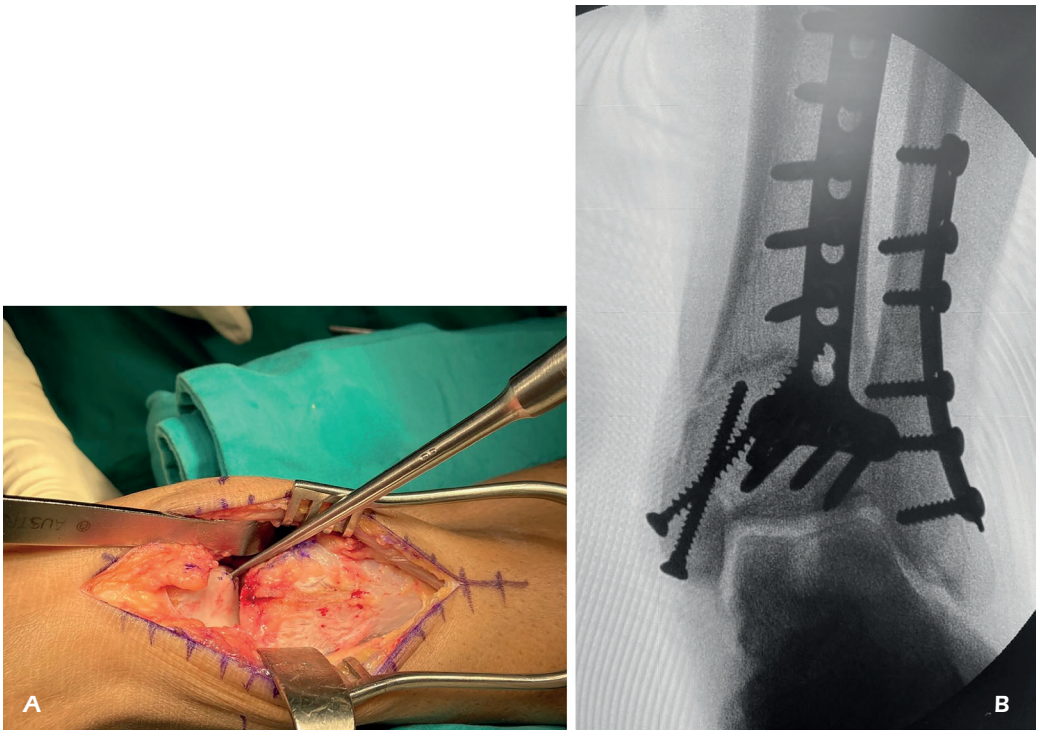

Figure 4. Intraoperative images revealing that the osteochondral lesion of the talus was debrided and curetted (**A**) and the completed supramalleolar osteotomy using the dome-shaped technique (**B**).

adequate when the matrix stayed in place at the lesion. Finally, PRP fibrin was used to cover the MSC-embedded matrix. A drain was placed outside the joint capsule, the skin was closed in layers of dressing, and a short leg slab was applied.

Results

The harvesting of ankle synovium provided a satisfactory source of MSCs with typical spindle-shaped morphology (Figure 6); the source-cell culture reached 30 million total cells in 4 weeks. The bacterial contamination and endotoxin tests were negative. Expression of surface markers indicative of multipotent MSCs was shown by flow cytometry (Figure 7). Multilineage differentiation potentials were confirmed by the formation of lipid droplets, proteoglycan aggregation and calcium mineralization in cultures grown in adipogenic, chondrogenic and osteogenic induction media, respectively (Figure 8).

The patient recovered uneventfully from his pain and other related symptoms in the postoperative period. At the follow up approximately 6 weeks later (Figure 9), the patient's score on Visual Analogue Scale-Foot and Ankle (VASFA)[6] had dropped from 79 preoperatively to 53.89 postoperatively, while the Short Form-36[7] score changed from 48.33 to 46.81. The mean VASFA pain subscales score improved from 75 preoperatively to 90 postoperatively. Further follow-up will be necessary to demonstrate the long-term results of this surgery.

Discussion

In this study, we have highlighted a potential role for ankle synovium in the treatment of OLT. Our novel harvesting technique generated synovial-derived MSCs that were shown to be multipotent MSCs useful for further chondrogenesis.

Baboolal et al[3] demonstrated that a cellular fraction mobilized during arthroscopy contained viable MSCs with proliferative potential. Trilineage differentiation capacities for bone, cartilage and fat lineages were shown, cultured daughter cells exhibited the standard MSC phenotype, and the mobilized synovial MSCs adhered to various fibrin scaffolds *in vitro* following culture passaging[3]. Their technique was simple and convenient, and not associated with any complications[3]. Re-

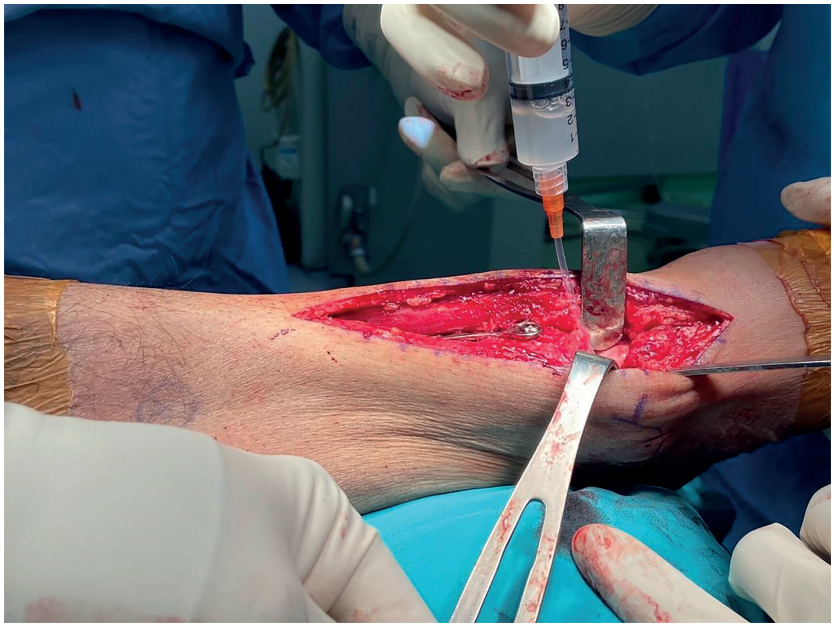

Figure 5. Injection of the synovial-derived mesenchymal stem cell solution into the surgical field.

garding the use of stem cells for OLT in the ankle, Richter et al[8] described promising results with a treatment involving matrix-associated stem cells sourced from a bone marrow aspirate concentrate. However, these authors did not report on the *in vitro* growth potential, quantification of MSC colony number, or MSC immunophenotype of the retrieved cells used in their study[7]. Buda et al[9] also described a one-step bone marrow-derived cell transplantation (BMDCT) for the treatment of OLT in concomitant ankle osteoarthritis. They found that BMDCT showed encouraging clinical and radiological outcomes at short-term follow-up[9]; however, similar to Richter et al[8], these

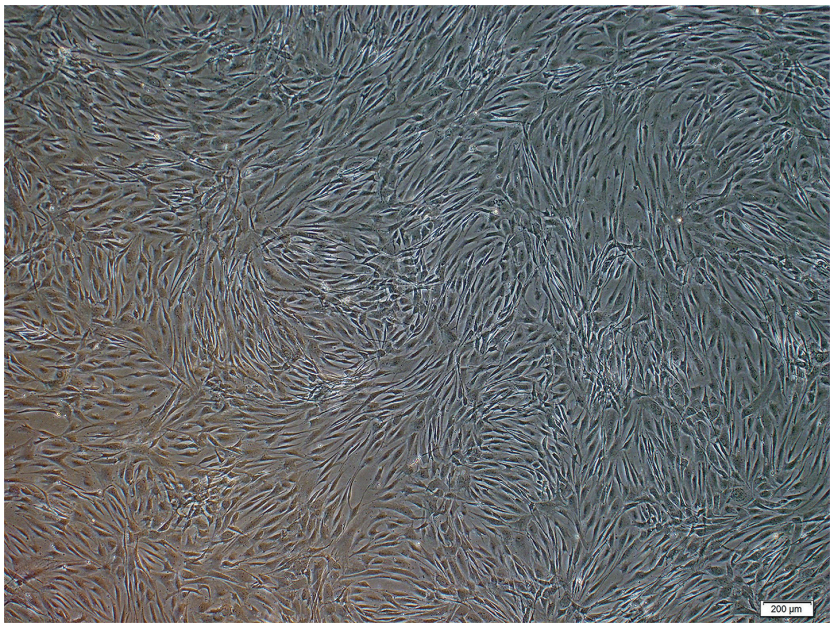

Figure 6. A bright-field microscopic image showing the typical spindle-shaped cell morphology of the cultivated cells. Scale bar = 200 µM.

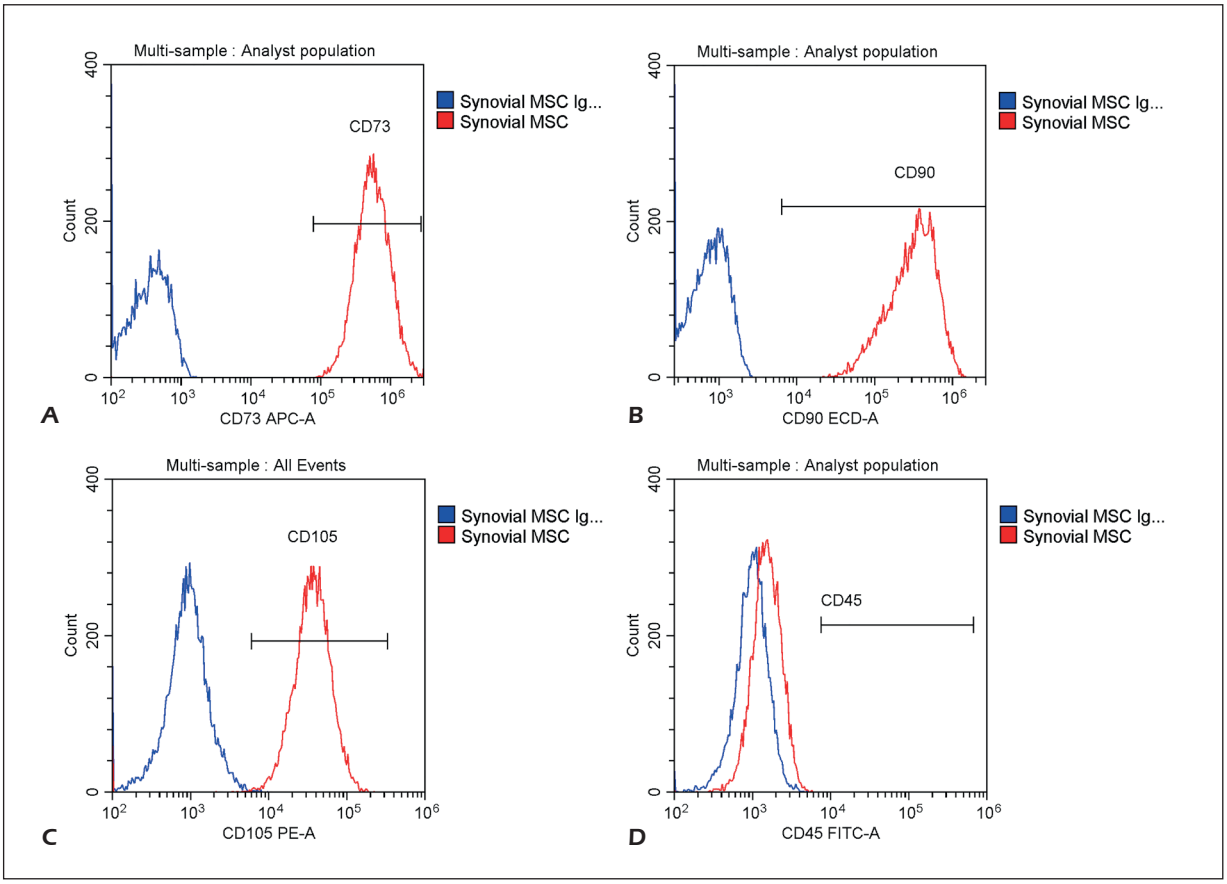

Figure 7. Fluorescence histogram of the cultivated synovial-derived cells showing positivity for mesenchymal stem cell markers CD73 (**A**), CD90 (**B**) and CD105 (**C**), and negativity for hematopoietic marker CD45 (**D**). The red line indicates fluorochrome-tagged antibody staining. The blue line indicates isotype-matched negative control staining.

authors did not provide *in vitro* growth, quantification of MSC colony number or immunophenotype in their study. Without those data, we can only speculate that their retrieved cells might have been multipotent MSCs[8,9]. The ISCT criteria are essential to prove that retrieved cells reflect the potential of differentiation into osteoblasts and chondrocytes[4]. In this study, we have shown conclusively that our harvesting technique for synovial-derived MSCs generated multipotent MSCs in accordance with these criteria. The harvesting technique was simple, convenient, and not associated with any complications in our patient. Although further follow-up is necessary to demonstrate the long-term results of the surgery, this study could be a model for a larger clinical study with a longer follow-up period.

The novelties of this research are two-fold: 1) the use of ankle synovium as a source of potential MSCs; and 2) a new harvesting technique for efficient collection of MSCs that involves minimally invasive surgery. At this stage, there is not adequate evidence to propose that MSCs obtained with this technique and derived from a different source could be used for other diseases or pathologic regions, such as osteoarthritis or cartilage lesion of the knee. Further study will be essential to apply our novel approach beyond OLT.

Conclusions

We have identified the ankle synovium as a novel source of synovial-derived MSCs for the treatment of OLT. Moreover, we developed an MSC harvesting technique that was minimally invasive, efficient, simple, convenient, and not associated with any complications. Our approach combining synovial-derived MSCs with collagen matrix therapy is encouraging for the field of stem cell therapy and must be investigated further to uncover its potential applications.

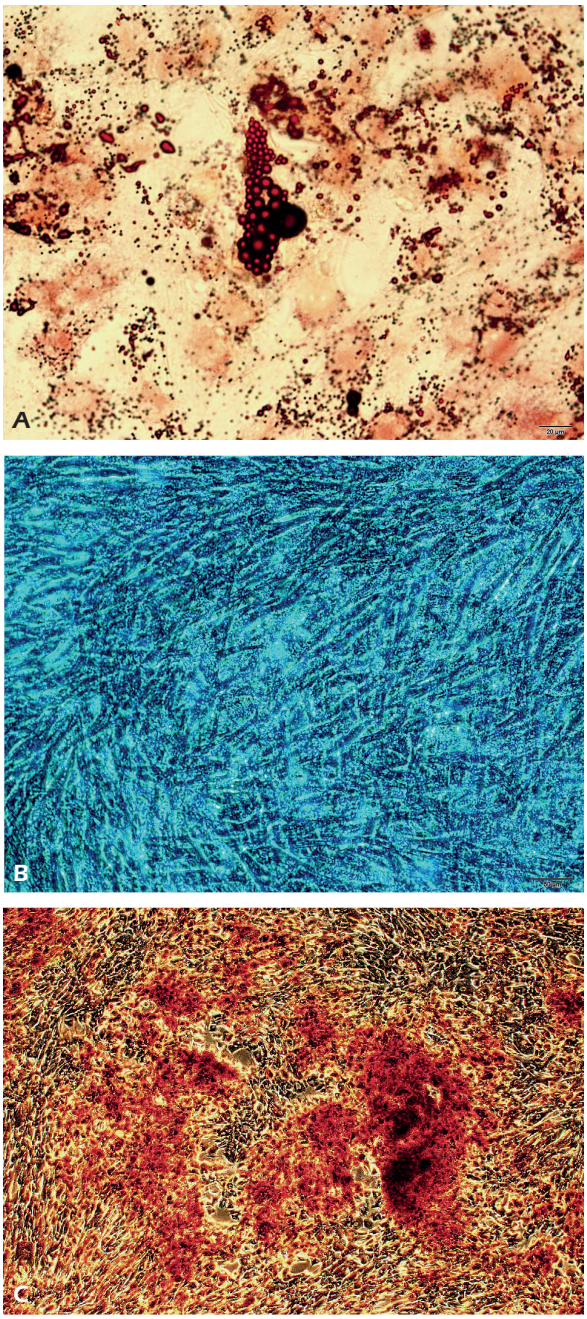

Figure 8. Mesodermal differentiation potential of cultivated synovial-derived mesenchymal stem cells cultured under three different induction media: adipogenic (**A**), chondrogenic (**B**), and osteogenic (**C**). Scale bars = 20, 50, and 200 μm, respectively.

Acknowledgments

Financial support from Medeze Research and Development for the laboratory equipment and facilities used in this research. We thank Michelle Kahmeyer-Gabbe, Ph.D, from Edanz Group (https://en-author-services.edanzgroup.com/) for editing a draft of this manuscript.

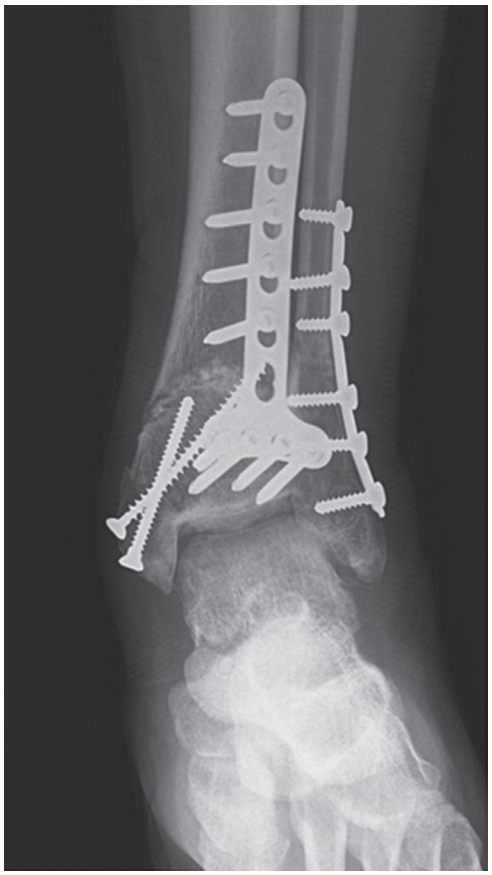

Figure 9. Anteroposterior radiograph of the left ankle approximately 6 weeks after surgery demonstrating the corrected alignment of the ankle with healing of the osteochondral lesion of the talus on the medial side.

Conflict of Interests

The authors declare the following potential conflict interest: technical support from Medeze Research and Development for the laboratory equipment and facilities used in this research.

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