Motorized Shaver Harvest Results in Similar Cell Yield and Characteristics Compared With Rongeur Biopsy During Arthroscopic Synovium-Derived Mesenchymal Stem Cell Harvest

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Purpose: To compare cell yield and character of synovium-derived mesenchymal stem cell (SDMSC) harvested by 2 different techniques using rongeur and motorized shaver during knee arthroscopy. **Methods:** This study was performed in 15 patients undergoing partial meniscectomy. Two different techniques were used to harvest SDMSCs in each patient from the synovial membrane at 2 different locations overlying the anterior fat pad, each within 1 minute of harvest time. Cell yield and proliferation rates were evaluated. Cell surface marker analysis was done after passage 2 (P2). Trilineage differentiation potential was evaluated by real-time quantitative polymerase chain reaction and histology. Statistical analysis between the 2 methods was done using the Mann−Whitney *U* test. **Results:** Wet weight of total harvested tissue was 69.93 (± 20.02) mg versus 378.91 (± 168.87) mg for the rongeur and shaver group, respectively ($P < .0001$). Mononucleated cell yield was 3.32 (± 0.89) versus 3.18 (± 0.97) × 10^3 cells/mg, respectively ($P = .67$). Fluorescence-activated cell sorting analysis revealed similar SDMSC-related cell surface marker expression levels in both groups, with positive expression for CD44, CD73, CD90, and CD105 and decreased expression for CD34 and CD45. Both groups showed similar trilineage differentiation potential in histology. Chondrogenic (SOX9, ACAN, COL2), adipogenic (LPL, PLIN1, PPAR-γ), and osteogenic (OCN, OSX, RUNX2) gene marker expression levels also were similar between both groups. **Conclusions:** No difference was observed between rongeur biopsy and motorized shaver harvest methods regarding SDMSC yield and cell characteristics. **Clinical Relevance:** The current study shows that both rongeur and motorized shaver harvest are safe and effective methods for obtaining SDMSCs. Motorized shaver harvest results in higher volume of tissue acquisition per time, thereby leading to higher number of SDMSCs which may be useful during clinical application.

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Recent advances in stem cell biology and tissue engineering have translated to clinical use of mesenchymal stem cells (MSCs) to treat a variety of knee joint conditions, including cartilage defects, meniscus tears, ligament injuries, and osteoarthritis.[1-6] Benefits of MSC use in knee surgery include histologically better repair outcomes, single-stage operations, and improved functional outcomes of patients.[7-9] MSCs are currently harvested from various tissues, including bone marrow, synovium, fat, and umbilical cord.[10,11] MSCs from different tissues, by definition, share similar characteristics of multipotency,

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plastic adherence, in vitro trilineage differentiation potential, and expression of specific cell surface markers.[12] Differentiation potential and therapeutic efficacy of MSCs, however, differ according to the site of harvest.[10]

Synovium-derived mesenchymal stem cells (SDMSCs) are cells isolated from synovium possessing characteristics of MSCs.[13] SDMSCs can be easily obtained during knee surgery with minimal morbidity.[14,15] Compared with bone marrow MSCs, SDMSCs have greater chondrogenic potential and their differentiation and proliferation capacity are not hindered by donor age or inflammation.[16,17] Due to these advantages, there is increasing interest and therapeutic applications of these cells for knee joint disorders.

Despite such clinical interest, there is very little research regarding the harvesting methods of these cells. A standardized and highly reproducible method of harvesting SDMSCs is essential, both for the safety of patients as well as to obtain a maximum number of high-quality cells.[18-20] Past research has suggested that the therapeutic effect of MSCs is highly dependent on the cell number of MSCs and, therefore, the need to obtain a high yield of cells during harvest.[21-23] Cell yield during harvest, in turn, is dependent on the method of harvest, as shown in past adipose and bone marrow MSC studies comparing various harvest methods.[21,23-25] Up to now, a number of different methods have been used for SDMSCs harvest during knee surgery, including arthroscopic biopsy, en bloc resection, motorized shaver, and customized devices, yet a comparison between harvesting methods in a well controlled clinical setting is lacking.[26-29] The purpose of this study was to compare cell yield and character of SDMSCs harvested by 2 different techniques using rongeur and motorized shaver during knee arthroscopy. We hypothesized that the arthroscopic shaver technique would result in a greater number of harvested cells and similar cell characteristics compared with the rongeur technique.

Methods

Study Design and Tissue Harvest

Study protocols were approved by our institutional review board (Ajou University Institutional Review Board, Suwon, Republic of Korea; AJIRB-BMR-SMP-20-170), and consent forms were completed by all participants. This descriptive laboratory study was performed in 15 patients aged 40 to 60 years old undergoing arthroscopic partial meniscectomy for degenerative medial meniscus posterior horn tears in osteoarthritic knees with synovitis. We excluded patients with end-stage radiographic osteoarthritis (Kellgren–Lawrence grade 4), previous history of knee surgery, intra-articular injection history within 6 months of operation, autoimmune disease–related arthritis, and trauma history. Arthroscopic partial meniscectomy was performed in patients with mechanical symptoms or failed conservative care for at least 3 months.[30,31] Harvest of synovial tissue was done after diagnostic arthroscopy and before meniscectomy by the corresponding author (D.Y.P.). For the rongeur biopsy technique, an arthroscopic rongeur (Raptor 0147, ACUFEX; Smith & Nephew, Andover, MA) was inserted through the anteromedial port, and harvest of tissue was done from the synovial membrane lateral to the anteromedial portal, overlying the medial half of the anterior fat pad under arthroscopic visualization. For the motorized shaver harvest technique, an arthroscopic shaver (4.2-mm, Large Hub, ReAct, Shaver, Cuda [42CUD-RA-ZZ], CONMED Linvatec, Utica, NY) was used in the oscillating mode at 2500 rpm without suctioning. Tissue was collected in a container attached to the outflow tube of the shaver (Fig 1). The shaver was inserted through the anterolateral portal, and harvest of tissue was done from the synovial membrane medial to the anterolateral portal, overlying the lateral half of the anterior fat pad under arthroscopic visualization. Each harvest method was done for 1 minute. Only synovial tissue showing signs of macroscopic synovitis was removed.[32] Care was taken not to infiltrate the fat pad during harvest.

Cell Isolation and Cell Counting

After harvest, tissue samples were immediately transferred to a laboratory within an aseptic container filled with normal saline for cell isolation. Tissue samples were rinsed in phosphate-buffered saline (PBS) and weighed. Tissues were treated with 0.1% type II collagenase (Gibco BRL, Carlsbad, CA) in serum-free Dulbecco's Modified Eagle's Medium-high glucose (DMEM-HG; HyClone, Logan, UT) at 37°C for 3 hours, and consequent homogenates were filtered with a 100-μm cell strainer and harvested by centrifugation at 480*g* for 5 minutes. The mononucleated cell (MNC) counting was done using a hemocytometer. Isolated cells were expended in α-modified Eagle's medium (α-MEM; HyClone) supplemented with 10% fetal bovine serum (FBS; HyClone) and 1% penicillin/streptomycin (Gibco BRL), and cultured at a density of 8×10^3 cells/cm^2.[33]

Colony-Forming Unit-Fibroblast (CFU-F) Assay

We conducted CFU-F assays to compare the cloning efficiency of SDMSCs harvested by the 2 different methods. CFU-F assay was performed using freshly digested cells seeded at a density of 1.5×10^3 cells into 100 mm culture dishes. After 14 days, the cultures were fixed with 100% methanol and stained with 1% crystal violet solution (Sigma-Aldrich, St, Louis, MO). The number of stained cell colonies was analyzed by selecting a size larger than 2.5 mm.[34-36]

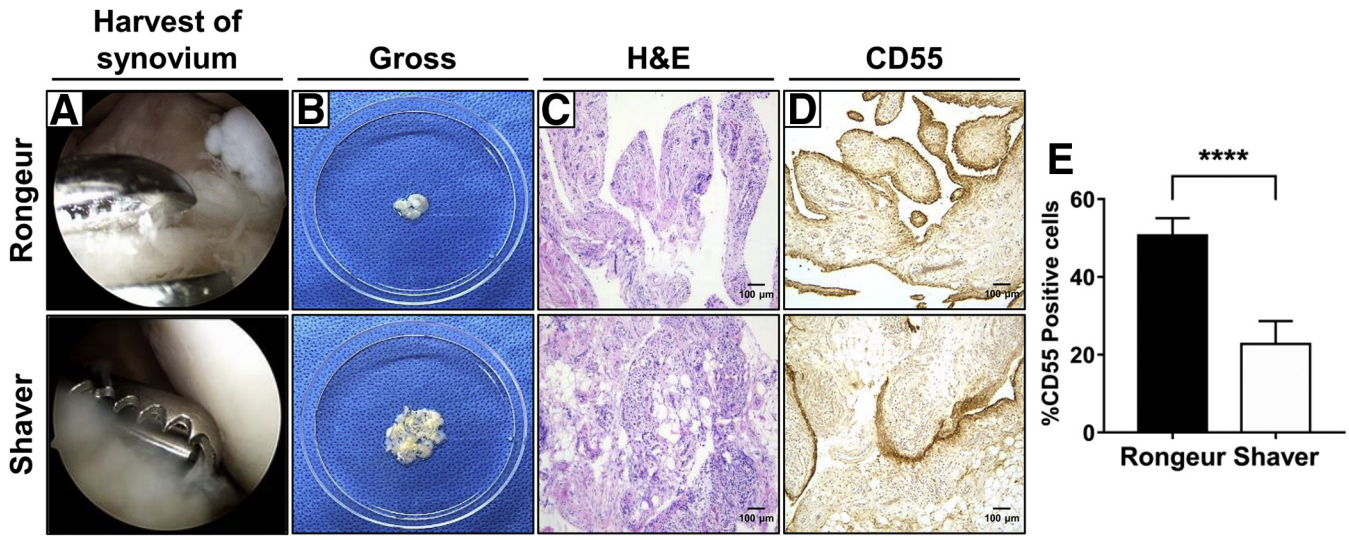

Fig 1. Arthroscopic tissue harvest by rongeur biopsy method and motorized shaver method. (A) Arthroscopic view of each harvest method in a left knee joint. Top-row photographs show the rongeur inserted through the anteromedial portal and a viewing portal through the anterolateral portal. The bottom-row photographs show the motorized shaver inserted through the anterolateral portal and a viewing portal through the anteromedial portal. (B) Gross morphology of harvested tissue. (C) Histology of harvested tissue, H&E staining. (D) Immunohistochemistry of harvested tissue of CD55 and (E) quantification of CD55 positive cells. Magnification, ×100. (H&E, hematoxylin and eosin.)

Histology and Immunohistochemistry (IHC)

Synovium samples from 5separate donors who met the inclusion criteria were fixed in 4% formalin for 3 days and processed for paraffin embedding and sectioning according to established procedures. Tissue section 4 μm in thickness were prepared and stained with hematoxylin-eosin for morphologic evaluation. For IHC analysis, tissue sections were stained with anti-human CD55 antibody (1:100; Abcam, Cambridge, UK; catalog No. ab133684). The CD55, a complement inhibitor, was chosen as a specific marker for the synovial membrane, the outermost layer of the synovium.[37] The IHC staining intensity was quantified using Image J software (National Institutes of Health, Bethesda, MD). The CD55 antibody staining intensity was quantified by the optical density values of DAB (brown) and hematoxylin staining (blue). Three different sections of each slide were averaged for each sample. A total of 5 samples were analyzed from each patient.

Proliferation Assay

To compare the proliferation ability of cells, the doubling time was analyzed during the culture expansion over P10. Synovium-derived cells harvested with a rongeur or shaver were seeded at a density of 1×10^4 cells/cm^2. When 80% confluence was reached, the cells were harvested and stained with 0.4% trypan blue solution. The doubling time was obtained by the formula TD = tplog2 / (logNt − logN0). N0 is the inoculum's cell number, Nt is the number of cells harvested after culture, and t is the time of the culture in days.

Differentiation Assays

Synovium-derived cells harvested with a rongeur or shaver at P2 were used to examine their ability to differentiate in vitro into chondrogenic, adipogenic, and osteogenic lineages as described herein. For chondrogenic differentiation, 3×10^5 cells were transferred to a 15-mL polypropylene tube and centrifuged at 500g for 10 minutes. The pellets were cultured in chondrogenic induction medium, which was changed every 3 to 4 days. The chondrogenic defined medium consisted of DEME-HG with 50 μg/mL ascorbate-2-phosphate, 100 nM dexamethasone, 40 μg/mL L-proline, insulin−transferrin−selenium mixture supplement, 1.25 mg/mL bovine serum albumin, 100 μg/mL sodium pyruvate (Sigma-Aldrich), and 10 ng/mL TGF-β3 (R&D Systems, Minneapolis, MN). After 3 weeks, the pellets were then harvested for mRNA extraction for real-time quantitative polymerase chain reaction (RT-qPCR), or embedded in paraffin, cut into 4-μm sections, and stained with Safranin-O (Sigma-Aldrich). For adipogenic differentiation, cells were plated at a density of 1×10^4 cells/cm^2. The adipogenic medium containing α-MEM supplemented with 10% FBS, 0.5 mM isobutyl-methylxanthine, 1 μM dexamethasone, 0.1 mM indomethacin, and 10 μg/mL insulin (Sigma-Aldrich). The cells were cultured in adipogenic medium, which was changed every 3 to 4 days. After 3 weeks, the cells were harvested for mRNA extraction for RT-qPCR or stained with Oil red O (Sigma-Aldrich). For osteogenic differentiation, cells were plated at a density of 5×10^3 cells/cm^2. The osteogenic medium

containing α-MEM supplemented with 10% FBS, 50 μg/mL ascorbate-2-phosphate, 100 nM dexamethasone, and 10 mM β-glycerophosphate (Sigma-Aldrich). The cells were cultured in osteogenic medium, which was changed every 3 to 4 days. After 3 weeks, the cells were harvested for mRNA extraction for RT-qPCR or stained with Alizarin Red S (Sigma-Aldrich).

RT-qPCR Analysis

RT-qPCR was performed according to previously described methods.[38] To summarize, RNA was extracted using TRIzol (Invitrogen, Grand Island, NY) and Total RNA of 1 μg was reverse-transcribed using a first strand cDNA synthesis kit (Bio-Rad, Hercules, CA) in the presence of specific primers. RT-qPCR was performed using the 1X SYBR Green Reaction Mix (Roche, Mannheim, Germany). The relative gene expressions of the samples were normalized to glyceraldehyde-6-phosphate dehydrogenase as an internal control and calculated by the comparative C_T method. The primers used are shown in Table 1.

Flow Cytometry

SDMSCs display several common surface antigens, such as CD44, CD73, CD90, CD105, and lack hematopoietic markers like CD34, CD45. The cells were harvested at P2 and washed twice with PBS containing 2% FBS. The cells were then fixed in 4% paraformaldehyde for 30 minutes at 4°C and stained with anti-CD44-FITC (catalog No. 555478), anti-CD73-FITC (catalog No. 561254), anti-CD90-FITC (catalog No. 555595), anti-CD105-FITC (catalog No. 555690), anti-CD34-FITC (catalog No. 555821), and anti-CD45-FITC (catalog no. 555482; BD Biosciences, Bedford, MA) for 40 minutes at 4°C in a darkroom. The cell–antibody complexes were washed twice with PBS and analyzed using a BD FACSvantage II flow cytometry analyzer (BD Biosciences).

Statistical Analysis

Data for the IHC, CFU-F, proliferation, differentiation, RT-qPCR, and flow cytometry were expressed as the mean ± 95% confidence intervals (CIs). Statistical significance between non-normal distributions of paired data from each patient was analyzed by Wilcoxon matched pairs signed rank test. A value of $P < .05$ was considered statistically significant ($^*P < .05$, $^{**}P < .01$, $^{***}P < .001$, and $^{****}P < 0.0001$).[38]

Results

Comparison of Synovium After Harvest and Isolation by Rongeur and Shaver Technique

For each patient (knee), 2 different techniques were used to harvest synovial tissue (Fig 1A). The average age of enrolled patients was 50.7 years old (range 43-59) with 11 female and 4 male patients. The radiographic Kellgren–Lawrence grade of harvested knees was grade 2 in 9 knees and grade 3 in 6 knees. As for meniscus tear pattern, 9 knees showed complex tears, 3 knees showed horizontal tears, and 4 knees showed combined root tears in the medial meniscus posterior horn region. Gross morphology differed between the 2 harvest groups, where tissue harvested by rongeur resulted in smaller quantity of tissue and slightly more red in color, while tissue harvested by motorized shaver was larger in quantity and more yellow in color (Fig 1B). Histology of rongeur harvested tissue

Table 1. Primers Used in RT-qPCR after the Reverse Transcription of mRNA

Primers	Gene Name	Sequences	Length, bp	Annealing Temp, °C
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	F: 5′-TGCACCACCAACTGCTTAGC-3′ R: 5′-GGCATGGACTGTGGTCATGAG-3′	87	60
OCN	Osteocalcin	F: 5′-AGCAAAGGTGCAGCCTTTGT-3′ R: 5′-GCGCCTGGGTCTCTTCACT-3′	63	60
OSX	Osterix	F: 5′-TGGCCATGCTGACTGCAGCC-3′ R: 5′-TGGGTAGGCGTCCCCCATGG-3′	146	60
RUNX2	Runt-related transcription factor 2	F: 5′-GGTTAATCTCCGCAGGTCACT-3′ R: 5′-CACTGTGCTGAAGAGGCTGTT-3′	143	60
LPL	Lipoprotein lipase	F: 5′-AGACACAGCTGAGGACACTT-3′ R: 5′-GCACCCAACTCTCATACATT-3′	137	60
PLIN1	Perilipin-1	F: 5′-GGACACAGTGGTGCATTACG-3′ R: 5′-GTCCCGGAATTCGCTCTC-3′	70	60
PPAR-γ	Peroxisome proliferator-activated receptor gamma	F: 5′-CAGGAAAGACAACAGACAAATCA-3′ R: 5′-GGGGTGATGTGTTTGAACTTG-3′	94	60
SOX9	SRY-related high-mobility-group box 9	F: 5′-CACACAGCTCACTCGACCTTG-3′ R: 5′-TTCGGTTATTTTTAGGATCATCTCG-3′	76	60
ACAN	Aggrecan	F: 5′-CCCCACTGGCCCCAAGAATCAAG-3′ R: 5′-CGCTGCGCCCTGTCAAAGTCG-3′	319	60
COL2	Collagen type 2	F: 5′-CTCCTGGAGCATCTGGAGAC-3′ R: 5′-ACCACGATCACCCTTGACTC-3′	152	60

RT-qPCR, real-time quantitative polymerase chain reaction.

showed synovial lining cells of 2 or 3 deep on the tissue surface, while shaver harvested tissue showed more adipose tissue under synovial lining cells (Fig 1C). IHC of tissue showed more CD55-positive cells in the rongeur group compared with the shaver group, indicating more synoviocytes from the membrane surface per tissue area in the rongeur group (Fig 1D). The percentage of CD55 positive cells was 50.67 ± 1.82, 95% CI versus 22.75 ± 2.43, 95% CI for rongeur group and motorized shaver group, respectively ($P < .0001$) (Fig 1E). The wet weight of rongeur obtained tissue was lesser compared with shaver-obtained tissue (69.93 ± 20.02 mg 95% CI vs 378.91 ± 168.87 mg 95% CI, $P < .0001$) (Fig 2A). Total MNC count after enzymatic digestion showed lower cell counts in the rongeur group compared with the shaver group ($0.65 \pm 0.34 \times 10^6$ cells, 95% CI vs $1.59 \pm 0.35 \times 10^6$ cells, 95% CI, $P < .0001$) (Fig 2B). The cell yield, determined as MNC number per tissue weight, however, was not different among both groups ($3.32 \pm 0.89 \times 10^3$ cells/mg, 95% CI vs $3.18 \pm 0.97 \times 10^3$ cells/mg, 95% CI, $P = .67$) (Fig 2C). The obtained SDMSC number after in vitro culturing for 4 days at P0 showed greater cell numbers in the shaver group compared with the rongeur group ($1.78 \pm 0.29 \times 10^6$ cells, 95% CI vs $6.63 \pm 0.78 \times 10^6$ cells, 95% CI, $P < .0001$) (Fig 2D). The number of CFU-F per 10^3 cells also was not different among both groups (67.80 ± 19.29, 95% CI vs 55.87 ± 22.15, 95% CI, $P = .13$) (Fig 2 E and F). Clinically, none of the participants reported any complications from the procedure, such as anterior knee pain, hemarthrosis, and arthrofibrosis.

Characteristics of SDMSCs After Expansion

After 7 days of culture, adherent cells obtained from the 2 harvesting methods showed similar morphology with elongated, fibroblast-like appearance (Fig 3A). Growth kinetics were similar between the 2 harvesting methods. The doubling time was 2.14 hours for the rongeur group compared with 2.29 hours for the motorized shaver group at passage 4 ($P = .75$) (Fig 3B). The phenotype of cells indicative of MSCs at passage 1 for both groups was similar, with less than 2% of cells

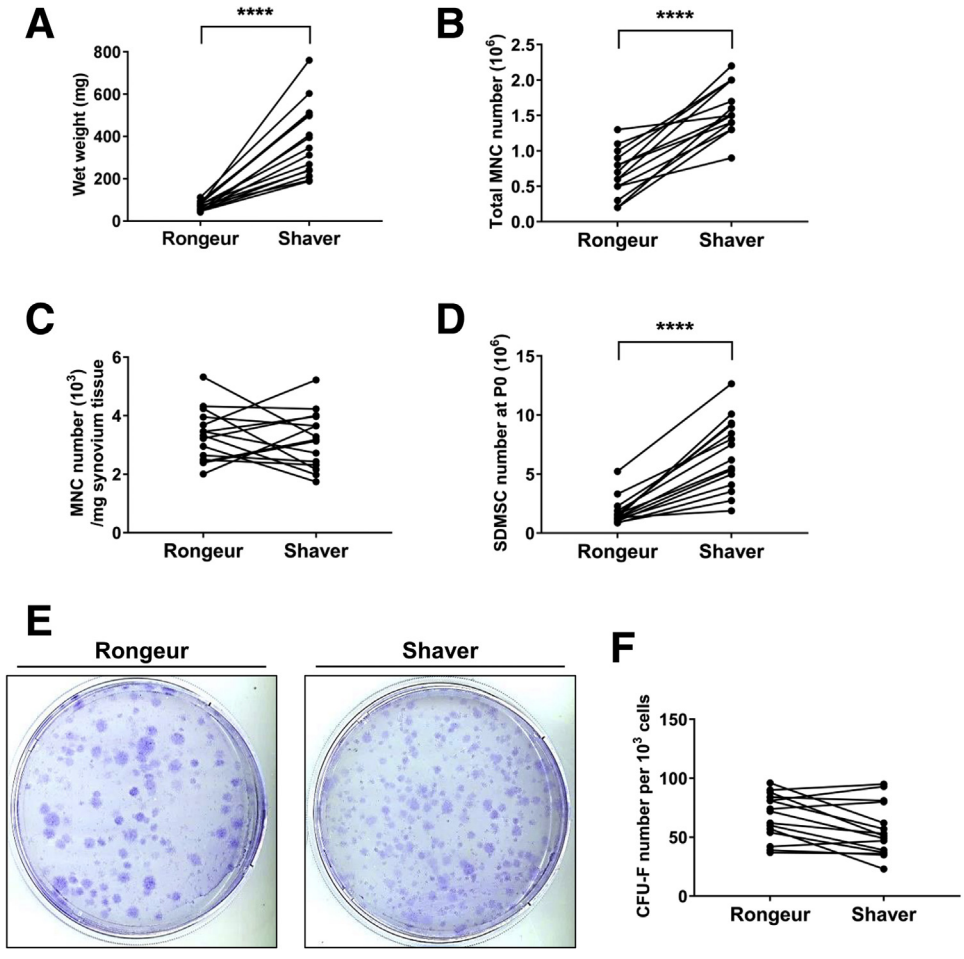

Fig 2. Cell isolation characteristics after arthroscopic tissue harvest. (A) Wet weight of harvested tissue. (B) Total mononucleated cell (MNC) number from harvested tissue. (C) MNC number/mg of harvested tissue. (D) SDMSC number after in vitro culturing for 4 days at passage 0. (E) Colony-forming units and (F) their count from harvested tissue. (SDMSC, synovium-derived mesenchymal stem cells.)

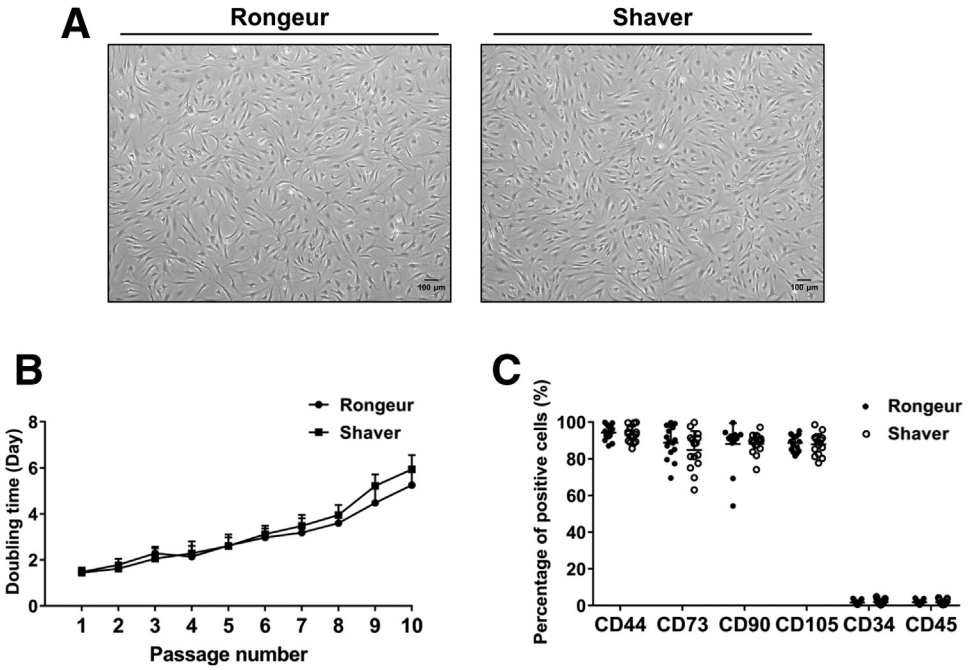

Fig 3. Cell characteristics after expansion. (A) Photograph of synovium-derived mesenchymal stem cells isolated by 2 different methods. Magnification, ×50. (B) Average doubling time over passages 10. (C) Immunophenotype of cells analyzed by flow cytometry, as expressed as a percentage of viable cells positive for the surface markers.

showing cell marker expression for CD34 and CD45, whereas 100% of cells showed cell marker expression for CD44 and CD73. As for CD90 expression, 88.12% of cells from the rongeur group and 88.20% from the shaver group were positive ($P = .98$). Finally, for CD105 expression, 88.67% of cells from the rongeur group and 87.87% from the shaver group were positive ($P = .67$) (Fig 3C).

Differentiation Potential of SDMSCs

Trilineage differentiation toward osteogenic, chondrogenic, and adipogenic lineage was analyzed for the rongeur group and motorized shaver group with histologic analysis and respective gene markers. As for osteogenesis, each group showed similar Alizarin Red S (+) staining, with both groups displaying red calcium granules (Fig 4A). Osteogenic gene markers of OCN (1.01 ± 0.06, 95% CI vs 0.95 ± 0.05, 95% CI, $P = .43$), OSX (1.42 ± 0.17, 95% CI vs 1.29 ± 0.09, 95% CI, $P = .50$), and RUNX2 (0.84 ± 0.07, 95% CI vs 0.67 ± 0.10, 95% CI, $P = .14$) were expressed in both groups, with both groups showing no difference (Fig 4B). As for adipogenesis, each group showed similar Oil Red O (+) staining (Fig 4C). Adipogenic gene markers of LPL (1.93 ± 0.10 , 95% CI vs 1.70 ± 0.11, 95% CI, $P = .13$), PLIN1 (1.46 ± 0.13, 95% CI vs 1.73 ± 0.12, 95% CI, $P = .14$), and PPAR-γ (1.95 ± 0.21, 95% CI vs 2.23 ± 0.27, 95% CI, $P = .42$) showed greater expression levels relative to glyceraldehyde-6-phosphate dehydrogenase in both groups, with no difference between both groups (Fig 4D). Finally, chondrogenic differentiation was observed for both groups, as shown in Safranin O stainings (Fig 4E). Chondrogenic gene markers of SOX9 (1.07 ± 0.10 , 95% CI vs 0.96 ± 0.07, 95% CI, $P = .67$), ACAN (1.03 ± 0.10, 95% CI vs 1.12 ± 0.13, 95% CI, $P = .61$), and COL2 (1.22 ± 0.09, 95% CI vs 0.99 ± 0.07, 95% CI, $P = .06$) were all expressed in both groups, with no significant difference between groups (Fig 4F).

Discussion

Comparison of rongeur biopsy and motorized shaver harvest for SDMSCs revealed no difference between the 2 techniques regarding cell yield and cell characteristics, with both groups showing surface marker profiles, proliferation abilities, and trilineage differentiation potentials compatible with SDMSCs. The major difference between the 2 techniques was the harvested tissue weight, total MNC number, and total SDMSC number after 4 days of culture, with the motorized shaver technique resulting in approximately 2.5 times and 3.7 times the number of MNCs and SDMSCs respectively over the same harvest time compared with rongeur biopsy.

Different harvest methods affect total cell quantity, cell yield, and cell characteristics during MSC harvest.[39,40] Previous studies have used various methods during SDMSCs harvest, including rongeur biopsy, motorized shaver resection, en bloc resection, and customized equipment.[26-28] Baboolal et al.[26] used a customized stem cell mobilizing device with saline irrigation to mobilize SDMSCs and resulted in greater cell yield compared with cytology brush. Arthroscopic shaver harvest has been compared with en bloc resection in dogs resulting in higher cell yields for the en bloc

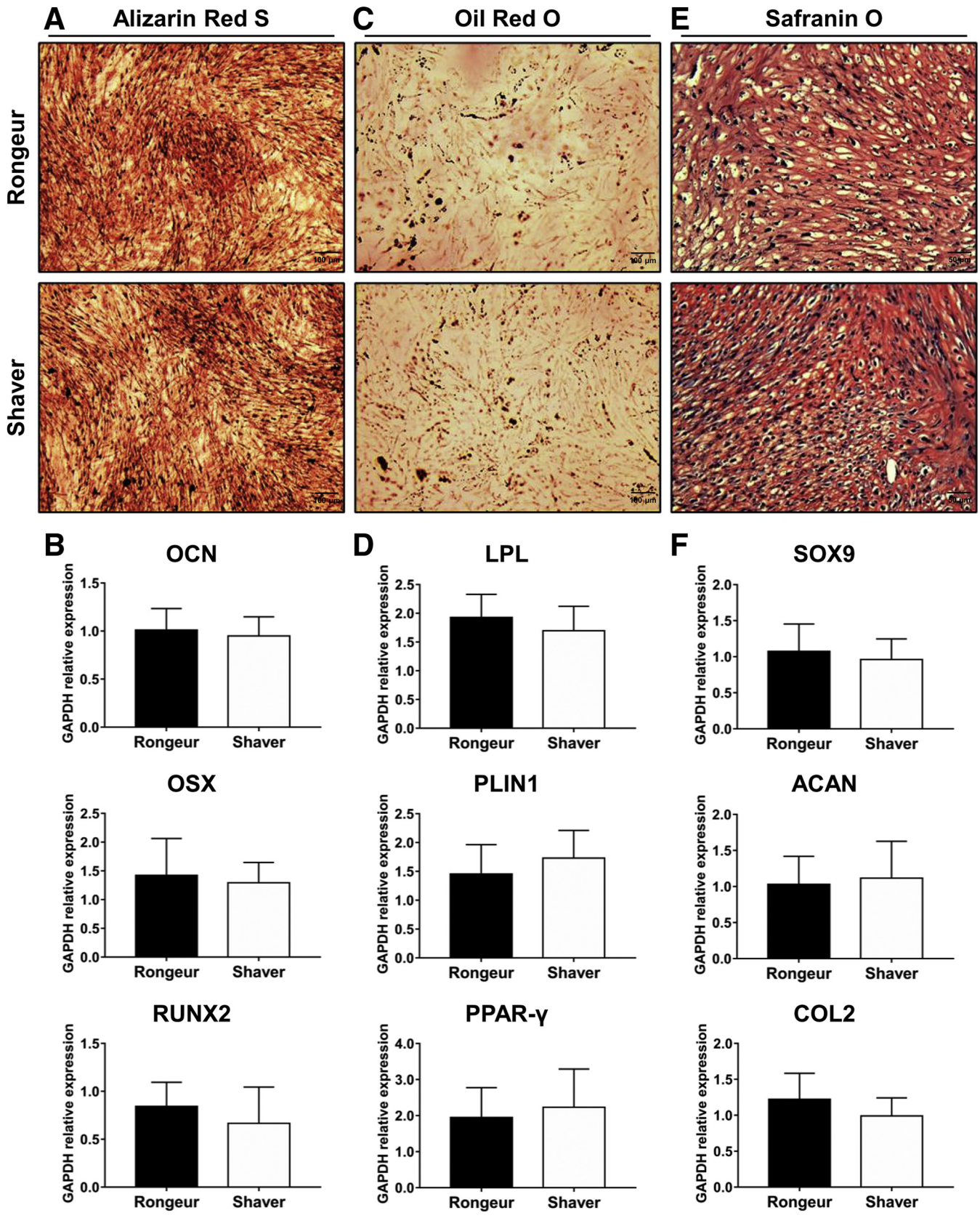

Fig 4. Trilineage differentiation of synovium-derived mesenchymal stem cells. (A) Alizarin Red S staining photographs showing osteogenic differentiation. (B) Osteogenesis-related gene markers. (C) Oil Red O staining photographs showing adipogenic differentiation. (D) Adipogenesis-related gene markers. (E) Safranin O staining photographs showing chondrogenesis. (F) Chondrogenesis related gene markers. Magnification, (A), (C) ×100 and (E) ×200.

resection, but both harvest techniques were performed in animals with two different pathologies.[41] Ferro et al.[42] recently reported a study regarding SDMSCs obtained from direct biopsy, motorized shaver, and synovial fluid samples. They reported similar MSC-like characteristics between harvesting methods, with the motorized shaver method showing the greatest MSCs-like cells isolated. This study, however, was done in a heterogeneous population of a wide age gap (20-78 years) with traumatic as well as degenerative conditions, and the 3 methods were not all completed for each patient. Harvest sites were also not specified for each method. Donor age, harvest site, and disease all affect SDMSCs harvest results.[43] Our study controlled these variables by including a more homogenous group and harvesting only from the synovium overlying the anterior fat pad. Both harvest methods were performed in each patient. Harvest time also was controlled, with both methods performed within 1 minute. In turn, direct comparison between the 2 methods was possible, with the motorized shaver method harvesting more synovial tissue and producing more MNC compared with the rongeur method during a fixed amount of time.

Among capsular tissues accessible during harvest, cells meeting the MSCs criteria recommended by the International Society of Cellular Therapy have been found in the synovial membrane, subintima, and infrapatellar fat pad.[37,44] While MSCs from all 3 regions show similar self-renewal capacity, multidifferentiation, and therapeutic applications,[45] recent evidence sheds light on the differences between these regions. Synovial membrane–derived MSCs are thought to be Ttype B synoviocyte subpopulation with MSCs like characteristics or immature fibroblasts.[13] Synovial membrane–derived MSCs expressed CD55 and showed greater chondrogenic potential compared with infrapatellar fat pad MSCs in some reports.[37,46,47] Subintimal MSCs show greater CD90 expression levels suggestive of a perivascular origin and show different responses toward chondrogenic growth factors compared with synovial membrane MSCs.[48] Infrapatellar fat pad MSCs share some similarities with adipose MSCs. They may be less affected by intra-articular inflammatory changes compared with synovial membrane MSCs.[45,49] Further investigations are required regarding cell markers and clinical efficacy for the respective regions to warrant specific harvest or sorting methods.[50]

In our study, we have used the term SDMSCs as proposed by Li et al.,[13] to designate cells that meet the MSCs criteria found in the synovium including the membrane and subintima. Currently, there is no terminology differentiating cells from the synovial membrane and subintima. The motorized shaver resects more tissue beneath the synovial membrane compared to the rongeur biopsy as seen in Fig 1 C and 1D. A larger percentage of CD55 (+) synovial lining cells were found in the rongeur biopsy samples, whereas fat and perivascular tissue typically found in the subintima was more abundant in the motorized shaver harvest group. This difference is likely due to the motorized shaver's mode of action, where tissue is siphoned in the shaver head before resection, resulting in more inclusion of the subintimal tissue compared to the rongeur biopsy method. Such difference, however, did not result in cell yield or cell surface marker expression levels such as CD90 between the 2 groups (Figs 2C and 3C). The motorized shaver group, nevertheless, probably harvests a more heterogeneous group of MSCs that includes the synovial membrane and subintima. In our study, we took care not to infiltrate the infrapatellar fat pad and did not use active suctioning during shaver use. Due to its proximity, however, fat pad tissue also may be harvested during motorized shaver use. Using a suctioning device during shaver use, a common practice during arthroscopy, may further increase the heterogeneity of the harvested tissue and affect final results. In all, motorized shaver use is more likely to harvest tissue beneath the synovial membrane, which may affect the homogeneity and therapeutic efficacy of the harvested cells.

Cell isolation is also an important factor affecting cell yield. Most previous studies used collagenase during cell isolation in varying protocols ranging from 0.02∼0.3% (vol/vol) in density with a treatment time ranging from 1 to 4 hours.[26,27,42,51] Studies using larger tissue samples from total knee arthroplasty required greater collagenase densities and longer treatment periods compared with our protocol that used 0.1% collagenase treatment for 3 hours.[52] Overall, our cell yield was $3.18 \pm 0.97 \times 10^3$ cells/mg for the motorized shaver group which is comparable to previous studies using motorized shaver harvest.[42,50]

Limitations

There are several limitations in our study. First of all, the sample size may not be enough to determine the statistical significance of our results. Second, we chose only the 2 most widely available methods of arthroscopic harvest, rongeur biopsy and motorized shaver, for analysis. SDMSCs also can be harvested using various devices such as specialized biopsy needles or cytology brush like devices, which may not be available in all hospitals.[29,33,42] Synovial fluid is another source of MSCs.[53] We did not analyze the synovial fluid, as not all patients presented with knee effusion, synovial fluid MSCs also may originate from other intra-articular structures other than the synovial membrane. Third, this study included patients with osteoarthritis and synovitis. Synovitis is known to increase quantity of SDMSCs as well as chondrogenic potential of

SDMSCs.[54-56] Lastly, the origin of SDMSCs may be differ between the 2 harvest methods, as aforementioned. SDMSCs harvested by motorized shaver may have MSCs of adipose origin. Delineation between adipose and synovial origin MSCs is currently not well established, and both cells exhibit similar characteristics. Researchers, however, should take into account the possible heterogeneity of SDMSCs during motorized shaver harvest.

Conclusions

No difference was observed between rongeur biopsy and motorized shaver harvest methods regarding SDMSCs yield and cell characteristics.

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