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Original Article

Optimization of human mesenchymal stem cell isolation from synovial membrane: Implications for subsequent tissue engineering effectiveness

Norihiko Sugita [a], Yu Moriguchi [a], Morito Sakaue [a], David A. Hart [b], Yukihiko Yasui [a], Kota Koizumi [a], Ryota Chijimatsu [a], Syoichi Shimomura [a], Yasutoshi Ikeda [a], Hideki Yoshikawa [a], Norimasa Nakamura [a, *]

[a] *Department of Orthopaedic Surgery, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita 565-0871, Japan*
[b] *McCaig Institute for Bone & Joint Health, University of Calgary, 3330 Hospital Drive Northwest, Calgary, Alberta T2N 4N1, Canada*

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ABSTRACT

Synovium-derived mesenchymal stem cells (SDMSCs) are one of the most suitable sources for cartilage repair because of their chondrogenic and proliferative capacity. However, the isolation methods for SDMSCs have not been extensively characterized. Thus, our aim in this study was to optimize the processes of enzymatic isolation followed by culture expansion in order to increase the number of SDMSCs obtained from the original tissue. Human synovium obtained from 18 donors (1.5 g/donor) was divided into three aliquots. The samples were minced and subjected to collagenase digestion, followed by different procedures: Group 1, Tissue fragments were removed by filtering followed by removing floating tissue; Group 2, No filtering. Only floating fragments were removed; Group 3, No fragments were removed. Subsequently, each aliquot was sub-divided into two density subgroups with half. In Group 1, the cell-containing media was plated either at high (5000 cells/cm^2) or low density (1000 cells/cm^2). In Groups 2 and 3, the media containing cells and tissue was plated onto the same number of culture dishes as used in Group 1, either at high or low density. At every passage, the cells plated at high density were consistently re-plated at high and those plated at low density were likewise. The expanded cell yields at day 21 following cell isolation were calculated. These cell populations were then evaluated for their osteogenic, adipogenic, and chondrogenic differentiation capabilities. The final cell yields per 0.25 g tissue in Group 1 were similar at high and low density, while those in Groups 2 and 3 exhibited higher when cultured at low density. The cell yields at low density were $0.7 \pm 1.2 \times 10^7$ in Group 1, $5.7 \pm 1.1 \times 10^7$ in Group 2, $4.3 \pm 1.2 \times 10^7$ in Group 3 (Group 1 vs Groups 2 and 3, $p < 0.05$). In addition, the cells obtained in each low density subgroup exhibited equivalent osteogenic, adipogenic, and chondrogenic differentiation. Thus, it was evident that filtering leads to a loss of cells and does not affect the differentiation capacities. In conclusion, exclusion of a filtering procedure could contribute to obtain higher number of SDMSCs from synovial membrane without losing differentiation capacities.

1. Introduction

Mesenchymal stem cells (MSCs) can be isolated from various tissues and have the potential to self-renew and differentiate into multiple lineages such as osteogenic [1], chondrogenic [2], adipogenic [3,4], myogenic [5] and neurogenic [6] specificities. Among the MSC sources, synovium-derived mesenchymal stem cells (SDMSCs) have been demonstrated to exhibit superior chondrogenic and proliferation potentials compared to MSCs derived from other tissue [7–9]. A considerable number of studies of cartilage repair have been conducted using SDMSCs with promising results [10–13]. For successful cell-based therapy, securing a sufficient number of cells is critical. It depends on the delivery method, specific to our "scaffold-

* Corresponding author.
E-mail address: norimasa.nakamura@ohsu.ac.jp (N. Nakamura).

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free tissue engineered construct" procedure [14], we need in average 1.3×10^8 cells for treatment cartilage defect and quality inspection in our clinical trial (UMIN000008266), [15]. However, optimized methods for culturing SDMSCs, including the isolation from synovial membranes have not been fully characterized. Most of the previous studies on the culture of SDMSCs reported the isolation of cells using a standard collagenase digestion followed by filtering to remove debris [7–13,16–23] before starting the primary culture. However, there may be a potential loss of additional MSCs in the filter-trapped undigested tissue fragments. We hypothesized that the use of undigested tissue fragments would lead to increases in the number of SDMSCs available from the original synovial tissue. Moreover, it was previously reported that plating density can influence the proliferation of MSCs [8,24]. Taken together, it was important to quantify how the filtering process, as well as the plating density of cells thereafter could affect the number of SDMSCs obtained within a clinically relevant duration of cell culture.

In the present study we aimed to maximize the yield of cultured human SDMSCs starting from equivalent weights of synovial membrane material. The results presented indicate that both the MSC isolation method, as well as the propagation density, significantly influences the assessed outcomes.

2. Materials and methods

2.1. Harvest of synovial membrane and isolation of cells

Our study protocol was approved by the institutional committee for medical ethics. Written informed consent was obtained from all patients. Human synovial membranes were obtained (1.5 g per patient) from 18 patients (10 male and 8 female donors; mean age, 25.5; range 16–48 years: Table 1) during arthroscopic surgery. Synovial tissues from each donor were divided into three aliquots (0.5 g each) and meticulously minced using surgical scissors. The minced tissues were then digested in a collagenase solution [440 u/ml collagenase A, Type AFA (Worthington Biochemical Corporation, Lakewood, NJ, USA)] in growth medium containing high-glucose Dulbecco's Modified Eagle Medium (DMEM, Wako Chemical Corp., Osaka, Japan), supplemented with 10% fetal bovine serum (FBS, Sigma–Aldrich, St. Louis, MO, USA) and 1% antibiotic–antimycotic solution (Sigma–Aldrich) according to the previously established protocol [12,16,25]. Specifically, we used the same, animal origin free, collagenase at the same concentration as we used in our clinical trial (UMIN000008266) according to the manufacturer's instruction. Following 3 or 16 h of incubation, undigested tissues were removed from the cell-containing liquid with a 70-μm nylon filter (BD Falcon, Franklin Lakes, NJ, USA) followed by centrifugation (1500 rpm for 5 min). Subsequently, the floating undigested tissue fragments were removed (Group 1). In Group 2, No filtering was performed but floating undigested tissue fragments were removed after centrifugation. In Group 3, No filtering and the floating undigested tissue fragments were not removed, and all components used for the subsequent cell culture. Therefore, Group 1 contained only cells; Group 2 contained cells and the precipitated undigested tissue fragments; while Group 3 contained cells plus both the precipitated and floating undigested tissue fragments (Fig. 1). In each group, all the contents were resuspended in 10 ml of complete media.

For microscopic observation, cell-containing media (10 μl) from Groups 1–3 were applied onto cell counter plates for analysis.

DNA was extracted from the cell-containing liquid (100 μl) after collagenase digestion in all groups with a DNeasy Blood & Tissue Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. Briefly, cells and small tissues were digested with lysis buffer and proteinase K, DNA was purified, and the DNA content was quantified in a spectrophotometer. Total DNA content per 0.5 g tissue was calculated according to the ratio of sampling volume (100 μl) to the total volume (10 ml) of the cell-containing suspension.

2.2. Plating and subsequent primary cell culture

Each aliquot (Groups 1–3) from individual donors was further divided into two subgroups (5 ml each) for subsequent plating at two different cell densities.

For Group 1, the cells were plated at high (5000 cells/cm^2) or low density (1000 cells/cm^2). For Groups 2 and 3, media containing cells and undigested tissues was plated onto the same number of culture dishes used in Group 1, either at high or low density. Cells were cultured in the growth medium containing 10% FBS at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The medium was replaced every 4 days.

2.3. In vitro expansion of cell populations

The cultured cells were subjected to passaging when reaching 80% confluency. Cells were harvested by treatment with trypsin–EDTA (0.25% trypsin and 1 mM EDTA; Gibco BRL, Life

Table 1
Synovial samples used in this study.

Sample number	Age	Sex	Diagnosis
1	16	Male	Anterior cruciate ligament injury
2	30	Male	Anterior cruciate ligament injury
3	23	Male	Synovitis (After anterior cruciate ligament reconstruction)
4	35	Female	Synovitis (After anterior cruciate ligament reconstruction)
5	30	Male	Anterior cruciate ligament injury
6	23	Male	Anterior cruciate ligament injury
7	44	Female	Anterior cruciate ligament injury
8	16	Male	Osteochondromatosis
9	20	Female	Anterior cruciate ligament injury
10	18	Female	Anterior cruciate ligament injury
11	44	Male	Anterior cruciate ligament injury
12	19	Male	Anterior cruciate ligament injury
13	23	Female	Anterior cruciate ligament injury
14	16	Male	Anterior cruciate ligament injury
15	17	Male	Meniscal injury
16	18	Female	Anterior cruciate ligament injury
17	19	Female	Meniscal injury
18	48	Female	Anterior cruciate ligament injury

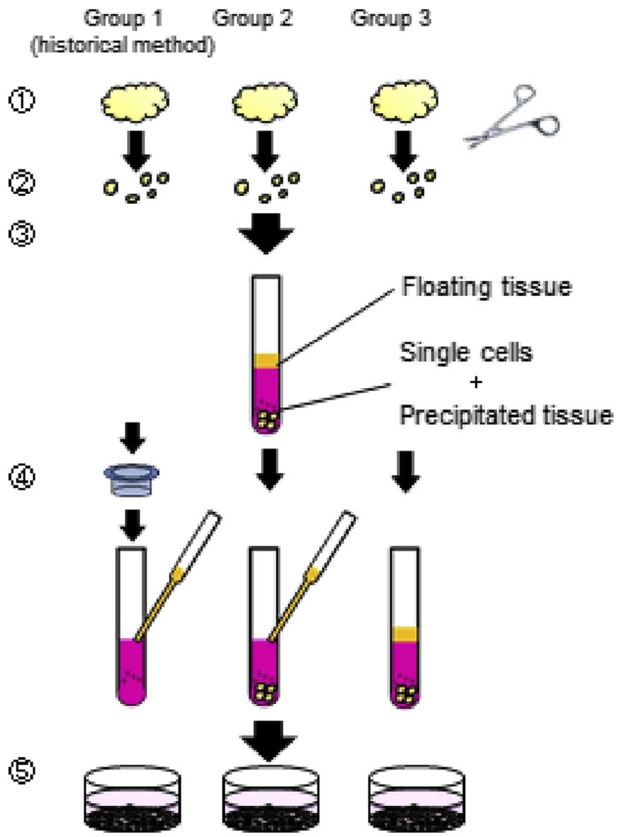

Fig. 1. Traditional process of enzymatic cell isolation from synovial membrane and grouping. ① Human synovial membranes were obtained from arthroscopic surgeries and divided into 3 groups (0.5 g for each group). ② The tissues were meticulously minced. ③ The fragments were digested in collagenase solution. ④ After 3 h digestion, Group 1 (Conventional method): precipitated tissues were removed by filtering followed by the removal of floating tissues after centrifugation; Group 2 No filtering. Only floating tissues were removed after centrifugation; Group 3: No filtering. No removal of floating tissue. ⑤ In Group 1, Nucleated cells are counted and plated in the dishes at high and low densities. In Groups 2 and 3, the media containing cells and undigested tissues was plated onto the same number of culture dishes used in Group 1.

Technologies Inc., Grand Island, NY, USA). After the first passage, the cells plated at high density were consistently re-plated at high density (5000 cells/cm^2) and those plated at low density were likewise re-plated at low density (1000 cells/cm^2) thereafter.

In our on-going clinical trial protocol (UMIN000001195), the duration for the expansion of SDMSCs was set at approximately 3 weeks [26], similar to another clinical trial using bone marrow derived MSCs [27]. Thus, we chose to set the duration for cell expansion at a clinically relevant 21 days, and the final cell number per 0.25 g tissue in each subgroup was calculated for comparison to assess optimal in vitro conditions.

2.4. In vitro differentiation protocols

Cells obtained after 21 days of culture for each low density subgroup were used for lineage-specific differentiation assays.

2.4.1. Osteogenic differentiation

2×10^4 cells in each subgroup were plated in 12-well plates and then cultured in complete medium for 2 days. The medium was then changed to osteogenesis medium (STEMPRO® Osteogenesis Differentiation Kit, Life Technologies, Grand Island, NY, USA) and cultured for another 21 days. The medium was replaced two times per week. After induction for 21 days, the wells were washed two times with PBS, fixed in 4% paraformaldehyde, and then stained with 0.5% Alizarin Red S.

2.4.2. Adipogenic differentiation

2×10^4 cells were plated in 12-well plates and cultured in complete medium for 2 days. The medium was then changed to adipogenesis medium (STEMPRO® Adipogenesis Differentiation Kit, Life Technologies) and cultured for another 7 days. The medium was replaced two times a week. After induction for 7 days, wells were washed two times with PBS and stained with Oil Red O.

2.4.3. Chondrogenic differentiation

2×10^5 cells were placed in 15 ml polypropylene tubes and centrifuged at 1500 rpm for 10 min. The cell pellets were cultured in complete medium for 2 days. The medium was then switched to chondrogenic medium, comprised of DMEM supplemented with 1% insulin–transferrin–selenium supplement (Corning® ITS Premix, Corning Life Sciences, Bedford, MA, USA), 0.2 mM Asc-2P (Sigma–Aldrich), and 200 ng/mL recombinant human BMP2 (Osteo Pharma, Osaka, Japan) for another 21 days. The medium was replaced two times per week. For histological analysis, two pellets were fixed with 4% paraformaldehyde, embedded in paraffin, cut into 5-μm sections, and stained with Safranin O. Nuclei were counterstained with hematoxylin. For quantifying the glycosaminoglycan (GAG) content, three pellets were digested with 0.4 M papain extraction reagent overnight at 65 °C, and the GAG content was measured by Blyscan sulfated GAG assay kit (Biocolor, Carrickfergus, Ireland).

2.5. Statistical analysis

Data was presented as the mean ± one standard error of the mean. Data input and calculations were performed with IBM SPSS Statistics 22 (IBM, Armonk, NY, USA). In all analyses, we used a linear mixed model. We included the combination of groups and densities as a fixed effect and a repeated effect. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. The effect of filtering following collagenase digestion

In Group 1, cell density appeared to be lower than in Groups 2 and 3, in which more cells along with cell aggregates and undigested tissues were observed (Fig. 2a). The total DNA content was 3.9 ± 7.2 μg in Group 1, 42.0 ± 7.2 μg in Group 2, 42.3 ± 10.0 μg in Group 3. There were significant differences between Groups 1 and 2 ($p < 0.01$) and between Groups 1 and 3 ($p < 0.05$). The differences for each comparison were approximately 10 fold. No significant differences between Groups 2 and 3 were detected (Fig. 2b).

Extending the initial collagenase digestion time to 16 h did not lead to any significant increases in cell numbers for Group 1 (data not shown) and therefore, it was likely that the cells populated within the undigested tissues were not released even by longer exposure to collagenase.

3.2. Comparison of cell numbers obtained after 21-day culturing of each sub-group

The cell number after 21 days of culture per 0.25 g tissue was calculated for each subgroup. There were no significant differences between the high and low density subgroups ($0.6 \pm 0.9 \times 10^7$ cells vs $0.7 \pm 1.2 \times 10^7$ cells) in Group 1, whereas more cells were obtained for the low density subgroups than the high density subgroups for Groups 2 ($5.7 \pm 1.1 \times 10^7$ cells vs 3.1 ± 1.0^7 cells, $p < 0.05$) and 3 ($4.4 \pm 1.2 \times 10^7$ cells vs $1.5 \pm 1.5 \times 10^7$ cells, $p < 0.05$) (Fig. 3).

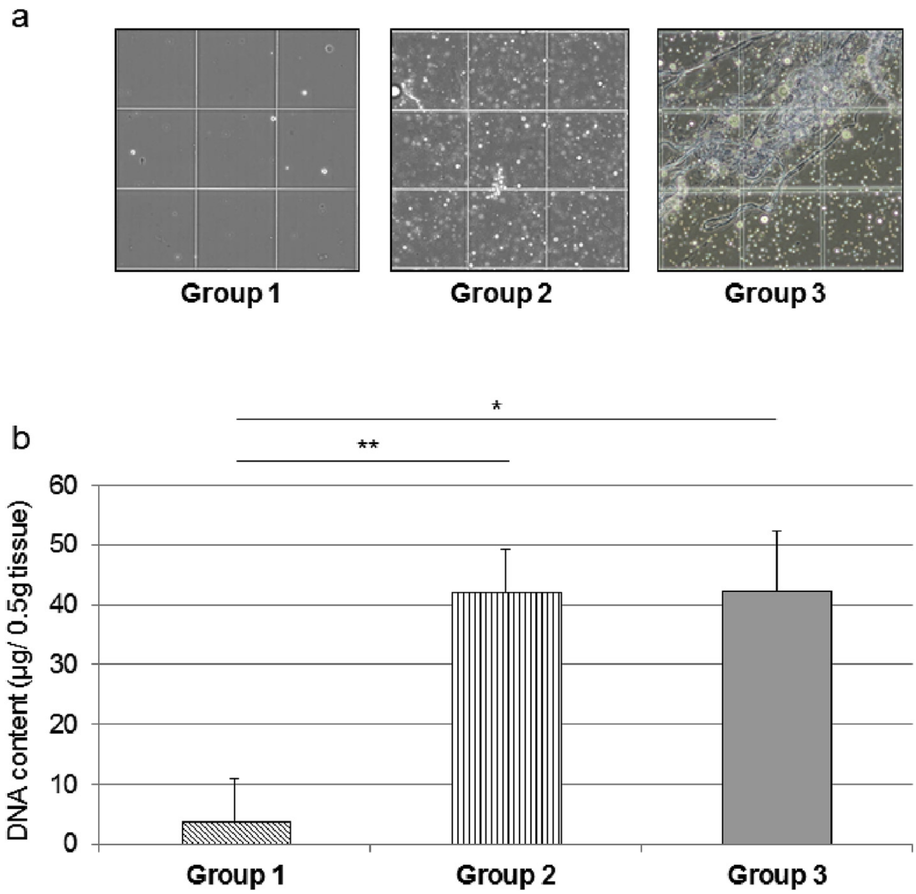

Fig. 2. Evaluation of the filter processing effect on cell-containing fluids. (a) The cell-containing liquids (10 μl) were applied onto cell counter plates (digested liquid from 0.5 g tissue/10 ml) in each group. 1 Grid = 0.25 mm. (b) The DNA content in the cell-containing liquids from 0.5 g tissue in each group. *$p < 0.05$, **$p < 0.01$.

The yields from the low density subgroups in Groups 2 and 3 were significantly greater than the values for Group 1 (Group 2 vs 1, >8-fold; $p < 0.01$, Group 3 vs 1, >6-fold; $p < 0.05$). The cell viability was over 90% after all the different protocols (data was not shown).

3.3. *In vitro differentiation and histological analyses of cells*

Since cell expansion in the low density culture subgroups showed enhanced results compared to the findings obtained at high density for all of the groups (1, 2 and 3), we focused on comparing the differentiation capacity of the expanded cells only among the lower density culture subgroups.

3.3.1. *Osteogenesis*

After 21 days of osteogenic induction, mineralization was similarly observed for all groups, with calcium deposition stained with Alizarin Red S (Fig. 4a).

3.3.2. *Adipogenesis*

Following 7 days of adipogenic induction, under light microscopy, Oil Red O-stained sections revealed similar formation of lipid vacuoles for cells of each group (Fig. 4b).

3.3.3. *Chondrogenesis*

After 21 days of chondrogenic induction, the cell pellets cultured in chondrogenic media showed intense staining for Safranin O in each group (Fig. 5b). Regarding GAG quantity, there was no significant statistical difference among the three groups (1, 2, 3) (Fig. 5c).

4. Discussion

In most of the previous studies used for the preparation of SDMSCs, a filtering process was included following collagenase digestion to remove undigested tissue fragments [7–13,16–23]. The present study has revealed that such filtering procedures result in a significant loss (approximately 90% by DNA content measurement) of the number of the cells obtained when compared with cultures not subjected to filtering. It was likely that not all of the cells contained in the collagenase-treated media adhered to the culture dish and participate in subsequent proliferation. Therefore, loss of 90% of cells by the comparison of DNA content may be an overestimation. However, it is reasonable to presume that the majority of the SDMSCs are still within the undigested tissues after collagenase digestion for 3 or 16 h.

Final cell yields after 21 days of culture in the non-filtered groups (i.e. Group 2 and 3) were higher than those of the filtered group (i.e., Group 1). As previously reported [8,24], the proliferative efficiency was higher at low density culture than at high density for the non-filtered groups (Group 2 and 3), and the final cell yield in Group 2 (low density culture subgroup) which exhibited the best yields in the non-filtered groups yielded 5-fold more cells than those in Group 1. Conversely, such differences based on culture cell density were not detected for the filtered group (Group 1). All these results very clearly indicate the negative effect of the filtering process.

The differences between Groups 2 and 3 were based on whether the floating undigested tissue fragments separated after centrifugation were utilized for the subsequent culture. Microscopic

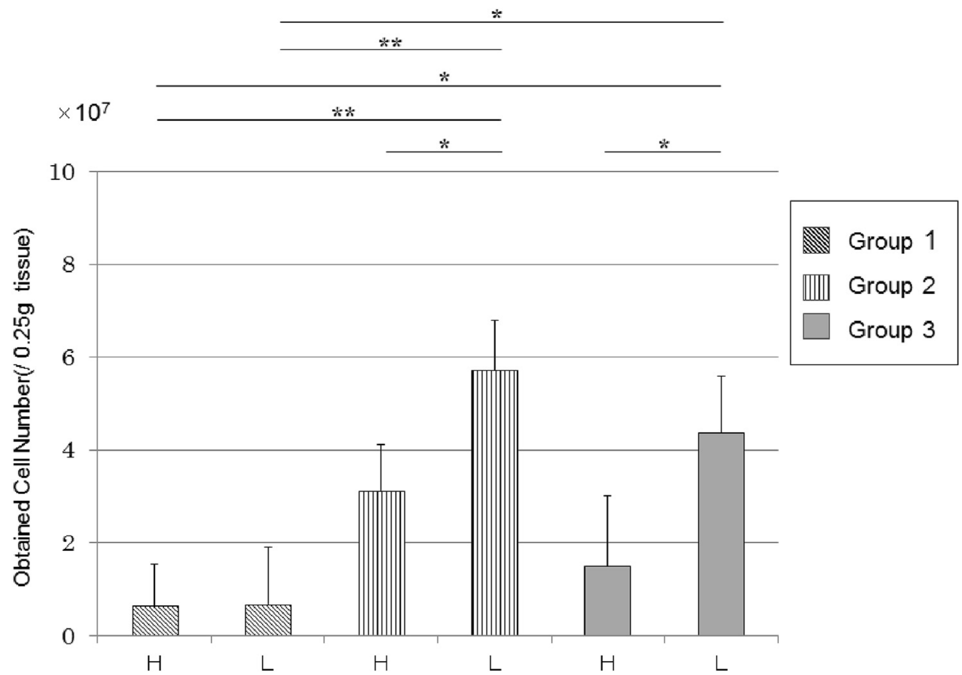

Fig. 3. Comparison of cell numbers from Groups 1–3 obtained after in vitro culturing for 21 days. Cell number calculated after 21 days for cells cultured at high density (H) and low density (L). $^{*}p < 0.05$, $^{**}p < 0.01$.

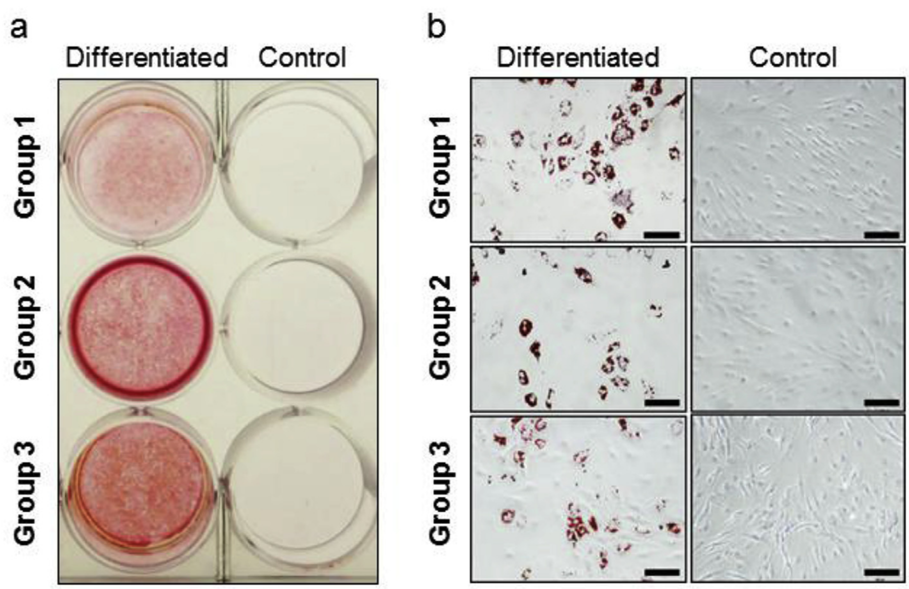

Fig. 4. Osteogenic and adipogenic differentiation capacity for cells in Groups 1–3. (a) Osteogenic differentiation: 21-day expanded cells cultured at low density replated in 12-well plates and osteogenesis induced for another 21 days for each group. Resultant cultures were then stained with Alizarin Red S. (b) Adipogenic differentiation: 21-days expanded cells at low density were then subjected to adipogenesis for another 7 days and were subsequently stained with Oil Red O. Scale bars = 100 μm.

observation further suggested that these floating tissue fragments are comprised mainly of adipose synovium, a finding consistent with the floating properties of the fragments, and whose strong chondrogenic differentiation capacity has been reported previously [28,29]. Based on these reports, we assessed whether retention of such floating tissue fragments plus the precipitated undigested tissue fragments at the subsequent culture stage would be beneficial on subsequent culture to obtaining better yields of SDMSCs. However, the results showed no such significant differences between Groups 2 and 3. It could be speculated that the floating undigested tissue fragments may not have become well adhered to the culture dish and thus, might have missed the opportunity for outgrowth of the SDMSCs. The preparation method for Group 2 is simpler than that for Group 3 because there is no need to carefully preserve the floating tissue fragments at the collagenase washing out process. Thus, both in terms of efficiency of cell expansion and simplicity in preparation method, we recommend the Group 2 method for the isolation of SDMSCs.

There was a possibility that isolating methods affect cell differentiation capacity, however three groups showed similar stain in

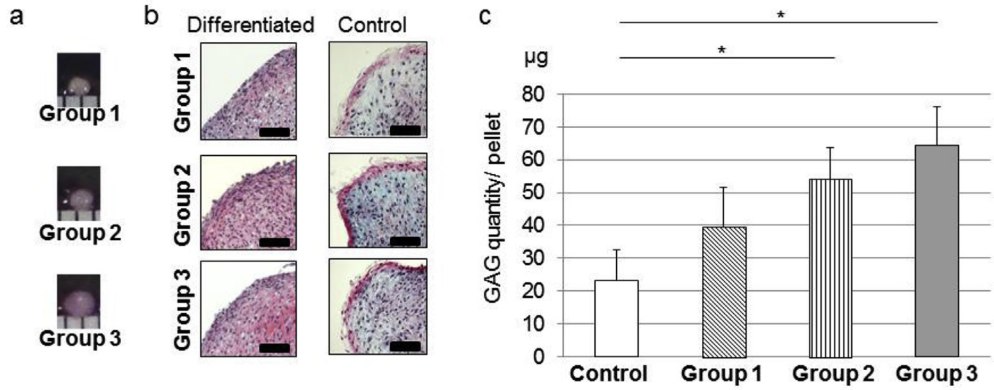

Fig. 5. Chondrogenic differentiation potential for cells from Groups 1–3. 21-days expanded cells at low density were pelleted by centrifugation and chondrogenesis subsequently induced for another 21 days. (a) Cell pellets in each group were measured against a 1-mm scale bar. (b) Histological appearance with Safranin O. Scale bars = 100 μm. (c) GAG content per cell pellet. The data for the uninduced control groups in relation to GAG content is shown as the mean for all three groups. $^{*}p < 0.05$.

osteogenic and adipogenic differentiation and there was no significant statistical difference among the three groups in GAG quantify. So we conclude that the cells in non-filter groups have equivalent differentiation capacities to those in filter group.

As another method of cell isolation from tissues, explant culture has been initially reported as adipose tissues [30], Wharton's jelly [31], and synovium [23]. In all these papers, explant culture was compared with an enzymatic method employing filtering; in the report about synovial tissues, the cell yield from explant culture was reported to be equal to that from the enzymatic method. Conversely, in the present study, cell yield from the enzymatic method without filtering was superior to that with filtering, and the cell differentiation capacity was not affected by the use of filtering or not. Although we did not directly compare our methods tested with explant culture, these finding suggest that the enzymatic method without filtering (enzymatic explant method) provides more cells than explant culture. The enzymatic digestion of collagenous matrix might have facilitated cellular expansion out of the matrix as compared with traditional explant culture.

One issue which still remains unclear was whether the MSCs readily released from the synovial membrane tissue by the collagenase treatment represent a unique subpopulation when compared to those that are retained in the undigested tissue fragments. As the number of cells released from the synovial membrane tissue was not increased when the collagenase treatment was extended to 16 h (data not shown), the digestion time more than 3 h was not a limiting factor and the cells released appeared to be a subset of the total cells available.

As a potential concern, the use of serum in our collagenase-based digestion media might have reduced the action of collagenase, leading to the presence of undigested tissue debris after digestion process. We included serum according to the protocols previously published [12,16,25], and did not investigate the experiments with serum-free digestion media. In the literature, there have been several reports of synovial digestion by collagenase without the use of serum [7,19,32]. In these studies, the duration of digestion was 3 h to overnight, and notably, all the reports included filtering process after collagenase digestion. This suggests the presence of residual debris (undigested tissue) after collagenase digestion regardless of the presence of serum. Thus, the presence of serum or not in the collagenase solution does not likely affect the major conclusion of the present study.

Recent study reported that more primitive progenitors are included in non-adherent cells [33,34], so there may be potential improvement of the quantity and quality of stem cell population if we follow the procedures. In the present study, we discarded medium that could potentially contain non-adherent cells at the time of medium change and passage and thus could not confirm the improvement. This issue needs to be clarified in the future study.

Some other limitations of the present study include its relatively small sample size, no comparison of gender, and individual differences. More donor samples are need to be assessed to further clarify these issues, as well as perhaps inclusion of clonal, genetic, and epigenetic analysis of the different cell populations.

5. Conclusions

We have developed a simpler and significantly more efficient method for SDMSCs isolation than the conventional isolation method which employs a filtering process. Without the use of any special equipment such as a bioreactor or the addition of biological reagents or growth factors, we succeeded in increasing the cell yield over 5-fold, a finding which is very relevant when considering future clinical applications to repair cartilage defects. In addition, this methodology may be applicable to MSC isolation from other tissue cell sources, but future research to examine the feasibility of applying this method to other MSC sources will be required.

Conflict of interest

Norimasa Nakamura was supported by a grant from the New Energy and Industrial Technology Development Organization, Japan. David A. Hart was supported by an AIHS CRIO Grant on MSC Bioengineering, the AIHS Team Grant in Osteoarthritis, and research funds from the McCaig Professorship.

References

[1] Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 1997;64:295–312.
[2] Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. Tissue Eng 1998;4:415–28.
[3] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science (New York, NY) 1999;284:143–7.
[4] Bianco P, Gehron Robey P. Marrow stromal stem cells. J Clin Invest 2000;105:1663–8.
[5] de la Garza-Rodea AS, van der Velde-van Dijke I, Boersma H, Goncalves MA, van Bekkum DW, de Vries AA, et al. Myogenic properties of human mesenchymal stem cells derived from three different sources. Cell Transplant 2012;21:153–73.

[6] Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 2000;164:247–56.
[7] Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 2005;52:2521–9.
[8] Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell Tissue Res 2007;327:449–62.
[9] Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, et al. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res 2008;333:207–15.
[10] Hori J, Deie M, Kobayashi T, Yasunaga Y, Kawamata S, Ochi M. Articular cartilage repair using an intra-articular magnet and synovium-derived cells. J Orthop Res Off Publ Orthop Res Soc 2011;29:531–8.
[11] Nakamura T, Sekiya I, Muneta T, Hatsushika D, Horie M, Tsuji K, et al. Arthroscopic, histological and MRI analyses of cartilage repair after a minimally invasive method of transplantation of allogeneic synovial mesenchymal stromal cells into cartilage defects in pigs. Cytotherapy 2012;14:327–38.
[12] Ando W, Tateishi K, Hart DA, Katakai D, Tanaka Y, Nakata K, et al. Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. Biomaterials 2007;28:5462–70.
[13] Shimomura K, Ando W, Tateishi K, Nansai R, Fujie H, Hart DA, et al. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. Biomaterials 2010;31:8004–11.
[14] Ando W, Tateishi K, Katakai D, Hart DA, Higuchi C, Nakata K, et al. In vitro generation of a scaffold-free tissue-engineered construct (TEC) derived from human synovial mesenchymal stem cells: biological and mechanical properties and further chondrogenic potential. Tissue Eng Part A 2008;14:2041–9.
[15] Lee WJ, Park JS, Jang SJ, Lee SC, Lee H, Lee JH, et al. Isolation and cellular phenotyping of mesenchymal stem cells derived from synovial fluid and bone marrow of minipigs. J Vis Exp JoVE 2016. http://dx.doi.org/10.3791/54077.
[16] De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928–42.
[17] Koga H, Muneta T, Ju YJ, Nagase T, Nimura A, Mochizuki T, et al. Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. Stem Cells (Dayton, Ohio) 2007;25:689–96.
[18] Koga H, Shimaya M, Muneta T, Nimura A, Morito T, Hayashi M, et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. Arthritis Res Ther 2008;10:R84.
[19] Mochizuki T, Muneta T, Sakaguchi Y, Nimura A, Yokoyama A, Koga H, et al. Higher chondrogenic potential of fibrous synovium- and adipose synovium-derived cells compared with subcutaneous fat-derived cells: distinguishing properties of mesenchymal stem cells in humans. Arthritis Rheum 2006;54:843–53.
[20] Moriguchi Y, Tateishi K, Ando W, Shimomura K, Yonetani Y, Tanaka Y, et al. Repair of meniscal lesions using a scaffold-free tissue-engineered construct derived from allogenic synovial MSCs in a miniature swine model. Biomaterials 2013;34:2185–93.
[21] Hatsushika D, Muneta T, Nakamura T, Horie M, Koga H, Nakagawa Y, et al. Repetitive allogeneic intraarticular injections of synovial mesenchymal stem cells promote meniscus regeneration in a porcine massive meniscus defect model. Osteoarthr Cartil/OARS, Osteoarthr Res Soc 2014;22:941–50.
[22] Dry H, Jorgenson K, Ando W, Hart DA, Frank CB, Sen A. Effect of calcium on the proliferation kinetics of synovium-derived mesenchymal stromal cells. Cytotherapy 2013;15:805–19.
[23] Lee DH, Joo SD, Han SB, Im J, Lee SH, Sonn CH, et al. Isolation and expansion of synovial CD34(−)CD44(+)CD90(+) mesenchymal stem cells: comparison of an enzymatic method and a direct explant technique. Connect Tissue Res 2011;52:226–34.
[24] Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells (Dayton, Ohio) 2002;20:530–41.
[25] Pei M, He F, Vunjak-Novakovic G. Synovium-derived stem cell-based chondrogenesis. Differ Res Biol Divers 2008;76:1044–56.
[26] Nakamura N, Hui J, Koizumi K, Yasui Y, Nishii T, Lad D, et al. Stem cell therapy in cartilage repair—culture-free and cell culture—based methods. Operative Tech Orthop 2014;24:54–60.
[27] Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. Am J Sports Med 2010;38:1110–6.
[28] Guilak F, Estes BT, Diekman BO, Moutos FT, Gimble JM. 2010 Nicolas Andry Award: multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. Clin Orthop Relat Res 2010;468:2530–40.
[29] Innes JF, Gordon C, Vaughan-Thomas A, Rhodes NP, Clegg PD. Evaluation of cartilage, synovium and adipose tissue as cellular sources for osteochondral repair. Vet J (London, England 1997) 2013;197:619–24.
[30] Busser H, De Bruyn C, Urbain F, Najar M, Pieters K, Raicevic G, et al. Isolation of adipose derived stromal cells without enzymatic treatment: expansion, phenotypical and functional characterization. Stem Cells Dev 2014;23(19):2390–400.
[31] Yoon JH, Roh EY, Shin S, Jung NH, Song EY, Chang JY, et al. Comparison of explant-derived and enzymatic digestion-derived MSCs and the growth factors from Wharton's jelly. BioMed Res Int 2013;2013:428726.
[32] Harvanova D, Tothova T, Sarissky M, Amrichova J, Rosocha J. Isolation and characterization of synovial mesenchymal stem cells. Folia Biol 2011;57:119–24.
[33] Di Maggio N, Mehrkens A, Papadimitropoulos A, Schaeren S, Heberer M, Banfi A, et al. Fibroblast growth factor-2 maintains a niche-dependent population of self-renewing highly potent non-adherent mesenchymal progenitors through FGFR2c. Stem Cells 2012;30:1455–64.
[34] Mehrkens A, Di Maggio N, Gueven S, Schaefer D, Scherberich A, Banfi A, et al. Non-adherent mesenchymal progenitors from adipose tissue stromal vascular fraction. Tissue Eng Part A 2014;20:1081–8.