Isolation of Adipose Tissue–Derived Stem Cells: Enzymatic Digestion in Combination with Mechanical Distortion to Increase Adipose Tissue–Derived Stem Cell Yield from Human Aspirated Fat

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Mesenchymal stem cells (MSCs) are of great interest due to their properties of immune modulation, tissue regeneration, and multipotent differentiation. Future developments of clinical applications, however, require a higher yield of MSCs, lower number of passages of cells in culture, and shorter time from harvest to use. Optimization and standardization of techniques for mesenchymal adipose tissue–derived stem cell isolation offers solutions to current bottlenecks as a larger amount of MSCs can be isolated. These improvements result in shorter expansion time, fewer passages, less donor material needed, and higher MSC yield. This paper describes an MSC isolation method combining enzymatic digestion with mechanic disruption. This protocol is a standardized and easy-to-implement method for reaching significantly higher MSC yields compared to conventional enzymatic isolation protocols. Based on the results presented, we hypothesize that the combined enzymatic and mechanical method increases the surface area of the adipose tissue, facilitating digestion by enzymes. This approach reduces the amount of adipose tissue and in vitro expansion time needed to reach sufficient amounts of MSCs for clinical purposes. Importantly, the method does not require increased amounts of collagenase, nor does it impair the viability or differentiability of the MSCs. Using this protocol increases MSC yield by a factor of three. As a consequence, these results indicate that the physiological concentration of MSCs in adipose tissue is higher than previously assumed.

Keywords: adipose tissue-derived stem cells • mesenchymal stem cell isolation • mesenchymal stem cell therapy

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INTRODUCTION

Optimization of MSC isolation is not only important in enhancing efficiency of the isolation, but also contributes to determining the physiological level of MSCs in adipose tissue. This is essential in guiding a therapeutic dose, e.g., in cell-assisted fat grafting. Varying efficiencies of MSC yields from human adipose tissue have been reported. Harvesting methods, harvesting area, isolation technique, and patient comorbidities all seems to influence MSC yield and viability (Bony et al., 2015; Choudhery, Badowski, Muise, Pierce, & Harris, 2014; Cianfarani et al., 2013; Faustini et al., 2010; Iyyanki et al., 2015; Keck et al., 2014; Oedayrajsingh-Varma et al., 2006; von Heimburg, Hemmrich, Haydarlioglu, Staiger, & Pallua, 2004). To optimize MSC extraction, different isolation techniques have been investigated (Domenis et al., 2015; Faustini et al., 2010), including enzymatic isolation, mechanical isolation, and automated systems. Only a single published study combines enzymatic and mechanical MSC isolation (Li et al., 2018), and these authors describe an increased yield of the stromal vascular fraction (SVF) from excised adipose tissue. However, excision of adipose tissue is a more complicated procedure than aspiration. Furthermore, a direct measurement of MSC recovery using a combination of enzymatic and mechanical isolation has yet to be performed.

This article describes protocols for isolation of the SVF from aspirated human adipose tissue and a direct precise measurement of the MSCs therein. This improved protocol for MSC extraction uses combined enzymatic digestion and mechanical disruption (see Basic Protocol 1) to increase the MSC yield from human adipose tissue compared to enzymatic digestion alone (see Alternate Protocol). To characterize and quantify the amount of MSCs in the SVF, we introduce a multicolor flow cytometric protocol (see Basic Protocol 2) for identification of MSCs complying with the guidelines from International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) (Bourin et al., 2013). In addition, protocols for determining MSC differentiation into adipocytes (see Basic Protocol 3) and osteocytes (see Basic Protocol 4) are described.

BASIC PROTOCOL 1

ISOLATION OF MSCs USING ENZYMATIC DIGESTION IN COMBINATION WITH GentleMACS-BASED MECHANICAL DISRUPTION

Briefly, adipose tissue is extracted by aspiration, washed by centrifugation, and mechanically disrupted using a GentleMACS Octo Dissociator in the presence of collagenase. After digestion and disruption, the SVF (including MSCs) is isolated by centrifugation. Erythrolysis is performed to remove erythrocytes before the SVF suspension is filtered through a 70-μm cell strainer to remove debris and obtain a single-cell suspension.

NOTE: All reagents and equipment used for extraction of the adipose tissue sample or that comes into contact with tissues or cells must be sterile. All manipulations are performed in a sterile laminar flow bench. In case of human studies, all experiments must be approved by the appropriate institutional or national review boards and, in addition, human subjects must give informed consent.

Materials

Aspirated human adipose tissue sample
Phosphate-buffered saline (PBS; Gibco, 70011-036)
RPMI-1640 (Gibco, 31870-025)
Collagenase type IV (Gibco, 17104-019)
MSCM (see recipe)
Erythrolysis buffer (see recipe)

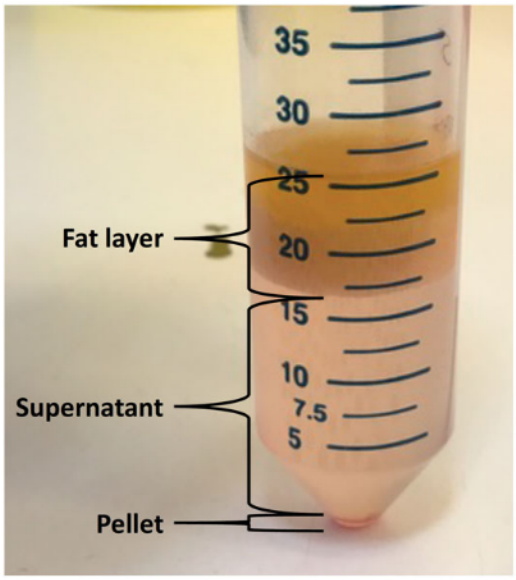

Figure 1 Discarding the supernatant and pellet during the tissue sample wash. It is important to minimize the loss of adipose tissue sticking to the pipet. This is accomplished by discarding the supernatant and pellet immediately after centrifugation in one attempt using a large 25-ml pipet.

50-ml Falcon tubes
5-, 10-, and 25-ml pipets
GentleMACS Octo Dissociator with C-tubes (Miltenyi)
70-µm cell strainer

Prepare adipose tissue samples

1. Measure the weight and volume of the adipose tissue sample.

   This protocol describes the isolation protocol for human aspirated abdominal adipose tissue samples. However, different species, harvesting methods, and harvesting areas can be used with similar results. We have successfully implemented this protocol in a porcine study. Note, if excised adipose tissue is used, prior coarse disruption using scalpels into 1- to 2-mm pieces is necessary.

2. Transfer 10 g sample to a 50-ml Falcon tube.
3. Wash sample by adding 10 ml PBS and mixing gently by careful shaking.
4. Centrifuge for 5 min at 850 × *g*, 18°C.
5. Remove the pellet and supernatant using a 25-ml pipet, leaving only the fat layer in the tube. Be careful to limit the sticking of adipose tissue to the pipet (Fig. 1).
6. Perform a second wash step by repeating steps 3-5.

Process adipose tissue samples

7. Transfer washed sample to two C-tubes. For optimal disruption, place a maximum of 5 g (pre-wash starting weight) tissue in each tube.
8. Add 10 ml RPMI-1640 to each tube, followed by 1500 U collagenase IV (final 300 U collagenase IV per gram adipose tissue).
9. Close the lid of the C-tubes and transfer to the GentleMACS Dissociator.

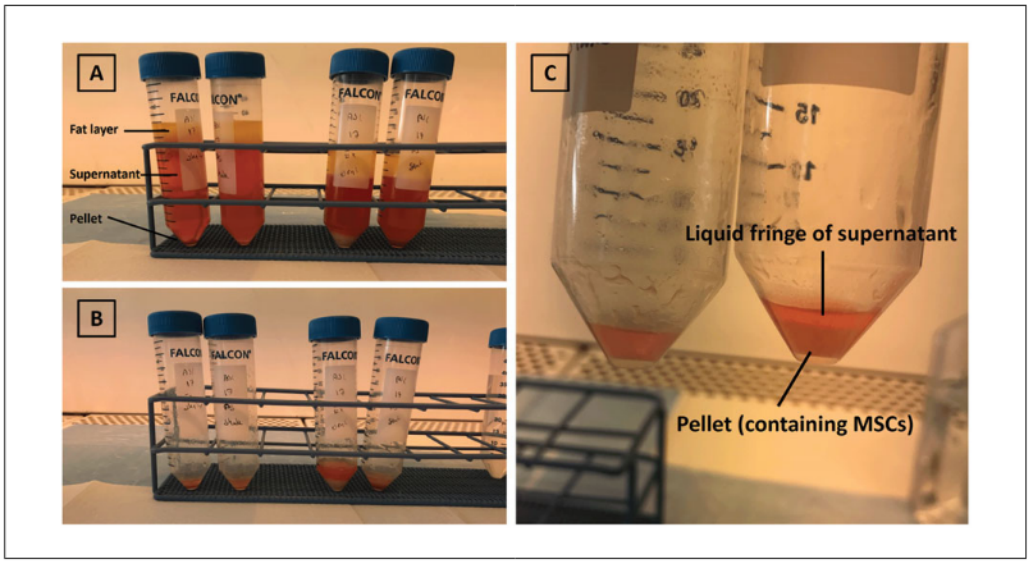

Figure 2 Discarding the fat layer and supernatant after tissue digestion. It is important not to discard any of the pellet, which contains the MSCs. This risk is minimized by discarding the fat layer and supernatant immediately after centrifugation. (**A**) Samples after centrifugation. (**B, C**) Pellets after discarding fat layer and supernatant. A liquid fringe of supernatant may be kept in the tubes.

10. Run program ADTAK 37°C on the GentleMACS:

    duration: 41 min and 10 sec
    clockwise rotation: 20 rpm
    at the 21- and 40-min markers, shifting clockwise and counter-clockwise rotation at 1323 rpm for 1 min.

11. Centrifuge C-tubes for 1 min at 100 × *g*, 18°C.

12. Transfer suspension from C-tubes to a new 50-ml Falcon (combining suspensions that were split in step 7).

    Extracellular matrix may adhere to the lid/auger of the C-tubes. To ensure all tissue is collected, wash C-tubes with 5 ml MSCM and add to the Falcon tubes.

13. Add 5 ml MSCM to each 50-ml tube.

    If the C-tubes were washed with 5 ml MSCM in step 12, another 5 ml is still added here.

14. Centrifuge for 10 min at 650 × *g*, 18°C.

Isolate SVF

15. Using a 25-ml pipet, discard the fat layer and supernatant (Fig. 2).

    It is crucial not to discard any of the pellet, as this contains the MSCs. The risk is minimized by discarding the fat layer and supernatant immediately after centrifugation. A liquid fringe of the supernatant may be kept in each tube.

16. Gently resuspend the pellet by tapping the tube.

17. Add 5 ml erythrolysis buffer. Wash the sides of the tube and mix the suspension by pipetting up and down.

18. Incubate for 10 min on ice.

19. Add 10 ml MSCM and mix gently.

20. Centrifuge for 10 min at 650 × *g*, 18°C.

21. Using a 25-ml pipet, carefully discard only the supernatant.
22. Add 5 ml MSCM and resuspend the cells by repeated pipetting.
23. Pass the suspension through a 70-µm cell strainer into a 50-ml Falcon tube.
24. Wash the strainer using 5 ml MSCM and collect in the same tube.

The sample must be analyzed immediately after isolation.

ISOLATION OF MSCs USING CONVENTIONAL ENZYMATIC DIGESTION

ALTERNATE PROTOCOL

This protocol describes the method used to isolate MSCs from adipose tissue using enzymatic digestion alone based on Zuk et al. (2001). The majority of the steps are similar to Basic Protocol 1.

Additional Materials (also see Basic Protocol 1)

Heated orbital shaker

1. Prepare adipose tissue samples as described (see Basic Protocol 1, steps 1-6).
2. Add 10 ml RPMI to the 50-ml Falcon tubes followed by 3000 U collagenase IV (final 300 U collagenase per gram adipose tissue).
3. Place lids on the Falcon tubes and transfer to a heated orbital shaker for 41 min of digestion at 37°C, 120 rpm.
4. Proceed with Basic Protocol 1 at step 13.

MULTICOLOR FLOW CYTOMETRIC CHARACTERIZATION OF MSCs

BASIC PROTOCOL 2

Using flow cytometry, the total SVF can be analyzed for the content of MSCs. Cells are stained with propidium iodide for viability assessment and a panel of monoclonal antibodies. Antibodies are chosen based on the absence or presence of their reactive antigen on the surface of MSCs. Using a multicolor antibody panel and stringent cell gating, it is possible to characterize and quantify the MSCs from the heterogeneous pool of cells in the SVF. Corresponding fluorescence minus one (FMO) controls must be used to control the compensation for spectral overlap and gating strategies.

Materials

Control compensation bead set (BD Biosciences, 552843)
FACSFlow Sheath Fluid (BD Biosciences, 342003)
Antibodies:
FITC anti-human CD31 (BioLegend Europe BV, 303104)
Brilliant Violet 421 anti-human CD34 (BioLegend Europe BV, 343610)
FITC anti-human CD45 (BioLegend Europe BV, 368508)
PE anti-human CD73 (Ecto-5′-nucleotidase; BioLegend Europe BV, 344004)
PE/Cy7 anti-human CD90 (Thy1; BioLegend Europe BV, 328124)
APC anti-human CD105 (BioLegend Europe BV, 323208)
Filtered SVF cell suspension (see Basic Protocol 1 or 2)
Propidium iodide (Sigma, P4864)

Plastic flow cytometry tubes
Flow cytometer (e.g., NovoCyte, ACEA Biosciences)

Prepare beads for compensation

1. From the control compensation bead set, mix 1 drop of positive beads and 1 drop of negative beads in a tube. Add 500 µl FACSFlow Sheath Fluid and vortex briefly at 1800 rpm.

The control compensation set contains two populations of bead microparticles: a population of anti-mouse Ig, κ particles, which bind any mouse κ light chain–bearing immunoglobulin, and a population of negative control particles, which have no binding capacity. When mixed together with a fluorochrome-conjugated mouse antibody, the compensation beads provide distinct positive and negative (background fluorescence) stained populations used to set compensations levels on the instrument software.

2. Add 100 µl bead mixture (BM) to five separate tubes.
3. Add the relevant amount of each antibody to single tubes, e.g.:

   5 µl anti-CD34 to tube 1
   5 µl anti-CD45 to tube 2
   0.16 µl anti-CD73 to tube 3
   0.5 µl anti-CD90 to tube 4
   5 µl anti-CD105 to tube 5.

   All volumes are based on dilution assays fitted to our laboratory. The concentration of antibodies needed to saturate the samples may vary depending on species, method of harvest, and type of adipose tissue. A preliminary dilution assay of the chosen antibodies can be performed to determine the optimal concentration of each antibody.

4. Vortex all tubes briefly, then incubate 15 min at room temperature in the dark.
5. Add 300 µl FACSFlow to each tube and vortex briefly.
6. Acquire signals for each fluorochrome by running tubes 1-5 and set the compensation levels on the flow cytometer.

Prepare sample for analysis

7. Prepare 8 tubes for cell analysis on the flow cytometer (tubes 6-13).
8. Add 100 µl SVF cell suspension (CS) to each tube. Set aside tube 6 as an unstained control sample.

   Five of these tubes are designated as FMO controls and used as controls for compensation and gating, another tube as the unstained control, and two tubes for the multicolored flow panel including a viability stain. See Table 1.

9. Prepare tube 7 as the FMO(FITC) by adding the recommended amount of all antibodies except those conjugated to the FITC fluorochrome.
10. Similarly, prepare tube 8 as FMO(BV421), tube 9 as FMO(PE), tube 10 as FMO(APC/Cy7), and tube 11 as FMO(APC). For anti-CD31, use 5 µl/tube.
11. Add all antibodies to tube 12 and 13.
12. Incubate tubes 6-13 for 30 min at room temperature in the dark.
13. Add 300 µl FACSFlow to tubes 6-13 and vortex briefly.
14. Run ~100 µl of sample from each tube on the flow cytometer in consecutive order. Vortex each tube briefly before analysis.

    Optimally, keep the flow rate below 1000 events/sec (depending on the flow cytometer) during acquisition.

Perform gating

15. Define the first gate (scatter gate) on the FSC-H versus SSC-H profile (Fig. 3A). Subsequently, remove duplets in a FSC-H to FSC-A plot (Fig. 3B).
16. Create three bi-exponential dot plots to gate for the relevant surface markers and define cell populations (Fig. 3C-E).

Table 1 Overview of Antibodies and Fluorochromes for Multicolor Flow Cytometric Analysis of MSCs[a]

	Compensation samples					Control	FMO controls					Samples	
Tube no.	1	2	3	4	5	6	7	8	9	10	11	12	13
	BV421	**FITC**	**PE**	**APC/Cy7**	**APC**		**÷FITC**	**÷BV421**	**÷PE**	**÷APC/Cy7**	**÷APC**		
AB	CD34	CD45	CD73	CD90	CD105		CD34	CD31	CD31	CD31	CD31	CD31	CD31
							CD73	CD45	CD34	CD34	CD34	CD34	CD34
							CD90	CD73	CD45	CD45	CD45	CD45	CD45
							CD105	CD90	CD90	CD73	CD73	CD73	CD73
								CD105	CD105	CD105	CD90	CD90	CD90
												CD105	CD105
Suspension	BM	BM	BM	BM	BM	CS	CS	CS	CS	CS	CS	CS	CS

[a]Fluorochromes for flow cytometric analysis may need to be changed to fit the flow cytometer in use, but the specificity of the antibodies is essential to define the MSC population. Abbreviations: AB, antibody; BM, bead mixture; CS, cell suspension; FMO, fluorescence minus one.

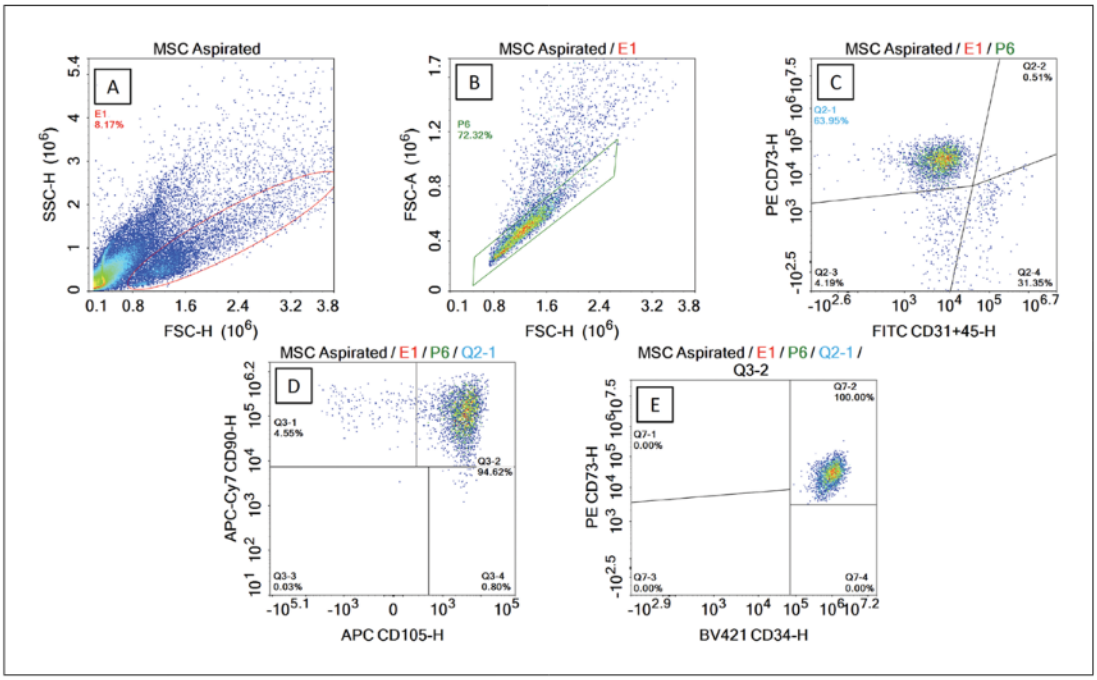

Figure 3 Multicolor flow cytometric analysis of MSCs. (**A**) Forward/side scatter. (**B**) Gating, removing duplets. (**C**) Gating of CD31$^-$ CD45$^-$ CD73$^+$ cells. (**D**) Further gating of CD90$^+$ and CD105$^+$ cells. (**E**) Gating of CD34$^+$ cells. Gating ports of (**C-E**) are determined by FMO controls.

Use the data from the FMO controls to define the negative controls for placement of gates and defining cell populations.

17. Add propidium iodide (8 µl/ml) to tubes 12 and 13. Vortex and run samples again.
18. Create a bi-exponential dot plot with propidium iodide and a random *x* axis to determine the relative amount of viable cells.
19. Use the NovoCyte flow cytometer absolute count function to determine the concentration of the gated cells in the suspension.

BASIC PROTOCOL 3

ADIPOGENIC DIFFERENTIATION ASSAY

This protocol describes the method used to examine the ability of MSCs to differentiate to adipocytes. The ability is verified by Oil Red O staining of intracellular lipid droplets in the cell culture.

Materials

MSCs (optimally at passage 3)
MSCM (see recipe)
Adipogenic differentiation medium (see recipe)
Phosphate-buffered saline (PBS; Gibco, 70011-036)
10% formalin
0.3% (w/v) Oil Red O stain (Sigma, O0625) in 99% isopropanol
60% (v/v) isopropanol

6-well culture dish
Light microscope

Induce adipogenic differentiation

1. Seed 50,000 MSCs per well in MSCM in a 6-well culture dish.

2. Culture in MSCM at 37°C and 5% CO_2 until 70-90% confluency is reached (2-3 days).
3. Change medium to adipogenic differentiation medium and culture for 14 days, refreshing the medium twice a week.

Stain with Oil Red O

NOTE: The following steps are performed at room temperature.

4. Aspirate differentiation medium from all wells and wash carefully three times with PBS.
5. Add 10% formalin to the wells and incubate for 30-60 min.
6. Dilute 3 parts of 0.3% Oil Red O stain with 2 parts distilled water. Incubate 10 min.

   This solution is stable for a maximum of two hours.
7. Remove formalin from the wells and wash with 2 ml distilled water.
8. Remove distilled water and add 2 ml 60% isopropanol. Incubate for 2-5 min.
9. Remove isopropanol and add 2 ml diluted Oil Red O solution. Incubate for 5 min.
10. Wash with water.
11. Inspect sample under a light microscope.

OSTEOGENIC DIFFERENTIATION ASSAY

BASIC PROTOCOL 4

This protocol describes the method used to examine the ability of MSCs to differentiate to osteocytes. The ability is verified by Alizarin Red S staining of calcium deposits in the cell culture.

Materials

MSCs (optimally at passage 3)
MSCM (see recipe)
Osteogenic differentiation medium (see recipe)
Alizarin Red S stain (Sigma, A5533)
0.5% NH_4OH or 1 M HCl
Phosphate-buffered saline (PBS; Gibco, 70011-036)
70% (v/v) ethanol, ice cold

6-well culture dish
Light microscope

Induce osteogenic differentiation

1. Seed 50,000 MSCs per well in MSCM in a 6-well culture dish.
2. Culture in MSCM at 37°C and 5% CO_2 until 70-90% confluency is reached (2-3 days).
3. Change medium to osteogenic differentiation medium and culture for 14 days, refreshing the medium twice a week.

Stain with Alizarin Red S

NOTE: The following steps are at room temperature unless otherwise indicated.

4. Make a saturated Alizarin Red S solution in milli-Q water (2 g in 100 ml) and adjust pH to 4.2 with 0.5% NH_4OH or 1 M HCl. Spin the solution briefly and use the supernatant only.

   As the pH is critical for this solution to work, a fresh batch should be made for each analysis.

5. Wash cells twice with PBS.
6. Fix cells for at least 60 min in ice-cold 70% ethanol at 4°C.
7. Discard the ethanol and add 2 ml saturated Alizarin Red S. Monitor staining by eye (5-10 min should be sufficient; stain maximally for 60 min).
8. Carefully wash three times with distilled water.
9. Inspect the sample under a light microscope.

REAGENTS AND SOLUTIONS

Adipogenic differentiation medium

MSCM (see recipe) containing:
20 nM dexamethasone (Sigma, D4902)
60 µM indomethacin (Sigma, I7378)
200 µM 3-isobutyl-1-methylxanthine (IBMX, Sigma, I5879)
Store up to 14 days at 4°C

Erythrolysis buffer

Erythrolysis buffer stock solution:
1.55 M ammonium chloride
100 mM potassium-H-Carb
1 mM EDTA sodium salt
Demineralized sterile water to bring stock buffer from 10× to 1×
Store up to 1 month at 4°C

MSC culture medium (MSCM)

Minimum essential medium, alfa modified (Sigma, 4526) containing:
15% fetal bovine serum (Hyclone, SV30160-03)
100 U/ml penicillin + 100 ug/ml streptomycin (Life Technologies, 15070-063)
2 mM glutamine (Life Technologies, 25030-024)
Store up to 14 days at 4°C

Osteogenic differentiation medium

MSCM (see recipe) containing:
5 mM β-glycerophosphate (Sigma, G9422)
50 µg/ml L-ascorbinsyrephosphate (Sigma, 49752)
20 nM dexamethasone (Sigma, D4902)
Store up to 14 days at 4°C

COMMENTARY

Background Information

Human adipose tissue is an abundant source for adipose tissue–derived stem cells, which are of great interest in immunomodulation, regenerative medicine, and aesthetic medicine. Their capacity for multipotent differentiation, tissue repair, and immune modulation, in combination with their easy accessibility, promise these cells to be great candidates of cell therapeutic applications (Abdi, Fiorina, Adra, Atkinson, & Sayegh, 2008; Le Blanc & Ringden, 2007; Ringden et al., 2007).

Zuk et al. (2001) described how mesenchymal stem cells from adipose tissue could be derived through enzymatic digestion. The product of adipose tissue digestion and

centrifugation is the SVF, which is a heterogeneous population of cells containing MSCs as well as leukocytes, fibroblasts, endothelial cells, and macrophages (Eto et al., 2009; Schaffler & Buchler, 2007).

Implementation of MSCs for several clinical treatments would require immediately available MSCs at the point of care, e.g., the operating room (OR). The procedure of MSC isolation, however, requires a more comprehensive laboratory setup than is possible to fit in the OR. Investigation of point-of-care mechanical and enzymatic isolation techniques for MSC extraction has been conducted, including automated enzymatic and mechanical systems (Domenis et al., 2015; Iyyanki et al., 2015; Keck et al., 2014; Lee et al., 2017; Oedayrajsingh-Varma et al., 2006). However, a shorter isolation procedure and point-of-care availability have proven less efficient, as these systems have not been able to reach yields comparable to the original enzymatic isolation.

The combination of enzymatic and mechanical isolation presented in this article exploits the different techniques used in current literature. Mechanical disruption finely dissociates the tissue and, by increasing the surface area of the tissue, provides a more efficient enzymatic digestion, resulting in an increased MSC yield (Table 2). The method is supported by a study investigating excised adipose tissue (Li et al., 2018), where the authors found an increase in SVF cell yield with prior tissue disruption for 15 sec before enzymatic digestion. Their study, however, reports specific yield of the heterogeneous SVF population and not a precise measurement of phenotypically characterized MSCs. The study also differed in methods between the examined groups, as they used more digestive enzymes in the disruption group. Without precise characterization of MSCs, it is difficult to determine if the introduction of mechanical disruption in fact increases the MSC yield. Our study presents a standardized setup investigating precise measurement of MSC yield from aspirated adipose tissue with and without mechanical disruption.

A limiting factor for implementation of MSCs for therapeutic use is to reach sufficient numbers in a practical way and in short time. Methods that increase the basic yield of MSCs will be beneficial both for treatments using fresh SVF isolate and for expanded MSC cultures to reach extensive amounts. This paper presents a method for MSC isolation combining enzymatic isolation and mechanical disruption. With the introduction of mechanical disruption, we were able to increase the MSC yield without increasing the concentration of digestive enzymes. This allows for less starting material and shorter time for in vitro expansion to reach required numbers of MSCs. Finally, this method is implementable for point-of-care use.

Critical Parameters and Troubleshooting

To implement the method described here, a tissue disruption device such as a GentleMACS Octo Dissociator is needed. We do not believe this specific product will be crucial to obtain higher MSCs yields, as other methods of mechanical disruption combined with enzymatic digestion will likely also significantly increase the MSC yield.

To maximize the potential yield of MSCs it is important to use freshly harvested adipose tissue. Storage of adipose tissue has been shown to reduce the yield and viability of MSCs (Eto et al., 2012; von Heimburg et al., 2004).

Critical steps of the isolation protocol involve discarding the supernatant. It is important to be careful not to discard the pellet and thereby discarding the MSCs (see Fig. 2). The risk is minimized by handling the suspension immediately after centrifugation.

We recommend running a pilot test of the flow cytometric analysis to predefine gates and cell populations. Furthermore, we recommend performing a dilution assay of chosen antibodies to evaluate the optimal concentration of each antibody and avoid nonspecific binding.

If one does not have access to a flow cytometer with an absolute count feature in order to determine the absolute MSC number directly, we recommend counting nucleated cells by a coulter counter or hemocytometer and performing a relative MSC quantification using multicolor flow cytometric analysis of surface markers.

Statistical Analyses

Data were analyzed after logarithmic transformation using Student's *t*-test. The assumption of normality was accessed using QQ-plots of log-transformed differences and averages, and variance of homogeneity was accessed using Bland-Altman plots. A p value <0.05 was deemed statistically significant. Statistical analysis was performed using Stata Statistical Software, Release 12.

Anticipated Results

Periumbilical adipose tissue samples harvested with a blunt cannula by Coleman's

Table 2 Yield of MSCs Isolated by Enzymatic Digestion Alone and in Combination with Mechanical Digestion

	Yield ($\times 10^3$)			
	Enzymatic alone (95% CI)	Enzymatic + mechanical (95% CI)	Median ratio	p value[a]
Number of samples	10	10		
MSCs/gram (95% CI)	22.7 (0.6-44.7)	52.8 (12.3-93.4)	2.73	0.0003
MSCs/ml (95% CI)	20.0 (0.7-39.3)	47.7 (11.2-84.1)	2.76	0.0003

[a]From Student's *t*-test.

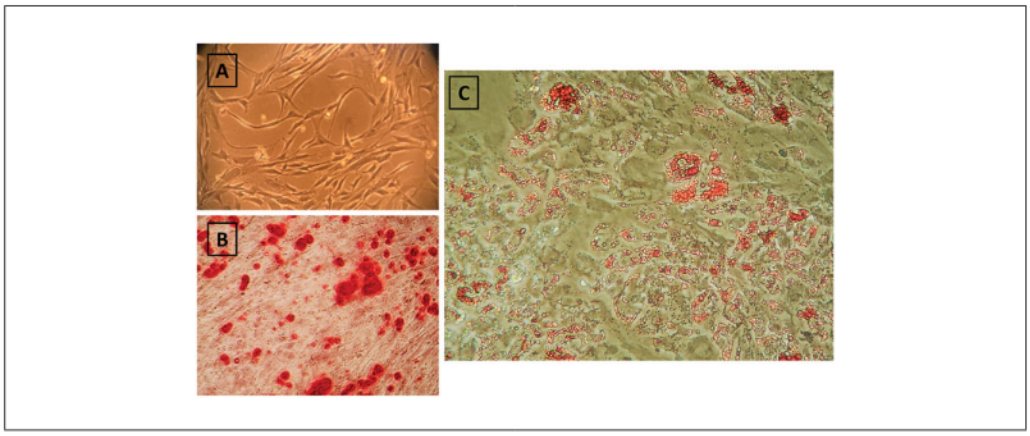

Figure 4 Inclusion of mechanical disruption did not decrease viability or differentiability of MSCs. (**A**) Morphology by light microscopy. (**B**) Adipogenic differentiation by Oil Red O staining. (**C**) Osteogenic differentiation by Alizarin Red S staining.

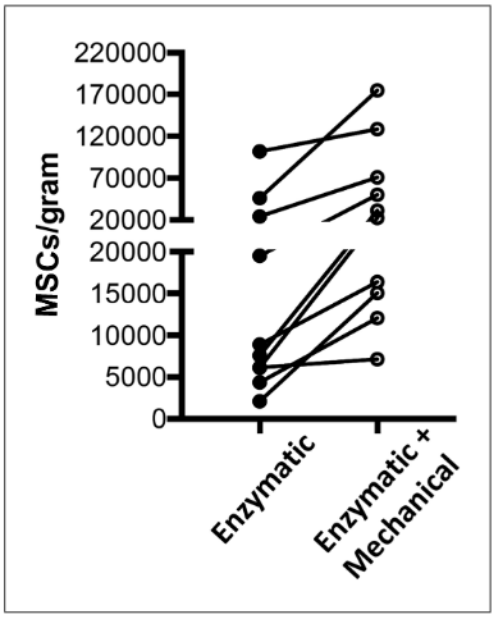

Figure 5 Difference in MSC yield between isolation techniques.

technique in duplicates for each of the ten individuals were compared in our study. Using the described combination of enzymatic digestion and mechanical disruption yielded ~53,000 MSCs/gram aspirated human adipose tissue. The method of isolation did not alter differentiability or viability of the MSCs (Fig. 4). Based on the results (Table 2 and Fig. 5), we anticipate that the revised method of isolation will increase MSC yield by a factor 3 compared to conventional isolation using enzymatic digestion alone. The results originate from ten patients undergoing post-bariatric abdominal surgery and thus reflect yield of MSCs in patients with associated comorbidities.

We hypothesize that the gain of including mechanical disruption in the isolation process is based on finely dissociating the adipose tissue increasing the surface area to facilitate the digestion by enzymes. These results were better than expected, as aspirated human adipose tissue is fluidic and therefore has a larger surface. We hypothesize that other firmer adipose tissue sources might benefit even more from mechanical disruption. Further, we have successfully implemented this method using subcutaneous porcine fat with similar results.

Time Considerations

Isolation of the SVF containing MSCs and characterization by flow cytometry takes about 4-6 hr.

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Conflicts of Interest

All authors declare no conflict of interest.

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