Asian Biomedicine Vol. 7 No. 3 June 2013; 333-338 **DOI: 10.5372/1905-7415.0703.184**

Original article

Simple lipoaspirate washing using a coffee filter

Jeanne Adiwinata Pawitan[a], Isabella Kurnia Liem[b], Des Suryani[c], Arleni Bustami[d], Reza Yuridian Purwoko[e]
[a]Department of Histology, [b]Department of Anatomy, [c]Biomedical Science Master Programme, [d]Integrated Laboratory, [e]Biomedical Science Doctoral Programme, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia

Background: Lipoaspirate contains noxious substances derived from liposuction. Therefore, extensive washing is recommended before the lipoaspirate is processed further for culture or fat grafting. Washing a small amount of lipoaspirate may not pose a problem, but washing a large volume of lipoaspirate may be cumbersome, time consuming, and requires a lot of phosphate buffered saline (PBS).
Objective: To introduce a simple method for lipoaspirate washing using fine-mesh stainless-steel tea or coffee filter, a small tea spoon, and a porcelain bowl.
Methods: The filter was used to collect the adipose tissue fragments. Further washing of the fragments was achieved by soaking the adipose tissue containing filter in a PBS containing porcelain bowl and stirring using a small tea spoon to transfer the contaminating materials to the PBS. Enzymatic processing to dissociate the cells from the tissue and primary cultures was conducted as usual in MesenCult.
Results: Using the equipment mentioned above, the adipose tissue fragments were readily separated from the blood, free lipids, anesthetics, and other noxious material in the liquid portion. This simple method saves time and PBS compared with previously described methods. Further enzymatic processing produced sufficient cells to be cultured, and culture results showed plastic adherent cells on day 2 that became confluent on day 6.
Conclusion: Lipoaspirate washing using a fine mesh stainless steel filter is time saving and produced cells that grow well in MesenCult.

***Keywords*:** Cell, culture, filter, lipoaspirate

Adipose tissue is a rich source of multipotent stem cells that are very promising for regenerative medicine because of the ease and minimal side effects in obtaining the tissue [1]. Adipose tissue derived stem cells may be obtained from adipose tissue excision or liposuction material that is processed and cultured to isolate and expand the stem cells.

Processing adipose tissue before culturing may require several steps. When the adipose tissue is obtained by excision, it should be minced to small fragments, while liposuction readily provides small fragments that are mixed with other materials, i.e. blood, debris, anesthetics, and other substances that are used in liposuction, and free lipid that is released from disrupted adipocytes. Therefore, processing lipoaspirate may include extensively washing the adipose tissue fragments to separate them from other materials [2-8], followed by dissociation of the cells either enzymatically [2-6] or mechanically [7], so that they are ready to be cultured. All of these processes may be conducted in a closed system device [8].

There are various methods to wash the adipose tissue fragments. In most methods, an equal volume of washing solution is added, mixed, and followed by either centrifugation [2, 3] or decantation after 3 to 30 minutes of sedimentation in bottles [4, 5], using a separation funnel [6] or a syringe [7]. Finally, the floating adipose tissue fragments are collected either directly [2, 3] or by removing the infranatant using a pipette [4], suction [5], or ejection from the separation funnel [6] or syringe [7].

In addition to providing adipose-derived stem cell culture, lipoaspirate washing is required in fat grafting. However, lipoaspirate washing in fat grafting should be gentle to avoid rupture of the mature adipocytes because the washed adipose tissue fragments will be reinserted into the patient, and therefore, the centrifugation method should be avoided [7, 9]. For fat grafting purposes, washing of adipose tissue

Correspondence to: Prof. Jeanne Adiwinata Pawitan, MD, PhD, Department of Histology, Faculty of Medicine, University of Indonesia, Jakarta 10430, Indonesia. E-mail: jeanneadiwip@fk.ui.ac.id, jeanneadiwip@gmail.com

fragments is preferably done by decantation [7, 9], or filtering using Telfa gauze (Kendall, Chicago, IL, USA) that is an ideal washing technique for fat graft survival [10, 11].

Washing a small amount of lipoaspirate may not pose a problem, washing a large volume of lipoaspirate may be cumbersome, time consuming, and requires a lot of washing solution. In particular, filtering using Telfa gauze is not suitable for large volume [11]. Therefore, we introduce a simple washing method using a fine mesh stainless steel tea or coffee filter to collect the adipose tissue fragments and then washing the fragments by soaking the filter in a washing solution and stirring using a small tea spoon to remove the contaminants.

Materials and methods

This technical study was conducted in the Integrated Laboratory, Faculty of Medicine Universitas Indonesia, from June to August 2012, and was approved by the ethics committee of the Faculty of Medicine University of Indonesia.

Lipoaspirate was obtained by tumescent liposuction and kept in a sterile bottle (**Figure 1**) at 4°C for no more than 24 hours. The source of lipoaspirate (gender, age, and site), liposuction device, lipoaspirate volume, and time interval between liposuction and processing was noted.

The lipoaspirate was poured gently onto a sterile fine mesh stainless steel filter with a diameter of 7 cm (tea or coffee filter, **Figures 2 and 3**) to retain the fat tissue fragments, and the filtrate collected into a sterile 250 ml glass beaker and discarded.

The fat tissue fragment containing filter was soaked in a sterile porcelain bowl (diameter 7.5 cm, **Figures 2 and 4**) that was half filled with phosphate buffered saline, pH 7.4 (PBS), and the tissue was gently stirred using a sterile stainless steel small teaspoon to wash the tissue and to remove blood, debris, free lipid, and other contaminating materials.

After washing, the buffer became turbid and lipid laden (**Figure 5**). The washing buffer was replaced several times until it remained clear, and the adipose tissue fragments appeared yellow and clean (**Figure 6**). Using the tea spoon, the fragments were transferred into sterile 50 ml centrifuge tubes and weighed.

Subsequently, 0.075% collagenase type I (Sigma, St. Louis, MO, USA) in PBS was sterile filtered into the tubes, until the lipoaspirate:collagenase solution ratio was 1:2. The tubes were incubated at 37°C for 1 hour and were agitated every 5 minutes. When the adipose tissue volume was reduced and appeared clear yellow (because of free lipid, **Figure 7**), the floating yellow free lipid and remnants of tissue fragments were removed, and the infranatant transferred into sterile 15 ml centrifuge tubes, and centrifuged at 800 ×*g* for 10 minutes. The pellets were washed with PBS, and reconstituted in a commercial complete medium (MesenCult, Stemcell Technologies, Vancouver, BC, Canada) and cultured in a twelve-well plate (seeding number around 170,000 viable cells per well). The cultures were inspected every day. The days when the cells were attached and further when the culture became confluent were noted.

Before culture, the viable and nonviable individual cells were counted and noted, and the amount of viable cells per gram of adipose tissue was calculated.

Figure 1. Lipoaspirate before washing

Figure 2. Equipment for lipoaspirate washing step

Figure 3. Lipoaspirate was poured on to a stainless steel coffee filter, and the fluid was collected in a glass beaker

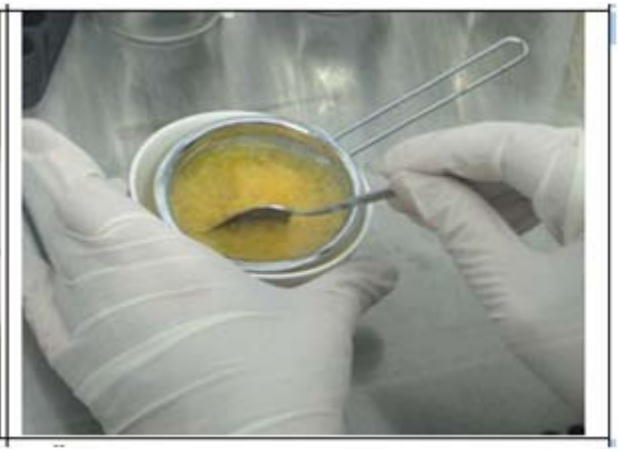

Figure 4. Lipoaspirate washing by soaking the filter and contents in phosphate buffered saline in a porcelain bowl

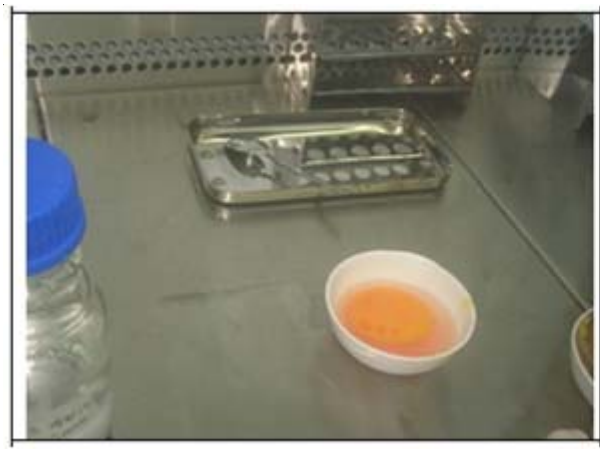

Figure 5. Free lipids and other contaminants were left in the porcelain bowl

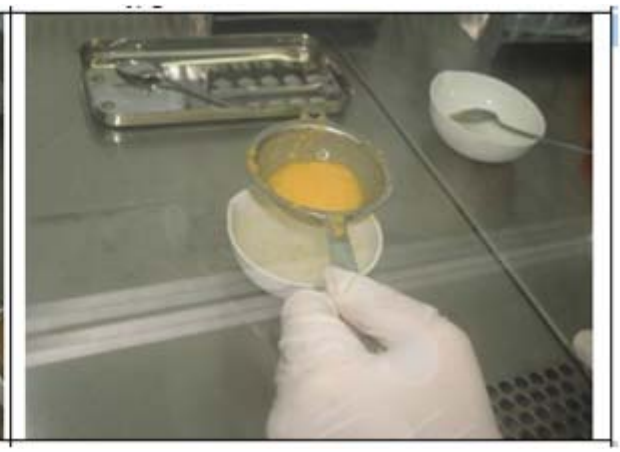

Figure 6. Contaminant free lipoaspirate

Results

Using a sterile fine mesh stainless steel filter, adipose tissue fragments were readily separated from the blood, free lipids, anesthetics, and other material contained in the liquid portion of the lipoaspirate. Unlike washing methods using suction or transferring the fragments using a pipette or decantation, washing using the filter did not leave any residual fluid that diluted the fragments.

Further, washing by soaking the filter and stirring gently using a sterile stainless steel spoon allowed the residual blood, free lipid, and other contaminating material to pass through the filter pores (**Figure 3**), while the adipose tissue fragments were retained (**Figure 4**).

Descriptions of the lipoaspirate source (gender, age, and site), liposuction method (device and mode), and time interval between liposuction and processing are shown in **Table 1**.

The filter pore size, volumes of lipoaspirate that were processed, weight of contaminant free adipose tissue fragments after washing, and viable individual cell count after collagenase treatment and washing are shown in **Table 2**.

Result of collagenase treatment is shown in **Figure 7**. Collagenase treatment yielded individual cells mixed with rope-like clusters of cells, but the cell count was conducted on individual cells. The cells were attached on day 2, and became confluent on day 6. The result of lipoaspirate derived cell culture on day 2 after washing is shown in **Figure 8**.

Table 1. Lipoaspirate source, liposuction method, and time between liposuction and processing

Sample	Lipoaspirate source				Liposuction method		Interval from liposuction to processing
	Age (y)	Gender	Site	BMI	Device	TLC	
1	52	F	Abd	29.62	PAL LipoSculptor cannula 3.0 mm*	NaCl, lidocaine, sodium bicarbonate, Epinephrine	12 hours
2	62	F	Abd	25.77			13 hours

Abd = abdomen, TLC = tumescent solution content, *Microaire, Charlottesville, VA, USA.

Table 2. Filter pore size, lipoaspirate volume, weight of contaminant free adipose tissue and viable cell count

Sample	Filter pore size	Lipoaspirate volume	Weight of adipose tissue* (gram)	Cell count		Viable cells/gram of adipose tissue*
				Viable (%)	Nonviable	
1	0.5 mm	200 mL	31.5	1,605,000 (59%)	1,110,000	51,004
2	0.5 mm	110 mL	16.9	882,000 (55%)	714,000	52,279

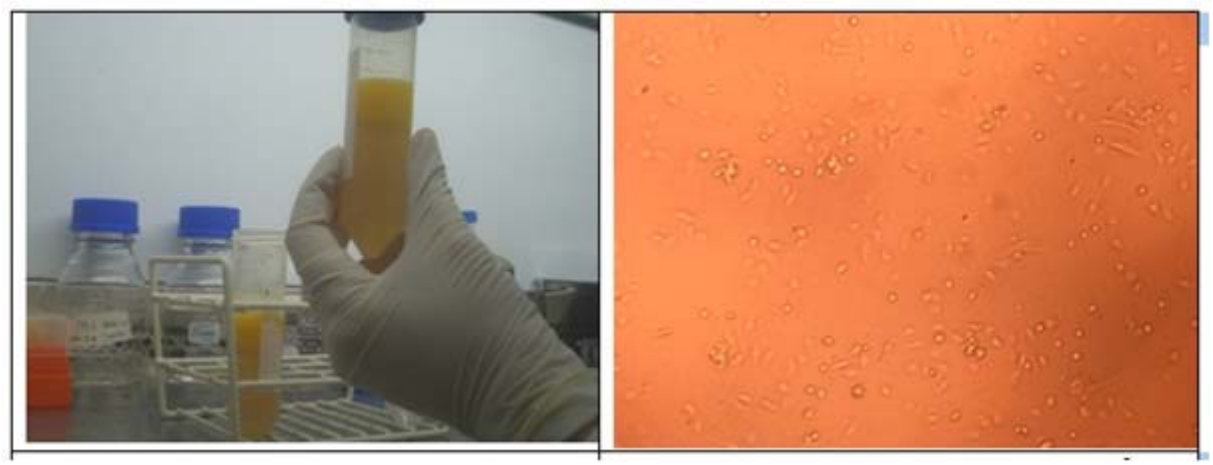

Figure 7. Lipoaspirate after collagenase digestion

Figure 8. Culture result of processed lipoaspirate in MesenCult (commercial medium): plastic adherent cells on day 2, after washing, 100× magnification

Discussion

Washing lipoaspirate before processing and culture is preferred, especially for lipoaspirate that is obtained under local anesthetics in combination with epinephrine to reduce bleeding, because certain anesthetics either alone or in combination with epinephrine are proven to reduce cell viability and preadipocyte differentiation capacity [12]. Moreover, lipoaspirate washing is highly recommended to avoid graft resorption as a result of unviable cell contaminants, when the adipose tissue is to be injected back to patients as an esthetic filler.

The entire processing of lipoaspirate until it is ready for culture is laborious, and may take 8–10 hours [13], especially for a large amount of lipoaspirate. Washing the lipoaspirate using a fine-mesh stainless-steel filter may save a lot of time, because the adipose tissue fragments are readily separated from the fluid portion, and washing is facilitated as it can be conducted by soaking the filter and its contents in a buffer and gently stirring the fragments. Moreover, the separated fluid portion may be processed further to obtain additional adipose derived stem cells [13, 14].

In this study, a cell count was conducted on individual cells because of the difficulty in counting the actual number of cells in clusters. However, the results of this mode of counting that are depicted in Table 2 are substantially less than the actual cell yield. Therefore, viable cell counts in this study are less compared with other studies. Zuk et al. [15] obtained $2–6 \times 10^8$ cells from 300 mL lipoaspirate after a shorter time (30 minute) of 0.075% collagenase digestion. Moreover, Oedayrajsingh-Varma et al. [16] obtained $0.7 \times 10^6 \pm 0.1 \times 10^6$ viable cells/g adipose tissue from the abdominal region.

This study shows that, after collagenase treatment, individual cells were mixed with cell clusters. To minimize cell clusters, the collagenase concentration needs to be increased, or treatment time needs to be prolonged, but increased concentration or prolonged collagenase treatment may increase the number of unviable cells, which may have adverse effects on culture results. Our study showed that 0.075% collagenase digestion for 1 hour yielded 55%–59% viable cells while another study achieved 81 ± 2% viable cells from 0.1% collagenase A digestion for 45 minutes [16].

Our results showed that although the individual viable cell counts and percentages of viable cells were lower, cells were attached on culture day 2 and confluence was attained on day 6 (5 days), which is faster compared with the study by Astori et al. [17], where the cells were attached after 4–7 days. Studies by Zuk et al. [15] and Mitchel et al. [18] showed that lipoaspirate-derived cell culture required 7–9 days and 6.0 ± 2.4 days to become confluent, respectively [15, 18].

The time needed to become confluent may be affected by the method of liposuction, the medium that is used to culture the cells and seeding density. Ultrasound-assisted liposuction derived cells require a longer time to become confluent compared with tumescent liposuction [16]. However, both our study and the study by Zuk et al. [15] used tumescent liposuction to obtain the lipoaspirate while the method of liposuction used by Mitchel et al. [18] was not known. Therefore, the faster cell confluence in our study might be the result of MesenCult medium used in our case compared with 10% FBS containing DMEM and DMEM/F12 by Zuk et al. and Mitchel et al., respectively [15, 18]. Moreover, Mitchel et al. used a seeding density of 48.180 ± 21.895 cells/cm^2 [18] that is lower compared with that in our study, and thus their culture took a longer time to attain confluence.

Conclusion

Lipoaspirate washing using a fine mesh stainless steel filter is time saving and cells obtained in this manner grow well in MesenCult medium.

Acknowledgment

This study was funded by the grant from the Directorate of Research and Community Service of Universitas Indonesia, Contract number 1594/H2.R12/HKP.05.00/2012. A preliminary study was reported as an oral presentation at the Scientific Meeting of the Indonesian Anatomist Association, which took place in Denpasar, Bali, Indonesia, October 12–13, 2012.

The authors have no conflict of interest to report.

References

1. Pawitan JA. Prospect of adipose tissue derived stem cells in regenerative medicine. Cell & Tissue Transplantation & Therapy. 2009; 1:7-9.
2. Sardjono CT , Setiawan M, Frisca, Saputra V, Aniko G, Sandra F. Application of a modified method for stem cell isolation from lipoaspirates in a basic lab. Med J Indones. 2008; 18:91-6.
3. Isolation of CD271 (LNGFR)$^+$ MSCs/ADSCs from human lipoaspirate. Macs Miltenyi Biotec. 2008. [Cited 2012 Jul 8]. Available from: http://www.miltenyibiotec.com/downloads/6760/6764/23629/23630/MSC_CD271_liposaspirate.pdf
4. Collaslab. Isolation of Stromal Stem Cells from Human Adipose Tissue. [Cited 2010 Apr 15]. Available from: http://www.collaslab.com/UserFiles/File/Adipose%20stem%20cell%20isolation.pdf
5. Yu G, Floyd ZE, Wu X, Halvorsen YC, Gimble JM. Isolation of human adipose-derived stem cells from lipoaspirates. In: Gimble JM, Bunnell BA, editors. Adipose-derived stem cells: methods and protocols. Methods Mol Biol. 2011; 702:17-27.
6. Isolation and culture mesenchyme cell from adipose. In: Rantam FA, Ferdiansyah, Nasronudin, Purwati, editors. Stem cell exploration. Methods of isolation and culture 1st ed. Surabaya: Airlangga Univ. Press; 2009.
7. Baptista L, Silva K, Pedrosa C, Borojevic R. Processing of lipoaspirate samples for optimal mesenchymal stem cells isolation. In: Serdev Nikolay, editor. Advanced techniques in liposuction and fat transfer. Rijeka: Intech; 2011.

8. Fraser JK, Hedrick MH. Systems and methods for treating patients with processed lipoaspirate cells. US Patent no. US 7,501,115 B2 (10 March 2001). [Cited 2012 Jul 8]. Available from: http://www.freepatentsonline.com/7501115.html
9. Cond□-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg. 2010; 63:1375-81.
10. Canizares O, Allen RJ, Scharf CL, Davidson EH, Nguyen PD, Tutela JP, et al. Shaken not stirred? The effect of processing techniques on fat graft survival. (Abstract) Plastic Surgery (The American Society of Plastic Surgeons annual meeting). 2009 Oct 23-27; Seattle, WA, USA. [Cited 2012 Jul 8]. Available from: https://asps.confex.com/asps/2009am/techprogram/paper_16619.htm
11. Fisher C, Grahovac T, McAtee J, Shippert R, Marra K, Rubin JP. A comparison of human fat graft processing methods: an animal study (abstract). The aesthetic meeting 2011: affirming the science of aesthetic surgery, 2011, May 6-11; Boston, MA, USA. [Cited 2012 Jul 8]. Available from: http://asaps.confex.com/asaps/2011/webprogram/Paper5077.html
12. Keck M, Zeyda M, Gollinger K, Burjak S, Kamolz LP, Frey M, et al. Local anesthetics have a major impact on viability of preadipocytes and their differentiation into adipocytes. Plast Reconstr Surg. 2010; 126: 1500-5.
13. Francis MP, Sachs PC, Elmore LW, Holt SE. Isolating adipose-derived mesenchymal stem cells from lipoaspirate blood and saline fraction. Organogenesis. 2010; 6:11-4.
14. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J Cell Physiol. 2006; 208:64-76.
15. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001; 7:211-28.
16. Oedayrajsingh-Varma MJ, van Ham SM, Knippenberg M, Helder MN, Klein-Nulend J, Schouten TE, et al. Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy. 2006; 8:166-77.
17. Astori G, Vignati F, Bardelli S, Tubio M, Gola M, Albertini V, et al. "In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells. J Trans Med. 2007; 5:55.
18. Mitchel JB, McIntosh K, Zvonic S, Garret S, Floyd ZE, Kloster A, et al. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells. 2006; 24: 376-85.