Cytotherapy, 2014; 16: 381–391

International Society for Cellular Therapy
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Enrichment isolation of adipose-derived stem/stromal cells from the liquid portion of liposuction aspirates with the use of an adherent column

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Abstract

Background aims. Adipose-derived stem/progenitor cells (ASCs) are typically obtained from the lipoaspirates; however, a smaller number of ASCs can be isolated without enzymatic digestion from the infranatant liposuction aspirate fluid (LAF). We evaluated the effectiveness of an adherent column, currently used to isolate mesenchymal stromal cells from bone marrow, to isolate LAF cells. *Methods.* We applied peripheral blood (PB), PB mixed with cultured ASCs (PB-ASC), and LAF solution to the column and divided it into two fractions, the adherent (positive) and the non-adherent (negative) fractions. We compared this method with hypotonic hemolysis (lysis) for the red blood cell count, nucleated cells count and cell compositions as well as functional properties of isolated mesenchymal cells. *Results.* The column effectively removed red blood cells, though the removal efficiency was slightly inferior to hemolysis. After column processing of PB-ASC, 60.5% of ASCs (53.2% by lysis) were selectively collected in the positive fraction, and the negative fraction contained almost no ASCs. After processing of LAF solution, nucleated cell yields were comparable between the column and hemolysis; however, subsequent adherent culture indicated that a higher average ASC yield was obtained from the column-positive samples than from the lysis samples, suggesting that the column method may be superior to hemolysis for obtaining viable ASCs. Mesenchymal differentiation and network formation assays showed no statistical differences in ASC functions between the lysis and column-positive samples. *Conclusions.* Our results suggest that a column with non-woven rayon and polyethylene fabrics is useful for isolating stromal vascular fraction cells from LAF solutions for clinical applications.

Key Words: *adherent column, adipose-derived stem cells, cell isolation, hemolysis, lipoaspirates, liposuction aspirate fluid*

Introduction

Subcutaneous adipose tissue is an abundant source of mesenchymal stromal/progenitor cells and is a promising cell source for clinical applications (1–3). Collagenase digestion of adipose tissue yields a heterogeneous stromal vascular fraction (SVF) that contains multipotent adipose-derived stem/stromal cells (ASCs) (1). ASCs in the SVF can be purified by cell sorting or by adherent culture, and the utility of the resulting purified cells has been demonstrated in numerous pre-clinical and clinical studies. Reported clinical applications include treatment of soft tissue and bone defects, chronic or irradiated ulcers, Crohn's disease, multiple sclerosis, graft-versus-host disease and myocardial infarction (2,3).

Although the floating adipose portion of liposuction aspirates is a major contributor of ASCs, the infranatant liquid portion (liposuction aspirate fluid: LAF) also contains ASCs (4). The SVF cells derived from the adipose portion are referred to as processed lipoaspirate cells (PLA cells) (5), whereas the SVF cells derived from the infranatant fluid are referred to as LAF cells (4). Total nucleated cell yield was comparative between PLA and LAF cells, but proportion of ASCs in LAF is smaller (3∼10%) than in PLA (15∼40%) (4). Thus, the total number of ASCs in LAF is small but not negligible.

Collagenase digestion is required to isolate PLA cells, and many countries regulate PLA cells because they do not pass minimal manipulation standards;

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(Received 23 January 2013; accepted 11 September 2013)

http://dx.doi.org/10.1016/j.jcyt.2013.09.002

this restricts the clinical use of PLA cells. LAF cells, however, are isolated without the use of enzymatic digestion. Therefore, freshly isolated LAF cells are considered minimally manipulated cells and are not generally regulated. Although LAF cells are not yet commonly used in clinical practice, establishing an efficient and standardized method of LAF cell isolation in a closed system (without excess manipulation) is important to ensure that they can be used clinically.

In the present study, we evaluated the effectiveness of an adherent column, which has been used previously to purify mesenchymal stromal cells from bone marrow (6), to purify ASCs from the LAF. We investigated the selectivity and efficiency of column isolation with the use of three different solutions, compared it with the conventional hemolysis procedure and evaluated its usefulness for isolation and purification of ASCs from LAF.

Methods

Human peripheral blood and adipose tissue samples

Human peripheral blood (PB) (1 mL) was harvested from healthy human male donors. Ethylene di-amine tetra-acetic acid disodium (1 mg; Wako Pure Chemical Industries, Ltd, Osaka, Japan) was added as an anticoagulant (7) and diluted to 50 mL with phosphate-buffered saline (PBS) to obtain a working solution. Aspirated subcutaneous adipose tissue was obtained from female patients undergoing liposuction surgery. Informed consent was obtained from each donor for the institutional review board–approved protocol.

Separation and processing of liposuction aspirates

Human liposuction aspirates were divided into two portions, the fatty supernatant (adipose portion, lipoaspirates) and the infranatant fluid (LAF), as previously described (4). The LAF is primarily composed of (i) a saline solution pre-operatively injected into the site to prevent nerve and blood vessel damage, (ii) peripheral blood and (iii) cells and tissue fractions derived from adipose tissue.

ASCs were isolated from the adipose portion of liposuction aspirates and used to prepare a peripheral blood–ASC mixture (PB-ASC), as described below. The aspirated fat tissue was washed with PBS and digested at 37°C in PBS containing 0.075% collagenase for 30 min on a shaker. The floating tissue (mature adipocytes and connective tissue) was separated from the cell pellet (corresponding to SVF) by centrifugation (800*g* for 10 min). The SVF cells were resuspended, filtered through a 100-μm mesh, plated at a density of 5×10^5 nucleated cells onto a 10-cm, non-coated tissue culture plastic dish and cultured at 37°C at 5% CO_2 humidified atmosphere. We cultured the cells in M199 medium (Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 IU penicillin, 100 mg/mL streptomycin, 5 mg/mL heparin, and 2 μg/mL acidic fibroblast growth factor. Media was replaced every 3 days. Primary cells were cultured for 7 days and were defined as passage 0. Cells were passaged every week by trypsinization. ASCs at passages 3–5 were used for experiments. We also used the fluid portion (LAF) as a material for cell processing; SVF cells isolated in this manner are referred to as LAF cells. Either column or hypotonic hemolysis was used to isolate LAF cells from the LAF.

Preparation of the PB and ASC mixtures

Two million ASCs were mixed with 1 mL PB to create a PB-ASC mixture. PB and ASC donors were not matched. To evaluate the efficacy of the column for removing contaminated red blood cells (RBCs) and white blood cells (WBCs), we prepared the mixture of PB and ASCs (as a simplified solution to simulate LAF) as well as the LAF; LAF solutions contain more materials than only PB and ASCs, such as cells and tissue debris. The ASC proportion in the PB-ASC mixture was increased compared with that of LAF to enable easier ASC detection.

Cell processing by adherent column or hypotonic hemolysis

Three kinds of cell solution (PB, PB-ASC and LAF solution) were used as a material for cell processing. The cell solutions were processed with either a hypotonic lysis method or a column method, and each solution, before processing, was called a native solution (native). The LAF solution was filtered with a 100-μm mesh (BD, Franklin Lakes, NJ, USA) before processing.

For hypotonic processing, the cell solution was centrifuged (800*g*, 5 min) to pellet the cells. After discarding the supernatant, we added 45 mL of sterile water to the remaining cell pellet and the mixture was manually shaken for 30 seconds, followed by addition of 5 mL of ×10 PBS to stop the hypotonic reaction. Most of the RBCs were selectively removed by hypotonic processing. The cell fraction obtained was washed with PBS and referred to as the hemolysis sample (lysis).

The column used was a filter constructed of rayon-polyethylene non-woven fabrics that have affinity to adherent cells (Kaneka Corp, Osaka, Japan) (Figure 1) (6). After the column was filled with PBS, 50 mL of each solution was applied to the column.