

RESEARCH ARTICLE

Isolation of Mesenchymal Stem Cells from Adipose TissueHarsan^{1,*}, Silmi Mariya², Dondin Sajuti², Andi Asadul Islam³, Eka Julianta Wahjoepramono¹,
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Abstract

BACKGROUND: In searching for the best source of stem cells, researcher found adipose stem cells as one of the ideal source due to its easiness in harvesting and its potential for differentiating into other cell lineage.

METHODS: We isolated stem cells from adipose tissue, cultured and confirmed its immunophenotype using polymerase chain reaction.

RESULTS: Cluster of differentiation (CD)44, CD73, CD90, CD105 were expressed, which represent immunophenotype of mesenchymal stem cells.

CONCLUSION: Mesenchymal stem cells can be isolated from adipose tissue.

KEYWORDS: adipose, mesenchymal stem cells, isolation, immunophenotype

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Introduction

Stem cell is a promising source for healing, especially for diseases, where destroyed cell or tissue is need to be repaired by new cells. Classic sources of stem cells are embryo and bone marrow, however harvesting these sources are facing problems. Harvesting embryo faces ethical problem, while harvesting bone marrow faces a technical problem, which needs invasive and painful procedures. In searching for the ideal source of stem cells, researcher found adipose tissue as an ideal source of stem cells. It is from adult tissue, without ethical problem, but easy for harvesting, without severe pain and low risk of morbidity even in some situation this tissue is discharged. Adipose stem cells have capability to differentiate into multiple lineages of cells and do not produce allergic reactions. This paper will discuss about the method to isolate stem cells from adipose tissue and its differentiation.

Methods**Isolation of Adipose Tissue**

Adipose tissue collection was performed by a veterinarian from PT Bimana Indomedical after all protocols were approved by the Animal Welfare Supervision Commission and Use of Research Animals, PT Bimana Indomedical, no. R.09-14-IR. Ten grams adipose tissue was collected and washed with phosphate buffer saline (PBS), which was supplemented with 1% antibiotics and 1% antifungal. Then, adipose tissue was chopped in sterile petri dish into small pieces, inserted into a tube containing 4 mL of 0.075% collagenase type II and incubated at 37°C for 30 minutes. Samples were centrifuged at 1200xg for 10 minutes. Resulted supernatant was discarded and pellet was resuspended in growth medium: Dulbecco's Modified Eagle Medium (DMEM) with 20% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin and

2% amphotericin B. Cells were grown with 10 various cell concentrations in a 6-well tissue culture plate.(1) Media was changed every 3-4 days, subculture was done. After the confluence was reaching 80%, cells were counted and regrowth in larger container, until it reached a concentration suitable for treatment (Figure 1).

mRNA Extraction and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Mesenchymal stem cells mRNA were extracted using kit RNAeasy (Qiagen, Maryland, USA), with the standard procedures available from the company. RT-PCR was performed to obtain cDNA from purified RNA samples, using Superscript III kit (Invitrogen, Carlsbad, USA) refers to the standard procedures from the company. Reverse Transcriptase PCR was performed to obtain a complimentary DNA (cDNA) from purified RNA samples, using Superscript III kit (Invitrogen, USA) refers to the standard procedures from the company. PCR amplification was carried out for cluster of differentiation (CD) 44, CD73, CD90, CD105, and glyceraldehyde-3-phosphate (GAPDH). For CD44, Forward primer: 5'CCCGGGGGCCACTAGCACCTCA3' and Reverse primer: 5'GCCTGGACCACGGGAACCTT3'; For CD73, Forward primer: 5'CCCGGGGGCCACTAGCA-CCTCA3' and Reverse primer: 5'GCCTGGACCACGGGA-ACCTT3'; For CD90, Forward primer: 5'TCAGGAAA-TGGCTTTTCCCA3' and Reverse primer: 5'TCCTCAATGAGATGCCATAAGCT3'; For CD105, Forward primer: 5'CTGGAGCAGGGACGTTGT3' and Reverse primer: 5'GCTCCACGCCTTTGACC3'; For GAPDH, Forward primer: 5'CCTCAACGACCACCTT-GTCA3' and Reverse primer: 5'TTACTCCTTGGAGG-

CCATGT3'. PCR reagents used were GoTaq Master Mix (Promega, Madison, USA). PCR reactions were performed 40 cycles, annealing temperatures for CD44 and CD 73 at 55°C, while for CD90, CD105 and GAPDH at 50°C. Visualization of the PCR results was performed by electrophoresis in 2% agarose gel with addition of 0.1 mg/mL ethidium bromide in Tris-acetate-EDTA buffer. Electrophoresis was performed at 100V for 45 minutes. GelDoc (Biorad, Hercules, USA) was used to visualize the electrophoresis result.

Results

The development of stem cells population was presented in Table 1 and PCR results were presented in Figure 2.

Table 1. Development of stem cells population.

Passage	Day	Cell Number/Container (10 ⁶ cells)	Container
P0	1	10	2 6-well plates
P1	7	0.6	1 T25 flask
P2	14	1	2 T25 flasks
P3	21	1.2	6 T25 flasks
P4	27	1.8	5 T25 flasks (+1 T25 flask for PCR)
P5	35	3.55	5 flasks T25 (+1 T25 flask for PCR)

Discussion

The classic source of stem cells is embryo, with its enormous potential. Nevertheless, the presence of ethical problems has led the searching for another source of stem cells that is bone marrow. However, bone marrow mesenchymal stem cells also has a problem with harvesting, it causes pain and low cell number that can be obtained. In this situation, adipose tissue becomes an interesting alternative source of stem cells. Stem cells from adipose tissue have the same characteristic with mesenchymal stem cells. These cells can be easily harvested, abundant and have the ability to differentiate into various tissues. As mesenchymal stem cells, these cells are also hypo-allergenic, that can be used on another host (allogeneic).

Stem cell from adipose tissue is one of the interesting sources of stem cells. According to Gimble, the ideal source of stem cells should be

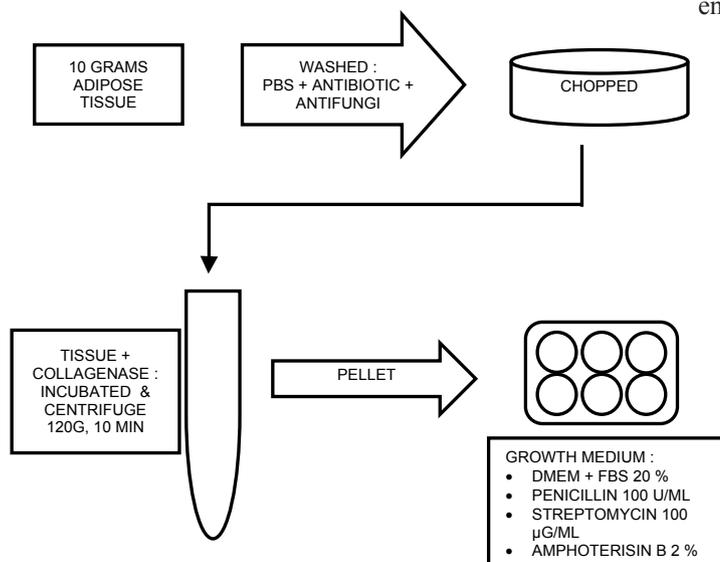


Figure 1. Schematic illustration of isolation of stem cells from adipose tissue.

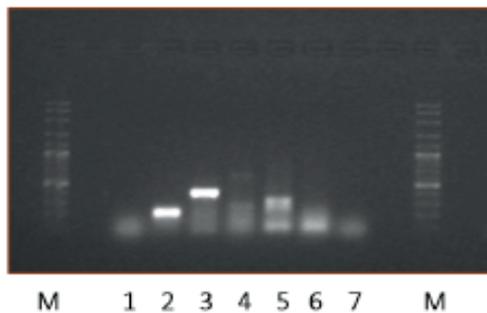


Figure 2. PCR analysis of adipose stem cells. M: Size Marker 100bp; 1: Control; 2: CD44 (213 bp); 3: CD73 (403 bp); 4: CD90 (100 bp); 5: CD105 (225 bp); 6: GAPDH (104 bp); 7: Control.

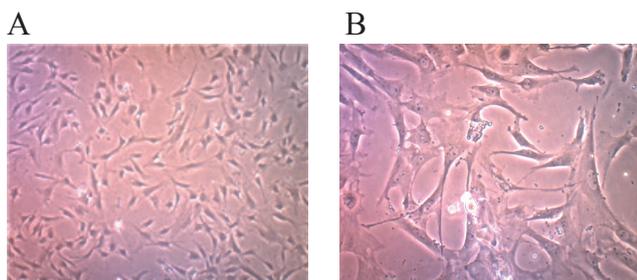


Figure 3. Eighty percent confluence of stem cells on culture dish. A: 32x magnification, B: 80x magnification.

available in large quantities, it can be easily harvested (with "minimal invasive" method), can differentiate into various types of cells, can be safely and effectively transplanted to the recipient either autologous or to others and can be developed following the guidelines of Good Manufacturing Practice. Stem cells from adipose tissue can meet all of the criteria of an ideal source of stem cells.(2) Adipose tissue can be harvested easily, but containing abundant of progenitor cells that have characteristic properties of stem cells. In some people, this tissue can even be removed and discharged (liposuction on obesity), harvesting this tissue can also be done in a simple and painless manner. This provides opportunity to utilize of adipose tissue as a source of stem cells.(3,4,5)

Evidence of stem cells in adipose tissue obtained from several pathological conditions, such as Progressive Osseous Heteroplasia, where on its subcutaneous tissue, osteoblast, chondrocytes and adipocytes were found. This disorder gives information that adipose tissue has the capability for adipogenic, chondrogenic and osteogenic differentiation. (6) In liposarcoma, there were activation of hormone receptor for adipogenesis, peroxisome proliferator-activated receptor (PPAR) γ . Also in obese patients, who experience a decrease in the amount of adipose tissue (after liposuction,

for example) there will be an activation on the stem cell population for generation of new adipocytes.(1)

In the human body, there are two types of adipose tissue, there are white and brown adipose tissues. The biggest part of adipose tissue in human body is white adipose tissue that serves as energy storage depot and an active endocrine organ. While brown adipose tissue contains many mitochondria and uncoupling protein 1 (UCP1), to produce ATP.(7) There are three types of white adipose tissues, these are deposit, structural and fibrous adipose tissues, which are named according to their functions.(8)

Another way of adipose tissue classification was proposed by Gimble which states that there are five types of adipose tissue, namely: bone marrow, brown, "mammary", mechanical and white. Each type has a specific biological function. Bone marrow adipose tissue occupies the space previously used by hematopoietic cells and serves as a reserve of energy, also a source of cytokines and hematopoietic osteogenesis process. Brown adipose cells found around vital organs such as heart, kidney, aorta, gonads and it has function to regulate temperature, especially found in infants and fade away when people got older. "Mammary" adipose tissue produce nutrients and energy during lactation, the existence of these cells are regulated by hormones associated with pregnancy. Mechanical adipose cells function as a network support for vital organs such as the eyes, hands and palms. White adipose cells serve as energy reserves and insulators and have many multipotent stem cells.(2)

There are several terms that are often used, such as: adipose-derived stem/stromal cells (ASCs), adipose-derived adult stem (ADAS) cells, adipose-derived stromal cells (ADSCs), adipose stromal cells (ASCs), adipose mesenchymal stem cells (AdMSCs), lipoblast, pericytes, preadipocyte, and processed lipoaspirate (PLA) cells. International Adipose Applied Technology Society (IADIPOSES) has made a consensus to use the term ASCs. (2)

ASCs have the morphological characteristics like fibroblasts, but without the adipose droplet, after the culture, the ASCs will display proteins and cytokines that are similar to the cells of the bone marrow and muscles. (3,4) Adipose tissue is rich in stem cells with the cell ratio can be attached to a petri 1:100-1: 1500, this figure is much better compared to mesenchymal cells from bone marrow. (3,4) Immunophenotype of stem cells from adipose tissue has similarities more than 90% compared to mesenchymal cells.(2)

The methods for isolation of stem cells from adipose tissue can vary between researchers. In principle, these

methods start with enzymatic and centrifugal separation of adipose tissue from stromal cells and vascular. The resulting pellet from this centrifuge process is known as stromal vascular fraction (SVF). This pellet contains blood cells, fibroblast, pericytes, endothelial cells and preadipocytes (adipocytes progenitor). This cells population can be maintained in undifferentiated state for some time until it is decided to be differentiated to a specific type of cells.(1) Zhu, *et al.*, improved the method to isolate stem cells from adipose tissue by digesting adipose tissue with collagenase and trypsin for several time, also exchange the medium for several time to clean the red blood cells instead of using chemical substance. Their isolation can obtain $1-2 \times 10^7$ nucleated cells and 5×10^5 stem cells from 400-600 mg subcutaneous tissue (about 1,5 mL).(9)

The Mesenchymal and Tissue Stem Cells Committee of the International Society for Cellular Therapy (ISCT) had proposed a position statement regarding minimal criteria to defined mesenchymal stem cells, the stem cells that developed from adipose tissue should also meet these criteria. The criteria including capability to adhere to plastic, specific surface antigen expression and multipotent differentiation potential.(10) Our cell culture showed its capability to adhere to plastic and underwent several passage. PCR on our cell culture had revealed expression of CD44, CD73, CD90 and CD105 which is characteristic of mesenchymal stem cells.

Efficacy of ASCs for therapy is showed in a research by Rehman, *et al.* This research showed that ASCs have potential to give angiogenic and antiapoptotic effect. (11) Another advantage of ASCs is not triggering allergic reaction, it can even suppress immunological reactions. Particularly in stem cells that have undergone more than one passage. Thus these stem cells can be used for allogeneic transplantation.(2) In the research conducted by McIntosh, *et al.*, expression of human leucocyte antigen (HLA)-DR were decreasing after first passage also if comparing with SVF, as the consequence these cells almost not stimulate T-cell response at all.(12)

Differentiation of ASCs to neuronal stem cells can be done by adding certain substances into the medium. Substance that is often used is butylated hydroxyanisole, valproic acid and forskolin.(13) After such induction, stem cells will express neuronal markers as Neuronal nuclear antigen (NeuN), Microtubule-associated Protein 2 (MAP2), tau, β -III tubulin and Neuron-specific Enolase (NSE), as well as glial marker Glial fibrillary acidic protein (GFAP), vimentin and S100. The expression of NeuN can reach 80% of the cell population.(4,13)

ASCs have some advantages compare to bone marrow stem cells. They can differentiate into neuron, glia and vascular elements to build a neurovascular unit, the emerging concept for stroke healing. ASCs had great advantages in cell harvesting, preparation and administration.(14)

Conclusion

Mesenchymal stem cells can be isolated from adipose tissue.

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