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SVF Isolation and Immunophenotyping using Flow Cytometry in Leprosy Patients

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Sondang P. Sirait[1], Kusmarinah Bramono[1], Sri Linuwih Menaldi[1], Jeanne Adiwinata Pawitan[2,3,4], Wresti Indriatmi[1], Tiara Aninditha[5]

[1]Dermatovenerology Department, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia;
[2]Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia;
[3]Stem Cells Medical Technology Integrated Service Unit, Dr. Cipto Mangunkusumo General Hospital/ Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia;
[4]Stem Cells and Tissue Engineering Research Center, Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia;
[5]Neurology Department, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

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Abstract

Adipose derived stromal vascular fraction (SVF) contains a heterogeneous population of mononuclear cells, progenitor cells and about 1-10% are mesenchymal stromal cells. These cells are an ideal candidate for regenerative medicine for peripheral neuropathy. Leprosy is a disabling disorder with neuropathy, usually with consequences of permanent disability of the extremities. Before we could inject the SVF, we need to learn whether the SVF is appropriate to be used for neuropathy treatment using the flow cytometry characterization. Liposuction was done to four leprosy patients and adipose tissue was processed into SVF with a closed system. Following the process, the SVF were characterized and counted the CD73, CD90, CD34, CD45, Lin-Negatif, and CD105 cells using flow cytometry, cell counting, sterility and presence of mycobacteria. The process was done by ProStem using a traditional flow cytometer, without blocking and negative isotypes. The expected results from these protocols are the appropriate amount of mesenchymal cells needed to meet the IFATS and ISCT standards for SVF.

Troubleshooting

Isolation of Abdominal Adipose Tissue

1 The following procedure is performed after patient identification and obtaining patient consent.

2 The area was marked by using skin marker. After aseptic and antiseptic procedures the area was covered with a sterile surgical drape.

3 The local anesthesia was done with pehacain 2% and a small skin incision (1–3 mm) was made with number 11 blade afterwards.

4 The tumescent solution (TLA) was injected using a blunt tip cannula in the form of a fan-shaped. The tumescent solution is made based on Lilis formula with 1.000 mL NaCl 0.9%, 50 mL lidocaine 2%, 10 mL sodium bicarbonate 8.4%, and 1 mL epinephrine 1:1000.

5 The adipose tissue was collected using Mercedes aspiration cannula that connected with the canister and suction machine. The cannula, which is 3 mm in diameter, was directed in a radial, fan-shaped pattern. The fat above Scarpa's fascia was sucked out through the cannula with criss cross technique. Up to 100 mL of adipose tissue was collected.

6 The remaining tumescent solution is pushed caudally by pressing the operation area while the patient is standing.

7 The incision area was sutured and covered with sofratulle, gauze, sanitary pad, and Hypafix®. A compression bandage was applied using a large sized Hypafix®.

Isolation of Stromal Vascular Fraction

8 The tissue collected was processed into SVF using Sepax2® method.

9 The liposuction sample was transferred to a sterile collection bag without contact with air using a luer lock to luer lock connector. Then 0.15% collagenase solution (Serva) was added and incubated at 37 ºC for 60 minutes.

10 Using CS-900.2 kit the incubated adipose tissue was connected to a Sepax2® device. Press “Start Procedure” to start a fully automated cellular separation.

11 For cell collection, the system diluted the residual pellets and extracted the content into the final bag, and automatically rinsed the chamber. After the procedure is completed, a

text “Remove Bags and Air Filter” will appear on the device. About twelve mL of SVF was collected in a sterile 20 mL syringe.

12 Samples were taken for count of live cells using Trypan Blue method, flow cytometric analysis, endotoxin testing using Lonza® kit, contamination assessments using Mycoplasma kit, and blood agar for bacterial culture.

Flow cytometry and cell counting

13 Cell count and viability are analyzed with **Automated Cell Counter Countess II** for enumerating the cells and cell counting.

14 After counting, 100.000 cells in a 100 µL medium were exported into the test tube.

15 The cells were added 20 µL positive antibody cocktail (contains CD90-FITC, CD73-APC, CD105-PerCP.Cy5.5, and 20 µL negative antibody cocktails (contains CD45-PE, CD34-PE, CD11b-PE, CD19-PE, and HLA-DR-PE.

16 Samples were incubated for 30 minutes and washed with Phosphate Buffer Saline (PBS) afterwards.

17 Samples in PBS were analyzed in Flow cytometer BD FACSCanto II using negative isotypes for the beads and positive isotypes for the CDs with enumeration control methods.

18 CD73+, CD90+, CD105+, Lineage Negative, CD34+, CD34+/CD45+ are counted and plotted into graphs using the methods.

19 The process was done by 1 clinical laboratory scientist, verified by another clinical laboratory scientist, and authorized by 1 clinical pathologist.