

SOP for isolation and culture of human SVF cells (JUMC)

The tissue collection:

Sterile adipose tissue (AT) samples are collected from healthy obese donors aged 20-40 years, undergoing liposuction in the local surgery hospital.

AT transported to the department in PBS with gentamicin. (50µg/ml) at the room temperature.

Reagents:

- (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid) sodium salt (HEPES) Sigma cat. No H-1016.
- basic Fibroblast Growth Factor (bFGF), Sigma cat No.F-0291
- biotin, Sigma cat. No B-4639
- bovine serum albumin (BSA) –Sigma, cat No A-7030
- collagenase type IA 0,1%, Sigma cat. No C-2674
- D-panthothenate, Sigma, cat No P-5155
- Dulbecco's Mod Eagle Medium (DMEM) Gibco cat. No. 41966-029
- fetal calf serum (FCS)
- gentamicin, Sigma cat. No G-1272
- human insulin, Sigma cat. No I-2767
- human transferrin, Sigma cat. No F-8158
- hydrocortisone , Sigma cat. NoH-0135
- NaHCO₃, POCH
- phosphate- PBS without Mg and Ca, Biomed, Lublin
- thiazolidinediones e.g ciglitazone , BIOMOL cat.No.A6064
- triiodo-L-thyronine, Sigma cat. No H-0135

Required equipment:

- shaking water bath
- instruments e.g. scalpel, pinsetter
- cell strainers (ø 70 µm and optionally 25 or 40 µm)

Solutions:

All materials must be handled under sterile conditions!

1. Basal medium (pH=7,4):

DMEM/ Ham's: F12 medium (50:50, v:v)

15 mM Hepes

15 mM NaHCO₃

33 µM biotin

17 µM D-panthothenate

2. Collagenase solution:

Collagenase at a final concentration 200U/ml and pH= 7,4, dissolved in phosphate-buffered saline (PBS) supplemented with 2% bovine serum albumin (BSA). The solution should be freshly prepared for every isolation.

3. Inoculation medium:

Basal medium supplemented with gentamicin (50 µl/ml) and 10% fetal calf serum (FCS).

4. Basal Adipogenic medium I:

Basal medium supplemented with human transferrin (10 µg/ml), 10nM hydrocortison, 66 nM human insulin and 1nM bFGF.

Adipogenic factors:

1 nM thiazolidinediones (1 µg/ml) -e.g. ciglitazone, rosiglitazone, troglitazone, pioglitazone.

5. Adipogenic medium II (final differentiation):

basal medium supplemented with human transferrin (10 µg/ml), gentamicin (50 µg/ml),), 1 nM triiodo-L-thyronine, 66nM human insulin and 100 nM hydrocortisone.

Procedure:

1. Cleanse carefully fat pads from remaining connective tissue and blood vessels.
2. Incubate the tissue with collagenase digestion approx.60-90 min. in shaking water bath at 37°C. A collagenase digestion should be performed using 200U/ml and 3ml/g tissue.
3. Centrifuge the sample at 600g for 10 min.
4. Discard the supernatant and dilute the pellet in inoculation medium.
5. Centrifuge the sample at 600g for 10 min.
6. Discard the supernatant and dilute the pellet in an appropriate volume of inoculation medium.
7. Use cell strainers (ø 70 µm) to eliminate others cells (optionally 25 or 40 µm).
8. Seed preadipocytes at a density 3x10⁴ cells/cm²
9. Culture the cells for 16-24 hours in inoculation medium.
10. After 16-24 hours wash cells 2x with PBS and culture with adipogenic medium I (change medium every day until confluence is reached- usually after 5-6 days)
11. After reaching confluence wash cells with PBS and incubate in adipogenic medium II. To induce differentiation use thiazolidinediones (1 µg/ml) for first 3 days. The medium should be renew 3x/week.

Visible lipid accumulation starts within 6-8 days under these conditions. Within 16 days, the differentiating cells are completely filled with lipids droplets, and have changed their morphology.

