STROMAL VASCULAR FRACTION ISOLATION PROTOCOL

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Safety regulations and important considerations

1. Always work within the laminar flow hood to prevent contamination.
2. Clean laminar flow hood periodically during the procedure to maintain an aseptic environment.
3. During washing and centrifugation, be sure to remove all traces of red and white blood cells.
4. Spray regularly with alcohol curtains laminar flow hood to maintain sterility.
5. Before reinjection, make sure filter out clots and other debris.
6. Do not let cellular polypropylene extractors come in contact with other elements to maintain sterility.
7. Keep all items covered in the laminar flow hood during the procedure.
8. Avoid hand movements on the tissue samples, place them next to the back wall of the hood when it is not necessary to work in them.
9. Keep close a deposit of waste for easy removal.

Materials

CELLGENIC SVF Isolation System

[1 kit per patient]

This kit contains all the consumables needed for the production of platelet-rich plasma and isolation of the SVF from adipose tissue

Other materials required but not included in the kit:

Gloves

Sterile gauze 4 x 4

Bottle of alcohol with chlorhexidine or similar

Plastic or steel basin

Rack to accommodate sample tubes"

NaCl 0.9% bag 500 cc

Regular IV set

Equipment

Centrifuge

Swing Out Rotor,with capacity to accommodate up to 50 ml tubes

Laminar flow hood with HEPA filter

Dual Photoactivator LED

Additional Equipment:

Autoclave

Mayo table

Walk or IV pole

Monitor pulse / blood pressure / saturation O2

Line Clearance and Documentation

1. Take from the laminar flow hood all materials related to former patients and carefully wipe the entire surface of it with rubbing alcohol.
2. Spray with alcohol to place the rack before inserting the tubes in the laminar flow hood.
3. Have the form-guide steps
4. Enter the name of the patient in tubes of 50 ml blue cap.

Washing of the tissue samples

It is essential to wash the tissue samples collected to remove most of the erythrocytes, and the remains of tumescence.

1. Transfer 60 ml of adipose tissue collected previously two 60 mL syringes with luer lock peak equally (30 cc solid fat in each syringe).

2. Add Normal Saline to each 60 ml syringe, filled to the limit, through IV bag.

3. Close the cap and gently shake the syringe manually, place in upright position and let it stand. Repeat the same process with the other syringe.

4. Dispose of infranatant layer on both syringes to remove traces of blood and tumescence. Leave only the layer of fatty tissue.

Note: Repeat three times steps 2 to 4, until a clean sample of solid fat in each 30 cc syringe.

Enzimatic digestion

1. Spray with alcohol and place 4 tubes 50 ml into the hood.
2. Place 15 ml of fat on each of the tubes.
3. Using a 60 ml syringe filed with 60 ml saline, reconstitute the collagenase.
4. Place the 0.2 micron filter in the syringe and express 15ml of the collagenase mixture on each of the tubes.
5. Shake vigorously for 15 seconds, let stand on the heater or place on shaker incubator
6. Incubate, shaking every five minutes, for a total period of 20 minutes.
7. Shake the tubes and place them in the centrifuge.

Centrifugation and filtration

1. Centrifuge to separate the cells by adjusting the centrifuge at 900 G (gravitational force) for a time of 5 minutes.
2. Remove the tube from the centrifuge and discard the excess content, taking care to keep the pellets at the bottom with at least 5 ml of fluid.
3. Using a pipette or polypropylene extractor and a 20 cc syringe and successively aspirate the fluid drop to eliminate sticking button (pellet) to the bottom of the conical tubes.
4. Pour the suspension into an empty sterile tube and fill it with saline to the 45 ml.
5. Repeat steps 3 to 4 for the second tube.
6. Centrifuge both tubes again at 900 G (gravitational force) for 5 minutes.
7. Remove the tubes from the centrifuge and discard the excess fluid to leave 5 ml.
8. Take a 20 cc syringe and sterile polypropylene extractor and repeating (step 15)

Aim to obtain a total of 10 cc.

Suspension of cells in platelet growth factors rich plasma and filtration process.

1. Reconstitute the stromal vascular fraction cells obtained from both tubes and proceed to mix with platelet growth factors rich plasma. (about 8 ml). Avoid aspirating the retracted clot.
2. Place the filter into a sterile 50 ml conical tube and gently inject the cells suspended in platelet growth factors rich plasma through the filter, so that small clots remain in the mesh.
3. Aspirate again the filtered cells , remove the pipette and cap the syringe using white cap.

Photo Activation

Activate the mixture obtained from cells and plasma introducing it in low level laser activator for a period of 10 minutes.

Reintegration

The final product obtained by this protocol is 30 to 60 million cells activated and suspended in 18 cc of fluid. The reintegration of these cells will be performed by specific protocol or medical necessity.