Protocol

Isolation of Progenitor and Stem Cells from Human Adipose Tissue

⚠ All procedures must be performed in biosafety hoods.

⚠ This procedure is intended for processing human lipoaspirate tissue only.

⚠ Lipoaspirate tissue can be used fresh or stored at 4˚C within 24 hours of procurement.

❶ Pre-warm the lipoaspirate tissue to 37˚C if it was stored at 4˚C. Pre-warm the processing solution (sterile PBS or sterile Lactated Ringer's) to 37˚C.

❷ Measure the tissue volume (without waste fluid); this will be used to calculate the processing solution volume and Celase® GMP volume.

❸ Wash the tissue with an equal volume of the processing solution 2 to 3 times or until the most of the red blood cells have been washed out; this is indicated by a clear/pinkish color of the processing solution after it separates from the tissue.

❹ Reconstitute the Celase GMP vial with 5 mL of the processing solution. Invert the vial several times to fully reconstitute the enzyme into a clear solution (reference Celase GMP IFU).

❺ Place the washed and drained tissue into a sterile container, usually 4 times the size of the original tissue volume. Add an equal volume of processing solution to the tissue and add the appropriate volume of Celase GMP to the container; tightly close the container.

Adipose Tissue	Celase GMP
100 mL	1.8 mL
120 mL	2.1 mL
135 mL	2.4 mL
150 mL	2.7 mL
170 mL	3.0 mL
190 mL	3.4 mL
205 mL	3.7 mL
220 mL	3.9 mL
240 mL	4.3 mL
260 mL	4.6 mL

Adipose Tissue	Celase GMP
270 mL	4.8 mL
290 mL	5.2 mL
305 mL	5.4 mL
320 mL	5.7 mL
340 mL	6.1 mL
360 mL	6.4 mL
375 mL	6.7 mL
390 mL	7.0 mL
410 mL	7.3 mL
425 mL	7.6 mL

❻ Place the container in a 37˚C shaking water bath for 25 ± 5 minutes until the tissue becomes visibly broken down. If a shaking water bath is not available, gently swirl the container contents by hand continuously or in 5 minute increments for 25 ± 5 minutes.

❼ Collect the non-buoyant solution into multiple 50 mL centrifuge tubes. Place the tubes in a centrifuge and concentrate the cells at 400 g for 5 minutes at room temperature with a low-medium brake speed.

❽ Carefully aspirate the top lipid, floating layer and the solution that contains Celase GMP.

⚠ Do not disturb the cell pellet and leave 5-10 mL of the solution in the tube.

❾ Resuspend the cell pellet in each 50 mL tube with the remaining solution. Combine the cell pellets from each tube and then wash with the processing solution 2 times to reduce residual enzyme. Resuspend the combined cell pellet with 25-30 mL of the processing solution and pass through a 100 µm strainer followed by a 40 µm strainer.

❿ Centrifuge 1 more time and resuspend with the remaining processing solution into the final desired volume for cell counting and further *in vitro* and *in vivo** studies.

⚠ For human lipectomy tissue, mince the dissected adipose tissue to a 3-5 mm^3 size, then follow the same protocol for tissue wash and digestion. The processing solution to tissue volume ratio may be increased to 2:1. The Celase GMP volume may be doubled. Follow the rest of the protocol until progenitor and stem cells for *in vitro* and *in vivo** studies are obtained.

References: Fraser JK, Zhu M, Wulur I, Alfonso Z. 'Adipose-derived stem cells.' *Methods Mol Biol.* 2008; 449:59-67

*NOT FOR HUMAN CLINICAL USE WITHOUT APPROPRIATE REGULATORY APPROVALS