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TITLE:

Human Adipose Tissue Micro-Fragmentation for Cell Phenotyping and Secretome Characterization

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KEYWORDS:

Micro-fragmentation, adipose tissue, mesenchymal stem cell, stromal vascular fraction, pericyte, adventitial cell

SUMMARY:

Here, we present human adipose tissue enzyme-free micro-fragmentation using a closed system device. This new method allows the obtainment of sub-millimeter clusters of adipose tissue suitable for in vivo transplantation, in vitro culture, and further cell isolation and characterization.

ABSTRACT:

In the past decade, adipose tissue transplants have been widely used in plastic surgery and orthopaedics to enhance tissue repletion and/or regeneration. Accordingly, techniques for harvesting and processing human adipose tissue have evolved in order to quickly and efficiently obtain large amounts of tissue. Among these, the closed system technology represents an innovative and easy-to-use system to harvest, process, and re-inject refined fat tissue in a short time and in the same intervention (intra-operatively). Adipose tissue is collected by liposuction, washed, emulsified, rinsed and minced mechanically into cell clusters of 0.3 to 0.8 mm. Autologous transplantation of mechanically fragmented adipose tissue has shown remarkable efficacy in different therapeutic indications such as aesthetic medicine and surgery, orthopedic and general surgery. Characterization of micro-fragmented adipose tissue revealed the presence of intact small vessels within the adipocyte clusters; hence, the perivascular niche is left

unperturbed. These clusters are enriched in perivascular cells (i.e., mesenchymal stem cell (MSC) ancestors) and in vitro analysis showed an increased release of growth factors and cytokines involved in tissue repair and regeneration, compared to enzymatically derived MSCs. This suggests that the superior therapeutic potential of microfragmented adipose tissue is explained by a higher frequency of presumptive MSCs and enhanced secretory activity. Whether these added pericytes directly contribute to higher growth factor and cytokine production is not known. This clinically approved procedure allows the transplantation of presumptive MSCs without the need for expansion and/or enzymatic treatment, thus bypassing the requirements of GMP guidelines, and reducing the costs for cell-based therapies.

INTRODUCTION:

Adipose tissue, long used as a filler in reconstructive and cosmetic surgery, has recently become more popular in regenerative medicine once recognized as a source for mesenchymal stem cells (MSCs)[1]. Lipoaspirates dissociated enzymatically into single-cell suspensions yield an adipocyte-free stromal vascular fraction (SVF) that is used unaltered in the patient or, more commonly, is cultured for several weeks into MSCs[2].

However, enzyme dissociation ruptures the tissue microenvironments, secluding neighboring regulatory cells from presumptive regenerative cells that become considerably modified by in vitro culture. To avoid such experimental artifacts and consequent functional alterations, attempts have been made to process adipose tissue for therapeutic use while maintaining its native configuration as intact as possible[3,4]. Notably, mechanical tissue disruption has started to replace enzymatic dissociation. To this end, the full immersion closed system micro-fragments lipoaspirates into sub-millimeter, blood- and oil-free tissue clusters (e.g., Lipogems) via a sequence of sieve filtration and steel marble induced disruption[3]. Autologous transplantation of micro-fragmented adipose tissue, using this closed system technology, has been successful in multiple indications, spanning cosmetics, orthopedics, proctology and gynaecology[4-13].

Comparison between human micro-fragmented adipose tissue (MAT) obtained with the closed system device and isogenic SVF revealed that with respect to vascular/stromal cell distribution and secretory activity in culture, MAT contains more pericytes, which are presumptive MSCs[14], and secretes higher amounts of growth factors and cytokines[15].

The present article illustrates the enzyme-free micro-fragmentation of human subcutaneous adipose tissue using a closed system device, and the further processing of such micronized adipose tissue for in vitro culture, immunohistochemistry and FACS analysis, in order to identify the cell types present and the soluble factors secreted (**Figure 1**). The described method safely generates adipose derived sub-millimeter organoids containing viable adipose tissue cell populations in an intact niche, suitable for further applications and studies.

PROTOCOL:

Ethical approval for the use of human tissues in this research was obtained from the South East Scotland Research Ethics Committee (reference: 16/SS/0103).

1. Subcutaneous abdominal adipose tissue collection

NOTE: All instruments used in the manual lipo-aspiration procedure are provided by the manufacturer of the micro-fragmentation device.

1.1. Maintain sterility for all fluids, containers, instruments, and the operational area throughout the experiment.

1.2. Place the abdominoplasty sample (procured by the surgical team in a sterile bag without any solution) on top of a surgical cloth with the skin facing upwards. No washing is required. For optimal use, keep the sample at 4 °C and process the adipose sample within 16 h of harvesting.

1.3. Inject 50 to 100 mL of 37 °C 0.9% NaCl solution, depending on the size of the tissue sample (use 50 mL for maximum a 15 cm^2 adipose tissue surface and increase the volume accordingly), using a disposable tissue infiltration cannula, into the subcutaneous adipose tissue. Handle the specimen by the cutaneous part.

1.4. Connect a 10 mL Luer lock syringe to a disposable liposuction cannula.

1.5. Carefully introduce the cannula inside the adipose tissue from the edges. Once inside, create the vacuum inside the syringe by pulling the plunger. Keep the plunger in position by hand to secure the vacuum suction.

1.6. Make radial movements inside the adipose tissue sample until the syringe is full of lipoaspirate. Carefully remove the cannula from the tissue and disconnect the syringe. Connect a new empty syringe.

1.7. Repeat the procedure until 60 mL of lipoaspirate have been collected.

NOTE: The maximum amount of lipoaspirate that can be processed changes according to the type of device used. Please refer to the manufacturer instructions (**Table of Materials**).

2. Micro-fragmentation of the lipoaspirate

NOTE: This protocol is meant for the research use only. Micro-fragmentation is performed with the help of a commercially available device (**Table of Materials**).

2.1. Remove the device from the package and verify that the main unit is connected to the waste bag. Place the waste collection bag on the ground and make sure that the valves are secured to the process unit caps.

2.2. Connect the terminal spike of the input line to the saline bag by piercing the bag connection port. Keep the saline bag higher than the processing unit.

NOTE: For the type of device used in this protocol, a 2,000 mL bag of sterile saline is recommended.

2.3. Verify that all 5 clamps connected to the tubes of the circuits are open. Place the processing unit in a vertical position with the gray cap upwards.

2.4. Allow the saline solution to fill the processing unit. Check that the flow reaches the waste bag. Remove all air bubbles from the processing unit by shaking it.

2.5. Place the processing unit in a vertical position with the blue cap upwards and close the clamp next to the input line.

2.6. Start injecting the lipoaspirate by connecting the syringe to the self-occluding valve of the blue input cap. Keep the processing unit vertical during this procedure and slowly pull the syringe plunger until all the lipoaspirate has been transferred to the unit. Repeat the process until all the lipoaspirate is inside the processing unit.

2.7. Open the input clamp to restore the saline flow.

2.8. Vigorously shake the processing unit for 2 min at least, regularly checking the saline solution flow into the waste bag.

2.9. When the saline solution in the processing unit turns transparent, after approximatively 2 min of shaking, place the unit with the gray cap upwards, and close the clamp located on the drain near the gray cap. The processed tissue will float at the top.

2.10. Fill a 10 mL Luer lock syringe with saline solution and connect it to the loading valve of the blue cap. Close the clamp located near the blue cap. Connect an empty 10 mL Luer lock syringe, with the plunger completely inserted, to the valve of the gray cap.

NOTE: Use Luer lock syringes only.

2.11. Firmly inject the saline into the processing unit from the blue cap. Ensure that the syringe connected to the gray valve is consequently being filled with the processed tissue. Once having injected all the saline, carefully remove both syringes.

2.12. Repeat steps 2.10 and 2.11 until all the processed tissue is collected.

NOTE: The average yield of MAT is 30 mL from 60 mL of manual lipoaspirate. Some tissue will remain inside the processing unit and some clumps might be present attached to the blue edge inside the unit.

2.13. At the end of the processing, remove all syringes, close all clamps, detach the saline bag

and dispose the device according to local protocols.

NOTE: The processing device cannot be re-used.

3. Fluorescence immunohistochemistry

3.1. Transfer 300-500 µL of MAT into a 1.5 mL tube. Add 1 mL of 4% buffered paraformaldehyde (PFA), mix gently by hand (no vortexing) and leave at 4 °C overnight (from 16 to 24 h).

3.2. Collect the specimen and transfer to a clean 1.5 mL tube containing 1 mL of PBS. Repeat the step twice to remove PFA excess.

3.3. Transfer the specimen in 15% (w/v) sucrose in PBS solution and incubate at 4 °C overnight (from 16 to 24 h).

3.4. Transfer the specimen in a well of a 24 well plate and add an embedding solution made of 15% sucrose and 7% gelatin (w/v) in PBS. Fill the well halfway. Incubate for 4 h at 37 °C.

3.5. Transfer the samples to 4 °C. After 2-4 h for the gelatin to solidify, fill up the well with the embedding solution and leave at 4 °C overnight.

3.6. Remove the samples from the wells. Remove excess embedding compound and freeze on dry ice. Store the samples at -80 °C.

3.7. Cut 8-10 µm thick sections using a cryostat. For optimal sectioning, use the cryostat at -30 °C. Slides can be stored at -80 °C.

3.8. Prior to proceeding with the immunofluorescence, fix the sections in 4% PFA for 7 min. Remove the PFA and wash twice with lukewarm PBS (37 °C) in order to remove gelatin from the sections.

3.9. Block non-specific antibody binding by incubating the slides with 10% goat serum in PBS (v/v) for 1 h at room temperature.

3.10. Dilute the primary antibodies in antibody diluent or 0.2% (w/v) bovine serum albumin (BSA) in PBS (w/v): mouse anti-human-NG2 (1:100, stock 0.5 mg/mL), rabbit anti-human-PDGFRβ (1:100, stock 0.15 mg/mL).

3.11. Remove the blocking solution and add the diluted primary antibodies. Incubate at 4 °C overnight.

NOTE: Perform all the incubation steps from now on in the dark.

3.12. Remove the primary antibodies and wash 3 times with PBS for 10 min each time.

3.13. Dilute the secondary antibodies in antibody diluent or 0.2% (w/v) BSA in PBS: goat anti-mouse- 555 (1:300), goat anti-rabbit- 647 (1:300). Incubate 1 h at room temperature.

3.14. Remove the secondary antibodies and wash three times with PBS for 10 min each time.

3.15. If a biotin conjugated lectin is used, use the avidin/biotin blocking kit. Incubate for 10 min with each reagent provided with two washes in between with PBS.

3.16. Repeat the blocking step by incubating the slides for 1 h at room temperature with 10% goat serum in PBS.

3.17. Add the biotinylated lectin, biotinylated Ulex europaeus lectin (1:200, stock 2 mg/mL), diluted either in 0.2% BSA (w/v) in PBS or antibody diluent. Incubate 1 h and 30 min at room temperature.

3.18. Remove excess biotinylated lectin and wash three times with PBS for 10 min each time.

3.19. Add the secondary streptavidin conjugated antibody diluted in either 0.2% (w/v) BSA in PBS or antibody diluent. Incubate for 1 h.

3.20. Remove the secondary antibody and wash three times with PBS for 10 min each time.

3.21. Counterstain nuclei with 4',6-diamidino-2-phenylindole (DAPI, 1:500, stock 5 mg/mL), diluted in 0.2% BSA (w/v) in PBS, for 10 min at room temperature.

3.22. Remove the DAPI solution and wash twice with PBS, 10 min each time.

3.23. Mount the slides with an aqueous mounting agent. Let it dry completely, either overnight on the bench in the dark or 1 h at 37 °C.

3.24. Keep the slides at 4 °C in the dark and acquire images on an epifluorescence microscope.

4. Digestion of the micro-fragmented adipose tissue and cell isolation

4.1. Transfer the micro-fragmented adipose tissue into a sterile container.

4.2. Freshly make up the digestion medium by dissolving type-II collagenase in DMEM at the concentration of 1 mg/mL.

4.3. Add the same volume of digestion medium to the micro-fragmented adipose tissue (i.e., for 30 mL of sample add 30 mL of digestion medium).

4.4. Place the sealed container in a 37 °C shaking water bath set to 120 rpm for 45 min. Please

note that the container should allow a proper shaking of the sample. If a large container is not available, use two 50 mL tubes (30 mL of sample/digestion medium mixture in each) placed horizontally.

4.5. After the incubation, block the digestion by adding an equal volume of blocking solution (2% (v/v) FCS/PBS) and filter sequentially through 100 µm and 70 µm cell strainers.

4.6. Centrifuge the filtered suspension for 5 min at 200 x *g*. Discard the supernatant.

4.7. Resuspend the pellet in approximately 5 mL of erythrocyte lysis buffer (155 mM NH_4Cl, 170 mM Tris, pH 7.65). The volume of buffer may vary according to the erythrocyte contamination observed in the cellular pellet. Incubate at room temperature for 15 min.

4.8. Add an equal volume of blocking solution and filter through a 40 µm cell strainer.

4.9. Centrifuge the cell suspension for 5 min at 200 x *g* and discard the supernatant.

4.10. Resuspend the pellet in blocking solution. Mix well by pipetting. Count viable cells with Trypan blue exclusion on a hemocytometer.

4.10.1. Mix an equal volume of the cell suspension with Trypan blue stain. Aspirate 10 µL of the solution and place it on a hemocytometer.

4.10.2. Count the number of live cells (bright and round) present in at least 5 squares and get the mean cell number. In order to include the stain dilution factor, multiply the mean cell number by 2. To get the total number of live cells per 1 mL of sample multiply the obtained number by 10^4.

NOTE: The obtained single cell suspension, namely the stromal vascular fraction (SVF), can be either seeded at 20,000 cells/cm^2 in perivascular cell growth medium (DMEM supplemented with 20% heat inactivated FCS, 1% penicillin/streptomycin and 1% L-glutamine) or used for flow cytometry analysis and sorting. The average yield of nucleated cells in the SVF is approximately 3 x 10^4 cells per mL of MAT. Sorting experiments require at least 8 x 10^5 cells to obtain enough perivascular cells for culturing.

5. Cell labelling and sorting

5.1. Centrifuge the cell suspension at room temperature for 5 min at 200 x *g*, and re-suspend the pellet in blocking medium at a concentration of 1 x 10^6 cells per 100 µL.

5.2. Aliquot at least 50,000 cells for the unstained control and the fluorescence minus one (FMO) controls into polystyrene round bottom flow cytometry tubes. Use the rest of the cells for multicolor staining by placing in another tube.

NOTE: Once the settings of the experiment have been established (i.e., laser intensity, gating

strategy), the number of cells used for the unstained control and the FMOs can be reduced, if, the antibodies used are the same.

5.3. Add CD31-V450 (1:400), CD34-PE (1:100), CD45-V450 (1:400) and CD146-BV711 (1:100) antibodies to the single cell suspension for multi-color staining. For the FMO controls, add: for the V450 FMO equivalent volumes of CD34-PE and CD146-BV711 plus V450 isotype control; for the PE FMO equivalent volumes of CD31-V450, CD45-V450 and CD146-BV711 plus PE isotype control; for the BV711 FMO equivalent volumes of CD31-V450, CD45-V450 and CD34-PE plus BV711 isotype control.

5.4. Gently pipette, or vortex slowly the solution in the tube to mix and incubate the tubes at 4 °C for 20 min in the dark.

5.5. Prepare compensation control beads. Add 15 µL of positive beads and 15 µL of negative beads to 70 µL of blocking medium in 3 polystyrene round-bottom flow-cytometry tubes. Add CD34-PE, CD31-V450 or CD45-V450, and CD146-BV711 antibodies, one per tube.

5.6. Gently pipette, or vortex slowly, to mix the antibodies with the beads and incubate all tubes at 4 °C for 20 min in the dark.

NOTE: The procedure described can be used either for flow cytometry analysis or cell sorting.

5.7. Prepare collection tubes by pre-wetting the inner surface of the tubes with endothelial growth medium (EGM), leaving approximately 50 µL of medium on the bottom of each tube.

5.8. After antibody incubation, remove the excess of antibody by washing the cells and the beads with 2 mL of blocking medium.

5.9. Remove the solution by centrifuging the tubes at 200 x *g* for 5 min and carefully aspirating the supernatant. Replicate the washing step.

5.10. Resuspend the cells in blocking medium at a final concentration of 1 x 10^6 cells per 250 µL. Resuspend the beads in 100 µL of blocking medium.

5.11. Transfer the cells into new polystyrene round-bottom flow cytometry tubes with cell strainer cap. This will allow the disruption of cell clumps.

5.12. Transport all cell and bead suspensions to the cell sorter on ice in the dark.

5.13. Run unstained control cells to establish the background fluorescence and set the voltages.

5.14. Run the compensation control tubes to set the fluorescence compensation.

5.15. Use forward scatter area (FSC-A) vs side scatter area (SSC-A) to identify cells, and then use

forward scatter area (FSC-A) vs forward scatter height (FSC-H) to select single cells.

5.16. Add DAPI, 1 µL of 5 µg/mL solution for each mL of sample, to the unstained control to set the gating for dead cells (**Figure 2**). No washing is required after DAPI staining.

5.17. Run isotype controls to establish background fluorescence thresholds related to non-specific binding and set the gating.

5.18. Run the multi-color stained sample. Exclude hematopoietic and endothelial cells by gating on CD31 and CD45 negative cells. Collect the pericyte ($CD146^+$ $CD34^-$) and adventitial cell ($CD146^-$ $CD34^+$) populations into collection tubes (**Figure 2**).

Note: Optimal viable cell yields are approximately 2% of the total live cell dissociation for pericytes and 1.5% for adventitial cells.

6. Cell culture

6.1. Seed freshly sorted pericytes and adventitial cells at a density of 30,000 to 40,000 cells/cm^2 on gelatin coated culture plates

6.2. To coat the culture plates, cover the plate area with sterile 0.2% (w/v) gelatin in PBS solution (approximately 100 µL of solution per cm^2). Incubate at room temperature for 10 min and remove the solution. Keep freshly sorted cells on ice during culture plate coating.

6.3. Centrifuge freshly collected cells at 200 x *g* for 5 min and gently re-suspend the cell pellet in an appropriate amount of endothelial growth medium (EGM). If the number of cells collected is very low, avoid the centrifugation and plate directly the collected cells.

6.4. Seed the cells on gelatin-coated plates. Incubate at 37 °C in 5% CO_2.

6.5. Once cells have settled and adhered to the plate (after at least 72 h), exchange EGM with perivascular cell growth medium (DMEM supplemented with 20% heat inactivated FCS, 1% penicillin/streptomycin and 1% L-glutamine) for both pericytes and adventitial cells.

6.6. Once pericytes and adventitial cells have reached 80%-90% confluence, dissociate cells using 0.05% trypsin-EDTA. Collect with 5% FCS/PBS, centrifuge at 200 x *g* for 5 min, re-suspend in perivascular cell growth medium, and then passage the cells from 1:3 to 1:5 ratio (or at approximately 7,000 cells/cm^2) onto uncoated polystyrene culture plates or flasks.

REPRESENTATIVE RESULTS:

Mechanical dissociation of manual lipoaspirates resulted in the production of micro-fragmented adipose tissue (MAT), consisting of an aggregate of adipocytes enveloping a microvascular network (**Figure 3**). Immunofluorescence analysis of gelatin-embedded and cryofixed MAT highlights this structure, showing the vascular network marked by the endothelial cell marker

Ulex europaeus agglutinin 1 (UEA-1) receptor mainly consisting of small, capillary-like vessels (**Figure 4**). Notably pericytes expressing NG2 or PDGFRβ are normally distributed, ensheathing endothelial cells (**Figure 4**). These data confirm that the mechanical micro-fragmentation process is not affecting the perivascular cell compartment. The presence of perivascular cells, both pericytes and adventitial cells, has been confirmed by flow cytometry. To select cells a forward scatter area (FSC-A) vs side scatter area (SSC-A) gate was used (**Figure 2A**). The identified cell population was further gated using forward scatter area (FSC-A) vs forward scatter height (FSC-H) to select single cells and live cells were identified as negative for DAPI staining (**Figure 2B-C**). CD31 and CD45 markers were used to exclude hematopoietic and endothelial cells (**Figure 2C**). Finally, pericytes were identified as $CD146^+$ $CD34^-$ and adventitial cells as $CD146^-$ $CD34^+$ (**Figure 2D**). The gating strategy is summarized in **Figure 2.** The same gating strategy was followed for sorting experiments. FACS-purified MAT pericytes and adventitial cells both exhibit a spindle to stellate morphology in culture. Culture for 8 days showed that MAT secretes more cytokines (adiponectin, CD14, CD31, CD154, chitinase 3-like 1, complement factor D, CD147, GDF-15, IGFBP-2, IL1RA, IP-10, M-CSF, MIF, CXCL9, CCL19, PDGFAA, CCL5, RBP-4, relaxin-2, ST2, TNF-α, CD87), and angiogenic factors (angiogenin, angiostatin, DPPIV, endoglin, HGF, leptin, PDGFAB/BB, PlGF, thrombospondin 2, TIMP4, uPA) in vitro than SVF. Moreover, cytokines and angiogenic factors produced by both MAT and SVF were more abundant in MAT supernatants. Accordingly, MAT digested with collagenase and placed in culture produced a secretome similar to that observed from conventional SVF[15]. Globally, all these data demonstrate that micro-fragmented adipose tissue is not only therapeutically advantageous, but also amenable to phenotypic and functional investigations. In particular, the small size of MAT clusters allows testing of secretory activity in culture, which would be more difficult with large adipose tissue chunks.

FIGURE & TABLE LEGENDS:

Figure 1. Schematic representation of the micro-fragmentation of subcutaneous adipose tissue. Lipoaspirate is collected from subcutaneous adipose tissue using a cannula. Collected lipoaspirate is micro-fragmented using the close system device. Resulting micro-fragmented adipose tissue (MAT) is composed by multiple cell types including perivascular cells and adipocytes. MAT can be used in multiple tests, including explant culture and immunohistochemistry, and for cell isolation.

Figure 2. Representative gating strategy for perivascular cell analysis/sorting from MAT.
A forward scatter area (FSC-A) vs side scatter area (SSC-A) gate is used to identify cells (**A**), followed by forward scatter area (FSC-A) vs forward scatter height (FSC-H) to select single cells (**B**). Viable non-endothelial and non-hematopoietic cells are gated as negative for DAPI, CD31-V450 and CD45-V450 respectively, in the same panel (**C**). Finally, perivascular cells are identified as pericytes ($CD146^+$ $CD34^-$) or adventitial cells ($CD146^-$ $CD34^+$) (**D**).

Figure 3. MAT morphology. Bright-field view of MAT cultured in basal medium. Scale bar: 1,000 µm.

Figure 4. Vasculature network in MAT. Endothelial cells are stained with UEA-1. Boxed areas in A are showed enlarged in B and C. Arrowheads indicate pericytes, which have been stained using antibodies against PDGFRβ and NG2. Scale bar: 50 µm.

DISCUSSION:

This paper describes the physical fractionation, using a closed system device, of human adipose tissue into small clusters displaying normal adipose tissue microanatomy.

Manually aspirated human subcutaneous adipose tissue and saline solution are loaded into a transparent plastic cylinder containing large pinball-style metallic spheres that, upon vigorous manual shaking of the device, rupture the fat into sub-millimeter fragments. Attached filters and outlet allow eliminating debris, blood and free lipids and MAT is collected in a syringe linked to the device. Here, MAT has been successfully further processed for immunohistochemistry, flow cytometry and culture, although this specific device was developed in a medical perspective, to replace in the theatre the enzymatically produced adipose tissue stromal vascular fraction or culture derived adipose stem cells, and indeed proved highly efficient in plastic surgery and diverse other cell-based therapies[4-13].

Therefore, adipose tissue long used intact as a mere filler[16] returns in its native 3-dimensional configuration, after years of enzymatic dissociation and in vitro culture. This follows a general trend to grow and study tissues in their innate multicellular arrangement rather than as dispersed cell populations, as illustrated by the growing popularity of organoids[17].

Adipose tissue fragmentation using this mechanical device has proven reliable and reproducible and no modification of the original protocol was ever necessary. The ordinary supply of human subcutaneous fat for experimental studies is a lipoaspirate collected for cosmetic reasons, which is loaded directly onto the fragmentation device. A limitation for users in the research laboratory, though, is that the adipose tissue to be processed must be gently aspirated by hand, to maintain tissue microarchitecture as intact as possible, and not suck out with a vacuum pump as is routinely done in the operating room. Unless a surgeon willing to collect fat manually can be identified, a freshly resected abdominoplasty residue can be used. The fat attached to the inner aspect of the abdominal skin can then be carefully and sterilely aspirated with the syringe provided with the kit. This is the reason why we have described this step at the beginning of the protocol (step 1).

What actually explains the therapeutic superiority of MAT over enzyme dissociated adipose tissue? Flow cytometry analysis of MAT enzymatically dissociated immediately after fractionation revealed a higher proportion of pericytes, known as innate forerunners of mesenchymal stem cells[14], as compared with total aspirated adipose tissue[15]. More directly, the comparative analysis of MAT and SVF culture supernatants showed that the former secretes, qualitatively and quantitatively, more growth factors and cytokines involved indirectly in tissue repair. These include pro-angiogenic factors, as well as diverse mediators of immuno-inflammation[15].

Beyond these recent results, much remains to be understood regarding the molecular basis of

MAT therapeutic potential. To this end, the method here described represents a simple, fast technique that is low-impact to the adipose niche and generates MAT ideal for further experimental manipulation. From an ancillary point of view, research is also conducted to improve the usability of MAT in the clinic; in this respect, determining whether micro-fragmented adipose tissue can be frozen for delayed autologous or allogeneic administration appears as a priority. Finally, it will be important to determine whether other tissues and organs such as the bone marrow, pancreas or skeletal muscle, to cite but a few, can be micro-fragmented with the same device and provide intact microvasculature associated cellular niches amenable to experimental intervention.

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DISCLOSURES:

CT is a founder of Lipogems.

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FIGURE 1 TOP

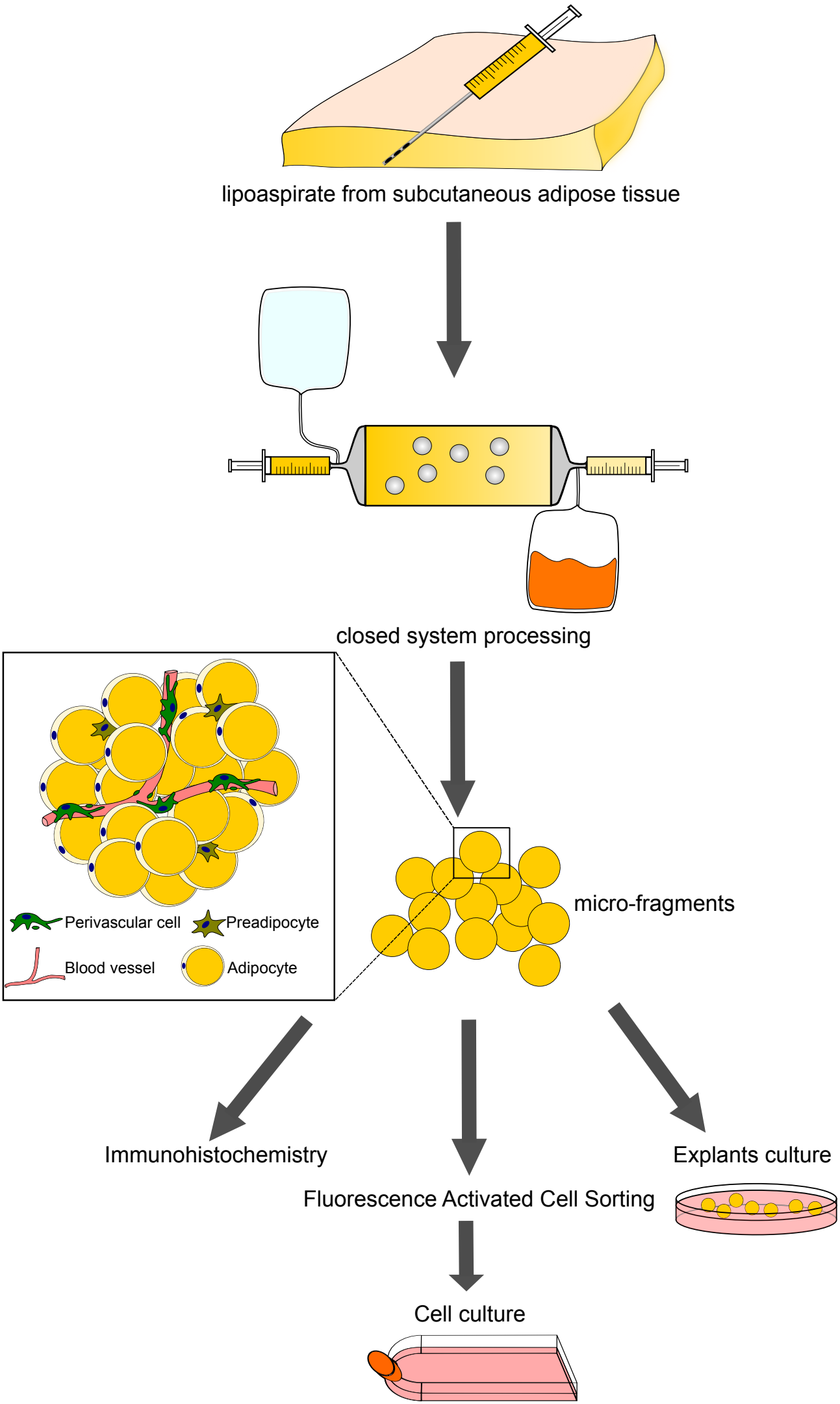

FIGURE 2, TOP

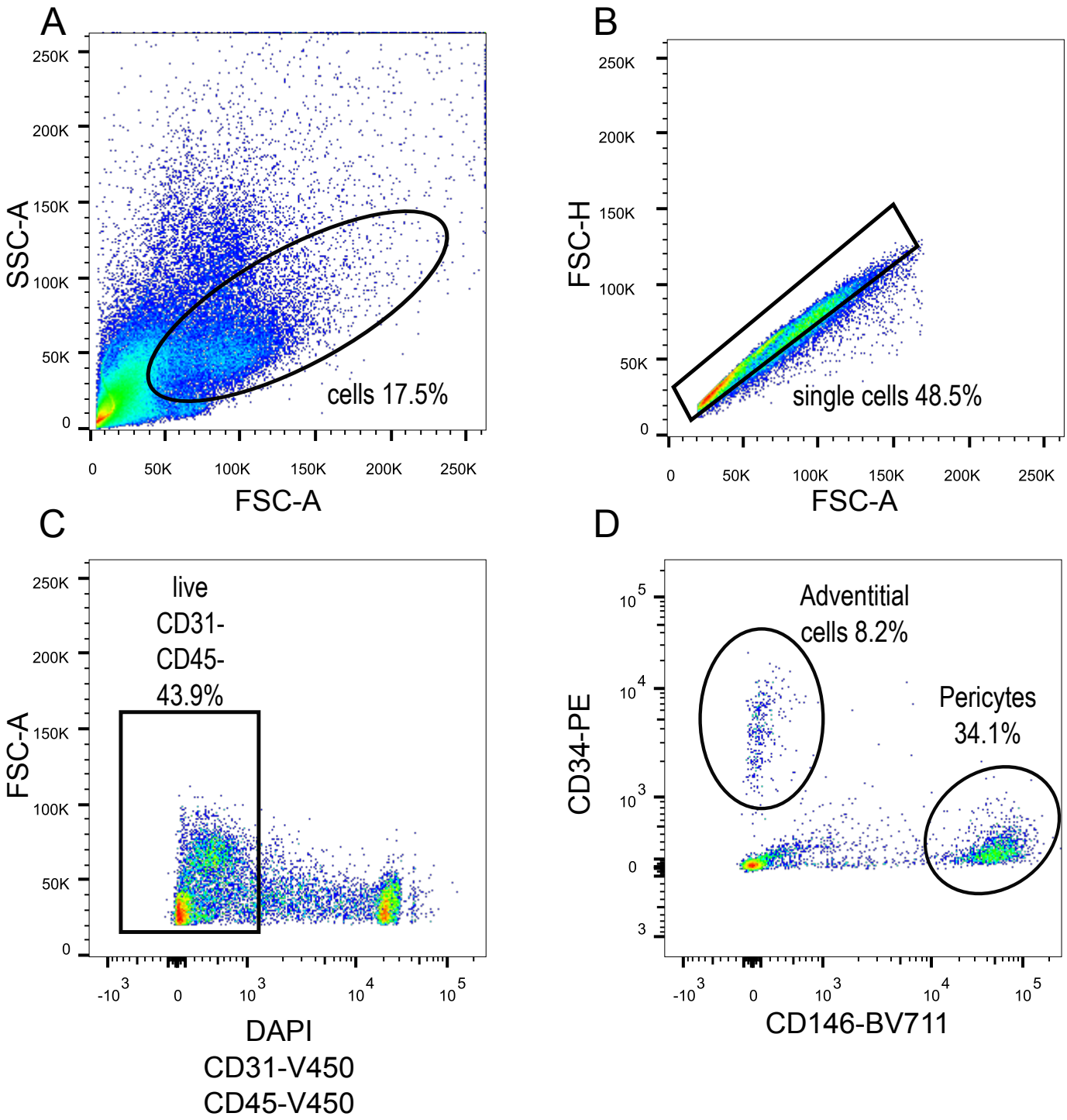

FIGURE 3, TOP

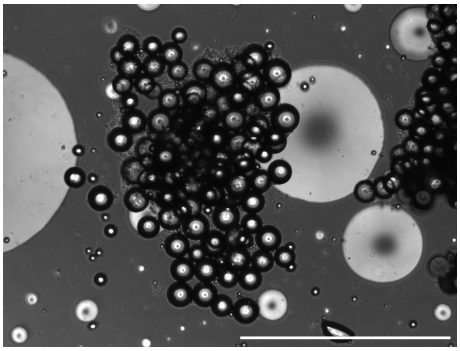

FIGURE 4, TOP

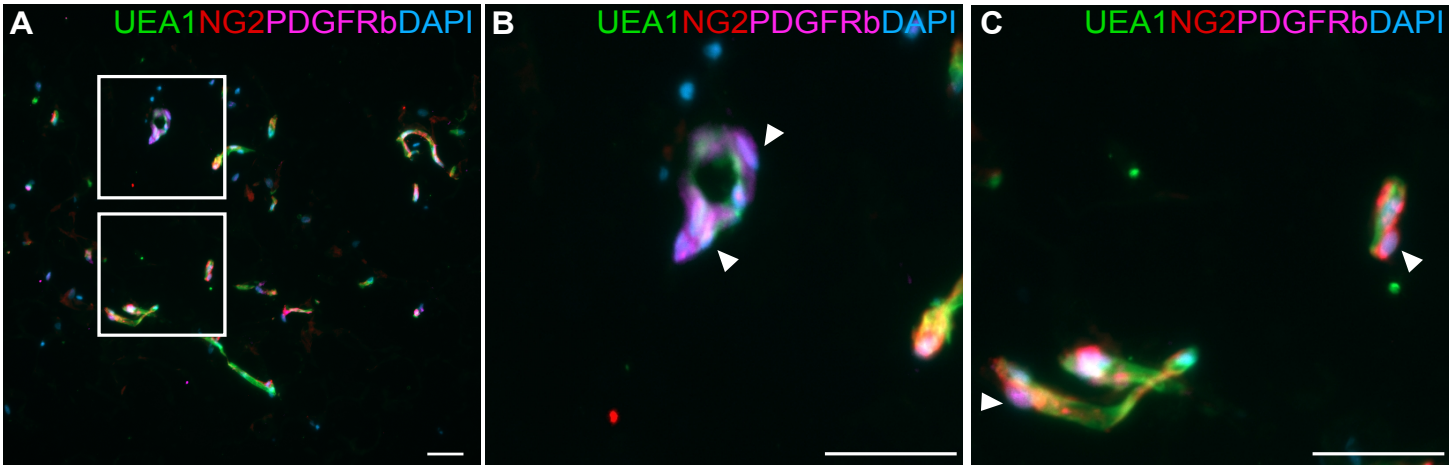

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Fig. 4 Vezzani et al..svg

Table of Materials

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Name of Material/ Equipment	Company	Catalog Number
4% Buffered paraformaldehyde (PFA)	VWR chemicals	9317.901
0.9% NaCl Solution	Baxter	3KB7127
AlexaFluor 555 goat anti-mouse IgG	Life Technologies	A21422
AlexaFluor 647 goat anti-Rabbit IgG	Life Technologies	A21245
Ammonium chloride	fisher chemicals	1158868
Antigent Diluent	Life Technologies	3218
Anti-Mouse Ig, κ/Negative Control (BSA) Compensation Plus	BD Biosciences	560497
Avidin/Biotin Blocking Kit	Life Technologies	4303
BD LSR Fortessa 5-laser flow cytometer	BD Biosciences	
Biotinylated Ulex europaeus lectin	Vector Laboratories	Vector-B1065
BV711 Mouse IgG1, k Isotype Control	BD Biosciences	563044
CD146-BV711	BD Biosciences	563186
CD31-V450	BD Biosciences	561653
CD34-PE	BD Biosciences	555822
CD45-V450	BD Biosciences	560367
DAPI	Life Technologies	D1306
Disposable liposuction cannula (LGI 13Gx185 mm – AR 13/18)	Lipogems	
Diva software 306 (v.6.0)	BD Biosciences	
DMEM, high glucose, GlutaMAX without sodium pyruvate	Life Technologies	61965026
EGMTM-2 Endothelial Cell Growth Medium-2 BulletKitTM	Lonza	CC-3156
Fetal Calf Serum (FCS)	Sigma-Aldrich	F2442
FlowJo (v.10.0)	FlowJo	
Fluoromount G	SouthernBiotech	0100-01
Gelatin	Acros Organics	410870025
Lipogems Surgical Kit	Lipogems	LG SK 60
Mouse anti human- NG2	BD Biosciences	554275
PE Mouse IgG1, κ Isotype Control	BD Biosciences	555749
Penicillin-Streptomycin	Sigma-Aldrich	P4333
Phosphate buffered saline (PBS)	Sigma-Aldrich	D8537

Polystirene round bottom 5 mL tube with cell strainer snap cap	BD Biosciences	352235, 25/Pack
Polystyrene round bottom 5 mL tubes	BD Biosciences	352003
Rabbit anti human - PDGFRb	Abcam	32570
Streptavidin conjugated-488	Life Technologies	S32354
Sucrose	Sigma-Aldrich	84100-5kg
Tissue infiltration cannula (17GX185 mm-VG 17/18)	Lipogems	
Tris base	fisher chemicals	BP152-500
Type- II Collagenase	Gibco	17101-015
V450 Mouse IgG1, κ Isotype Control	BD Biosciences	560373
Widefield Zeiss observer	Zeiss	
Zeiss Colibri7 LED light source (LEDs: 385, 475, 555, 590, 630 nm)	Zeiss	

Comments/Description
Laser 405nm (violet)/375nm (UV) – filter V450/50 for DAPI and V450 antibodies; Laser 561nm (Yellow-green) – filter YG582/15 for PE antibodies; Laser 405nm (violet)/375nm (UV) – filter V710/50 for BV711 antibodies
stock concentration: 5mg/mL
provided in the Lipogems surgical kit
stock concentration: 0.5 mg/mL

stock concentration: 0.15 mg/mL
provided in the Lipogems surgical kit
Objective used: Plan-Apo 20x/0.8
DAPI: UV, excitation 385nm; 488: Blue, excitation 475nm; 555: Green, excitation 555nm; 647:Red, excitation 630nm

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Author(s): BIANCA VEZZANI, MARIO GOMEZ-SALAZAR, JOAN CASAMITJANA, CARLO TREMOLADA, BRUNO REAULT.

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