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Research Article

Study and Assessment of a New Stromal Vascular Fraction Harvest Technique: Subcutaneous Tissue Source Harvesting with One STEP™ Technique

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Abstract

Stromal Vascular Fraction (SVF) harvesting using a conventional liposuction technique is a traumatic technique that increases cellular apoptosis of regenerative components and requires the use of enzymes and cell culture. The study describes and assesses, the effectiveness in the number and viability of total Stromal Vascular Fraction (SVF) cells obtained, using an innovative single-step photo stimulation technique, the One STEP™ technique, classified as minimal-grade manipulation for SVF harvesting of the subcutaneous adipose tissue. A portable cell counter device (Luna Stem™) in the operating room was used to analyze the samples, by fluorescence. Total cells, nucleated, non-nucleated, and viability were studied. The study was performed on eight healthy patients who underwent a liposculpture, and two samples were collected from each patient (n=16) all operated with the One STEP™ harvest technique between January 2020 and December 2021.The periumbilical area was selected as the donor area and the abdominal wall was divided into the right and left side. After infrared light emission, 40 cc of adipose tissue on each side was collected in a closed system (syringe). Simple centrifugation, without manipulation or enzyme used after adipose tissue was aspirated, was necessary to obtain 10 ccs of the stromal vascular fraction. The One STEP™ technique allows harvesting of adipose tissue preserving the stromal vascular fraction containing all regenerative stromal elements as the extracellular matrix without processing or manipulation; takes 20 minutes with a simple centrifugation protocol and non-enzymatic digestion process to preserve the stromal elements of the sample. The total number of cells obtained is between 1.06×10^7/ml and 2.11×10^7/ml with 92.5% viability and 0-5 death cells according to the tables and histogram obtained. The One STEP™ technique with selective photo-stimulation properties liberates the adipocytes and the stromal vascular fraction from the structural connective tissue (collagen fibers) without manipulation. Obtaining a high number of stromal vascular fraction cells, with high viability for potential regenerative therapy. This innovative technique (photo- stimulation) could change the concept and improve stromal vascular fraction harvesting, following the parameters of the "minimal grade manipulation" process.

Keywords: Laser; Stromal vascular fraction; Photo-chemical stimulation

Introduction

The recent discovery of Adipose Tissue (AT) as a source of multi potent stem cells has opened new alternatives in regenerative medicine [1,2]. Mesenchymal Stem Cells (MSC) can be easily expanded and have a capacity for differentiation into cells of multiple mesenchymal lineages that can be used to form bone, cartilage, muscle, and adipocytes both *ex vivo* and *in vivo* [3-6].

AT as a metabolic-endocrine-immune organ, has forced us to study and focus on the cellular scale and should be seen as a system of its own that constitutes sophisticated micro-ecosystems depending on highly complex, but structured interactions between different, organized sets of cells and their microenvironments [7]. The very complex chemical binding of cells and tissues due to the cell-to-cell or cell-to-matrix attachments is a challenge in choosing an adequate harvest technique (16). Changes in the quality and quantity of MSC in the SVF are crucial for a regenerative response [3]. Therefore, if these 2 factors are optimized, mesenchymal stem cells' quantity and quality will have an adequate and desired regenerative response and will have a tool to compare different harvest techniques.

In most cases, the harvest of adipose tissue uses the Suction Assisted Lipectomy (SAL) described by Illuoz, known as the conventional liposuction technique, previously associated with the tumescent infiltration technique. Its mechanism of mechanical disruption requires an energetic and traumatic suction with different cannulas and suction devices, increasing the apoptosis of adipose tissue and regenerative components. After SAL is applied many maneuvers/manipulation processes were described, including filtering, decanting, or emulsification, but the same mechanical disruption mechanism is used for adipose tissue harvesting, despite an enzymatic or mechanical stromal vascular fraction isolation being used.

An innovative concept was applied in stromal vascular fraction cells harvesting contained in the Subcutaneous Tissue (SCT), a technique based on a new concept, which mechanism is as elective photo stimulation using specific infrared light, applied direct to the subcutaneous tissue (adipose tissue and perivascular regenerative cells) that is aimed to minimize tissue trauma, preserving all regenerative elements (extracellular matrix, paracrine factors,

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mesenchymal stem cells, exosomes, cytokines) and obtain a high yield of surviving fat tissue rich in mesenchymal stromal stem cells as demonstrated in early analysis of the present technique [8-10], this stromal vascular fraction isolation method is considered as Minimal-Grade Manipulation [11].

The objective of this paper is to describe and asses, the effectiveness in the number and viability of total SVF cells obtain, using a portable Cell Counter device in the operating room, from an innovative single-step technique, the One STEP™ technique, classified as Minimal-grade Manipulation for SVF harvesting of the subcutaneous adipose tissue.

Methods

Study design

A Prospective study of the application of a new technique without a control group.

Study population

The study was performed on a group of patients undergoing One STEP™ liposuction between January 2020 and December 2021, a total of 8 patients from a private clinic in Peru. Their cells were isolated from the stromal vascular fraction according to the One STEP™ technique. All patients gave written informed consent to study the fat tissue extracted from them and went through blood analysis surgical risk tests.

For each patient, 2 sample units, in a total of 16 fat processing units, were collected for analysis on the Luna Stem Counter™ cell in the operating room.

The materials used in the One S.T.E.P™ procedure and the analysis of SVF by Luna-STEM are described in the same technique. Total cell number will be counted in millions per milliliter and viability will be the difference between dead and live total SVF cells.

Variables

The study variable was the selective tissue-engineered photo stimulation technique (One S.T.E.P™). The other variables are cells per gram of adipose tissue, dead cells, extracellular matrix, processing time, and viability obtained using the Luna-STEM Counter™.

Procedures (One STEP™ technique)

The technique is performed as follows: under regional anesthesia and sedation. Periumbilical marks, dividing on the right and left side are made where adipocytes and SVF cells will be harvested. Cold saline solution (4 degrees Celsius+adrenaline 1:500,000 is used and infiltrated deep above the superficial fascia using a 2 mm Klein cannula (wet technique.) Special care is taken not to tunnel (infiltrate) the subcutaneous cellular tissue so that the histological architecture is preserved. The emission of infrared laser energy was then started, using a Medilaser™ diode laser with a novel 1210 nm wave length-DMC Group (Brazil), with the preset: Mesenchymal Stem Cell harvesting.

Isolation of the stromal vascular fraction follows the protocol described by the author: Once the AT is obtained. A 40 cc sample is taken from the fat, which is distributed 10 cc into tubes (Figure 1A) and then placed in the Spin Plus digital table top centrifuge at 2200 rpm- 2000 rpm for 5 minutes, respectively.

After completing the centrifugation process, the stromal vascular fraction button is obtained at the bottom of the tube (Figure 1B) and aspirated.

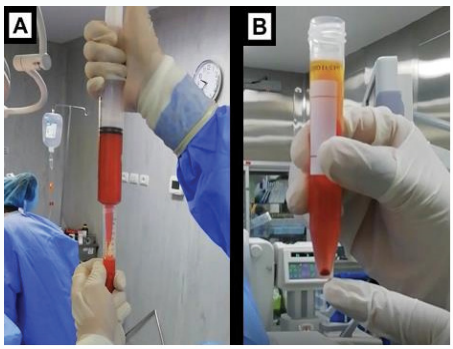

Figure 1: A) 50cc syringe containing the extracted fat. B) Stromal vascular fraction is deposited on the bottom of the fat processing units (red pellet).

Analysis of the SVF using a cell counter (Luna-STEM™) is an automated cell counter that integrates dual fluorescence optical components to provide advanced cell counting functionalities, and measure the number as well as the viability of cells (live/dead/total cells or nucleated/non-nucleated). Two fluorescences were used; acridine orange (green) stains all nucleated cells and propidium iodide (red) stains all dead nucleated cells. For analysis, a fraction of the sample is taken, which is used for final counting on the Luna-STEM according to the company's protocol (Logos Bio systems, South Korea) to analyze the quantity and viability of SVF cells.

Luna-STEM cell counter process was used, for this it was transferred 2 µl of acridine orange/propidium iodide stain was to a new 1.5 ml micro centrifuge tube, 12 µl of cell sample was into the tube, and 6 µl of buffer solution, and mixed by pipetting up and down or shaking the bottom of the tube. Holding the edge of the slide, load 10 µl of the mixed cell sample into the inlet of a chamber of the counting slide, tilt the pipette about 45~60 degrees, the loaded slide is inserted into the slide port of the instrument, and wait for about 60 seconds to check whether all the cells in the preview screen are immobile. Then press the count button in approximately 30-60 seconds, and the image and data will be displayed on the screen. The results report the total cell concentration, nucleated cell concentration, non-nucleated cell concentration, cell viability, and mean cell size. The data were analyzed using the mean, according to the results of the Luna STEM cell counter.

Results

Eight patients under went liposuction using One S.T.E.P technique, infrared laser (Figure 2A). For each patient, 2 different samples (left and right hemiabdomen area) making a total of 16 fat processing units were collected.

Once the process is finished, a 10 µl analysis unit is transferred from the stromal vascular fraction with a micropipette to be analyzed by the Luna STEM cell counter. The number of cells per gram of adipose tissue was 10.6-21.1 million cells per gram of adipose tissue, the average viability was 92, 5% (Table 1), and 0%-5% of dead cells were found. The time needed for each procedure was 10 minutes.

The bivariate analysis was represented in histograms. The variables analyzed were cell concentration, size of nucleated cells, and size of non-nucleated cells. The average size of the total mesenchymal stem cells was 7.05 mm. In the histograms, it is observed the sizes of both nucleated and non-nucleated cells are between 5 m and 10 m (Figure 3).

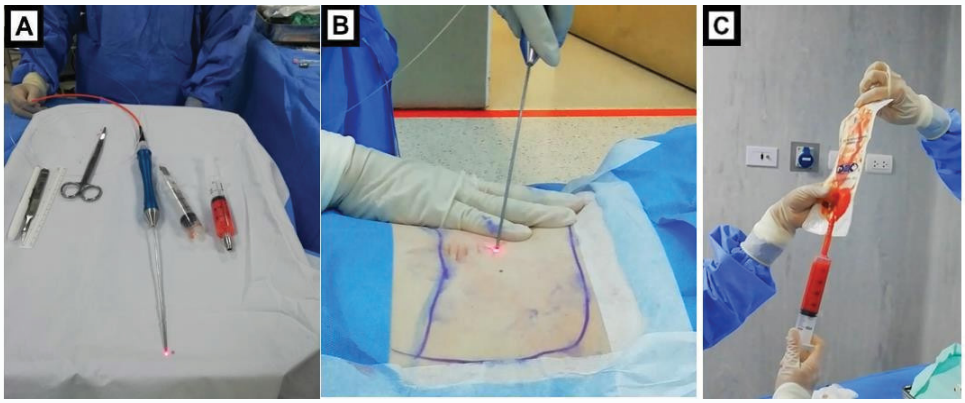

Figure 2: A) Non-destructive surgical liposuction diode laser uses an optical fiber through a 2 mm cannula, with a wavelength of 1210 nm, B) The laser is passed through adipose tissue, releasing and preserving adipocytes and mesenchymal stem cells, "laser fat preservation." [11]. C) Sterile collection bag, closure system. Refers to this final product as Pico Graft™ [8].

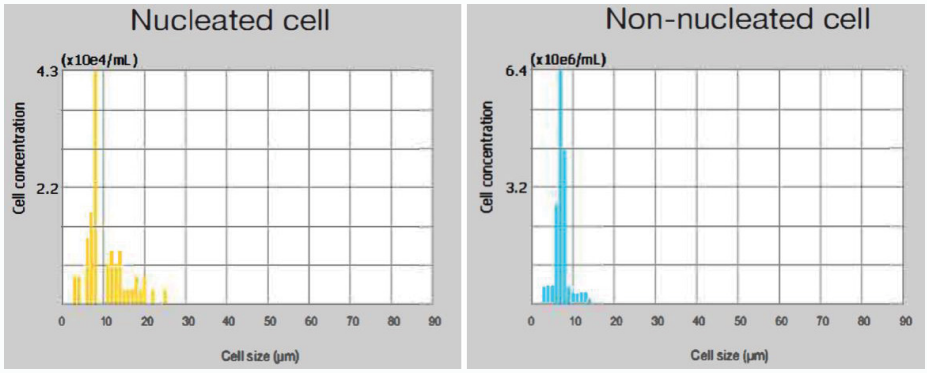

Figure 3: Cell size histogram expressed by cell concentration. It was observed in the histogram (nucleated cell) that the highest concentration of mesenchymal stem cells was between 0 and 10 m, but there were very few mesenchymal stem cells that passed 20 µm. In the histogram (Non-nucleated cell) there is no cell exceeding 20 µm.

Table 1: Results of the SVF cells isolation technique using the One STEP™ technique.

Mesenchymal stem cells insulation technology	One S.T.E.P™
Cells per gram of Adipose T	10.6-21.1 million cells per ml of adipose t
Dead Cells	0-5%
Extra-Cellular Matrix	Preserved
Processing time	<20 min
Viability	92.5% ± 7.5%
Mean	15.64 million cells

Discussion

The main findings would be that by using the One STEP™ technique with selective photo stimulation effect, a high number of total cells from the vascular stromal fraction are obtained, with high viability for potential regenerative therapy.

Discussing SVF harvesting from subcutaneous tissue requires analyzing important topics, which will influence the result of regenerative response, according to the harvest technique used.

The topics are 1) How to treat the donor area, the infiltration technique, and the extraction mechanism used. 2) Aspiration method. 3) Processing of the aspirated tissue. In topic 1, there are several mechanisms of action of technologies, from the most used and recognized as the gold standard technique whose mechanism is the mechanical disruption of Suction Assisted Lipectomy (SAL), which is very traumatic for adipose tissue as has been demonstrated previously [8,12], Carpaneda C in 1996 present the histologic alterations of adipose tissue after SAL technique, this technique is associated with the application of a tumescent factor infiltrative technique that will dilute the concentration and proportion of all the regenerative elements present in the stromal fraction, affecting the regenerative response.

There is ultrasound technology or VASER (commercial name) that according to its defenders would preserve both the adipocytes and the mesenchymal cells in the aspirated material, must be mentioned that this mechanism emulsifies the adipocytes destroying them and the increase in temperature created by this technology creates a thermal factor extra that nevertheless affects the components of adipose tissue.

Likewise, a tumescent infiltration technique must be associated, with diluting the concentration of mesenchymal stem cells and all the regenerative components.

The efforts at using mechanical separation (ultrasound, nutational, emulsification efforts) have not proven to be able to separate the very complex chemical binding of the stem-capable group due to the cell-to-cell or cell-to-matrix (ECM and periadventitial) attachments [13].

It has developed the mechanism of "Selective Tissue Engineering Photo Stimulation" (S.T.E.P.) with infrared light of 1210 nm wavelength, with specific parameters [12] whose mechanism is

photo chemical that intervenes in the activation of enzymes such as endogenous collagenase that is in a quiescent state as well as Cytochrome C oxidase present in the mitochondria of mesenchymal stem cells, as a result of this photochemical stimulation mechanism, the connective tissue made up mainly of collagen and reticulin fiber is modified, altering the adhesion property, which releases the regenerative elements of the stroma as well as the parenchyma of the fat lobules without damage and also greater production of energy in the mitochondria of the mesenchymal stem cells respectively as was proved in early analysis of the present technique before [8,12], another important difference is that it uses a wet infiltration technique only under the deep adipose tissue (above the muscular fascia) preventing the dilution of the regenerative elements of the perivascular stroma.

In the clinical experience, it was observed that if it is transplanted without damage and preserving the proportion and concentration of all regenerative components of the stromal vascular fraction-the "regenerative orchestra" preserved in the receipt area, improving the bio signaling between the donor cell and resident cells, obtaining a modulation of repairing and restoring biological process [14].

In topic 2, syringes or a suction machine are used, differing in the negative pressure exerted and a closed or open system, respectively. Open systems are known to expose the aspirated tissue to the air, which increases apoptosis, hence the use of closed systems is recommended. A 50 cc syringe was used as a close system connected to a 2.5 mm cannula. Topic 3 presents various techniques or maneuvers to be carried out, especially when applying the SAL technique and its mechanism of mechanical disruption: decantation, filtration, centrifugation, emulsification, and fragmentation techniques are the most applied. At this point, manipulating the aspirated tissue affects viability and concentration and increases apoptosis, resulting in a "poor concentration of mesenchymal stem cells" and increased apoptosis of adipocytes too [8,12].

Applying the One STEP™ technique, it is not necessary to carry out many processes (hence its name) since viable tissue is harvested without the presence of collagen or reticulin fiber, 98% preserved adipocytes, preserved elements of the stromal vascular fraction into, mesenchymal stem cells with an important angiogenic property factor, the CD marker 105+ an endoglin [9,10,13], in a closed system, a final product named as Pico Graft™. Only a simple centrifugation protocol, described by the author, without enzyme or manipulation (decanting, filtering, and emulsification) is necessary to separate the stromal vascular fraction from the adipose tissue (Figure 1 and 3).

To define whether a subcutaneous tissue harvesting technique that offers volumetric and regenerative treatment is adequate or optimal, one must refer to the number (quantity) of mesenchymal stem cells, their viability and their phenotype (quality), and the adipocyte viability and apoptosis in the harvested tissue.

Comparison of SVF cell isolation techniques for stromal vascular fraction separation and analysis using the same Luna Cell Counter™, Table 2 shows the comparison of different stromal vascular fraction isolation techniques, using the Luna Cell Counter™ device, to analyze the number of total cells, dead cells, and viability. Observed comparatively the results in favor of the One STEP™ technique and its selective photochemical stimulation property [15,16].

Previous studies demonstrated the viability of adipocytes at 98% (for volumetric treatment) after applying the One STEP™ technique in fresh samples and culture for 12 hours, including their behavior after grafting [8,12]. A recent thesis (17) and publication (15) confirm the analysis of mesenchymal stem cell harvest with the One STEP™ technique, which fulfilled the expression of surface markers (phenotype), their multiplication in culture, and their differentiation in chondrocytes, adipocytes, and osteocytes, fulfilling everything required by the International Society of Cell Therapy (ISCT) to confirm that it has been harvested optimal mesenchymal stem cells, contained in the SVF.

The present study, wants to exhibit the analysis of the number of total cells of the stromal vascular fraction as well as its viability (live/dead/total cells nucleated/non- nucleated), using immunofluorescence technology for analysis in the operating room during the same fatty tissue harvesting procedure, applying a one-step photo chemical procedure with innovative infrared light technology, and avoiding the need for a laboratory, which benefits time and costs, and as safe and minimal procedure harvest technique [17].

Conclusion

The One Step™ technique obtained a high number of total cells from the stromal vascular fraction, with fewer death cells and, high viability of >85%, the One STEP™ technique is a minimal manipulation harvest technique, making laboratory processing of the stromal vascular fraction unnecessary, the photochemical light property is realized under the skin in a single one-step technique, with a selective photo-stimulation concept and no enzyme or chemical reagent and no manipulation maneuvers are required to eliminate connective tissue, after subcutaneous aspirate collagen-free tissue.

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Table 2: Comparison of different SVF isolation techniques considered as Minimal-Grade manipulation, using the Luna Cell Counter™ device, to analyze the number of total cells, dead cells, and viability.

SVF cells insulation technology	One S.T.E.P™	Sonication Direct ultrasonic cavitation [6]	Enzymati c Collagena se enzyme [15]	Celution®800/C RS [16]
Cells per gram of Adipose Tissue	10.6-21.1 million cells per gram of adipose tissue	0.5-1million cells per gram of adipose tissue	0.4-0.5Millioncells per gram of adipose tissue	3,29 million cells per gram of adipose tissue
Dead cells	0-5%	5-15%	15-30%	-
Extra- Cellular Matrix	Preserves	Preserves	Digests	Digest
Processing time	<20 min	<40 min	>120 min	Approx. 60 min
Viability	85%-100%	90%	-	64.60%

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