Evaluation of utilizing microfat wash liquid as a potential source of mesenchymal stem cells in clinical applications

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Summary

Objective: To evaluate the quantity and quality of mesenchymal stem cells (MSCs) present in the wash liquid of the microfat obtained through the Lipogems kit. *Subject and method:* Samples were collected from three patients undergoing liposuction at the IMAGE Regenerative Clinic (Milan, Italy). The harvested Coleman fat was processed with the Lipogems kit to produce microfat. Three types of samples were collected and analyzed: (i) enzymatically digested Coleman fat, (ii) wash liquid from the Lipogems kit, and (iii) tumescent fluid. Evaluation parameters included total nucleated cell count, MSC count, and MSC quality, assessed by the expression of characteristic surface markers. *Result:* The microfat wash liquid also contained a substantial number of MSCs, representing approximately 41% of the values obtained from enzymatically digested Coleman fat. It is also worth noting that, on average, the microfat wash liquid exhibited a superior MSC percentage as compared to the conventional enzymatically digested samples. As anticipated, the tumescent solution contained negligible numbers of MSCs, confirming its limited regenerative potential. *Conclusion:* The wash liquid derived from microfat processed with the Lipogems kit represents a rich and clinically valuable source of high-quality MSCs.

Keywords: Lipogems, mesenchymal stem cells, microfat.

I. BACKGROUND

Over the past few decades, liposuction has become one of the most widely used plastic surgery procedures all over the world. Its main product - autologous fat - is not only an optimal filler material in reconstructive surgery, owing to its high biocompatibility, but has also been proven to be a rich source of mesenchymal stem cells (MSCs) with several potential applications in the fields of tissue engineering and regenerative medicine. Compared to traditional sources of MSCs, namely bone marrow, embryonic tissue or umbilical cord blood, fat tissue offers a range of advantages, most notably its ease of collection and minimally invasive procedure while maintaining a significant MSCs yield [1, 2, 3].

Besides the traditional Coleman fat grafting method, autologous fat can also undergo an additional microfragmenting step, often through a system of meshed filters, in order to break down the fat tissues into smaller particles, which improves their viability and graft compatibility. Depending on the size of these fragmented particles, the end product can either be labeled microfat or nanofat. However, this procedure involves a large volume of wash liquid, which is often discarded afterwards. [4]

This points to a number of research interests: Does the microfragmenting and fat washing procedures have any effect on the MSC stores in the

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original fat tissues? If so, can the wash liquid be salvaged as an alternative MSC source? In order to find an answer to these questions, we have decided to conduct this research, which aims to evaluate the possibility of utilizing microfat wash liquid as a potential source of MSC in clinical applications.

The objectives of this study are, therefore, to quantify the MSCs obtained from the microfat wash liquid using the Lipogems kit and analyze them qualitatively, with a view to assess their potential as a viable and easily salvageable source of MSCs.

II. SUBJECT AND METHOD

2.1. Subject

9 samples of tissues and liquids (Coleman fat, microfat wash liquid, tumescent solution) were collected from 3 female patients with an average age of 56 (±18.5), who underwent liposuction at the IMAGE Regenerative Clinic (Milan, Italy) from August to October 2024.

Selection criteria: Patients are aged between 37-74 years, present no evidence of active disease, and haven't undergone chemotherapy, radiotherapy and/or drug therapies in the two months prior to the time of research.

Exclusion criteria: Patients with BMI > 30, diabetes, hypertension, or a history of alcohol and/or nicotine abuse.

Samples were collected and labeled as follows: Coleman fat (#1), microfat wash liquid through the Lipogems kit (#2), and tumescent solution obtained during liposuction (#3).

2.2. Method

2.2.1. Location

Milan and Novara, Italy

2.2.2. Study design

Descriptive, cross-sectional study

2.2.3. Parameters

The research subjects' general information, namely age and gender, is recorded to provide a basic demographic background. Also collected are the defining parameters of MSC quality and quantity, including nucleated cells count, MSC count, and MSC percentage.

2.4. Data collection methods

Sample collecting procedure:

First, an informed consent is signed by patients at the IMAGE Regenerative Clinic, Milan. Following the liposuction procedure, samples are stored in sterile syringes and wrapped in three layers of specialized bags for the storage and transport of biological hazards. Samples are kept at room temperature and processed within 24 hours of collection at the University of Piemonte Orientale, Novara.

Nucleated cells extraction:

The fat tissues and liquids in all three samples (#1,2,3) are processed to extract their nucleated cells for further analysis. Coleman fat (#1) undergoes 1st centrifuge, then enzymatic digestion by collagenase enzyme, then a 2nd centrifuge, followed by erythrocytes lysate by Sodium chloride 0.3%. Similarly, the microfat wash liquid (#2) and tumescent solution (#3) also go through centrifuge (only once) and erythrocytes lysate in order to separate the nucleated cells.

Cell culture and nucleated cell count:

Cell culture is conducted in Fetal bovine serum (FBS) 50% in DMEM F12 culture medium, 37^{0}C/5% CO_2 for 2 weeks. Then, erythrocytes are removed by washing with PBS, the adherent nucleated cells are detached using trypsin, and cell counting is performed in the Neubauer Chamber after trypsinization.

Specific surface marker (CD31, CD44, CD45, CD73) analysis:

In order to confirm MSC characterization, nucleated cells are continuously passaged until the required cell number is achieved. Then, the cells are stained using fluorescence immunostaining with antibodies anti-CD31, anti-CD44, anti-CD45, and anti-CD73. Within one week after staining, the samples are analyzed in a flow cytometer using the Fluorescence-Activated Cell Sorting (FACS) method.

2.3. Data handling

SPSS 20.0 and Graphpad 10.1

2.4. Research ethics

The research design has been approved by the Ethics Committee of the University of Medicine and Pharmacy - Thai Nguyen University (TUMP). However, since the biological samples used in the research are considered waste material of the liposuction procedure and will not be reintroduced into the human body, they do not warrant as rigid bioethics adherence as normally required. Therefore, for a higher research standard and minimal impact from external factors, TUMP has collaborated with the IMAGE Clinic, where the Lipogems kit is readily available and handled by the very experts who developed it, as well as the University of Piemonte Orientale, whose Tissue Regeneration Laboratory houses state-of-the-art equipment and facilities for cell culture and analysis. In addition, the close proximity between these two institutions ensured that the samples can be processed within 24 hours of collection.

III. RESULT

3.1. Nucleated cell count comparison

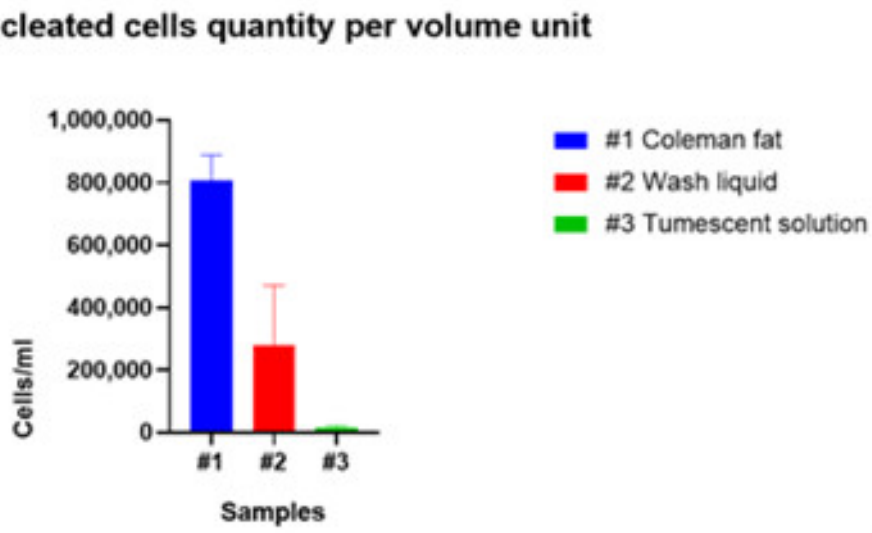

Figure 1. Nucleated cell counts in: 1ml of enzymatically digested Coleman fat (#1), the wash liquid corresponding to 1ml of microfat (#2), and 1ml of tumescent solution (#3).

Results (Figure 1): After 2 weeks of culture, the enzymatically digested Coleman fat showed a markedly higher yield of nucleated cells. It is worth noting that the microfat wash liquid also contained a noticeable number of nucleated cells, up to 35% of the standard method (enzymatic digestion of Coleman fat). On the other hand, the tumescent solution only yielded under 2% that of the standard method.

The quality of the stem cell population can be investigated by the stem cell's purity, their potency in immunomodulation, and cell viability. In this research, cell population purity is used as a standard for cell quality. The nucleated cells were immunostained using different antibodies to identify a number of positive and negative specific MSC surface markers (CD31, CD45, CD44, and CD73).

2.2. MSC quality comparison

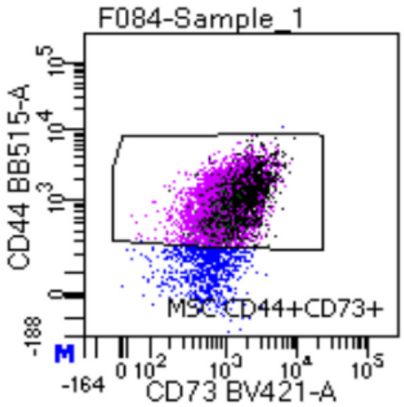

Figure 2. CD44 and CD73 expression in a Coleman fat sample from a 57-year-old female patient

CD44 and CD73 are broadly expressive markers in stem cells and other cell types.

CD44 expresses in hematopoietic stem cells and mesenchymal stem cells, leukocytes, epithelia, neurons, and cancer cells.

CD73 expresses in mesenchymal stem cells and haematopoietic stem cells, immune cells, endothelia and epithelia, and cancer cells.

Both are widely recognized as positive markers of mesenchymal stem cells. [5]

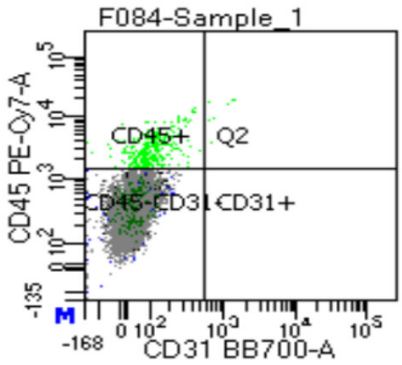

Figure 3. CD31 and CD45 expression in a Coleman fat sample from a 57-year-old female patient

CD31 is a marker of endothelia and blood cells (Platelet, Leucocyte, Hematopoietic stem cell).

CD45 is a hematopoietic marker, especially leukocytes.

Mesenchymal stem cells, in general, should not express these markers, which is why they are treated as MSC negative markers. [6]

Therefore, the formulation to identify mesenchymal stem cells in our research is:

MSC = CD31- CD45- CD44+ CD73+

The FACS reading are demonstrated in Figure 4 and Table 1.

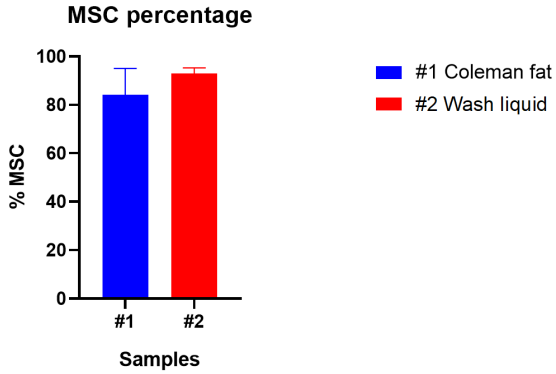

Figure 4. MSC percentage in lysated Coleman fat and microfat wash liquid after 4 passages.

Table 1. MSC percentage in Coleman fat and microfat wash liquid after 4 passages

Sample	#1 Coleman fat	#2 Microfat wash liquid
No. of samples (n)	3	3
% MSC (mean)	84.20	92.83
Std. Deviation	10.83	2.479

The findings show that in both samples, over 80% of the nucleated cells are MSC, and the average percentage of MSC present in the microfat wash liquid sample are higher than that of lysated Coleman fat. This suggests that microfat wash liquid might be a source of higher-quality MSC.

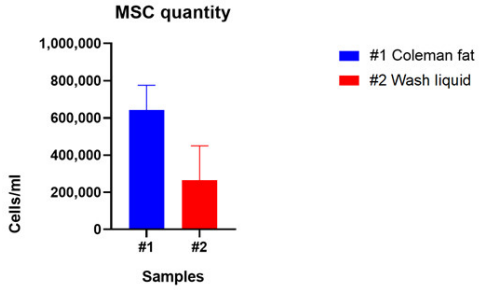

Figure 5. MSC count in lysated Coleman fat and wash liquid after 2 weeks of culture

3.3. MSC count comparison:

MSC count after 2 weeks of culture can be extrapolated, based on the nucleated cell count in the Neubauer chamber after 2 weeks of culture and the MSC percentage obtained through FACS analysis after 4 passages, and is illustrated in Figure 5 and Table 2.

Table 2. MSC count in lysated Coleman fat and microfat wash liquid after 2 weeks of culture

Sample	#1 Coleman fat	#2 Microfat wash liquid
No. of samples (n)	3	3
MSC/ml (mean)	641,439	263,711
Std. Deviation	133,910	185,950

Similar to the nucleated cell count results, enzymatic digestion of Coleman fat remains the 'golden standard' of MSC extraction. However, microfat wash liquid (#2) also continued to provide a rich source of MSC, amounting to 41% of that of lysated Coleman fat (#1). This is strong evidence of its potential clinical applications as a non-enzymatic MSC extraction method.

IV. DISCUSSION

The research subjects were selected from patients who underwent liposuction at the IMAGE Clinic from August to October 2024. The fact that all three patients were female reflects a practical aspect where the need for cosmetic surgery are often higher among female patients than their male counterparts. In addition, since IMAGE is a clinic specialized in regenerative medicine procedures such as intra-articular autologous fat and microfat injection for treatment of osteoarthritis, the research subjects would more than often fall under the middle-aged and elderly categories.

The findings in this study have shown that, despite being previously regarded as a biomedical waste, the microfat wash liquid obtained through the Lipogems kit is, in fact, a rich source of Mesenchymal stem cells (MSCs), with comparable yield (Table 2) and a surprisingly higher MSC purity (Table 1) as opposed to the traditional enzymatically digested Coleman fat. This suggests that among the different types of nucleated cells, MSC might be more vulnerable to collagenase enzyme, and that mechanical MSC extraction methods, while offering a lower yield, improve MSC viability and require less time as enzymatic digestion is not needed. Moreover, such non-enzymatic approaches promote a safer and simpler procedure that can applied at any point of care, without the need for sophisticated equipment or GLP standards. This, in turn, provides a competitive edge for stem cell therapy to become a more widespread and convenient clinical practice.

Another noteworthy point is that, according to Piccino MS [7], a higher percentage of MSCs in the fat tissue is associated with higher post-graft tissue viability. This puts an emphasis on MSC preservation during microfat processing, especially when the regenerative capacity of the graft is of the essence, thus calling for the optimization of the aforementioned procedure. In the specific case of the Lipogems kit, we would advise that clinicians

perform a single centrifuge on the wash liquid to salvage the MSC therein, before infusing it with the microfat for the final, MSC-enriched graft. For simple mechanical filters, it is advisable that the fat tissue be washed before going through the mesh, while avoiding further washing after microfragmenting, to minimize MSC loss.

An additional observation made during the experiment was that, in culture dishes with high erythrocyte concentration, the viability of nucleated cells significantly decreased compared to samples with low erythrocyte concentration. While this suggests a negative correlation between erythrocytes and MSCs survivability, future studies with a larger sample size are needed to clearly evaluate the effect of the erythrocyte components. This will allow for a more concrete conclusion regarding the necessity of removing erythrocytes using a hypotonic lysis in the MSC isolation protocol.

Although the comparative results in this study showed promising potential for microfat wash liquid to serve as a rich source of MSCs, the interpretation of these results should be conducted with caution due to the risk of data bias arising from multiple factors, both subjective and objective. These factors include the individual characteristics of the patient (gender, age, medical history, prior liposuction interventions), the skill level of the physician performing the liposuction, the discretion during sample processing and cell analysis, as well as the accuracy of cell quantification and FACS analysis methods.

One potential source of bias in this study stems from the limited immunophenotyping panel employed for MSC identification. According to the International Society for Cell & Gene Therapy (ISCT), MSCs are typically defined by positive expression of CD73, CD90, and CD105 and negative expression of CD34, CD45, CD14/CD11b, CD79a/CD19, and HLA-DR. Due to logistical constraints related to funding, time, and reagent availability, our analysis was limited to CD44, CD73, CD31, and CD45. While this panel is sufficient to identify an MSC-enriched cell population, it may not completely exclude all non-MSC cell types, and this limitation has been acknowledged in the interpretation of our findings. In addition, MSC proliferation and survival are influenced by intercellular communication mediated by cytokines and exosomes, as well as by initial cell density during culture [8]. Together with the small sample size, these factors may partly explain the observed variability in MSC yield. Depending on whether the use is for adipose tissue grafting or cell therapy, the microfat processing protocol needs to be optimized to either maximally preserve the number of MSCs in the adipose tissue, or enhance the efficiency of stem cell extraction. Future studies with a larger sample size and better control over influencing factors will help increase the accuracy when comparing different approaches, contributing to the refinement of the MSC isolation protocol for applications in regenerative medicine.

V. CONCLUSION

A study of 9 fat tissue and liquid samples from 3 liposuction patients found that the microfat wash liquid obtained from the Lipogems kit contained a significantly higher number of MSCs than the traditional Coleman fat. This can be considered an abundant and high-quality source of mesenchymal stem cells, and can be effectively utilized in clinical applications.

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