Alternative procedure for obtaining nanofat: analysis, culture, and clinical application

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Background: The mechanical extraction of body fat can alter the structure of adipose tissue, and its primary effect on transfer to other sites may be affected. In addition, it appears to be related to the activity of mesenchymal stem cells. Since its introduction, nanofat has been used for various regenerative treatments, such as scar improvement, aesthetic treatments, burns, irradiated tissue, and chronic wounds.

Objective: The main objective of this study is to present an alternative procedure for obtaining nanofat, its viability analysis, culture, and clinical application.

Materials and methods: A novel procedure for obtaining nanofat was developed. Each of the steps of the procedure is described, and the microscopic analysis of the mesh used and histology of the nanofat samples were also performed. In addition, mesenchymal stem cell derivation and immunophenotypic identification and their clinical application were carried out.

Results: The proposed procedure allowed obtaining nanofat in a closed manner, systematizing each step. Many mesenchymal stem cells with adequate results in their clinical application were identified. The study found that nanofat therapy significantly improved skin texture and radiance.

Conclusions: The proposed procedure is an alternative for obtaining nanofat and mesenchymal stem cells for their clinical application and in research studies in tissue engineering and regenerative medicine therapies.

Keywords: Nano, adipose tissue, mesenchymal stem cells.

After the first adipose tissue-derived stem cells (ADSCs) were discovered in 2001 by Zuk et al., they have been used for various purposes, including tissue regeneration and clinical applications in rejuvenation (1).

In 2013, the term "Nanofat" was introduced by Tonnard and Verpaele, reporting improved skin quality after intradermal injection of nanofat in the lower eyelids, tear troughs, perioral region, glabellar area, and décolletage wrinkles (2). The mechanical processing used to obtain fat alters the structure of adipose tissue, and its primary regenerative effect appears to be related to the activity of mesenchymal stem cells (ADSCs) (3). Since its introduction, nanofat has been utilized in several regenerative treatments, including scar revision, burns, irradiated tissue, and chronic wounds (4,5,6).

Subsequently, multiple devices have been developed to obtain nanofat, which share the common feature of providing semi-closed or closed systems. These minimize direct handling and reduce environmental contamination, thereby improving product safety. Additional desired features of these methods include reproducibility, cost-efficiency, and optimal mesenchymal stem cell yield. Furthermore, these systems aim to offer a cost-effective mechanical alternative to enzymatic digestion for ADSC isolation, while maintaining compliance with the FDA's "minimal manipulation" criteria (7,8,9).

This background motivated the development of an alternative procedure for the extraction and clinical application of nanofat.

The main objective of this study is to present an alternative procedure for obtaining nanofat, including analysis, culture, and clinical application.

Methods

A quasi-experimental study was conducted. Informed consent was obtained from all participants for the extraction and application of nanofat.

A novel nanofat extraction protocol was developed. Each step of the procedure was described and analyzed. Microscopic mesh evaluation and histological analysis using hematoxylin-eosin staining of macrofat, microfat, and nanofat samples were conducted at the pathology laboratory of the 20 de Noviembre National Medical Center, Mexico City. ADSC derivation and immunophenotyping were

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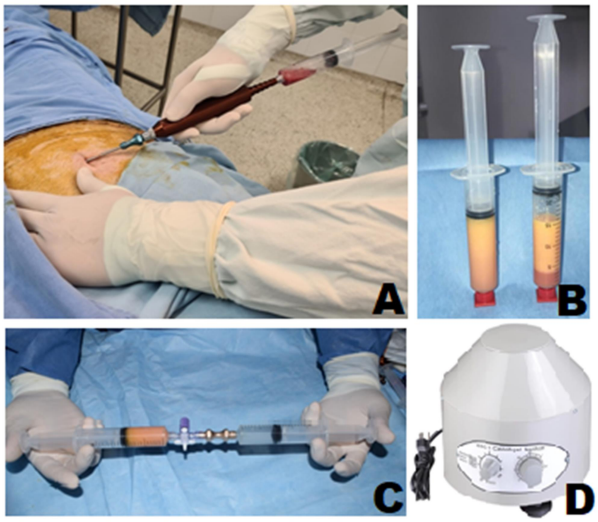

Figure 1. A) Fat harvesting using a 3 mm cheese-grater cannula. B) Decantation of the lipoaspirate post-extraction and after 5-minute washing. C) Washing the lipoaspirate with 1:1 saline solution after decantation. D) Centrifugation at 1500 rpm for 2 minutes (450g) after the second decantation.

performed at the tissue engineering and regenerative medicine laboratory of the same institution. This study followed the ethical principles of the Declaration of Helsinki and the Mexican General Health Law.

Technique Description

Aseptic preparation of the donor area (lower abdomen) was performed using chlorhexidine and sterile drapes. The lower abdominal area was infiltrated with a solution (800 mg lidocaine/1L saline + 1 mL of 1:1,000,000 epinephrine) using a 3 mm infiltration cannula. Twenty milliliters of lipoaspirate were harvested using a 3 mm cheese-grater cannula with 1 mm lateral holes, employing negative pressure from a 20 mL syringe.

The lipoaspirate was decanted for 5 minutes and then washed with saline in a 1:1 ratio, followed by another 5-minute decantation. It was subsequently centrifuged at 1500 rpm (450g) for 2 minutes. Mechanical emulsification was performed by shifting the fat between two 10 mL syringes connected through a three-way stopcock and serial Luer-lock connectors (1.2 mm and 2.4 mm). Thirty passes were performed. Next, a 200 µm nylon mesh was connected to the stopcock and filtration was performed. After removing the mesh, the product was transferred to 1 mL syringes for intradermal injection using 30G needles.

Samples from each procedural stage were sent to the tissue engineering and regenerative medicine laboratory for analysis and explant-based ADSC derivation, without enzymatic digestion. Immunophenotypic identification of adipose-derived mesenchymal stem cells was also performed.

ADSC Derivation: Non-Enzymatic Culture and Expansion

Samples were washed three times with PBS (Gibco, USA). After removing the majority of red blood cells, the explant technique was applied, placing 3–5 mm tissue fragments at the center of 10 cm^2 culture wells. 0.5 mL of DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, and 1% antibiotic-antimycotic was added and incubated for 3 hours. Subsequently, an additional 0.5 mL of complete medium was added. Medium was renewed every 72 hours.

ADSCs were cultured at 37°C, 5% CO_2, and 95% relative humidity. Upon reaching 80% confluence, subculturing was performed using 1% trypsin and a cell scraper.

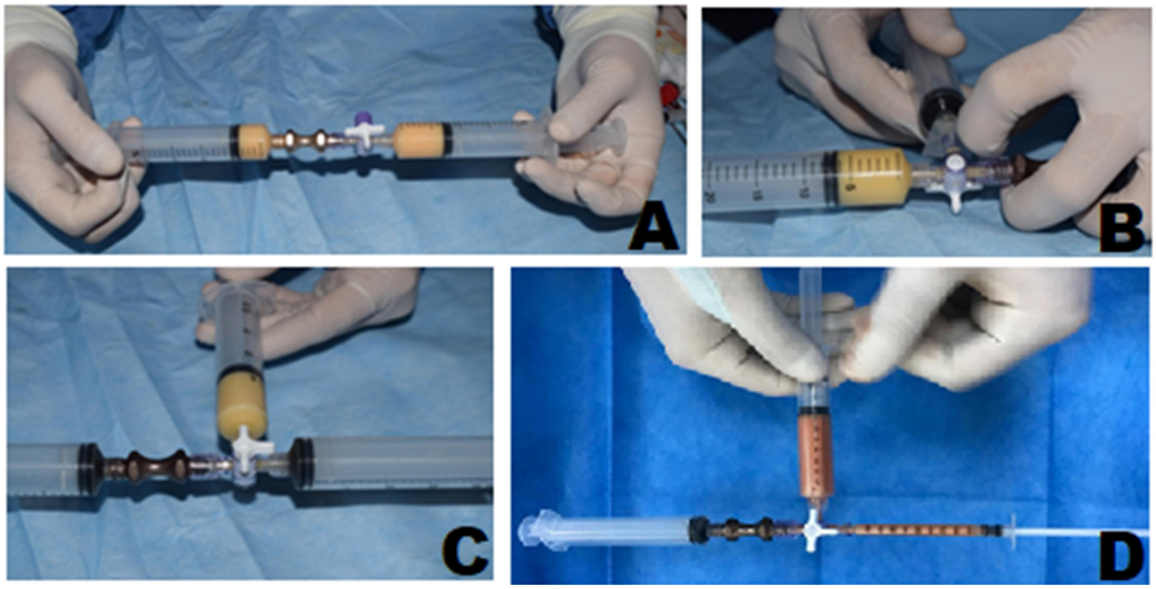

Figure 2. A) The centrifuged lipoaspirate is emulsified by 30 intersyringe passes. B) Placement of the nylon mesh between the three-way stopcock and syringe. C) Filtration of the lipoaspirate through the mesh. D) Transfer of nanofat into 1 mL syringes.

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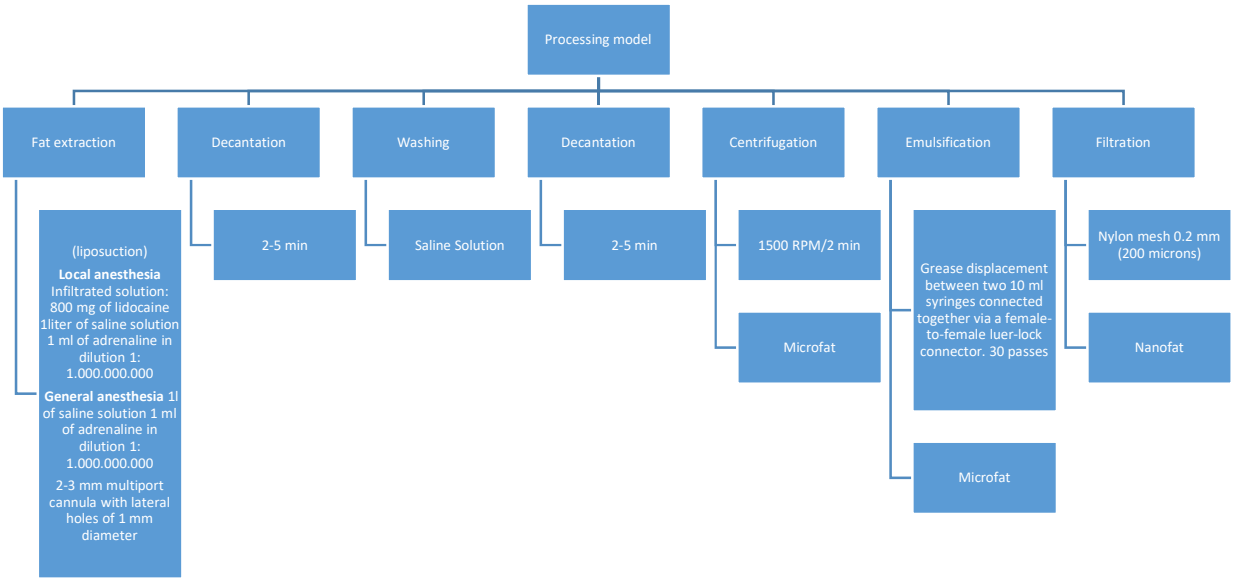

Figure 3. Flowchart summarizing the nanofat harvesting procedure.

Phenotypic Analysis by Immunofluorescence

Immunofluorescence was used to identify the presence of mesenchymal stem cell markers CD90 and CD105. Cells were fixed with 4% paraformaldehyde, washed with PBS, and permeabilized using 0.05% Triton-X (Sigma, USA). Blocking was performed with 5% goat serum and 0.25% Triton X-100 in PBS for 30 minutes. Primary antibodies (CD90 1:150, CD105 1:250; Millipore, Mexico) were applied for 1 hour, followed by washing and incubation with secondary antibodies (FITC and TRITC, Abcam, USA) for 1 hour. Nuclear staining was achieved using DAPI (300 mM; Abcam). Fluorescent images were acquired using an Olympus IX71 inverted epifluorescence microscope.

Results

Figure 3 outlines the procedural flowchart for nanofat extraction. Briefly: fat harvesting → serum decantation (2–3 minutes) → washing with saline → second decantation → centrifugation at 1500 rpm for 2 minutes to obtain microfat → mechanical emulsification and 200 µm mesh filtration to obtain nanofat.

Microscopy of the nylon mesh confirmed a 200 µm pore size. Fresh histology of nanofat samples revealed no viable adipocytes—only a lipid emulsion.

ADSC Derivation: Non-Enzymatic Culture and Expansion Results

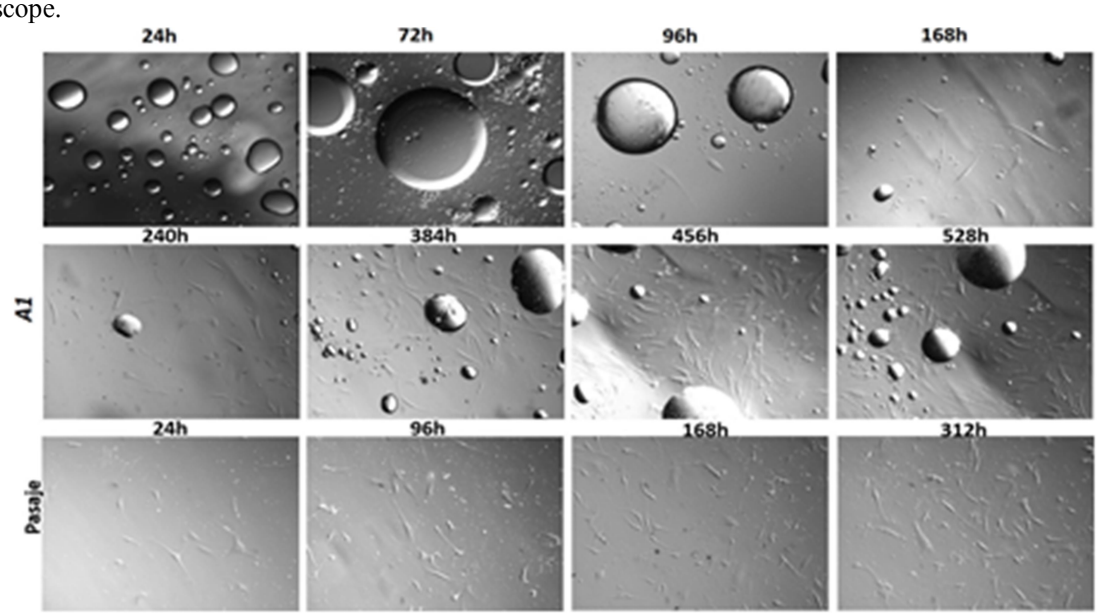

Figure 4. Nanofat isolation and expansion. Typical fusiform morphology of this cell lineage is observed. ADSCs can be seen at 168 hours, with increased numbers at 528 hours, and post-first passage at 312 hours.

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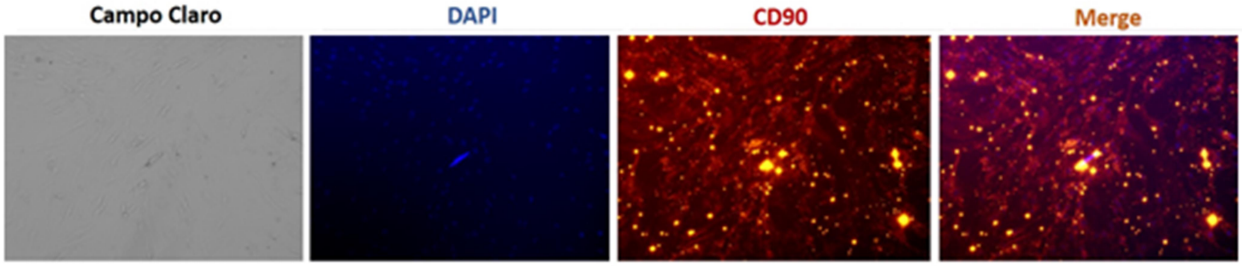

Figure 5. Phenotypic characterization of ADSCs derived from nanofat. Shown are brightfield view, nuclear staining (DAPI), CD90-positive marker, and merged DAPI-CD90 image after 456 hours in culture.

Among all samples, only nanofat yielded mesenchymal stem cells. These exhibited a fibroblastic morphology (Figure 4). Immunofluorescence confirmed that 90% of fusiform cells were CD90+ (Figure 5), validating their mesenchymal lineage.

Although no standardized patient satisfaction evaluation was performed, clinical nanofat application showed observable improvements in skin quality and brightness at 3- and 6-months post-treatment (Figures 6 and 7).

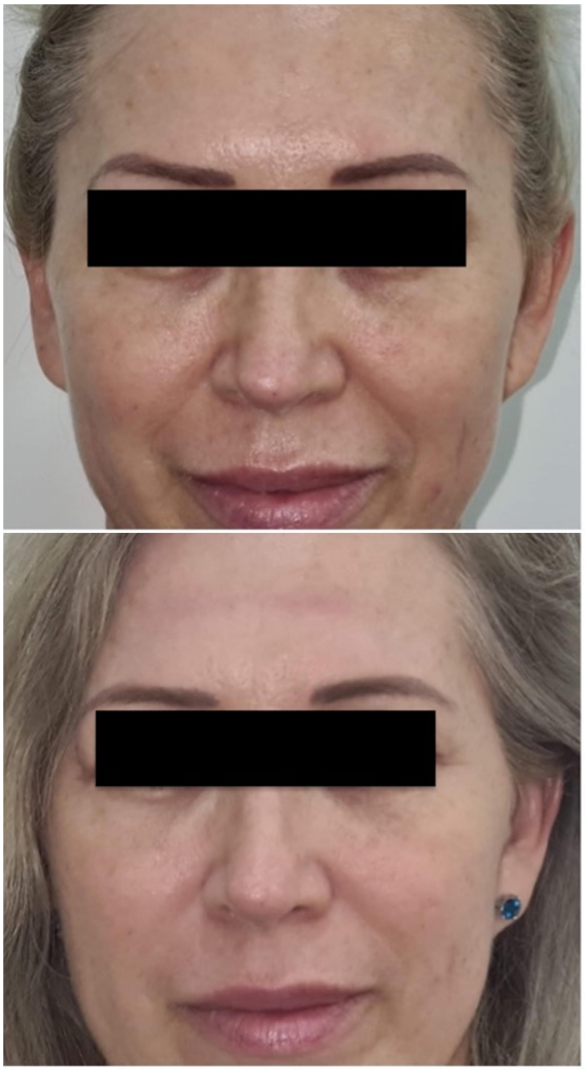

Figure 6. Frontal view demonstrating pre- and post-treatment (3 months) nanofat application to the malar region.

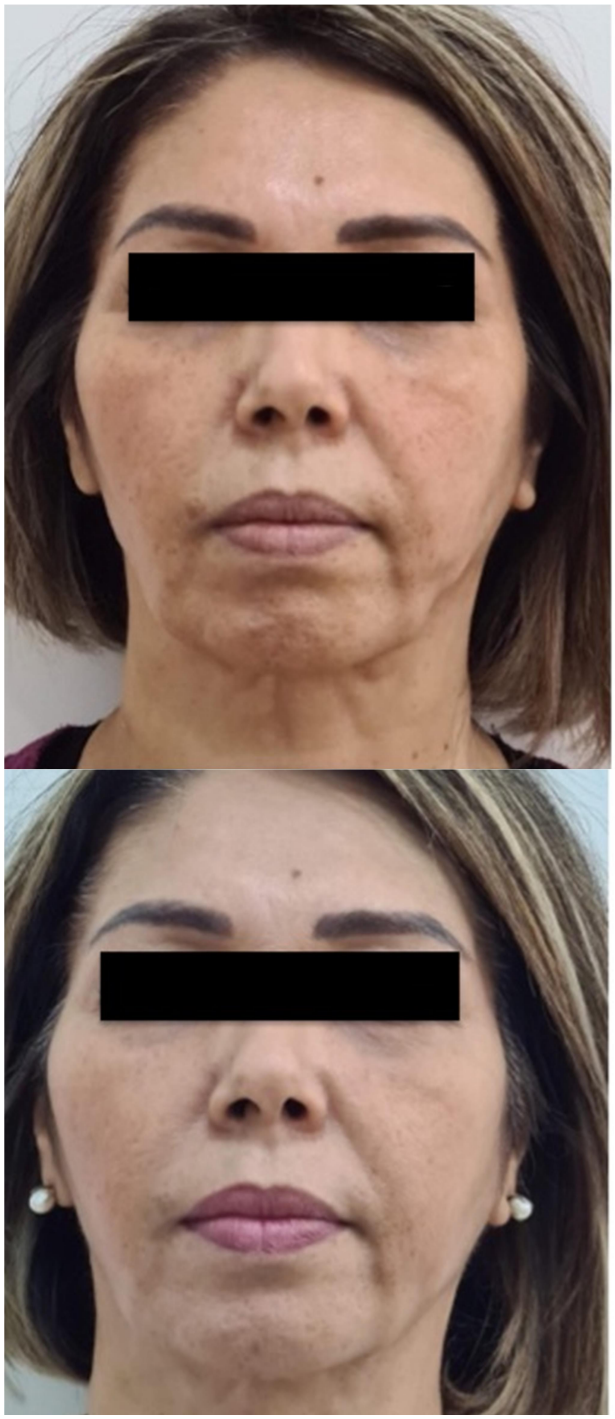

Figure 7. Frontal view demonstrating pre- and post-treatment (3 months) nanofat application to the malar region.

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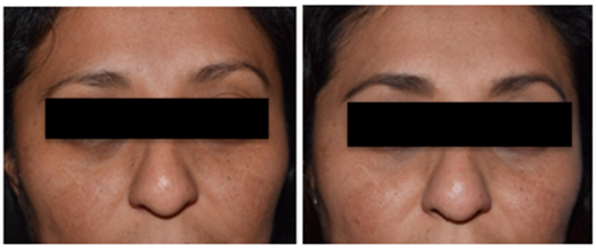

Figure 8. Frontal view demonstrating pre- and post-treatment (6 months) nanofat application to the malar and lower eyelid regions.

Discussion

Since their isolation and characterization over a decade ago, adipose-derived mesenchymal stem cells have become one of the most extensively studied adult stem cell types for soft tissue engineering and regenerative medicine. Compared to other sources, ADSCs offer advantages such as autologous availability, minimally invasive harvesting (liposuction), high proliferative capacity, and multilineage potential.

Decanting, washing, centrifugation, and transfer did not compromise MSC viability or quantity, as supported by prior literature (10,11).

The mesh used in this study had a pore size of 200 µm—smaller than the 500 µm mesh described by Tonnard et al. (2). Microscopy confirmed the absence of adipocytes after a single filtration step. Additionally, the use of 30G needles, compared to 27G in previous protocols, minimized trauma and enhanced injection control.

This closed-system technique prevents environmental contamination and manipulation of the adipose product. Although enzymatic methods may yield higher ADSC counts, they are associated with regulatory constraints, higher contamination risks, and greater cost due to required laboratory infrastructure (12).

The described mechanical method provides an accessible means to isolate ADSCs suitable for tissue engineering and regenerative medicine protocols. Several studies report successful clinical application of nanofat, with high acceptance and skin rejuvenation outcomes (13).

Conclusion

The proposed nanofat harvesting protocol allows for the collection of mesenchymal stem cells using a closed system and at lower cost. It offers a viable alternative for clinical and research applications in tissue engineering and regenerative medicine. However, randomized controlled trials are necessary to strengthen the evidence. A limitation of our study is the absence of validated patient satisfaction questionnaires to quantify therapeutic efficacy.

Conflicts of interests

The authors declare that they have no conflicts of interest related to the content of this article for publication in this journal.

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