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Case report

A new filling technique with vital micrograft from adipose tissue: A Lipo-Stem-Exos method. A case report

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Abstract. *Aim:* Through this clinical case report, we have demonstrated that the periocular area can be effectively corrected using a single filling session with vital micrografts derived from microfiltrated, fragmented adipose tissue. Over time, vital micrografts have allowed us to maintain a high correction capacity. The technique that we have defined Lipo-Stem-Exos has allowed us to obtain an excellent correction of the volume and the improvement of the Dark Circle, improving the general appearance of the gaze with excellent patient compliance. *Background:* The eyelid tissue is very thin and during aging, in addition to the loss of elasticity, the eye can experience a depression due to a decrease in periocular volumes with an exposure of the bones that make up the anatomical area and a deficit in the tear trough area. This phenomenon appears to be physiological in all individuals; however, in some cases, it may constitute a genuine cosmetic concern. *Method:* We subjected a patient to a nasolacrimal sulcus filling treatment with a technique that we defined Lipo-Stem-Exos. After the collection of adipose tissue, we fragmented it and selected viable micrografts with a size between 20 and 40 microns. Filtration of adipose tissue through 20–40 μm filters enabled the isolation of tissue progenitor cells from the side population under hypoxic conditions, while effectively excluding inflammatory contaminants. *Results:* With this technique we observed and reported excellent long-lasting results. The Lipo-Stem-Exos technique took us about 45 minutes with complete compliance from the patient. *Conclusion:* We observed the improvement of the periocular area with a clinical maintenance of the results even beyond 240 days from the session. These results suggest that micrografts extracted at 20–40 μm under hypoxic conditions retain viability and contribute to the improvement of the treated anatomical area.

Key words: Vital micrografts, microfiltration, side population, ADSCa, exosomes, lipo-stem-exos

Introduction

Correction and improvement techniques for the periocular anatomical area include the use of biocompatible materials such as free or cross-linked hyaluronic acid, collagen, PRP and calcium hydroxyapatite[1]. Clinical experience demonstrates that adipose tissue processed according to Tonnard (2013) can be used effectively in aesthetic procedures. Tonnard described the use of even finer injection needles, up to 27-gauge, for fat grafting. This approach can serve as an alternative to traditional fillers, both for restoring lost volume and correcting dark lower eyelids. Furthermore, the tissue progenitor cells contained within the grafts may also improve the skin texture in areas adjacent to the treated region[2,3] . The Tissue Progenitors of the adipose tissue of the Side Population have the same characteristics as the Adult Mesenchymal Stem Cells, as they possess the same surface markers CD44, CD73, CD90 and CD105 and have a size that varies from 17.9 microns in the smallest cells and 30,4 microns in the largest ones[3]. This population of cells is also defined as the

Side Population because it is the population of cells that best expresses the characteristics of Adult Mesenchymal Stem Cells with smaller dimensions and greater cytoplasmic complexity[3]. Once transferred into the recipient tissue, they promote a paracrine effect with improvement of tissue homeostasis through their cyclin-dependent duplication and the release of vesicles containing regenerative MicroRNAs[4] .

The periocular area is covered by very thin, delicate tissue, and exposure to ultraviolet (UV) radiation and reactive oxygen species (ROS) contributes to the loss of elasticity and turgor. The normal aging process contributes to enhancing the sunken eye, which despite not being able to go beyond a certain limit, contributes to highlighting the cosmetic blemish which is often a cause for concern for the patient, seeing as it can contribute to a fatigued appearance. Changes in the proportions of the periocular area, driven by normal aging as well as by reactive oxygen species (ROS) and ultraviolet (UV) exposure, result in the downward displacement of the superficial tissues over the superciliary arch, a descent of the supraorbital notch, and thinning of the lower infraorbital margin—commonly referred to as the "tear trough" - which is particularly susceptible to these alterations. The relevant supporting bone districts are the frontal bone superiorly, the zygomatic bone laterally, and the maxillary bone in its zygomatic-orbital portion infero-medially. Periocular aging is often associated with a fatigued appearance, and addressing this concern is a highly desired outcome for patients presenting with this aesthetic issue.

To restore both cosmetic and physiological harmony to the nasolacrimal sulcus over time, we elected to use exclusively vital micrografts derived from adipose tissue, sized between 20 and 40 μm. This approach was employed to achieve both volume restoration and tissue regeneration at the implantation site. Classical lipoaspirate is a rich and accessible source of tissue progenitors that can be successfully used due to the characteristic of the plasticity of those cells, that is, the ability to direct themselves towards the destiny of the recipient tissue. These cells are potentially able, after implantation in the recipient tissue, to correct texture defects as well as volumetric ones due to their self-renewal and differentiation abilities[5] . Tissue progenitor cells can be isolated from adipose tissue via microfiltration at appropriate sizes. Immunofluorescence and flow cytometry analyses demonstrate that the majority of these cells are of mesodermal or mesenchymal origin[3].

Adipose tissue is readily accessible and serves as a rich source of progenitor cells capable of differentiating into multiple cell lineages[6]. Once isolated and cultured, these cells form fibroblast-like colonies that can be maintained for extended periods with continued population doubling and minimal senescence[7]. Additionally, they actively release exosomes expressing characteristic markers, such as CD8[8]. They are able to promote neovasculogenesis, neocollagenogenesis and new extracellular matrix (ECM)[3]. Being mediators of tissue trophism, they participate in the regulation of tissue homeostasis, in the down regulation of inflammation, in the epigenetic reprogramming of immune cells by promoting the lineage of M1 macrophages through direct cell-cell contact and through their secretom[8,9].

Tissue progenitor cells promote tissue regeneration by stimulating resident fibroblasts[3] and initiating and maintaining key factors involved in the regenerative process[10]. Using vital micrografts sized to preserve tissue progenitors, it is possible to address both volumetric deficits[5] and the decline of adult stem cell niches[7], leading to progressive tissue improvement over time[11]. These progenitors exhibit the characteristics of adipose-derived stem cells (ADSCs), including identical surface markers, and when isolated from adipose tissue, they adhere to the adipose matrix[12]. They also demonstrate contact inhibition, enhancing the safety of the procedure[13].

Compared to bone marrow, adipose tissue allows extraction of approximately 100 times more adult mesenchymal stem cells per unit volume, with significant proliferative potential[14]. From one gram of adipose tissue, roughly 5×10^3 progenitor cells can be isolated - 500 times more than an equivalent volume of bone marrow[15]. These cells participate in physiological processes including migration, proliferation, extracellular matrix deposition, and remodeling of implanted tissue[16].

The filling technique employed, termed Lipo-Stem-Exos, involves the selective removal of fibrous strands and cellular debris from adipose tissue

processed according to Tonnard (2013)[2], preserving clusters of progenitor cells while excluding interfering material. These progenitor clusters demonstrate enhanced proliferative capacity[17], as they are free from inflammatory components such as non-functional cellular debris and fibrous tissue, which can activate the Toll-like receptor 4 pathway present in all cells[18]. The use of extremely small clusters, combined with maintenance under hypoxic conditions in the syringe, promotes exosome release containing microRNAs with regenerative potential[4,8]. This approach is referred to as Lipo-Stem-Exos.

Patient and Method

This case report describes a 40-year-old female patient who presented to the clinic seeking improvement of the periocular area and nasolacrimal fold. The patient underwent the Lipo-Stem-Exos technique, a volumetric correction procedure utilizing a hypoxic microfiltrate of fragmented adipose tissue sized between 20 and 40 µm. The aim of the study was to assess the long-term improvement of the treated anatomical area. The procedure was conducted in accordance with the standards of the local ethics committee and the Declaration of Helsinki (2000), following the acquisition of informed consent. The female patient did not present specific pathologies of the dermis or other systemic pathologies. The adipose tissue was extracted using a Notrox 2.5x150 multi-hole cannula mounted on a 20 ml Luerlock® syringe (Figure 1) which allows the extraction of an excellent quality of cell vitality[19] and an optimal quantity of cells even in modest quantity samples[3]. Before the extraction, a local anesthesia with Klein solution was performed on the donor area. This area was identified by us in the supratrochanteric area. We extracted approximately 10 cc of lipoaspirate and left it to settle to eliminate the anesthesia fluids for 15 minutes, from which we obtained approximately 7 ml of adipose tissue. The adipose tissue was disaggregated according to Tonnard (2013) and subsequently filtered to a size range of 20–40 µm to better preserve the side population[20] (Figure 2). The resulting micrograft was transferred into two separate 2.5 mL Luerlock® syringes to facilitate the injection

Figure 1. Collection.

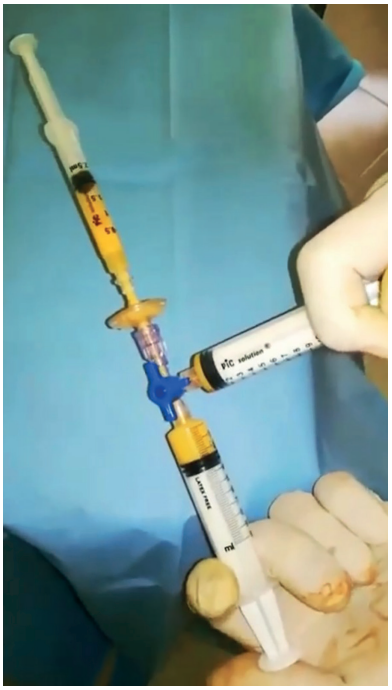

Figure 2. Fragmentation and filtration 20/40 microns in hypoxia.

process. Although some vital elements are lost during disaggregation and filtration[21], the therapeutic potential of the micrograft is enhanced[17], as fibrous strands (Figures 3, 4, 5) and cellular debris (Figure 6) are

effectively removed[18]. Microfiltration also protects the recipient areas by eliminating components that could activate an inflammatory response via the Toll-like receptor 4 pathway[3,18].

The adipose microfiltrate, sized between 20 and 40 μm (Figure 3), was maintained under controlled hypoxic conditions in a syringe for 240 seconds to enhance cell viability and promote exosome release[22]. Injection was performed using a 2.5 mL Luerlock® syringe fitted with a Notrox 0.7–1.2 × 70 mm cannula, employing a retrograde injection and release technique until volumetric deficits were corrected (Figure 5), avoiding hypercorrection. The use of nanofat prepared according to Tonnard (2013), with inflammatory elements removed via microfiltration and progenitor cell sizes preserved, led us to hypothesize that the procedure would yield positive outcomes with minimal complications. The entire procedure was completed in approximately 45 minutes.

Results

From the analysis of the results obtained, we obtained an excellent result with the technique defined as Lipo-Stem-Exos, that is the technique that involves the separation of the tissue progenitors from the interfering materials and the very brief hypoxia induced before the transfer of the tissue suspension to promote exosomal budding[8].

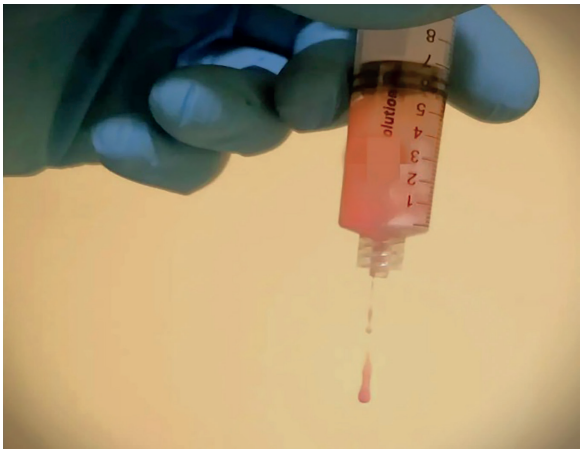

Figure 3. Fibrous shoot.

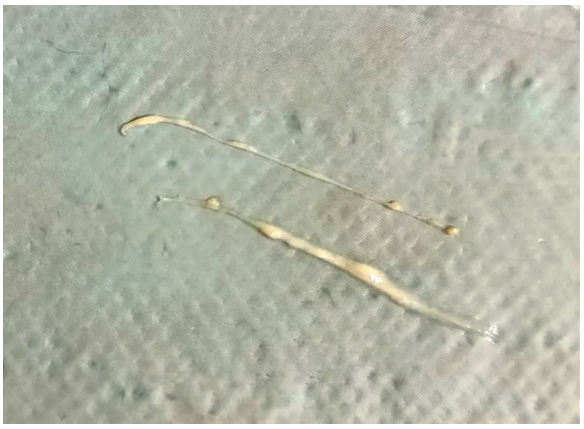

Figure 4. Fibrous Connective Tissue.

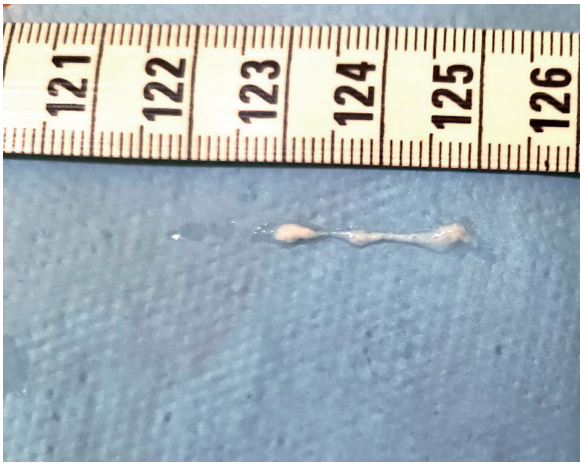

Figure 5. Fibrous Connective Tissue.

Figure 6. Cell shells captured by the filter.

Table 1. Vancouver Modified Scale.

Baseline				**Follow up 240 days**
Vascularity:	Red	Purple	Grey	Normal
Pigmentation:	Hyperpigmentation	Hypopigmentation		Normal
Pliability:	Firm	Ropes		Normal/Supple

The patient reported improvement in periocular wrinkles even at 240 days post-procedure, as assessed using the Berardesca scale[23] and the Vancouver Modified scale.

At the eight-month follow-up after the initial procedure, the patient assessed their satisfaction regarding volumetric filling and wrinkle correction using a 0-4 scale for each criterion (0 = unsatisfactory; 4 = satisfactory), as described by Berardesca et al.[23]. The results are summarized in Figure 9.

Peripalpebral skin tissue was also evaluated using the Vancouver Modified Scale, with results presented in Table 1. Representative outcomes are illustrated in Figures 7 and 8.

Figure 7. Before treatment.

Figure 8. Eight months after treatment.

Discussion

Adipose tissue represents a viable alternative to traditional fillers, with the added advantage of containing tissue progenitor cells[6]. These cells are capable of replenishing lost tissues by restoring niches that typically decline, particularly after the third decade of life[7], and maintain key factors essential for tissue regeneration[10]. They exhibit multipotent differentiation potential[11], can reprogram resident fibroblasts[4], and induce production of new extracellular matrix (ECM) with physiological collagen deposition and early neovasculogenesis[3].

Recognizing the potential for improved outcomes, we employed a technique involving fragmentation and microfiltration of adipose tissue to preserve tissue progenitor size, combined with brief maintenance under hypoxic conditions prior to implantation. This approach was applied in a single-session volumetric correction of a challenging anatomical area, the periocular region, with results as summarized in the accompanying tables and figures.

Conclusion

This clinical evaluation of case reports demonstrated that correction using fragmented and microfiltered adipose tissue sized between 20 and 40 μm yielded excellent outcomes in terms of periocular tissue texture and volume. The observed effects can be attributed to the selection of viable tissue and the brief hypoxic conditions applied. Specifically, isolating vital micrografts of 20–40 μm eliminates fibrous strands and cellular debris that can trigger inflammatory responses

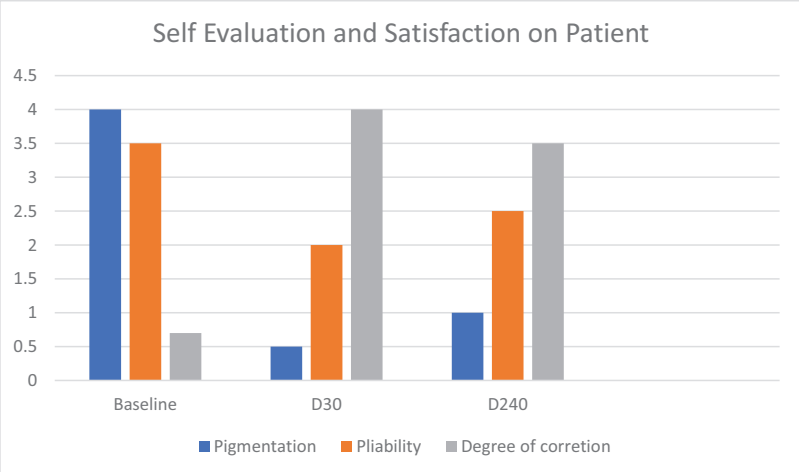

Figure 9. Berardesca's Scale.

via the Toll-like receptor 4 pathway, thereby preserving clusters of tissue progenitors[3,18]. Short-term hypoxia further stimulates exosome release[4,8], contributing to favorable cosmetic results.

Injection of the microfiltrate also promotes regulation of tissue plasticity through autocrine and paracrine signaling pathways[10,16] and mitigates reactive oxygen species (ROS) in the extracellular matrix[11,24]. Moreover, this approach supports the physiological formation of new niches for adult mesenchymal stem cells, enhancing their protective effects[3], prolonging their persistence at the injection site, and facilitating tissue regeneration via paracrine activity and microRNAs released from exosomes derived from tissue progenitors[4,8,20].

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