

# Fat Grafting for Facial Rejuvenation with Nanofat Grafts



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## KEYWORDS

• Nanofat • Needling • Rejuvenation • Stem cell • Regenerative • Antiaging

## KEY POINTS

- Nanofat has no filling capacity. It is a highly concentrated solution of progenitor cells and has no viable adipocytes.
- The mechanical protocol for nanofat preparation is essential. It consists of a 2-step emulsification technique followed by filtration.
- Striking improvement on the skin quality is perceived 6 to 8 months after nanofat grafting. Association of hyaluronic acid, botulin toxin, and vitamin C provides shorter-term enhancement of the skin quality.
- Grafting can be performed by a nanofat intradermal injection or by a microneedling delivery system. Nanofat cream is used as an adjunct after the procedure to further maximize benefit and to moisturize the skin during initial healing.

 Video content accompanies this article at "<http://www.plasticsurgery.theclinics.com>".

## INTRODUCTION

During the past 20 years, lipofilling has emerged as a fundamental component in a facial rejuvenation procedure (Video 1). Currently, facelifts and lipofilling can be almost considered inseparable: facelift techniques address the sagging process, whereas the lipofilling focuses on the deflation. First described in 2001,<sup>1</sup> adipose-derived stem cells (ADSCs) and their therapeutic potential may be the tool researchers and clinicians were looking for to address the third component of the aging process: the structural changes of the skin. For the past decade, these fat tissue cells have shown not only biocellular regenerative potential in vitro

but also striking improvement of the quality of skin in clinical cases.<sup>2-4</sup> Adipose tissue is easily harvested by minimally invasive liposuction and a large number of mesenchymal stem cells are obtained in the lipoaspirate. The wide availability of adipose tissue combined with the straightforward mechanical protocol to process the fat into a highly concentrated solution of progenitor cells brings regenerative medicine into real-life clinical practice. In 2013, Tonnard and colleagues<sup>2</sup> described the procedure to produce so-called nanofat out of harvested microfat by a simple mechanical emulsification.

The mechanical protocol consists of emulsification of the harvested microfat, followed by

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filtering.<sup>2</sup> The result is a whitish liquid with no viable adipocytes but with a large number of good-quality mesenchymal stem cells. Although adipocytes represent 80% to 90% of the volume of the harvested fat, they only represent 25% of the cell count.<sup>5</sup> The remaining 75% is called the stromal vascular fraction (SVF). This contains a large amount of ADSCs, as well as endothelial cells, monocytes, macrophages, granulocytes, and lymphocytes.

The regenerative mechanism by which nanofat works is still not completely understood. Studies suggest that the multiple cell types in the SVF might work in combination to provide a microenvironment where numerous markers come together and trigger the regenerative capacity of the stem cell and of the tissue itself.<sup>4</sup> Recent research has shown that the mechanical shear stress imposed on the fat during preparation of nanofat generates an upregulation of signaling pathways to enhance multipotent and pluripotent stem cells' capacity to regenerate.<sup>6</sup> The results of these complex intercellular crosstalking reactions were reported to range from enhanced collagen deposition and skin elasticity to formation of new blood vessels, tissue remodeling, thickening of the dermis, and downregulation of melanogenic activity.<sup>7-11</sup> This wide range of regenerative properties brings nanofat grafting into focus for both cosmetic and reconstructive surgery.<sup>12</sup>

This article describes the techniques and the authors' experiences in nanofat grafting, as well as its potential new applications in regenerative medicine.

## PREOPERATIVE EVALUATION AND SPECIAL CONSIDERATIONS

With the clinically perceived benefit of nanofat and its applications, the authors are presently offering it to all our patients in whom at least 1 of the following items is present:

1. Trophic skin changes associated with age and photodamage
  - Thin dermis
  - Fine wrinkles
  - Craquelé surface (ie, neck, décolleté, perioral)
  - Mucosal aging (ie, dry lips, genital)
2. Pigmentary conditions
  - Pigmentary changes (ie, face, hands)
  - Dark circles under the eye in cases of thin dermis associated with increased melanin pigment deposits
3. Scarring and atrophy
  - Scars
  - Radiodermatitis.

There are no specific contraindications of its use and only transient yellowish staining is sometimes seen in the first postoperative week, followed by a mild inflammation that can last up to 3 weeks.

Nanofat does not have filling capacity. Thus, the idea in nanofat grafting is injecting regenerative cells and extracellular elements to promote tissue regeneration and remodeling. Clinically, nanofat grafting can be done alone or in association with other rejuvenation procedures (ie, facelift and lipofilling). Indications for nanofat extend beyond facial rejuvenation procedures; areas such as the neck, décolleté, and hands can also benefit from its use. Overall improvement of the quality of the overlying skin can be perceived after 6 to 8 months. Reported changes range from improvement in elasticity, pigmentary conditions, texture, and fine wrinkles.<sup>2,3,8,10-12</sup> Its versatility, simplicity, and safety made nanofat an integral component of the authors' clinical practices.

## SURGICAL PROCEDURE

### *Harvesting*

After infiltration of modified Klein solution (1:1.000.000), microfat is harvested under full-force vacuum aspiration into a sterile canister. The use of a fine 2.4-mm cannula with 20 1-mm sharpened holes (Tulip Medical, San Diego, CA, USA) was associated with an increased rate of ADSCs in the microfat when compared with conventional harvested fat.<sup>13,14</sup> The lipoaspirate is then rinsed with saline or Ringer's lactate over a sterile nylon cloth with 0.5-mm perforations mounted on a sterile canister. After removal of fibrous remnants with a mosquito, the obtained microfat is transferred to 10-mL Luer-Lok syringes.

### *Nanofat Preparation by Intersyringe Shuffling*

The mechanical protocol of nanofat preparation consists of a 2-step emulsification process followed by filtration. The first step is performed by vigorous shifting of the microfat from a full 10-mL syringe to an empty one through a 2.4-mm female-to-female Luer-Lok connector (Tulip Medical, San Diego, CA, USA). After 30 passes, the same process is repeated with a 1.2-mm female-to-female Luer lock connector for another 30 vigorous passes. This progressive emulsification process is important to guarantee that all adipocytes are destroyed. At the end of this emulsification process, a whitish discoloration of the fat is seen. The fat is now passed through a double 400-micron or 600-micron filter (disposable filter or permanent strainer cartridge) to remove connective tissue remnants (Tulip Medical, San Diego,

CA, USA). When nanofat needling is planned, for every 10 mL of nanofat obtained, 1 mL of hyaluronic acid (HA) filler (cost  $\pm$  €80), 100 mg of vitamin C, and 50 units of botulin toxin (cost  $\pm$  €60) are added and mixed together. Preparation of the nanofat cream consists of mixing equal volumes of cream and nanofat.

### **Preparation of Recipient Site**

When performed under local anesthesia, nanofat injections and nanofat needling to the face are well-tolerated after facial nerve blocks using lidocaine 1% (supraorbital, supratrochlear, infraorbital, and mental nerves). For neck and décolleté treatment, topical anesthesia (lidocaine 5% cream applied for 40 minutes before procedure) is usually sufficient. Under general anesthesia, no extra infiltration or block is needed. Marking from the earlier microfat grafting is removed before the needling to avoid transcutaneous tattooing.

### **Combining Treatment Modalities**

Clinically, improvement of the quality of skin after nanofat grafting can be perceived after a minimum of 6 to 8 months. In an attempt to provide a shorter term enhancement of skin quality, the authors used a novel delivery system and used microbotox, skin booster HA, and vitamin C in our clinical practices, with obvious immediate results.

### **Microneedling**

The authors first described the method of delivering nanofat through intradermal injection using 27-gauge needles. Although this approach is very effective for small areas, such as upper lip and eyelids, addressing bigger areas is time-consuming and it may be difficult to deliver the nanofat emulsion evenly throughout the area. Full-face treatment, as well as neck and décolleté treatment, benefit from a different delivery method.

Verpaele and colleagues<sup>15</sup> recently described a novel method of nanofat delivery using a microneedling device (**Fig. 1**), the Hydra Needle 20 (Guangzhou Ekai Electronic Technology Co Ltd, Guangzhou, China). This method not only allows uniform delivery of nanofat in the created microchannels but also associates with the benefits of the microneedling itself. Microneedling is a well-established skin treatment that works by percutaneous collagen induction through activation of a series of growth factors.<sup>16</sup> Combining the regenerative capacity of the nanofat with the additional stimulus of collagen production and scarless healing provided by the needling may work synergistically to optimize results.<sup>12</sup> The device contains 20 1.5-mm needles and is used by repetitive tapping



**Fig. 1.** Hydra Needle 20 (Guangzhou Ekai Electronic Technology Co Ltd, Guangzhou, China) microneedling device. The 8-mL vial has a pump-system that delivers microdroplets of nanofat after each tapping motion. The device's lid contains 20 1.5-mm needles.

motions to create the microchannels. The papillary dermis is reached as reflected by the punctuate bleeding obtained. The pump system in the needling device delivers the content of each bottle (up to 8 mL each) in thousands of tiny droplets of nanofat into the dermis. Needling is normally performed for 20 minutes, the time to empty the 8 mL vial.

### **Microbotox**

The concept of microbotox was introduced by Wu<sup>17,18</sup> in 2001. Instead of acting in deep muscles, the idea of microbotox is to act on the quality of the skin itself. Through a very superficial and uniform technique of injection into the dermis, the botulin toxin acts by decreasing sweat, oil, and sebum production.<sup>18,19</sup> Improvement of the appearance of open pores, acne, rosacea, and fine lines are perceived without paralysis of deep muscles.

With the uniform and superficial delivery made possible by the microneedling device, the authors use botulin toxin to promote the microbotox effect.

Indeed, a faster change of the appearance of the skin was perceived when compared with nanofat delivery only. Thus, the use of botulin toxin provided a short-term result while nanofat regenerative capacities were still not perceivable.

### **Skin booster**

Similarly, the authors use a skin booster HA, Belotero Hydro (Merz Aesthetics, Germany), in the mixture with nanofat, botulin toxin, and vitamin C to obtain a short-term enhancement of skin quality before the effect of the nanofat kicks in. The ability of HA to moisturize the skin by attracting water to the tissues is well-known.<sup>20,21</sup> Also, antioxidant effects of HA have been associated with local stimulation of collagen production and regenerative properties.<sup>22,23</sup>

Clinically, a better overall quality of the skin is perceived shortly after the microneedling procedure. After only 5 to 7 days, petechiae produced by the microneedling are gone and patients experience a difference in their skin quality. The authors think that botulin toxin and HA together, rather than just 1 of them, play a role in these short-term changes in skin condition.

### **Vitamin C**

Vitamin C is also added to the mixture together with nanofat, botulin toxin, and HA filler. In vitro and in vivo studies have shown that, when added into the media, vitamin C enhances the viability, survival, and regenerative potential of ADSCs in a dose-dependent manner.<sup>24–27</sup> Also, it is an antioxidant agent that acts as photoprotection from ultraviolet A and B, treatment of hyperpigmentation, and is an important cofactor in collagen synthesis.<sup>28,29</sup> By delivering all these elements to the skin, the authors aim to address a wide range of the structural changes of the aging skin process.

### **Nanofat cream**

Although channels remain open for 30 minutes after needling,<sup>30</sup> a higher permeability of the skin is reported for up to 40 hours after the procedure (depending on the extent and depth of needling).<sup>31</sup> Therefore, in order to maximize potential benefit from nanofat and the other cofactors, and to moisturize the skin during initial healing, the authors developed nanofat cream, a mixture of 50% of emulsifying cream and 50% of nanofat, which is processed at the end of surgery. Patients are instructed to keep it in the refrigerator and use it 5 to 6 times a day during the healing process (normally 5–7 days).

The emulsifying cream components are

- Emulsifying cetostearyl alcohol type B, 3.6 g
- Macrogol cetostearyl ether, 0.9 g

- White petroleum jelly, 7.5 g
- Liquid paraffin, 3 g
- Purified water ad 50 g.

### **Nanofat Grafting**

For small areas, injection of nanofat is easily performed through fine 27-gauge needles. The aim is to inject it intradermally until a blanching is reached. For dark circles in lower eyelids, the aim is to stay above the orbicularis oculi. Typically, blanching will disappear within the hour after the treatment. In mucosal areas, the submucosal plane is the goal. Injection is performed on withdrawal of the needle in a fan-shaped pattern. The needle can be bent 60° to facilitate manipulation. Typically, 1 mL of nanofat can cover a 1-cm by 1-cm area. A lower eyelid needs an average of 2.5 mL of nanofat.

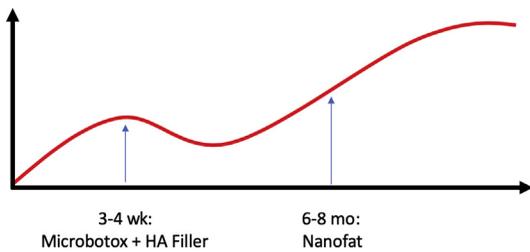
When nanofat needling is planned, quick tapping motions deliver the 8-mL volume of the vial through thousands of microdroplets. The average time to deliver the full volume of the vial is 20 minutes. The authors estimate that the rate of tapping motions performed during needling is 3 per second. Because each tap creates 20 microchannels, 72,000 microchannels are created during the 20-minute treatment. When the full face, neck, and décolleté are addressed, 1 bottle is used for the face only and another is used for the neck and décolleté. At the end of the nanofat needling, a diffuse punctate bleeding shows that the papillary dermis was reached. The nanofat is then left on the skin for another 10 minutes before cleansing the treated areas with saline. After cleansing, nanofat cream is applied.

### **POSTOPERATIVE CARE**

Both nanofat injection with a fine needle and the nanofat needling cause little downtime. In cases of fine-needle injections, minimal yellowish staining should be expected for the first 7 to 10 days and, in cases of needling, erythema, ecchymosis, and petechiae can persist for 5 to 7 days. During this period, patients are advised to use the nanofat cream 5 to 6 times a day. Cream should be kept in the refrigerator in the meantime. With makeup, patients can easily resume their activities 2 days after the procedure.

### **EXPECTED OUTCOME AND MANAGEMENT OF COMPLICATIONS**

With the use of HA filler, botulin toxin, and microbotox, a short-term improvement of skin quality can be perceived after only 2 weeks. The regenerative capacity of the nanofat by itself is expected



**Fig. 2.** Clinical perception of increase in skin quality when combining treatments.

to take 6 to 8 months to be clinically perceivable (**Fig. 2**). Long-term benefit has been observed from the delivery of living SVF cells into the skin.

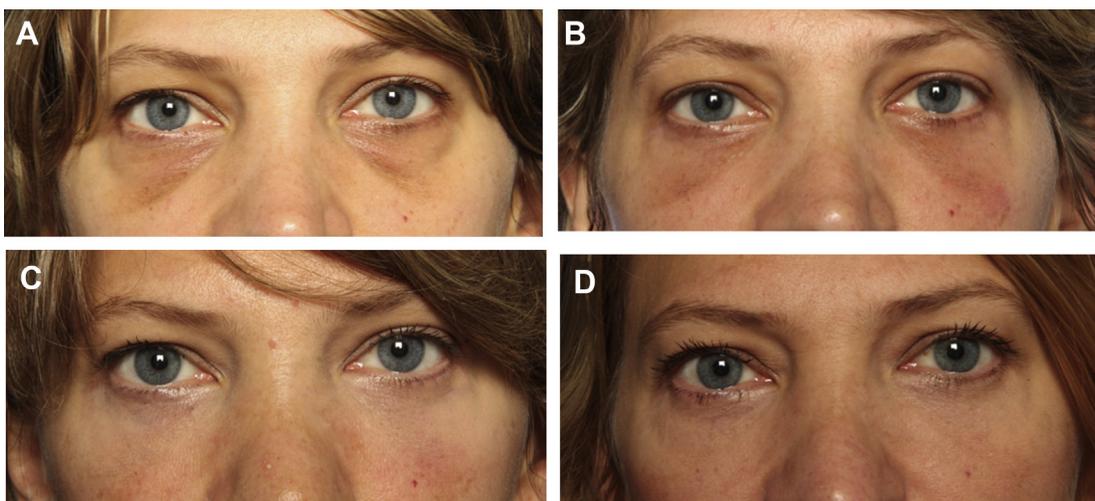
### REVISION OR SUBSEQUENT PROCEDURES

For the treatment of dark circles under the eyes, subsequent procedures may be necessary. In the authors' early series, we had a few cases with persistent yellowish discoloration of the sites of injection for which a corrective procedure with laser resurfacing was needed. This was probably due to inadequate emulsification and incomplete destruction of adipocytes. After the introduction of the 2-step emulsification process followed by filtering, there were no new cases. This mechanical protocol is essential to destroy all the adipocytes and to prevent this complication.

### CASE DEMONSTRATIONS

#### Case 1

See **Fig. 3**.



**Fig. 3.** (A) A 33-year-old patient with dark pigmented circles since childhood. Treatment consisted of a lower fat redraping blepharoplasty through a subciliary approach and 1.6 mL of nanofat injection into each pigmented region. (B) At 4 months postoperatively, moderate erythema is still present. (C) At 7 months postoperatively, redness has disappeared. (D) At 5 years postoperatively, the appearance remains stable. (From Tonnard P. Centrifacial Rejuvenation. Pg 122–23. Thieme: New York. 2017.)

#### Case 2

See **Fig. 4**.

#### Case 3

See **Fig. 5**.

#### Case 4

See **Fig. 6**.

#### Case 5

See **Fig. 7**.

### DISCUSSION

As the awareness of the structural changes of the skin aging process came to light, the new frontier in rejuvenation became the search and development of new tools to address and reverse these changes. Resurfacing procedures, such as ablative lasers and peeling, have been implemented in the practice of many surgeons; however, the risk of persistent erythema, postinflammatory hyperchromia, permanent hypopigmentation, or (even worse) scars may limit their use, especially in Fitzpatrick skin types III to V. Another limiting factor is their considerably long downtime and recovery period.

The wide availability and ease of processing the fat and the striking clinical results after 6 to 8 months of the treatment have stimulated a shift from ablative therapies to a more regenerative approach. Limited side effects and absence of



**Fig. 4.** (A, C) A 41-year-old patient with signs of photodamage. Treatment consisted of upper and lower augmentation blepharoplasty and full-face nanofat needling (with botulin toxin). (B, D) At 12 months postoperatively, noticeable improvement of quality of skin and pigmentation is seen. (From Tonnard P. *Centrofacial Rejuvenation*. Pg 122–23. Thieme: New York. 2017.)

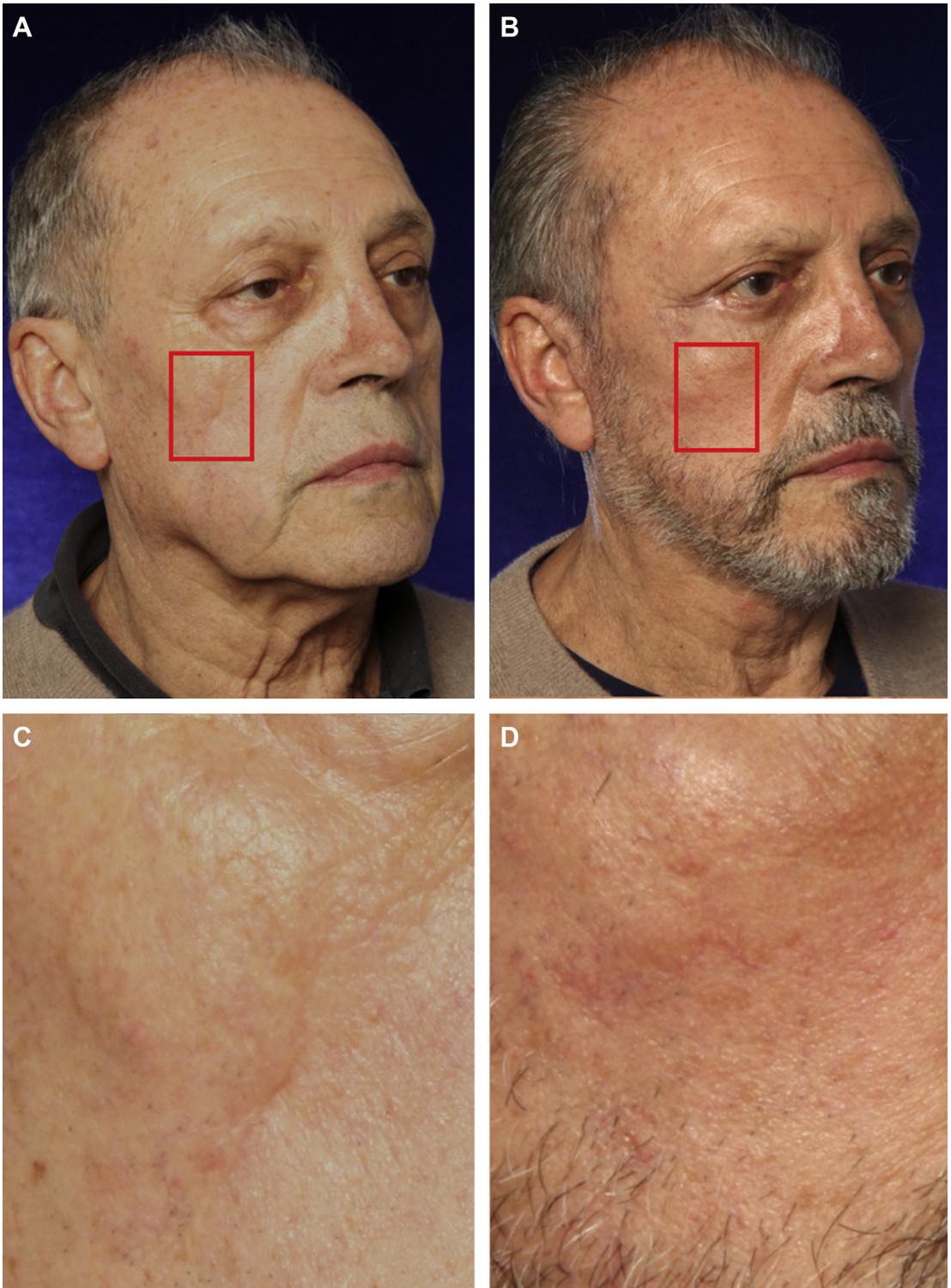
contraindications bring antiaging medicine into real-life clinical practice.

Clinically, the combination of the power of nanofat with the other described treatment modalities has shown an obvious short-term enhancement of the quality of skin. This encourages clinicians

in taking a more proactive posture with regard to the aging process. Either combined with other surgical rejuvenation procedures or alone, nanofat is a tool for plastic surgeons not only looking to reverse structural changes of the aging skin but also looking to prevent them.



**Fig. 5.** (A, C) A 65-year-old patient with craquelé sun-damaged skin in the face and neck. Treatment consisted of a MACS-lift, temporal-lift, centofacial lipofilling, and nanofat needling to face and neck. (B, D) At 6 months post-operatively, noticeable improvement of quality of skin and pigmentation is seen. MACS, Minimal Access Cranial Suspension.



**Fig. 6.** (A, C) A 72-year-old patient with pigmentary changes on face and a deep scar on right malar area. Treatment consisted of a MACS-lift, temporal-lift, augmentation blepharoplasty, and nanofat injections to scar on right malar area. (B, D) At 12 months postoperatively, improvement of quality of skin and pigmentation, as well as softening of the scar is noticeable.



**Fig. 7.** (A) Perioral rejuvenation. A 65-year-old patient with trophic skin changes over the perioral region. Treatment consisted of a MACS-lift and perioral microfat, SNIF, and nanofat injections. (B) At 8 years follow-up, noticeable improvement of quality of skin and is seen. SNIF; Sharp Needle Intradermal Fat grafting.

## SUMMARY

The cumulative regenerative properties of nanofat encourages clinicians to offer patients an annual or biannual boost of regenerative cells to halt or even reverse the structural changes of the aging skin. Nevertheless, further studies and longer follow-ups are needed. The potential new applications of nanofat are vast.

## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at <https://doi.org/10.1016/j.cps.2019.08.006>.

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