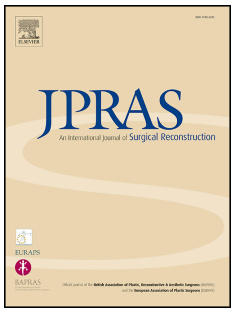
Accepted Manuscript



Facial skin rejuvenation by autologous dermal microfat transfer in photoaged patients: clinical evaluation and skin surface digital profilometry analysis

Angelo Trivisonno, Alfredo Rossi, Massimo Monti, Donato Di Nunno, Christophe Desouches, Claudio Cannistra, Gabriele Toietta

PII: S1748-6815(17)30155-9

DOI: 10.1016/j.bjps.2017.04.002

Reference: PRAS 5288

To appear in: *Journal of Plastic, Reconstructive & Aesthetic Surgery*

Received Date: 5 June 2016

Revised Date: 2 February 2017

Accepted Date: 14 April 2017

Please cite this article as:
Trivisonno, A.; Rossi, A.; Monti, M.; Di Nunno, D.; Desouches, C.; Cannistra, C.; Toietta, G. Facial skin rejuvenation by autologous dermal microfat transfer in photoaged patients: Clinical evaluation and skin surface digital profilometry analysis. Journal of Plastic Reconstructive and Aesthetic Surgery 2017, 70, 1118-1128.
DOI: 10.1016/j.bjps.2017.04.002
http://www.sciencedirect.com/science/article/pii/S1748681517301559

TITLE PAGE

MANUSCRIPT TITLE

Facial skin rejuvenation by autologous dermal microfat transfer in photoaged patients: clinical evaluation and skin surface digital profilometry analysis

AUTHORS

Angelo, Trivisonno[1]; Alfredo, Rossi[2]; Massimo, Monti[1]; Donato, Di Nunno[3]; Christophe, Desouches[4]; Claudio, Cannistra[5]; Gabriele, Toietta[6]

AUTHOR INSTITUTIONAL ADDRESSES

[1] Department of Surgical Science F. Durante, Policlinico Umberto I, University of Rome "La Sapienza", Rome, Italy.

[2] Department of Internal Medicine and Medical Specialties, Clinic of Dermatology, University of Rome "La Sapienza", Rome, Italy.

[3] Department of Medicine, Unit of Dermatology, Military General Hospital Celio, Rome, Italy.

[4] Centre Médical Alliance, Marseille, France.

[5] Department of General Surgery, Bichat C. B. University Hospital, Paris, France.

[6] Department of Research, Advanced Diagnostic and Technological Innovation – Translational Research Area, Regina Elena National Cancer Institute, Rome, Italy.

CORRESPONDING AUTHOR CONTACT

Gabriele Toietta

Department of Research, Advanced Diagnostic and Technological Innovation – Translational Research Area

Regina Elena National Cancer Institute

via E. Chianesi, 53

00144 - Rome, Italy

Phone: ++39-06-52662604 Fax: ++39-06-52662505

e-mail: gabriele.toietta@ifo.gov.it

Presented at the 14th Aesthetic & Anti-aging Medicine World Congress, March 31-April 2, 2016, Monte Carlo, Monaco.

SUMMARY

Cumulative, long-term exposure to solar ultraviolet radiation promotes premature skin aging characterized by wrinkle formation and reduced skin elasticity. In this study, we assessed whether microfat transfer could improve dermal and subcutaneous tissue thickness loss associated with photoaging. Twenty one patients affected by facial photoaging (photodamage grade II-IV; age range 35-62 years; 19 females, 2 males; all of Caucasian origin) were treated using minimally-invasive autologous dermal white fat transfer harvested with a recently designed microcannula. The results were determined by clinical assessment, patient self-evaluation and quantified by the Antera 3D® dermal digital device for non-invasive, objective, reliable and accurate assessment of facial skin texture, color and wrinkle characteristics. Compared with the pretreatment condition, the increment in soft tissue volume and improvement in skin quality and texture was assessed by a dermatologist after treatment. In addition, instrumental evaluation by digital skin profilometry of the treated areas revealed: a 41% reduction in average wrinkle depth ($7.29 \pm 1.04 \times 10^{-2}$ mm pre-treatment vs. $4.31 \pm 1.16 \times 10^{-2}$ mm at 90 days post-treatment; $p < 0.001$), improved skin texture, more homogeneous and uniform skin color and declined facial hemoglobin and melanin concentrations. The majority of patients (above 90%) reported improvements in self-perception. No significant complications were reported throughout the study. In conclusion, by using digital profilometry analysis as an objective and innovative tool to determine the outcome of treatment, we demonstrated that autologous microfat transfer is a safe and well-tolerated procedure with measurable beneficial effects on facial skin aging.

KEYWORDS: Adipose tissue; autologous microfat transfer; dermal digital imaging; facial skin rejuvenation; photoaging; skin aging.

INTRODUCTION

Given the increase in average life expectancy in industrialized Countries, the aging population is rapidly growing. Even if it represents only approximately 8% of total body mass, skin is the most noticeable indicator of age. Facial skin aging is characterized by fine wrinkling and increased skin laxity caused by muscle atrophy and soft tissue volume loss and redistribution.[1] Several factors, including tobacco smoke, excessive alcohol consumption, diet, exposure to chemical substances and environmental pollution have an effect on skin aging.[2] In addition, over time, ultraviolet light exposure induces premature skin damage referred to as photoaging. In particular, ultraviolet radiation A is responsible for increased production of reactive oxygen species (ROS) which cause oxidative damage to cellular components such as DNA, lipids, proteins and mitochondria.[3] Clinical manifestation of skin photoaging includes rhytids, coarse texture, loss of translucency, pigmentation heterogeneities, elastosis and reduced turgor correlated with elastin and collagen degradation, decreased dermal and epidermal thickness owing to dermal cell and fibroblast apoptosis, precancerous lesions, and skin cancer.[3]

The first line of defense against photoaging remains skin protection during sun exposure. Symptomatic treatments include topical administration of retinoids, neuro-modulators, dermal fillers and the use of visible light devices.[4] Lipotransfer is a practice by which autologous fat is harvested from a donor site and subsequently re-injected into areas of soft tissue volume loss.[5] Fat transfer is rapidly becoming an emerging method among the most innovative approaches for facial rejuvenation.[6-8] Compared with administration of synthetic dermal fillers, fat transplant is a safer method to achieve natural-appearing volumetric rejuvenation[6] since the use of autologous material presents no biocompatibility problems.[9] Moreover, implanted adipose tissue exerts some beneficial effects on tissue surrounding the transplant area. How adipose tissue transplantation exerts its beneficial effects on skin quality and texture is not completely understood.[10] Therefore, further studies aimed to define valid means to improve fat graft survival and to evaluate its effects on skin aging are required. A key role in tissue regeneration has been ascribed to soluble factors produced by implanted fat and to the presence of adipose-derived stromal and stem cells (ASCs) able to promote soft tissue remodeling. In fact, adipose tissue has acquired new interest not only as an energy store but also as an endocrine organ and as an accessible source of mesenchymal stromal cells.[11, 12] Adipose tissue has endocrine capacities through

the production of adipokines, which are key metabolism and inflammation mediators. Moreover, adipose tissue contains ASCs, that can activate paracrine signaling through growth factors, cytokines and exosomes which influence the migration, proliferation and homing of different target cells. In particular, paracrine factors produced by ASCs have a role on angiogenesis, protection from oxidative stress, dermal fibroblast and keratinocyte migration, proliferation, collagen synthesis and cellular matrix protein production.[13 14]

Several studies have been performed in order to assess the efficacy of autologous fat transplantation to promote skin rejuvenation.[6, 7] Nonetheless, the results have not always been predictable since the success of the procedure is influenced by many factors, including the site and the techniques used for fat harvesting as well as the method of placement.[15] In addition, the absence of a standardized, objective, non-invasive method to evaluate the outcomes of fat transplant makes it difficult to compare results obtained following different procedures or the effects of similar methods on different patients.[16, 17].

The the ideal site for harvesting adipose tissue suitable for isolation of ASC and/or lipofilling procedures is a relevant but unresolved question.[15] In fact, the yield of isolated ASCs and fat graft survival could be influenced by differences in the structural and metabolic functions of distinct adipose tissue depots[18]. Sbarbati[19] distinguished three types of subcutaneous white adipose tissue characterized by different organization and composition of the lipid and the stromal-vascular fractions: i) deposit, ii) fibrous and iii) structural white adipose tissue. Deposit white adipose tissue is found in large depots in the abdominal area. It works mainly as lipidic storage and it is poorly vascularized. Fibrous fat is situated in regions undergoing mechanical stress, such as the heel. It is characterized by the presence of fibrous components and it has a prominent mechanical function. Structural white adipose tissue is located in superficial areas in the limps and hips. It has a structural function and it is enriched in stromal and vascular components.[19] ASCs mainly derive from perivascular cells anatomically and functionally associated with the blood vessels which are enriched in the more vascularized depots of dermal structural white adipose tissue compared to deeper layers of fat. Dermal adipose tissue could therefore have improved intrinsic regenerative capabilities and the ability to engraft on transplant. Superficial liposuction, which removes the structural fat underlying the reticular dermis defined as dermal white adipose tissue,[20] leads to improved recovery of ASCs compared with conventional suction-assisted liposuction, which removes fat from a deeper layer.[21,22] In

the present work we refined the method of dermal white adipose tissue harvesting using an innovative designed microcannula and then by non-invasive skin profilometry, assessed the efficacy of autologous fat transfer to promote facial skin rejuvenation.

METHODS

Patients

The study protocol was approved by the Institutional Review Board (n°1794/15, 13/02/2015) and was performed in accordance with the principles of Good Clinical Practice expressed in the Declaration of Helsinki. Each subject gave her/his written informed consent to participate in the study.

The study involved 21 patients affected by facial photoaging, displaying photodamage grade II to IV according to the Glogau global photodamage scale (see Supplementary Table 1).[23] The age range was 35-62 years; 19 patients were females and all subjects were of Caucasian origin. Exclusion criteria were: immunological diseases, inflammatory cutaneous diseases, anticoagulant therapies. Patients subjected to permanent fillers, polylactic acid or hyaluronic acid treatment within the previous 12 months were excluded from the study.

Clinical assessment

Patients consulted a dermatologist twice before the treatment and then 30 and 90 days after microfat transplant. During clinical assessments photographic records of the affected areas were acquired and digital imaging skin surface profilometry analysis was performed (see *Skin surface digital profilometry analysis* section). Clinical assessment of the facial area was performed using the following a 3-point scales. Skin roughness score: 1 = mild; 2 = moderate; 3 = severe; skin texture homogeneity score: 1 = slightly inhomogeneous; 2 = uneven; 3 = very uneven; irregular skin melanin pigmentation score: 1 = mild; 2 = moderate; 3 = severe; skin redness score: 1 = mild; 2 = moderate; 3 = severe.

Subcutaneous lipoaspirate harvest and transplant

Lipoaspirate collection sites were located in the thighs and hip regions. Under local anesthesia the collection sites were infiltrated with tumescent solution (adrenaline 0.5 mg, 20 mg lidocaine, physiological solution 250 ml), as previously described. [21] A rounded-tip infiltration cannula, 200 mm-

long, 2.1 mm in diameter, multiperforated (4 round ports with diameter 1 mm, placed in a single row along the side of the distal cannula shaft), was used for adipose tissue harvest (Trivisonno Micro Harvester™, Tulip Medical Products, San Diego, CA) (see Supplementary Figure 1). The cannula was connected by a Luer-Lock to a 5-ml syringe and pushed through the site of access at the most superficial level considered necessary. Negative pressure was then manually applied to the syringe, and 20-30 ml of fat was harvested. Lipoaspirates were allowed to decant for 20-30 minutes, then the oil and aqueous phases were discarded. Autologous lipoaspirate was then diffusely administered in the facial area by intradermal injection using a 1-ml syringe equipped with a 23 G needle. A total of 12-18 ml of tissue was administered in small aliquots in each patient.

Skin surface digital profilometry analysis

Skin surface profilometry was performed using the Antera 3D® multi-spectral analyzer (Miravex Limited, Dublin, Ireland) before treatment (baseline) and 30 and 90 days after microfat transfer. Antera 3D® is a versatile instrument for the accurate measure of several parameters related to skin roughness, including: average wrinkle depth; indentation index of fine lines (lateral size below 1.5 mm), folds (lateral size below 2.5 mm) and wrinkles (lateral size below 5.0 mm); texture roughness of fine lines, folds and wrinkles. Moreover, Antera 3D® assesses by multi-spectral analysis the average concentration and uniformity of melanin and hemoglobin concentrations, determining pigmentation and redness.[24] For consistency in instrument positioning, profilometry measures were performed in all patients on the same side of the face. Subsequently, the identical area was automatically identified and specifically matched in the follow-up images and values calculated by Antera 3D® software.

Questionnaires

Patient questionnaires are considered a valuable tool to measure the success of facial aesthetic procedures.[25] All participants were asked to subjectively evaluate the effects of the treatment on wrinkle soothing with a score ranging from 0 to 4 (0: worsened; 1: poor/no change; 2 moderate; 3: good; 4: excellent). Moreover, any self-evaluated adverse effect was also reported.

Statistical analysis

Results are expressed as means ± standard deviation (SD). Data analysis and comparisons between groups were performed using INSTAT software (GraphPad, San Diego). The significance of differences was assessed with a two-tailed Student's *t*-test for unpaired data. Statistical significance level was set at $p < 0.05$.

RESULTS

Dermal white fat harvesting and autologous facial lipotransfer

A total of 21 subjects with clinical evidence of facial photoaging, including the presence of areas with significant roughness and coarse lines, expressed their willingness to participate in the study. The study flow chart is illustrated in Figure 1. On enrollment, a dermatologist consulted the patients and performed baseline clinical assessments, acquisition of photographic records and digital imaging skin surface profilometry analysis of the affected areas, as described in the Methods section. Patients then submitted to dermal white adipose tissue collection from the hip and thigh regions, as previously described.[21] After decantation, autologous microfat was diffusely distributed in small volumes (less than 0.1 ml per pass) in the facial area via multiple passes by intradermal injection.

Treatment assessment by clinical, profilometric and self-evaluation analysis

Follow-up analysis was performed at 30 and 90 days after autologous dermal white microfat transfer (Figure 1). Throughout the study the same investigators evaluated the outcome in each patient by subjective clinical estimations through observation and palpation of the treated areas and photographic comparison with pretreatment records (Figure 2). Overall, a general increment in soft tissue volume, a reduction in cheek rhytides, and improvements in the overall appearance of facial skin were assessed in all patients, at both analyzed time points. In particular, based on clinical evaluation on a 3-point scale, pre and 90 days post treatment skin parameters taken into account showed a statistically significant improvement ($p < 0.05$; Table 1). In details, 6 patients had a 2-point reduction in the skin roughness scores, 12 patients had a 1-point reduction, and 3 patients were stable. Similarly, skin texture homogeneity improved by 2 points in 7 patients, by 1 point in 12, and was stable in 2.
The significant decline of wrinkle depth compared with pre-treatment values was confirmed by objective analysis with non-invasive skin profilometry analysis and multi-spectral analysis performed

with the Antera 3D® optical skin scanning device at 30 and 90 days after autologous microfat transfer (Figure 3). All patients responded to the treatment with a marked reduction in wrinkles depth beginning at the first time point of analysis, i.e. 30 days after treatment, and further improvement at 90 days (see Supplementary Table 2). In particular, compared with pretreatment values (baseline) a 20% and 40% reduction in average wrinkle depth was assessed 30 and 90 days after autologous fat transfer, respectively (Figure 3; raw data are reported in Supplementary Table 2). The difference between pre- and post-treatment values reached statistical significance (in particular, two-tailed $p < 0.001$). Moreover, improvements in indentation index (Figure 4) and skin texture (Figure 5) were also determined (see Supplementary Table 2). As expected, the reductions of these parameters were more pronounced for fine lines, reaching at the end of follow-up, a reduction of above 25% compared with baseline levels. Nonetheless, a significant modulation of the indentation index and texture were correspondingly determined for folds and wrinkles (Figures 4 and 5).

Irregularities of pigmentation were also, to some extent, recovered. In general, an overall reduction of skin color was assessed during follow-up using Antera 3D® as colorimeter (Figure 1, top panels). In particular, we determined by multi-spectral analysis that the average concentrations of both hemoglobin and melanin in the treated areas were statistically significantly reduced at the end of the follow-up compared with baseline levels (Figure 6 and Supplementary Table 3). These results are in accordance with the clinical evaluation using a 3-point scale, indicating that irregularities in melanin distribution were reduced by 2 point in score in 5 patients, by 1 point in 14 and were stable in 2. Moreover, skin redness was improved considerably (2 point reduction in score) in 3 patients, satisfactorily (1 point reduction) in 17 and was stable in 1 (Table 1).

Postoperative swelling is considered unavoidable in fat transplantation procedure[10] and it was observed to different extents in all of the patients participating in the study. Swelling spontaneously resolved in approximately 2-3 weeks. Four patients reported circumscribed areas of bruising that resolved in 7-14 days. No substantial adverse events or additional significant complications were reported throughout the study, indicating that the treatment was generally safe and well-tolerated.

At the end of the study patients were asked to self-evaluate the appearance of the treated areas. The efficacy of the treatment on wrinkle smoothing was positively (score ≥ 2) deemed by the majority (more than 90%) of the patients, while no one considered the treatment detrimental (score = 0: worsened).

DISCUSSION

The demand for plastic surgery procedures has constantly grown over the years, reaching 15.6 million cosmetic procedures performed in the United States in 2014 according to the American Society of Plastic Surgeons. In particular, from 2013 to 2014 a 5% increase for liposuction and 2% in the use of fat in minimally invasive procedures have been assessed. Despite these rising numbers, the efficacy of fat transplant for facial rejuvenation has been questioned due to the lack of objective corroborating data.[6, 10] As a matter of fact, the outcome of fat transplant is unpredictable, concomitantly depending on many parameters that are difficult to evaluate.[10, 26] For instance, anatomical locations of the fat depots and the methods used for adipose tissue harvest, processing and re-injection can interfere with the success of lipotransfer.[27, 28] In particular, adipose tissue from the superficial adipocyte layer has distinctive metabolic functions and morphological structures compared with the deeper adipocyte layer.[20] In the present study, using a recently designed microcannula, we collected superficial dermal structural white fat, which provides higher yields of adipose-derived stromal cells.[21, 22] The tissue collected by this method can be diffusely injected through a 23 G needle, allowing for precise implantation at the correction site. In addition, administration of small volumes (less than 0.1 ml per pass) of fat and multiple passes ensures improved fat graft take, since implanted tissue is easily vascularized and not exposed to prolonged hypoxia.[10, 29, 30]

Cell-assisted lipotransfer, a procedure in which stromal vascular fraction cells are isolated and then mixed with adipose-tissue before re-injection, has been suggested to improve graft take.[31] However, the process to isolate ASCs from adipose tissue by collagenase digestion cannot be considered as “minimal manipulation” and requires compliance with good manufacturing process technical standards and regulatory limitations.[32] In this respect, lipotransfer is less expensive and time consuming and can be performed in a single surgical procedure.[7] This study provides evidence that lipotransfer restores volume and enhances facial skin texture. The study was designed to evaluate the improvements in autologous dermal white microfat transfer on facial photoaging by means of subjective and objective methods. We followed up the patients for 90 days after the treatment and all of the analysis methods confirmed detectable improvements of skin appearance and texture after the treatment compared with pre-treatment values.

Defining the quality of aging skin and the response to clinical treatment is an extraordinarily challenging task.[33] In fact, there is no broad consensus on the use of a validated facial grading scale

and patient self-assessment is subjective and not always reliable. Other objective methods have been considered, including histologic and microscopic analysis[34] requiring collection of skin biopsy samples and non-invasive procedures such as evaluation of silicone replicas and spectrophotometric analysis.[35] A novel 3D method for fat graft volumetric analysis has been recently reported.[36] In the present study, we describe the use of the Antera 3D® digital device for non-invasive, reliable, and reproducible assessment of facial skin texture, color and wrinkle characteristics in photoaged patients. Antera 3D® has been recently utilized for the evaluation of the efficacy of botulin toxin injection[37] but has never been used for the assessment of photoaged skin in response to autologous lipotransfer treatment. The major advantage of digital skin profilometry is that the method is objective, non-invasive, fast, and it can be easily repeated, providing the possibility for longitudinal analysis of each patient. The Antera 3D® device determines different parameters including size and depths of fine lines, folds and wrinkles; skin texture and color; local hemoglobin and melanin concentrations. Conversely, compared with more invasive approaches, this strategy provides limited information on the molecular and cellular mechanisms through which lipotransfer exerts its beneficial effect on skin texture. Reactive oxygen species generated from oxidative cell metabolism play a major role in photoaging, triggering cellular senescence and inflammation.[38] In this regard, evaluating strategies to counteract oxidative stress may be relevant not only for the treatment of photoaging but also for other ROS-mediated age-related pathologies. Antioxidant activity by secreted factors produced by the transplanted material could play a crucial role in mitigating the effect of photoaging,[14] but additional research is needed to support this hypothesis.

Another limitation of this study is that we have yet to quantify the long-term results of the procedure. As a matter of fact, this study was performed to assess the feasibility, safety and relatively short term outcome of the procedure (Figure 1). Even if longer follow up analysis was not within the main scope of the study, we have been able to include the results of Antera 3D® analysis performed in one patient approximately 1 year after the treatment (Supplementary Figure 2). Although partial, these data suggest that autologous dermal microfat transfer has the potential to promote long-term enhancement of skin quality in photoaged patients. This is in agreement with retrospective review of published results which revealed improvements following fat transfer that lasted as long as 3 years.[39] Since the procedure is safe and well-tolerated the possibility to repeat the process could subsequently be feasible. On this point, recent experimental studies explored the possibility to perform transplantation

of cryopreserved dermal adipose tissue, opening the perspective view of possible repeated administrations of material harvested in a single procedure and cryopreserved in several aliquots for subsequent use.[40]

CONCLUSION

We have provided objective and quantitative data supporting the beneficial anti-wrinkle effect of autologous dermal white adipose tissue transfer in facial skin aging. The procedure is safe so clinical translation of autologous microfat transplant can represent a suitable clinical option for facial rejuvenation supported by evidence-based medicine.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests to disclose.

ACKNOWLEDGEMENTS

The authors would like to thank Giuliana Di Rocco for critical discussion and advice.

FUNDING

This work was supported by a grant to G.T. from Ministero della Salute (RF-2011-02347907) and, in part, by IRE 5x1000.

REFERENCES

1. Donofrio LM. Fat distribution: a morphologic study of the aging face. Dermatol Surg 2000: 26: 1107-12.
2. Kohl E, Steinbauer J, Landthaler M, Szeimies RM. Skin ageing. J Eur Acad Dermatol Venereol 2011: 25: 873-84.
3. Han A, Chien AL, Kang S. Photoaging. Dermatol Clin 2014: 32: 291-9, vii.
4. Stern RS. Clinical practice. Treatment of photoaging. N Engl J Med 2004: 350: 1526-34.
5. Hsu VM, Stransky CA, Bucky LP, Percec I. Fat grafting's past, present, and future: why adipose tissue is emerging as a critical link to the advancement of regenerative medicine. Aesthet Surg J 2012: 32: 892-9.
6. Marten TJ, Elyassnia D. Fat grafting in facial rejuvenation. Clin Plast Surg 2015: 42: 219-52.
7. Charles-de-Sá L, Gontijo-de-Amorim NF, Maeda Takiya C, et al. Antiaging treatment of the facial skin by fat graft and adipose-derived stem cells. Plast Reconstr Surg 2015: 135: 999-1009.
8. Rigotti G, Charles-de-Sá L, Gontijo-de-Amorim NF, et al. Expanded stem cells, stromal-vascular fraction, and platelet-rich plasma enriched fat: comparing results of different facial rejuvenation approaches in a clinical trial. Aesthet Surg J 2016: 36: 261-70.
9. Coleman SR. Structural fat grafting: more than a permanent filler. Plast Reconstr Surg 2006: 118: 108S-20S.
10. Coleman SR, Katzel EB. Fat grafting for facial filling and regeneration. Clin Plast Surg 2015: 42: 289-300, vii.
11. Trivisonno A, Abecassis M, Monti M, Toietta G, Bachir A. Adipose tissue: from energy reservoir to a source of cells for epithelial tissue engineering. In Shiffman MA, Di Giuseppe A, Bassetto F, ed. Stem Cells in Aesthetic Procedures. Springer Berlin Heidelberg, 2014: 303-26.
12. Mailey B, Hosseini A, Baker J, et al. Adipose-derived stem cells: methods for isolation and applications for clinical use. Methods Mol Biol 2014: 1210: 161-81.
13. Kim WS, Park BS, Kim HK, et al. Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. J Dermatol Sci 2008: 49: 133-42.

14. Kim WS, Park BS, Sung JH. Protective role of adipose-derived stem cells and their soluble factors in photoaging. Arch Dermatol Res 2009: 301: 329-36.

15. Geissler PJ, Davis K, Roostaeian J, et al. Improving fat transfer viability: the role of aging, body mass index, and harvest site. Plast Reconstr Surg 2014: 134: 227-32.

16. Yu NZ, Huang JZ, Zhang H, et al. A systemic review of autologous fat grafting survival rate and related severe complications. Chin Med J (Engl) 2015: 128: 1245-51.

17. Zielins ER, Brett EA, Longaker MT, Wan DC. Autologous fat grafting: the science behind the surgery. Aesthet Surg J 2016: 36: 488-96.

18 Wronska A, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. Acta Physiol (Oxf) 2012: 205: 194-208.

19. Sbarbati A, Accorsi D, Benati D, et al. Subcutaneous adipose tissue classification. Eur J Histochem 2010: 54: e48.

20. Driskell RR, Jahoda CA, Chuong CM, Watt FM, Horsley V. Defining dermal adipose tissue. Exp Dermatol 2014: 23: 629-31.

21. Trivisonno A, Di Rocco G, Cannistra C, et al. Harvest of superficial layers of fat with a microcannula and isolation of adipose tissue-derived stromal and vascular cells. Aesthet Surg J 2014: 34: 601-13.

22. Di Taranto G, Cicione C, Visconti G, et al. Qualitative and quantitative differences of adipose-derived stromal cells from superficial and deep subcutaneous lipoaspirates: a matter of fat. Cytotherapy 2015: 17: 1076-89.

23. Glogau RG. Physiologic and structural changes associated with aging skin. Dermatol Clin 1997: 15: 555-9.

24. Matias AR, Ferreira M, Costa P, Neto P. Skin colour, skin redness and melanin biometric measurements: comparison study between Antera(®) 3D, Mexameter(®) and Colorimeter(®). Skin Res Technol 2015: 21: 346-62.

25. Kosowski TR, McCarthy C, Reavey PL, et al. A systematic review of patient-reported outcome measures after facial cosmetic surgery and/or nonsurgical facial rejuvenation. Plast Reconstr Surg 2009: 123: 1819-27.

26. Gir P, Brown SA, Oni G, et al. Fat grafting: evidence-based review on autologous fat harvesting, processing, reinjection, and storage. Plast Reconstr Surg 2012: 130: 249-58.

27. Kaufman MR, Miller TA, Huang C, et al. Autologous fat transfer for facial recontouring: is there science behind the art? Plast Reconstr Surg 2007: 119: 2287-96.
28. Lin JY, Wang C, Pu LL. Can we standardize the techniques for fat grafting? Clin Plast Surg 2015: 42: 199-208.
29. Mashiko T, Yoshimura K. How does fat survive and remodel after grafting? Clin Plast Surg 2015: 42: 181-90.
30. Lindenblatt N, van Hulle A, Verpaele AM, Tonnard PL. The role of microfat grafting in facial contouring. Aesthet Surg J 2015: 35: 763-71.
31. Sterodimas A, de Faria J, Nicaretta B, Boriani F. Autologous fat transplantation versus adipose-derived stem cell-enriched lipografts: a study. Aesthet Surg J 2011: 31: 682-93.
32. Banyard DA, Salibian AA, Widgerow AD, Evans GR. Implications for human adipose-derived stem cells in plastic surgery. J Cell Mol Med 2015: 19: 21-30.
33. Baillie L, Askew D, Douglas N, Soyer HP. Strategies for assessing the degree of photodamage to skin: a systematic review of the literature. Br J Dermatol 2011: 165: 735-42.
34. Hughes MC, Bredoux C, Salas F, et al. Comparison of histological measures of skin photoaging. Dermatology 2011: 223: 140-51.
35. Wheller L, Lin LL, Chai E, et al. Noninvasive methods for the assessment of photoageing. Australas J Dermatol 2013: 54: 290-5.
36. Zhu M, Xie Y, Zhu Y, Chai G, Li Q. A novel noninvasive three-dimensional volumetric analysis for fat-graft survival in facial recontouring using the 3L and 3M technique. J Plast Reconstr Aesthet Surg 2016: 69: 248-54.
37. Cavallini M. Preliminary report on an objective, fast, and reproducible method to measure the effectiveness of botulinum toxin type A. Aesthet Surg J 2015: 35: 715-20.
38. Fisher GJ, Kang S, Varani J, et al. Mechanisms of photoaging and chronological skin aging. Arch Dermatol 2002: 138: 1462-70.
39. Meier JD, Glasgold RA, Glasgold MJ. Autologous fat grafting: long-term evidence of its efficacy in midfacial rejuvenation. Arch Facial Plast Surg 2009: 11: 24-8.
40. Conti G, Jurga M, Benati D, et al. Cryopreserved subcutaneous adipose tissue for fat graft. Aesthetic Plast Surg 2015: 39: 800-17.

FIGURE LEGENDS

Figure 1. Study design flow chart. Long-term follow-up was performed on one patient approximately one year after the treatment (see Supplementary Figure 2).

Figure 2. Macroscopic imaging of facial skin.

General appearance of a portion of the cheek of a selected patient before treatment, 1 month, 3 months and approximately 1 year after autologous microfat transplant.

Figure 3. Autologous dermal microfat facial transfer reduces average wrinkle depth.

Skin profilometry analysis was performed with the Antera 3D® optical skin scanning device at baseline and repeated 1 and 3 months after autologous fat transfer. (**a**) Profilometry analysis of a portion of the cheek of a selected patient before treatment (D0), 30 days (D30), and 90 days (D90) after autologous microfat transplant. The color bar indicates the range of wrinkle depth from minimum (0.00 mm, white) to maximum (0.15 mm, violet). The bar graph (**b**) shows the average wrinkle depth assessed in all 21 patients enrolled in the study. Values are means ± standard deviations and are expressed as percentage relative to baseline (D0) values. An asterisk (*) denotes $p < 0.01$ versus the baseline value (D0) assessed by Student's *t*-test for unpaired data.

Figure 4. Autologous dermal microfat facial transfer reduces indentation index of fine lines, folds and wrinkles.

Skin profilometry by Antera 3D® provides a three-dimensional digital reconstruction of the skin. At baseline areas of analysis (indicated in the grey circles in the figure) were selected. Software-assisted identification of the same area made longitudinal analysis at subsequent time points possible. According to the developer, Antera software accuracy is within a ± 5% margin of error. Profilometry of a selected patient before treatment (D0), 30 days (D30) and 90 days (D90) after autologous dermal white fat transplant for analysis of indentation index of (**a**) fine lines, (**b**) folds and (**c**) wrinkles. The color bar indicates the range of indentation index expressed in arbitrary units from minimum (white) to maximum (violet). The bar graph (**d**) shows the analysis of wrinkle indentation index of all 21 patients enrolled in the study. Values are means ± standard deviations and they are expressed as percentage

relative to the baseline (D0) values. *$p < 0.01$ versus the baseline value assessed by Student's *t*-test for unpaired data.

Figure 5. Autologous dermal microfat facial transfer improves texture and roughness of fine lines, folds and wrinkles.

Profilometry of a selected patient before treatment (D0), 30 days (D30) and 90 days (D90) after autologous dermal white fat transplant for analysis texture of (**a**) fine lines, (**b**) folds and (**c**) wrinkles. The color bar indicates the range of values expressed in arbitrary units from minimum (white) to maximum (violet). The bar graph (**d**) shows the analysis of skin texture values assessed in all 21 patients enrolled in the study. Values are means ± standard deviations and are expressed as percentage relative to the baseline (D0) values. *$p < 0.01$ by Student's *t*-test for unpaired data in comparison with pretreatment (D0) values.

Figure 6. Autologous dermal microfat facial transfer reduces hemoglobin and melanin concentrations in the treated area.

Multi-spectral analysis was performed with an optical skin scanning device at baseline (D0) and repeated 1 (D30) and 3 (D90) months after autologous fat transfer. Figure top panels (**a**) refer to acquisitions for hemoglobin and lower panels (**b**) to melatonin analysis from a selected patient. Grey circles indicate areas of examination, automatically determined by Antera software. The color bars indicate the concentrations expressed in arbitrary units. The range values are 0.0 (white) to 4.5 (violet) for hemoglobin and 0.0 (white) to 1.0 (black) for melanin. Relative concentrations of hemoglobin and melatonin in all 21 patients are represented in the corresponding bar chart graphs (**c**). Values are means ± standard deviations and are expressed as percentage relative to the baseline (D0) values. *$p < 0.05$ by Student's two-tailed *t*-test for unpaired data in comparison with pretreatment (D0) values.

SUPPLEMENTARY DATA LEGENDS

SUPPLEMENTARY TABLE 1. Severity of photoaging according to Glogau scale in all 21 patients enrolled in the study.

SUPPLEMENTARY TABLE 2. Profilometry readings: topography measurements in all 21 patients enrolled in the study.

SUPPLEMENTARY TABLE 3. Multi-spectral analysis: chromophore concentration in all 21 patients enrolled in the study.

SUPPLEMENTARY FIGURE 1. Rounded-tip, multiperforated cannula (200 mm-long, 2.1 mm in diameter) used for adipose tissue harvest (Trivisonno Micro Harvester™, Tulip Medical Products).

SUPPLEMENTARY FIGURE 2. Analysis performed on a selected patient approximately one year after the treatment. Profilometry analysis of indentation index of (**a**) fine lines and (**b**) folds. Multi-spectral analysis of (**c**) hemoglobin and (**d**) to melatonin.

TABLE

Table 1: Clinical assessment graded on a 1–3 point scale

	D0	D90
Skin roughness score	2.33 ± 0.73	1.19 ± 0.40*
Skin texture homogeneity score	2.43 ± 0.68	1.19 ± 0.40*
Irregular skin melanin pigmentation score	2.33 ± 0.58	1.24 ± 0.44*
Skin redness score	2.29 ± 0.64	1.14 ± 0.36*

Average scores ± standard deviation; D0: baseline before the treatment; D90: 90 days after autologous microfat transplant. *$p < 0.05$ versus baseline value assessed by Student's *t*-test for unpaired data.