

Standardized Anatomic and Regenerative Facial Fat Grafting: Objective Photometric Evaluation From 1-19 Months After Injectable Tissue Replacement and Regeneration (ITR2)

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Level of Evidence: 4 (Therapeutic)

Abstract

Background: A standardized technique for facial fat grafting, Injectable Tissue Replacement and Regeneration (ITR²), was developed to address both anatomic volume losses in superficial and deep fat compartments as well as skin aging, incorporating newer regenerative approaches.

Objectives: The authors sought to track the short and long terms effects of a new standardized technique for facial fat grafting in the midfacial zone across a 19-month time period.

Methods: Twenty-nine female were analyzed for mid-facial volume changes after autologous fat transfer with ITR². Across 19 months, volumes were evaluated using the Vectra XT 3D Imaging System to calculate differences between a predefined, 3-dimensional mid-facial zone measured preoperatively and serially after fat grafting with novel approach using varying fat parcel sizes.

Results: Patient data was analyzed collectively as well as separately by age (< and > 55 years). Collective analysis revealed a trend of initial volume loss within the first 1-7 months followed by an increase within the 8–19-month range, averaging 56.6% postoperative gain and ending at an average of 52.3% gain in volume by 14-19 months. A similar trend was observed for patients <55 years of age, but to a greater extent, with a 54.1% average postoperative gain and final average of 75.2%. Conversely, patients above 55 years of age revealed a linear decay beginning at 60.6% and steadily declining to 29.5%. Multiple regression analysis revealed no statistically significant influence of weight change during the study duration.

Conclusions: Preliminary evidence shows a dynamic change in facial volume, with an initial decrease in facial volume followed by a rebound effect that demonstrated improvement of facial volume regardless of patient weight change or amount of fat injected 19 months after treatment. Volume improvement occurred to a greater extent in patients under 55 years old, whereas in patients older than 55 volume gradually decreased. To our knowledge, this study represents the first time that progressive improvement in facial volume has been shown 19 months after treatment with a new standardized technique of fat grafting.

Since its first reported description in 1893 by Neuber, Autologous Fat Grafting (AFG) has undergone several advancements in both its procedural methodology and biological understanding¹. For the majority of the early 1900s, fat grafting was primarily confined to treating specific facial deficits including malar region and chin¹. By the 1980s, AFG was introduced to aesthetic surgery by a number of individuals such as Illouz who utilized injectable fat grafting following liposuction, and Ellenbogen who used it to treat facial atrophy wrinkles, nasolabial folds, and chin augmentation²⁻⁶. The basis for AFG was standardized by Coleman, who defined specific steps and equipment for harvesting, centrifugation, cleaning, and injecting microfat to the face. Additionally, Coleman and Grover outlined the basic findings of aging including decreased skin elasticity, bone resorption and remodeling, tissue atrophy, and ptosis⁷⁻⁸. The phenotypic effects of aging have led many practitioners to incorporate AFG in facelift procedures to counter volume loss in soft tissue and bone⁹. Biological advancements for AFG were largely attributed to the discovery of stem and regenerative cells in adipose tissue by Zuk et al in 2001 and confirmed by Rigotti's observations of neo-angiogenesis and histological signs of reversal of architectural changes of aging in elastin and collagen by a mechanically obtained stromal vascular fraction and expanded mesenchymal stem cells¹⁰⁻¹¹. These findings coupled with the detailed three-dimensional (3D) description of anatomical facial fat compartments by Rohrich and Pessa led to the development of a new standardized technique that extends Coleman's report¹².

Injectable Tissue Replacement and Regeneration (ITR²) extends the technique described by Coleman and incorporates a novel treatment that uses varying fat parcel sizes to address losses in deep fat and bone, superficial fat, and to stimulate regeneration in skin.¹³ ITR² strategically utilizes millifat (2 mm parcel size) as structural fat parcels in the deep compartments, microfat (1 mm parcel size) as smaller parcels for superficial compartments, and a cell optimized nanofat (500 micron parcel size) as mechanical stromal vascular fraction product for skin regeneration applied intradermally or as a biological cream. With the use of facial topography and proportion analysis, individual-specific treatment can be achieved addressing not only the 2-dimensional dermal and the superficial musculoaponeurotic system (SMAS) fascial layers, but also the 3-dimensional volume loss in both superficial and deep structural compartments of the face. At the same time, again with the patient's fat, skin aging is improved by combinations of nanofat microneedling, intradermal injection and topical application of nanofat biocream.

Presently, the most popular means of facial volume restoration is with a variety of synthetic fillers with some limited biological effects. Fat is not a substitute for fillers, but rather a foundational approach in facial aging to address specific anatomic losses and regenerate skin. Prior to the introduction of microfat, millifat, and nanofat, fillers were the only means of contouring both fine lines and larger atrophic fat deficits¹⁴. Autologous fat transfer may not only reverse the effects of facial volume loss, but also may regenerate blood supply to sustain the longevity of the tissue¹⁵⁻¹⁶. Unfortunately, a major factor in the results of such procedures could be patient age as endothelial dysfunction, which leads to decreased angiogenesis, steadily increases over time. Moreover, adipose cells, SVF fraction cells and adipose stem cells eventually become senescent and lose some of their effects with age.¹⁸

Previously, we reported progressive improvement in mid-facial volume, up to 24 months following ITR² when combined with facelift surgery¹⁹. The basis of the present study is to evaluate the effects of this standardized anatomic and regenerative technique on patients receiving solely facial fat grafting. Accordingly, a 3D photometric analysis was used to prospectively track mid-facial volume changes over a 19-month period in 29 patients undergoing ITR².

The objectives of this report are to describe a new standardized technique of facial fat grafting that incorporates anatomic replacement of lost fat and bone as well as regeneration of facial tissues and determine its effect on mid-facial volume using photometric analysis. The paper attempts to demonstrate how topographical analysis of the face can be used to determine precise areas of volume loss from skin to bone.

METHODS

We prospectively evaluated mid-facial volume in 29 female patients from 1 to 19 months after ITR² using 3D Photometric Imaging (Canfield Scientific Inc., Oarsippany, NJ), between February 2017 to February 2020. A consent form, subject's bill of rights, and media authorization form were obtained from all patients in accordance with the Declaration of Helsinki. 3D analysis of the mid-facial region was chosen because this area of fat grafting received the largest volume of the three different fat grafts and was the same area studied in our earlier report combining fat grafting with facelift surgery¹⁸. After tumescent fluid was injected, the fat was harvested from the inner and outer thighs and/or flanks and abdomen with a 2.7 mm diameter cannula with hole sizes of 2x1mm (Khoury cannula, Marina Medical, Stuart, FL) inserted through a dilated, 14-gauge needle puncture. The fat was rinsed with Ringer's lactate and decanted. The fat was then made into 3 product sizes: millifat, microfat, and a cell optimized, nanofat using LipocubeNanoTM (Lipocube, Inc. London, UK)²⁰. Based on topographical analysis of the face, millifat grafting was performed as indicated into the deep fat compartments, pre-periosteal level in the pyriform aperture, zygoma and maxilla, the medial and lateral sub-orbicularis oculi fat (SOOF) compartments, and into the deep medial cheek fat compartment utilizing an 18-gauge side port cannula through an 18-gauge needle incision in the nasolabial fold. Millifat was also grafted into the buccal space, using an 18 Gauge needle incision at the oral commissure and tunneling the 18 Gauge cannula submucosally into the buccal fat compartment, Millifat was also used in the deep temporal region and the pre-periosteal lateral brow as well as into the upper and lower lip, chin, mandibular border and gonial angle, when necessary, based on topographical analysis of areas of fat and bone loss. In our procedures, the mid-facial grafting was relatively consistent. Microfat was injected into the superficial compartments of the mid-face as needed and Nanofat was microneedled throughout the mid-facial region as well as the entire face, neck and chest as indicated. Lastly, a Nanofat biocreme made by centrifugation of the Nanofat, removal of excess fluid and compounding with a liposomal transport agent along with arnica and a cucumber smell, was given to the patient on discharge to be kept refrigerated and used two to three times a day until it was gone.²¹

Mid-facial volume was measured preoperatively and postoperatively with the Vectra XT 3D Imaging System (Canfield Scientific Inc., Parsippany, NJ). Similar to our earlier reports¹⁹, pre- and postoperative photos were overlaid and aligned according to consistent anatomical points and rigid structures of the face that remained invariable over time. (Fig. 1) Once the photos were overlaid, volume changes in the mid-facial zone were measured. The

lateral portion of the nasolabial fold, the inferior border of the zygomatic arch, and the superior border of the mandible anatomically defined the perimeter of the buccal space.

Facial volume was measured at different intervals from 1 to 19 months in all 29 patients. In addition, facial volume data was evaluated over time in patients <55 years of age (n=15) and >55 years of age (n=14). As in our previously published study¹⁸ individual patient measurements could not always be collected at consistent time periods. Therefore, in order to track average mid-facial volume changes over the 19-month period, the volumetric data from each of these three groups was further subdivided into four month-categorized subgroups: (1) 1-3 months, (2) 4-6 months, (3) 7-13 months, (4) 14-19 months. A 2-tailed, repeated measures t-test was conducted for each subgroup. Statistical significance was defined as $P < 0.05$. In addition, multiple regression analysis was conducted to measure how age, BMI, weight change during the study period, initial fat in ml injected, and months since the operation may have influenced volume over time.

RESULTS

Patients ranged in age from 38 to 70 years (average= 52.9 years). All of the patients were females. The average preoperative weight of patients was 145.3 pounds, and the average preoperative BMI was 22.5 kg/m². Patients' weight change was negligible, averaging 0.31 pounds gained postoperatively. 3D analysis of volumetric changes in the mid-facial region of the 29 patients revealed an improvement in facial volume at 12 to 19 months. Postoperative facial volume improvements over preoperative volume measurements averaged 56.6% at the 1 – 3-month range. By 4 – 7-months, improvement in midfacial volume dropped to an average of 32.1%, and then steadily increased to 46.6% by the 8 – 13-month period. By the 14–19-month time period, the average leveled off at about 52.3% (Fig. 2). 3D photo measurements revealed that all patients experienced an increase in midfacial volume from the preoperative volumes at some point during the study period. In the analyzed midfacial zones, facial volume appeared to initially decline (average decline, 56.6% of original midfacial volume), troughing in the 4 - 13-month range, but later increased (average increase in volume retention, 52.3% of original midfacial), peaking at around 14 months (range, 7-19 months). Parametric tests proved that the observed decline within the 4-7-month range was statistically significant when compared to the 1–3-month subgroup ($P < 0.05$). The 2-tailed repeated measures t-test for all 4 month-categorized subgroups revealed that all average volumes were good indicators of central tendency within each group ($P < 0.05$). Human error in 3D

photometry was calculated to be 0.2187 mL. No surgical complications occurred in any of these 29 patients.

When separating patients based on age (above and below 55 years), two different trends were observed (Fig. 3). Patients under 55 years of age exhibited the same dynamic changes that the collective analysis demonstrated, but to a greater extent. The midfacial volume initially declined (average decline, 54.1% of original midfacial volume) in the 1–3-month group, troughing at 6 months (range 4–13 months), but later increased (average increase, 75.3% of original midfacial volume), peaking at around 14 months (range, 7-19 months). 95% confidence intervals (CI) for each month subgroup were calculated and it was found that the observed decline in the 4–6-month range was unique and excluded from both the first and last month subgroups' CIs (4–6-month, CI = 9.67% - 31.9%). Patients above 55 years of age, on the other hand, exhibited a linear decay beginning with initial volume retention averaging 60.6% and steadily declining to 29.5% by the 14–19-month range. The 2-tailed repeated measures t-test for all 4 month-categorized subgroups revealed that all average volumes were good indicators of central tendency within each group ($P < 0.05$). CIs for this group steadily declined initially indicating a 95% confidence between 20% to 94% volume retention in the 1-3 month period and dropping to 14% to 41% volume retention by the 13-19 month period. Additionally, the final month-categorized subgroup (14-19-month) for the <55 age group and >55 age group revealed a statistically significant difference in average midfacial volume retention.

The multiple regression analysis measuring the effects of age, BMI, weight change, initial fat volume in ml injected, and months since procedure revealed that none of these factors played a significant role in average volume retention at the 14–19-month range when evaluating all patients together ($n=29$). Similar results were obtained with multiple regression analysis when selectively evaluating patients <55 or >55 years of age.

DISCUSSION

Previous studies conducted by our group have observed the effects of utilizing anatomic and regenerative fat grafting in combination with deep plane facelifts.¹⁹ However, no study of isolated facial fat grafting using this standardized technique (ITR²) has been reported. To our knowledge, this study is first to prospectively evaluate dynamic changes in midfacial volume using 3D photometry after standardized anatomic, tissue plane specific regenerative fat grafting. Present standardized techniques for facial fat grafting have generally been designed to provide mere facial volume augmentation. Observations on skin regeneration, resulting in

rejuvenated appearance, have been reported, but often as a by-product of the fat graft rather than a primary concern. Potential regenerative effects that have not been mentioned are restoration of the “functional matrix”, which in theory may have beneficial effects on craniofacial bone aging. In addition, progressive improvement of midfacial volume in <55-year-old patients 19 months after treatment may indicate a “trophic effect” on the facial tissues that supports the idea that facial tissue atrophy has been reversed to some extent, at least for a period of time, actually temporarily reconstructing youthful tissues.²²

Interestingly, our clinical data may offer some insight into fat graft remodeling and survival. Given that mid-facial volume is likely to be a reflection of fat graft survival, it's dynamic changes over 19 months with a gradual loss of facial volume and then what appeared to be a recovery, especially in the under 55 year age group, appears to support Yoshimura's graft replacement theory.³⁰⁻³² Several theories have been presented pertaining to the survival of fat grafts, particularly the host replacement theory, the cell survival theory, and more recently, the graft replacement theory.

The first theory for fat graft survival was the host replacement theory reported in 1923 by Neuhof and Hirschfiel²³⁻²⁵. This theory suggested that the grafted adipose tissue immediately dies upon transplantation and subsequently becomes the scaffolding for recruitment of host adipose and connective tissue cells. The cell survival theory was postulated by Peer in 1950, and assumes that grafted adipocytes are able to survive transplantation by simple diffusion, competing for more favorable positions within the host before microvascular anastomoses occur.²⁶⁻²⁷ The findings of Peer have been bolstered by a number of other researchers, but more recent discoveries prompted Yoshimura to propose a new theory that focuses on the role of adipose-derived stem cells²⁸⁻³⁰. The graft replacement theory, proposed by Yoshimura, connects the cell survival theory with the findings of Zhao et al, who discovered that grafted fat eventually survives through neovascularization²⁸. Although, Yoshimura found that it was only a small set of adipocytes that undergo neovascularization and neo-angiogenesis as the majority of the cells die due to the hypoxic environment of the transplantation site. It was postulated that only adipose-derived stem cells are able to survive and upon differentiation, subsequently are able to replace the dead adipose cells³⁰⁻³².

The graft replacement theory is also supported by Kato et al. description of the three graft zones that determine the differential fates of adipocytes. The first zone was termed the surviving zone, which is the superficial layer of the fat graft and adjacent to the host tissue, measuring only 100 to 300 µm in thickness. In this zone, both adipocytes and adipose-derived

stem cells are able to survive via plasmatic diffusion as suggested by the cell survival theory. The second zone was termed the regeneration zone, where all grafted adipocytes die, but the adipose-derived stem cells which are tolerant of low oxygen tensions, survive. The final layer was labeled the necrotizing zone as both adipocytes and adipose-derived stem cells are not viable.³³⁻³⁵ Zone modeling of fat grafts (microribbon, fluid accommodation, external volume expansion models) introduced by Khouri et al. linking fat graft survival theories to the common ground of oxygen diffusion and graft perfusion essentially reaffirms Kato et al. hypothesis⁴¹. Graft dispersion increases the coefficient of graft cells – host tissue contact, therefore, the technique with the most dispersed graft form (nanofat) delivered to the least perfused layer (skin) is logical as it potentially increases fat survival.

Through clinical observation using 3d photometry, we provide data that appears to support the graft replacement theory and kin part the host replacement theory. The initial decline from 56% to 32% volume retention observed in the 1-6-month follow-up period for total patients falls in line with the mouse model observations made by Yoshimura. According to Yoshimura, the first 3 months following the injectable fat transfers are demarcated by the replacement of dead adipocytes by adipose-derived stem cells and by the slow lipid absorption of adipose tissue that may persist for up to 12 months. These same results are evident when observing the <55 age group data that saw a similar decline from 54% to 21% in the 1 - 6-month groups. The initial volume observed in the 1 - 3-month groups could be attributed to necrotic adipose tissue and latent swelling and the steady decline in the following months is most likely a result of the lipid droplet absorption that was described as potentially occurring for up to 12 months. The wave of volume restoration observed in the 7 - 14+ month time period is a novel discovery yet to be observed in other research. However, these studies did not include adequate control groups and it was postulated that implied superiority of stem cell-assisted (enhanced) lipotransfer over traditional lipofilling should be investigated more and this is why standardization of techniques (including that offered by our methodology) is important.³⁶⁻³⁷

In the retrospective study conducted by Wang et al., 10 clinical studies using various methods of fat processing for autologous fat transfers were compared to observe the differing degrees of volume retention in a 12-month period. Of the selected studies, all exhibited linear decay of volume from the 1–3-month period to the 6–12-month period, similar to the trend observed for >55 patients³⁸. No other studies were found that depicted the subsequent increase trend of volume retention that we observed in the cumulative patient analysis and in the <55 patient analysis.

While one of the strengths of this research was an age range-balanced study population with n=15 patients below 55 years of age and n=14 patients above 55 years of age, one of the limitations to the study is the small sample size (n=29) which was all female. The use of a larger study population as well as the incorporation of male patient data could result in more definitive results. Possible age-differential in adipose cell fat behavior and regenerative capability validates the Hayflick limit concept with cells division potential ceasing once telomeres shorten to a critical length. Stem cells seem to be endowed with an increased proliferative potential than differentiated cells but with general aging up-regulation of telomerase activity (alternative lengthening of telomeres dependent on homologous recombination).³⁹ Molecules secreted by adipocytes and adipose tissue derived stem cells (secretome) are necessary for tissue remodeling and regeneration. The predisposition of age, inflammation, stress and genotoxic induced senescence, more persistent and perhaps more difficult to reverse in seniors, may result in progression of signs of aging. The quality and perhaps quantity of adipocytes secretome allows adipocytes to influence surrounding tissues (e.g., improve quality of overlying skin).^{22,40} Consequently, concepts of delay or reversal of senescence and aging in general, including the fate of lipotransfer, may apply or focus on different components of tissue homeostasis.²²

Additionally, inconsistent time periods for volume retention measurements across individual patients could be a limitation as individual progression could not be tracked steadily across the entire 19-month study. In order to counter the inability to measure all patients at consistent time periods, patients' volumes were inputted into four month-categorized subgroups which were subsequently compared to each other. Another limitation to the research could be the applied methodology for calculating volume differences in the predefined midfacial zones of patients. Consistency between calculated values was maintained by assigning groups of patients to individual researchers and by ensuring each measurement was taken following an exact protocol.

CONCLUSION

Two important findings were seen in this study. Using a similar fat grafting protocol (ITR²), anatomic losses were addressed and a cell-optimized nanofat was used for regenerative effects. Three types of fat parcels were made: Millifat which was a 1.5-2 mm parcel, microfat, a 1 mm parcel and cell optimized nanofat which was a 500-micron product. This made sense based on using smaller parcels closer to the surface so they would not show and larger, more structural grafts under the muscles and on the bone. Most working in the field acknowledge that higher doses of regenerative cells and growth factors are associated with more effects. Hence, the use of a cell optimized nanofat which is purely for skin regeneration and has no augmentation effects. The two findings were that there seemed to be less improvement in mid-facial volume in patients over 55 years of age. Some of this may have been related to soft tissue laxity as well as to gradual loss of the effects of the fat graft. In younger than 55-year-old patients, improvement in mid-facial volume was actually close to 80% at 14-19 months. We speculate that this may be related to using younger tissues and cells. The use of this anatomic and regenerative fat grafting technique has led to high patient and surgeon satisfaction rates.

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REFERENCES

1. Van de Graaf RC, Korteweg SFS. Gustav Adolf Neuber (1850–1932) and the first report on fat auto-grafting in humans in 1893. *Hist Plast Surg*. 2010;1(1):7–11.
2. Illouz YG. L'avenir de la réutilisation de la graisse après liposuccion. *Rev Chir Esthétique Lang Fran*. 1984;36: 13–14.
3. Agris J, Illouz YG, Pitanguy I. *Liposuction: The Franco- American Experience*. Medical Aesthetics, Incorporated. *Am J Cosmet Surg*. 1987;(4)2:89–94.
4. Illouz YG. The fat cell “graft”: a new technique to fill depressions. *Plast Reconstr Surg*. 1986;78(1):122–123.
5. Illouz YG. Present results of fat injection. *Aesthetic Plast Surg*. 1988;12(3):175–181.
6. Ellenbogen R. Fat transfer: current use in practice. *Clin Plast Surg*. 2000;27(4):545-556. PMID: 11039888
7. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg*. 1995;19(5):421–425. <https://doi.org/10.1007/s00266-020-01847-3>
8. Coleman SR. The technique of periorbital lipoinfiltration. *Oper Tech Plast Reconstr Surg*. 1994;1(3):120–126. [https://doi.org/10.1016/S1071-0949\(10\)80002-2](https://doi.org/10.1016/S1071-0949(10)80002-2)
9. Guerrerosantos J. Simultaneous rhytidoplasty and lipoinjection: a comprehensive aesthetic surgical strategy. *Plast Reconstr Surg*. 1998;102(1):191–199. DOI: 10.1097/00006534-199807000-00032
10. 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(3):261–270. <https://doi.org/10.1093/asj/sjv231>
11. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211–228. <https://doi.org/10.1089/107632701300062859>
12. Rohrich RJ, Pessa JE. The fat compartments of the face: anatomy and clinical implications for cosmetic surgery. *Plast Reconstr Surg*. 2007; 119:2219–2228. doi: 10.1097/01.prs.0000265403.66886.54
13. Coleman SR, Grover R. The anatomy of the aging face: volume loss and changes in 3-dimensional topography. *Aesthet Surg J*. 2006;26(1S):S4–S9. <https://doi.org/10.1016/j.asj.2005.09.012>
14. Winters R, Moulthrop T. Is autologous fat grafting superior to other fillers for facial rejuvenation? *Laryngoscope*. 2013;123(5):1068–1069. <https://doi.org/10.1002/lary.23614>

15. 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(3):261–270. <https://doi.org/10.1093/asj/sjv231>
16. 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(4):999–1009. doi: 10.1097/PRS.0000000000001123
17. Matz, R.L., Andriantsitohaina, R. Age-Related Endothelial Dysfunction. *Drugs Aging* **20**, 527–550 (2003) <https://doi.org/10.2165/00002512-200320070-00005>
18. Cohen SR, Fireman E, Hewett S, Saad A. Buccal fat pad augmentation for facial rejuvenation. *Plast Reconstr Surg*. 2017;139(6):1273e-1276e. DOI: <https://doi.org/10.1097/PRS.00000000000003384>
19. Cohen SR, Hewett S, Ross L, Fischer M, Saad A, Teubel S, Delaunay F. Progressive Improvement in Midfacial Volume 18 to 24 Months After Simultaneous Fat Grafting and Facelift: An Insight to Fat Graft Remodeling. *Aesthet Surg J*. 2020 Feb 17;40(3):235-242. doi: 10.1093/asj/sjy279. PMID: 30335128. <https://doi.org/10.1093/asj/sjy279>
20. Steven R Cohen, MD, FACS, Tunç Tiryaki, MD, Hayley A Womack, BS, Serli Canikyan, BS, Kai Uwe Schlaudraff, MD, Michael Schefflan, MD, Cellular Optimization of Nanofat: Comparison of Two Nanofat Processing Devices in Terms of Cell Count and Viability, *Aesthetic Surgery Journal Open Forum*, Volume 1, Issue 4, December 2019, ojz028, <https://doi.org/10.1093/asjof/ojz028>
21. Cohen SR, Goodacre AK, Womack H, Delaunay F, Wood D, Wesson T, Tiryaki T. Topical Nanofat Biocrème Improves Aesthetic Outcomes of Nonablative Fractionated Laser Treatment: A Preliminary Report. *Aesthet Surg J*. 2020 Jul 13;40(8):892-899. doi: 10.1093/asj/sjz240. PMID: 31504170.
22. Galanin I, Nicu C, Tower JI. Facial Fat Fitness: A New Paradigm to Understand Facial Aging and Aesthetics. *Aesthetic Plast Surg*. 2021 Feb;45(1):151-163. doi: 10.1007/s00266-020-01933-6. Epub 2020 Sep 10. <https://doi.org/10.1007/s00266-020-01933-6>. PMID: 32914326.
23. Gause TM 2nd, Kling RE, Sivak WN, Marra KG, Rubin JP, Kokai LE. Particle size in fat graft retention: a review on the impact of harvesting technique in lipofilling surgical outcomes. *Adipocyte*. 2014;3(4):273–279. <https://doi.org/10.4161/21623945.2014.957987>
24. Patel AJ, Benson JR, Malata CM. Chapter 29: The science of autologous fat grafting. In: Querci della Rovere G, Benson JR, Nava M, eds. *Oncoplastic and Reconstructive Surgery*

- of the Breast. Boca Raton, FL: CRC Press; 223–233. <http://dx.doi.org/10.3109/9781841847610-30>
25. Neuhof H, Hirshfeld S. *The transplantation of tissues*. New York, NY: Appleton, 1923.
 26. Peer LA. loss of weight and volume in human fat grafts: with postulation of a cell survival study. *Plast Reconstr Surg*. 1950;5(3):217–230.
 27. Peer LA. Cell survival theory versus replacement theory. *Plast Reconstr Surg (1946)*. 1955;16(3):161–168.
 28. Zhao J, Yi C, Li L, et al. Observations on the survival and neovascularization of fat grafts interchanged between C57BL/6-gfp and C57BL/6 mice. *Plast Reconstr Surg*. 2012;130(3):398e–406e. doi: 10.1097/PRS.0b013e31825dbfd3
 29. Doi K, Ogata F, Eto H, et al. Differential contributions of graft-derived and host-derived cells in tissue regeneration/remodeling after fat grafting. *Plast Reconstr Surg*. 2015;135(6):1607–1617. doi: 10.1097/PRS.0000000000001292
 30. Suga H, Eto H, Aoi N, et al. Adipose tissue remodeling under ischemia: death of adipocytes and activation of stem/progenitor cells. *Plast Reconstr Surg*. 2010;126(6):1911–1923. doi: 10.1097/PRS.0b013e3181f4468b
 31. Eto H, Kato H, Suga H, et al. The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. *Plast Reconstr Surg*. 2012;129(5):1081–1092. doi: 10.1097/PRS.0b013e31824a2b19
 32. Yoshimura K, Eto H, Kato H, Doi K, Aoi N. In vivo manipulation of stem cells for adipose tissue repair/reconstruction. *Regen Med*. 2011;6(6 Suppl):33–41. <https://doi.org/10.2217/rme.11.62>
 33. Kato H, Mineda K, Eto H, et al. Degeneration, regeneration, and cicatrization after fat grafting: dynamic total tissue remodeling during the first 3 months. *Plast Reconstr Surg*. 2014;133(3):303e–313e. doi: 10.1097/PRS.0000000000000066
 34. Doi K, Ogata F, Eto H, et al. Differential contributions of graft-derived and host-derived cells in tissue regeneration/remodeling after fat grafting. *Plast Reconstr Surg*. 2015;135(6):1607–1617. doi: 10.1097/PRS.0000000000001292
 35. Pu LL. Mechanisms of Fat Graft Survival. *Ann Plast Surg*. 2016;77(Suppl 1):S84–S86. doi: 10.1097/SAP.0000000000000730
 36. Gaur M, Dobke M, Lunyak VV. Methods and Strategies for Procurement, Isolation, Characterization, and Assessment of Senescence of Human Mesenchymal Stem Cells from Adipose Tissue. *Methods Mol Biol*. 2019;2045:37–92. doi: 10.1007/7651_2018_174. PMID:

30838605. https://doi.org/10.1007/7651_2018_174

37. Trojahn Kølle SF, Oliveri RS, Glovinski PV, Elberg JJ, Fischer-Nielsen A, Drzewiecki KT. Importance of mesenchymal stem cells in autologous fat grafting: a systematic review of existing studies. *J Plast Surg Hand Surg*. 2012 Apr;46(2):59-68. doi: 10.3109/2000656X.2012.668326. <https://doi.org/10.3109/2000656X.2012.668326>
38. Wang GH, Zhao JF, Xue HY, Li D. Facial aesthetic fat graft retention rates after filtration, centrifugation, or sedimentation processing techniques measured using three-dimensional surface imaging devices. *Chin Med J (Engl)*. 2019;132(1):69-77. doi:10.1097/CM9.0000000000000016
39. Shay JW, Wright WE. Hayflick, his limit, and cellular ageing. *Nat Rev Mol Cell Biol*. 2000 Oct;1(1):72-6. doi: 10.1038/35036093. PMID: 11413492.
40. Lunyak VV, Amaro-Ortiz A, Gaur M. Mesenchymal Stem Cells Secretary Responses: Senescence Messaging Secretome and Immunomodulation Perspective. *Front Genet*. 2017 Dec 19;8:220. doi: 10.3389/fgene.2017.00220. PMID: 29312442; PMCID: PMC5742268.
41. Khouri RK, Khouri RE, Lujan-Hernandez JR, Khouri K, Lancerotto L, Orgill DP. Diffusion and Perfusion: The Keys to fat Grafting. *Plast Reconstr Surg Glob Open*, 2014;2:e220 doi: 10.1097/GOX.0000000000000183
42. Gaur M, Amaro-Ortiz A, Dobke M, Jordan IK, Lunyak V. Acute genotoxic stress-induced senescence in human mesenchymal cells drives a unique composition of senescence messaging secretomes (SMS). *J Stem Cell Res Ther* 2017; 7 (8): 396-410). DOI: 10.4172/2157-7633.1000396

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Figure Legend

Figure 1. (A, B) Midfacial volume calculated by 3D analysis with VECTRA XT 3D Imaging System (Canfield Scientific, Parsippany, NJ, USA). Preoperative and postoperative patient images overlaid and aligned according to rigid structures of the face. Midfacial zone is anatomically defined by the lateral portion of the nasolabial fold, the inferior border of the zygomatic arch, and the superior border of the mandible.

Figure 2. Average facial volume retained for all patients (n=29) across 4 month-categorized subgroups (1-3 months, 4-6 months, 7-13 months, 14-19 months).

Figure 3. (A, B) Average facial volume retained in age separated groups (<55 years, n=15; >55 years, n=14) across 4 month-categorized subgroups (1-3 months, 4-6 months, 7-13 months, 14-19 months).

Figure 4. (A, E, I, M, Q) Preoperative photos of a 46-year-old, female patient who received 9mL of fat to the target area and a total of 58mL of fat to the face (B, F, J, N, R) Patient photos 4 months after ITR2 procedure (C, G, K, O, S) Patient photos 1.8 years after ITR2 procedure. (D, H, L, P, T) Patient photos 2.6 years after ITR2 procedure. (U) This patient received a total of 19.25mL of millifat, 6mL of microfat, and 4mL of nanofat.

Figure 5. (A, D, G, J, M) Preoperative photos of a 38-year-old, female patient who received 20mL of fat to the target area and a total of 58mL of fat to the face. (B, E, H, K, N) Patient photos 10 months after ITR2 procedure. (C, F, I, L, O) Patient photos 2.5 years after ITR2 procedure. (P) The patient received a total of 49mL of millifat, 8mL of microfat, and 1mL of nanofat.

Figure 6. (A, E, I, M, Q) Preoperative photos of a 60-year-old, female patient who received 23mL of fat to the target area and a total of 87 mL of fat to the face. (B, F, J, N, R) Patient photos 1-months after ITR2 procedure. (C, G, K, O, S) Patient photos 4-months after ITR2 procedure. (D, H, L, P, T) Patient photos 8-months after ITR2 procedure. (U) This patient received a total of 48mL of millifat, 36mL of microfat, and 3mL of nanofat.



Figure 1A



Figure 1B

Average Facial Volume Retained

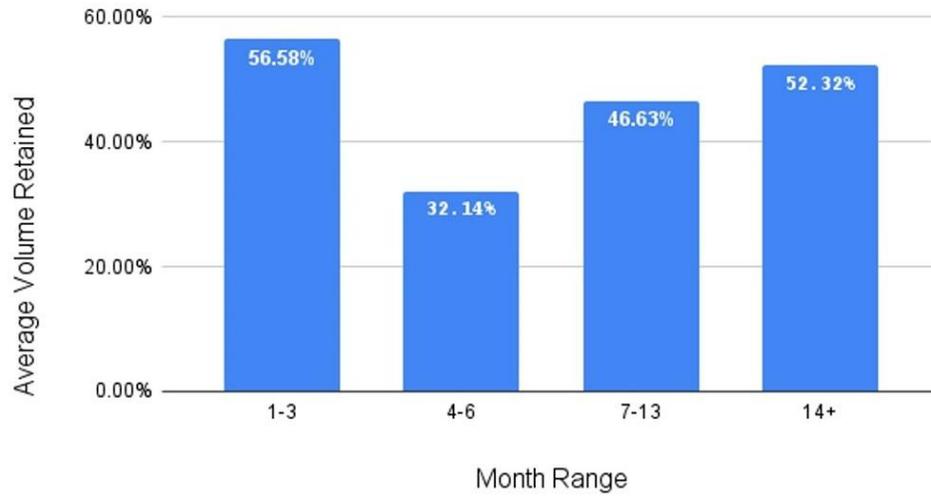


Figure 2

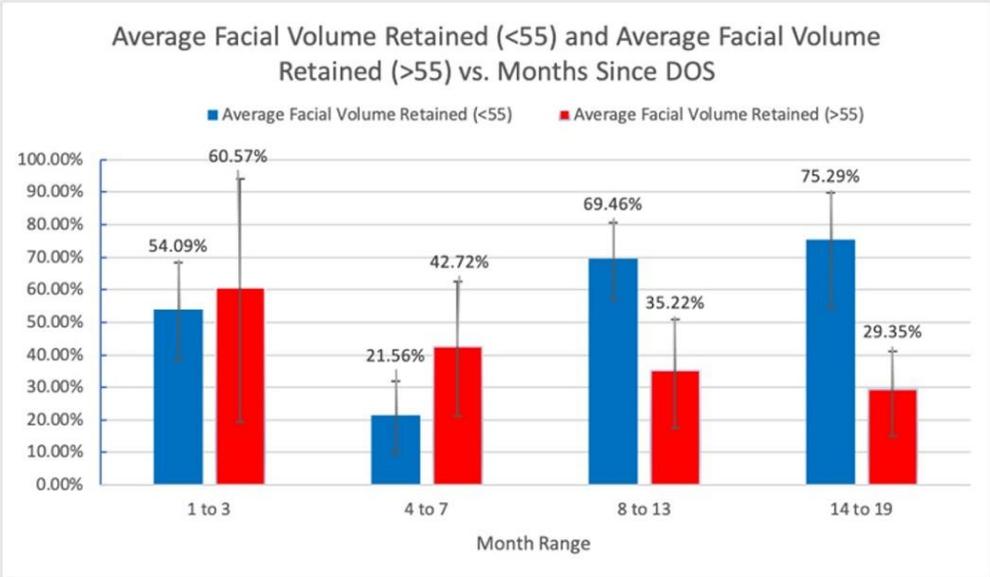


Figure 3A

Average Facial Volume Retained (<55) and Average Facial Volume Retained (>55) vs. Months Since DOS

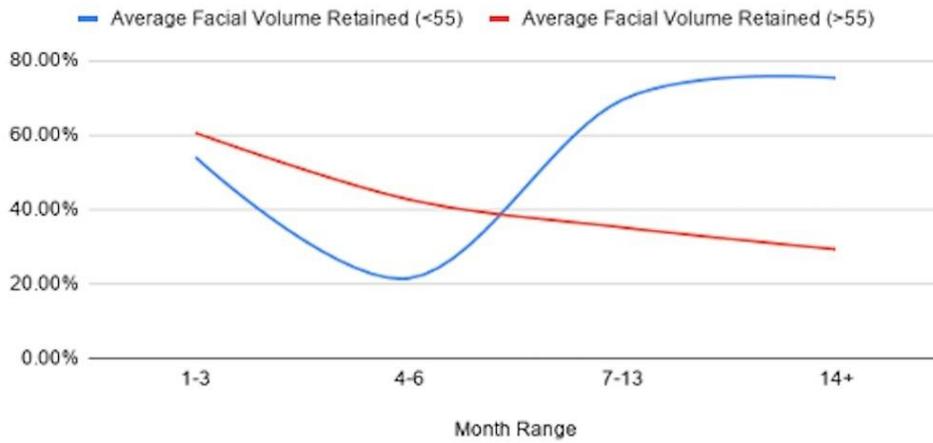


Figure 3B



Figure 4A



Figure 4B



Figure 4C



Figure 4D



Figure 4E



Figure 4F



Figure 4G



Figure 4H



Figure 4I



Figure 4J



Figure 4K



Figure 4L



Figure 4M



Figure 4N



Figure 40



Figure 4P



Figure 4Q

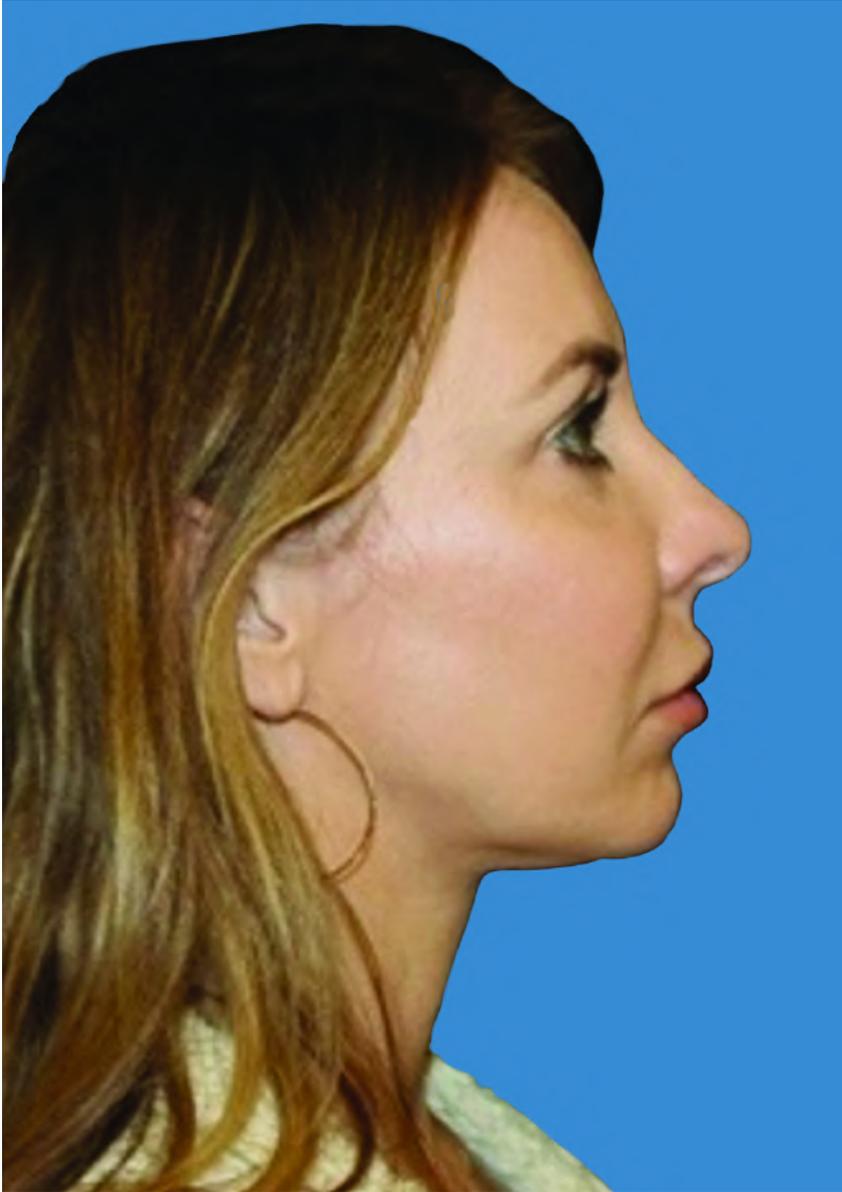


Figure 4R



Figure 4S



Figure 4T

PATIENT: _____

TOTAL VOLUME: 29.25cc

ITR² DATA
ANATOMIC PLACEMENTS & VOLUMES OF FAT

Pre-Skeletal

Millifat

Brows
L: ___/R: ___

Dorsum

Nasal Tip
0.25ml

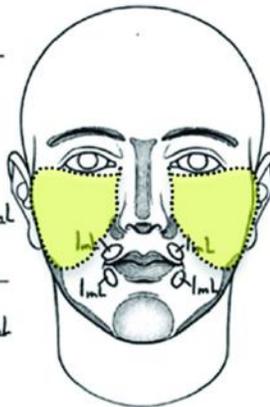
Pyrimids
L: 1.5/R: 1.5

Lips
U: ___/L: ___

Mandibles
L: 2ml/R: 2ml

Chin

Supraparosteal



*Deep Fat Compartments
Below Muscle*



Millifat

Temporals
L: ___/R: ___

SOFs/Cheeks
L: 4ml/R: 4ml

Sup. Orbit Sulc.
L: ___/R: ___

Buccal Fat
L: ___/R: ___

Microfat

Forehead
L: ___/R: ___

Temporals
L: 2ml/R: 2ml

Infraorbitals
L: ___/R: ___

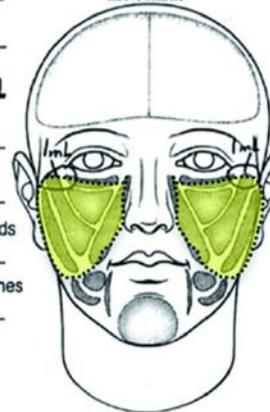
Cheeks
L: ___/R: ___

Nasolabial folds
L: ___/R: ___

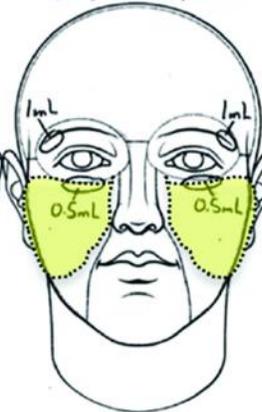
Marionette Lines
L: ___/R: ___

Chin

*Superficial Fat Compartments,
Above Muscle*



*Epithelium, Dermis,
and/or Superficial Fat Compartment*



Nanofat

Tear Trough
L: ___/R: ___

Perioral Region

Cheeks
L: 0.5ml/R: 0.5ml

Microneedling
(Face, Neck, Chest)

Biocream
(20.0 cc)

Figure 4U



Figure 5A



Figure 5B



Figure 5C



Figure 5D



Figure 5E



Figure 5F



Figure 5G



Figure 5H



Figure 51



Figure 5J



Figure 5K



Figure 5L



Figure 5M



Figure 5N



Figure 50

PATIENT: _____

TOTAL VOLUME: 58cc

ITR² DATA
ANATOMIC PLACEMENTS & VOLUMES OF FAT

Pre-Skeletal

Millifat

Brows

L: ___ / R: ___

Dorsum

Nasal Tip

3ml

Pyriforms

L: 2ml / R: 2ml

Lips

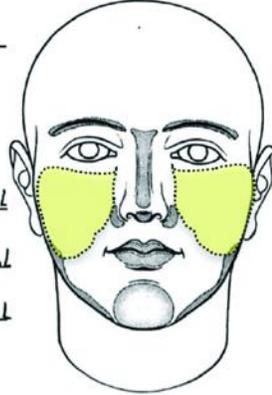
U: 2ml / L: 2ml

Mandibles

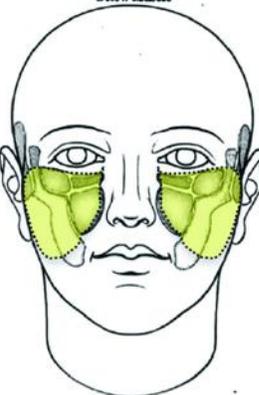
L: 2ml / R: 2ml

Chin

Supraparotical



*Deep Fat Compartments
Below Muscle*



Millifat

Temporals

L: 7ml / R: 7ml

SOOFs/Cheeks

L: 8ml / R: 8ml

Sup. Orbit Sulc.

L: ___ / R: ___

Buccal Fat

L: 2ml / R: 2ml

Microfat

Forehead

L: ___ / R: ___

Temporals

L: ___ / R: ___

Infraorbitals

L: ___ / R: ___

Cheeks

L: ___ / R: ___

Nasolabial folds

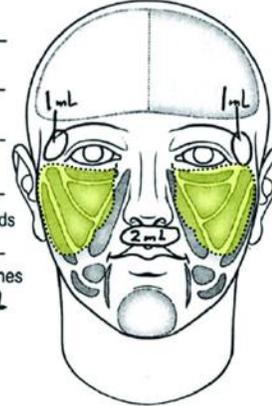
L: ___ / R: ___

Marionette Lines

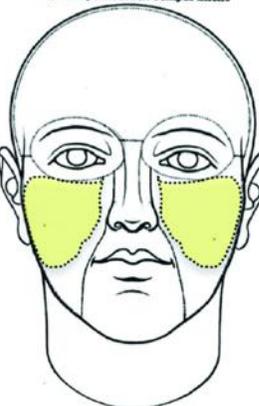
L: 2ml / R: 2ml

Chin

*Superficial Fat Compartments,
Above Muscle*



*Epithelium, Dermis,
and/or Superficial Fat Compartment*



Nanofat

Tear Trough

L: 0.5ml / R: 0.5ml

Perioral Region

L: ___ / R: ___

Cheeks

L: ___ / R: ___

Microneedling

(Face, Neck, Chest)

Bioream

(20.0 cc)

Figure 5P



Figure 6A



Figure 6B



Figure 6C



Figure 6D



Figure 6E



Figure 6F



Figure 6G



Figure 6H



Figure 61



Figure 6J



Figure 6K



Figure 6L



Figure 6M



Figure 6N



Figure 60



Figure 6P



Figure 6Q



Figure 6R



Figure 6S



Figure 6T

PATIENT: _____

TOTAL VOLUME: 87ml

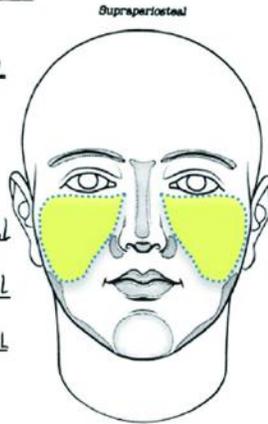
ITR² DATA
ANATOMIC PLACEMENTS & VOLUMES OF FAT

Pre-Skeletal

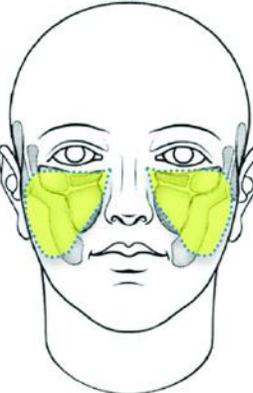
Millifat

Brows
L: 1ml / R: 1ml
Dorsum
2ml
Nasal Tip

Pyriforms
L: 2ml / R: 2ml
Lips
U: 4ml / L: 4ml
Mandibles
L: 5ml / R: 5ml
Chin
2ml



**Deep Fat Compartments
Below Muscle**

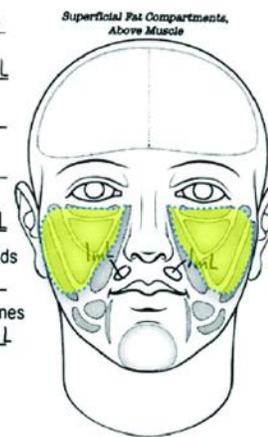


Millifat

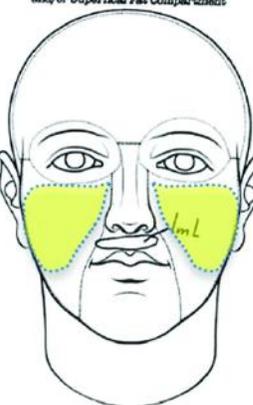
Temporals
L: 2ml / R: 2ml
SOOFs/Cheeks
L: 8ml / R: 8ml
Sup. Orbit Sulc.
L: _____ / R: _____
Buccal Fat
L: _____ / R: _____

Microfat

Forehead
L: 4ml / R: 4ml
Temporals
L: _____ / R: _____
Infraorbitals
L: _____ / R: _____
Cheeks
L: 3ml / R: 3ml
Nasolabial folds
L: _____ / R: _____
Marionette Lines
L: 7ml / R: 7ml
Chin
6ml



**Epithelium, Dermis,
and/or Superficial Fat Compartment**



Nanofat

Tear Trough
L: 0.5ml / R: 0.5ml
Perioral Region

Cheeks
L: 0.5ml / R: 0.5ml
Microneedling
(Face, Neck, Chest)
Biocream
(20.0 cc)

Figure 6U