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THE "CELL ENHANCED REGENERATIVE FACELIFT: C.E.R. FACELIFT. " EVIDENCE FOR LONG TERM RESTORATION OF AGE RELATED FACIAL VOLUME AND SKIN REJUVENATION WITH MINIMALLY INVASIVE TRANSPLANTATION OF ADIPOCYTE CELL COMPLEX AND STEM CELL SPECIFIC GROWTH FACTORS COMBINATIONS.

Cosmetology

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ABSTRACT

The employment of adipose tissue in cosmetic procedures is well stablished, nevertheless the usual methods have their disadvantages. There is evidence that stem cells contained within adipose tissue can successfully be used in cosmetic practices. This paper emphasizes in a new developed technique that optimizes a unique and highly innovative adipogenic growth factor "cocktail", described further in the article, that promotes subsequent engraftment upon transplantation and that induces adipogenic differentiation with great efficiency. This technique was employed for a facelift procedure, performed in six steps with significant results in skin quality improvement, volume data and patient satisfaction. This was demonstrated on patients with acne scars or even burn scars, or for rejuvenation purposes.

KEYWORDS

stem cells, facelift, regenerative, skin treatment, anti-aging

INTRODUCTION

Adipose tissue is commonly employed in plastic surgery and is an essential element in many forms of reconstructive surgery, such as facial reconstruction for trauma and congenital or inherited deformities like Parry Romberg's Disease. In most cases, adipose tissue is acquired from fat deposits in the body by liposuction. However, a major issue with past fat grafting is reabsorption and degeneration; which results in lack of permanent volume enhancement.

With the relatively new discovery of adult stem cell pools contained within adipose tissue, the perspective on what a fat graft/transfer can accomplish cosmetically has evolved. For example, *Kim et al.* published interesting data which suggested that adipose-derived stem cell (ADSC) needle transplantation is useful in aging skin because it results in increased dermal thickness and increased fibroblast numbers; as well as increased dermal angiogenesis (Kim et al., February 28, 2011).

Moreover, stem cells are important for maintaining the highly dynamic state of adipose tissue throughout the lifetime of an individual (Zeve et al. 2009). These stem cells are critical for the formation of new adipocytes needed for tissue regeneration and their activity is under constant control by both endogenous factors in tissues as well as by circulating hormones and growth factors. For this reason, the authors have developed a novel growth factor formulation for use with autologous adipose tissue to induce the regeneration of human skin and soft tissues *in vivo,* in order to restore the age related tissue losses that normally occur in the face and body without evidence of malignant transformation in *in vitro* (D. Rodgerson, V. Giampapa et al. 2013) and *in vivo* studies (Isakova et al., 2007) as well as no clinical evidence of abnormal cell transformation over a 6 year period.

METHODOLOGY

The Technology and Technique

The lead author has developed and optimized a unique and highly innovative adipogenic growth factor "cocktail" that promotes subsequent engraftment upon transplantation and that induces adipogenic differentiation with great efficiency. This novel "growth treatment cocktail" is composed of a mixture of factors known to individually promote stem cell differentiation and to exert beneficial effects on adipogenic cell populations, without the complications of tumorigenesis or abnormal cell line differentiation (D. Rodgerson, V. Giampapa et al. 2013).

The mixture includes hyaluronic acid, insulin, dexamethasone, FGF, indomethacin, T3/T4 and dimethyl amino ethanol in a sterile water medium. These components are all known to positively affect adipocytes and stem cells. Specifically, hyaluronic acid is used as a gel matrix scaffold to deliver the growth factors. It also binds CD44 expressed by MSCs which has been found to stabilize stem cells (D. Rodgerson, V. Giampapa et al., 2013).

Additionally, hyaluronic acid is FDA approved for treating wounds, as a dermal filler, and in adipose tissue regeneration (Altman et al. 2010; Chung et al. 2010; Cervelli et al. 2010; Beer, 2009).

Procedure

The procedure is simply performed under local anesthesia in the office setting:

1. Oral stem cell releasing compounds are used by the patient one week prior to the procedure to increase the number of circulating stem cells from the bone marrow. This is supplied by a commercial over the counter source (STEMKIND INC). Two tablets are taken daily in the morning for 7 days prior to the procedure.
2. Fat tissue is extracted by liposuction under local anesthesia using a sodium bicarbonate buffered 0.5 % lidocaine with epinephrine 1:200000 solution, preferably from the medial thighs or lower abdomen.
3. The aspirated fat is then placed on Telfa absorbent pads to remove blood, serum and excess oil.
4. Adipose tissue is then treated with the specific growth factors as seen in **Table 1** in a sterile kidney basin.
5. They are then placed in a 3cc syringe with a blunt 18 gauge needle for transplantation to the desired sites in the midface and other facial regions.

Table 1. Growth factors and concentrations

Components	Fat Factor
Hyaluronic Acid	20 mg/mL
T3	0.09 µg/mL
T4	0.27 µg/mL
Insulin	0.18 U/mL
Dimethyl amino ethanol	0.09 gm/mL
Indomethacin	0.71 mg/mL
Dexamethasone	0.051 mg/mL
Fibroblast Growth Factor-2	10 ng/mL

6. Finally, superficial Trichloroacetic acid (TCA) is applied to the full face to help improve superficial skin damage and improve skin appearance, as well as to stimulate the transplanted adipocyte cell growth factor complex in the subdermal plane.

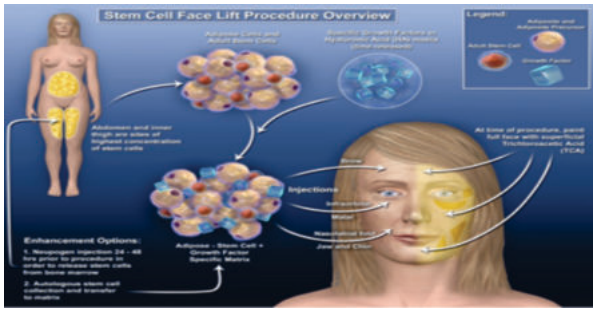


Figure 1. Procedure illustrated

The key to this procedure is the correct sites of injection in the face; this is done in three steps:

1. Using fingertip palpation technique, the lipoaspirate with its cellular elements with growth factors combination is infiltrated at the level of the infraorbital rim onto the periosteum. A blunt 18-gauge needle is introduced via the piriform aperture and placed onto the infraorbital rim at the periosteal level and the periosteal level in the nasolabial fold. Approximately 0.5 cc of the complex is deposited in four or five passes along the orbital rim deep to the orbicularis muscle. This corrects the tear-trough deformity, and hollowness under the eyes, as well as decreases the nasolabial fold prominence.

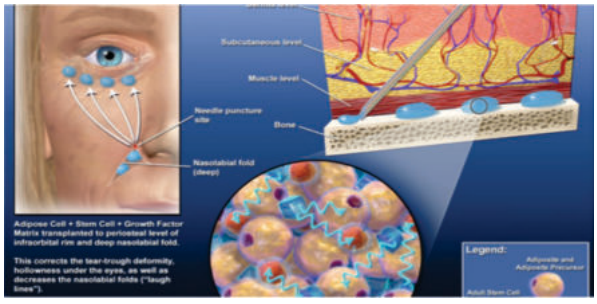


Figure 2. Site of injection

2. A linear transfer of the aspirate is applied to the deep muscular component of the malar eminence as well as the malar mound structure level. Here, approximately 4 to 6 cc of the complex is injected with the 18-gauge blunt needle in linear "rivelets" to allow for optimal growth of neighboring blood vessels into the transplanted matrix, and to avoid local avascular necrosis of the transplanted fat cells. This improves midface volume and projection and creates a more youthful facial contour, as well as decreases the nasolabial folds (laugh lines).

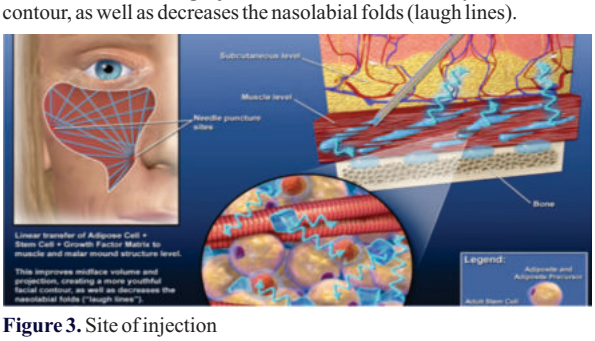


Figure 3. Site of injection

3. A superficial injection at the level of the dermal subcutaneous junction is then completed to stimulate the regeneration of dermal elements, collagen, elastin, and HA production. This is accomplished via a 3cc syringe which contains a growth factor combination to encourage collagen formation and HA production over an extended period of time. This step is designed to stimulate the transplanted adipose cell matrix as well as stimulate the dormant local stem cells present. This also can improve the quality and texture of the skin, reducing wrinkles under the eyes and at the crows-feet area and the nasolabial folds.

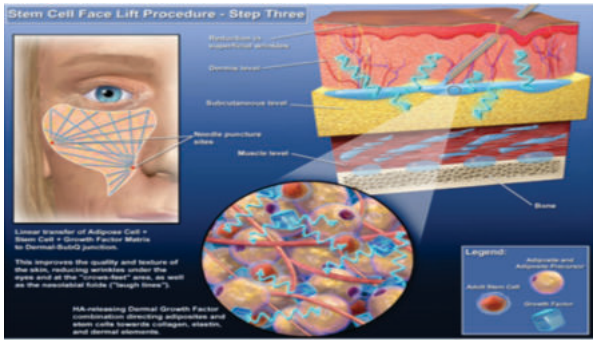


Figure 4. Site of injection

The effectiveness of this new approach to enhance adipocyte survival and create adipocytes that have a younger gene expression pattern, which produces enhanced elements to improve skin quality and skin vitality, was measured in 210 patients, 80% women and 20% men, ages 30 to 65, who underwent the procedure between January 2016 and January 2019. The Visa Software was implemented to determine an objective improvement in skin parameters after one year from the initial procedure. Additionally, volume of the adipose graft and skin quality were evaluated 1 year post-operatively by blinded, independent surgeons via questionnaires.

RESULTS

Skin Quality Improvement

As illustrated by a Visia computerized skin analysis of skin surface quality markers (**Figure 5**), a substantial improvement in all measured parameters was seen after "growth factor enhanced fat grafts" as compared to the pre-procedure status and the pretreatment computerized skin measurements. In general, the quality of the skin in those patients who had undergone the growth factor enhanced fat grafting procedure continued to improve for up to a year.

Figure 5. Visia results pre and 12 months post procedure

Volume Data

Clinical results regarding volume of the growth factor enhanced fat graft patients were evaluated 1 year post-operatively. The growth factor enhanced fat grafted patients documented no abnormal cell development. The volume improvement appeared to be most effective in the mid facial region. In a subpopulation of the initial group, 5 female patients (ages 53 to 65 years old) were randomly chosen to be followed up for one year to determine improvement in volume in distinct regions of the midface. This was done by 3 blinded, independent experienced plastic surgeons via questionnaires comparing growth factor treated adipose grafts to traditional autologous fat grafted patients (controls) for midfacial rejuvenation effects. The midfacial zones evaluated were tear trough, malar eminence, submalar region, and nasolabial crease. These patients were

rated from 0 to 2 (0 for no improvement; 1 for mild improvement; and 2 for marked improvement). Specifically, the volume of the growth factor enhanced adipose tissue was judged almost 2-fold greater than the volume of a traditional autologous graft as seen in **Figure 6**.

These investigative results indicate that the growth factor treated fat grafts appear to survive much better than the non-treated fat grafts. This result is unusual in that, unless adipose grafts are replenished regularly, they usually show significant degrees of fat tissue degeneration. Long term natural appearing results were seen during the following 6 year follow up by the author with no noted adverse events, calcification or localized cosmetic deformaties.

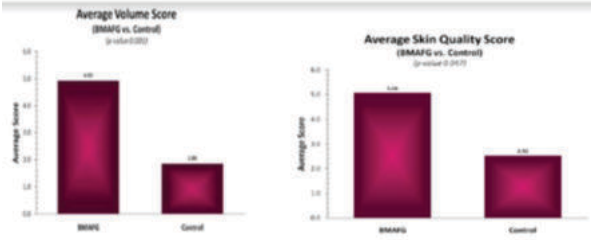


Figure 6. Stem cell facelift vs regular facelift volume and skim quality scores

Patient Satisfaction

Overall, patients did notice an improvement in the five major areas evaluated. The greatest improvement perceived by patients was facial volume with 91% of noticeable or significant changes compared to pre-procedure; this was followed by improved skin quality with an 88% improvement.

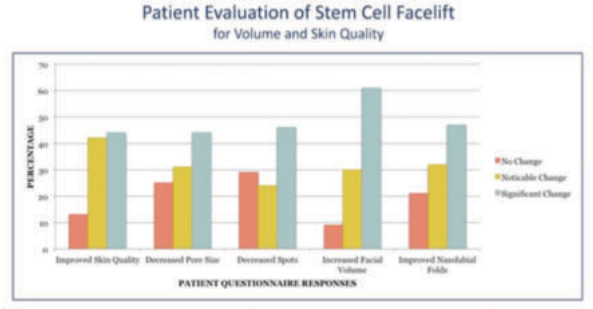
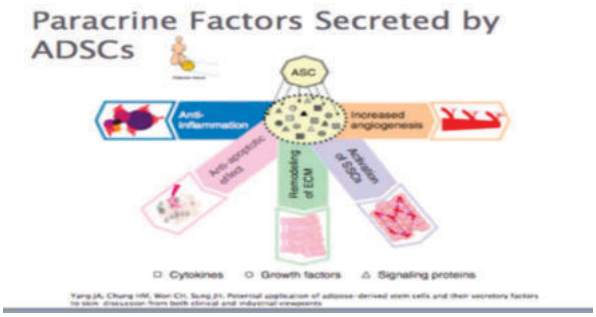


Figure 7. Patient satisfaction evaluated through subjective questionnaire for volume and skin quality

DISCUSSION

It is theorized that the improvement in facial volume and skin quality is due to the endogenous growth factors that are being produced and secreted by the newly transformed MSCs to young adipocytes. It is well know that young adipocytes have a different gene expression profile and that they are capable of producing an enhanced amount of cytokines and growth factors that produce more collagen and key factors that enhance skin quality as seen in **Figure 8** (Yang, J.A. et all, 2010). There may also be local stimulation effects of the resident older adipocytes after a successful transplantation of the fat and growth factor combination. The combined complex of fat, growth factors, and MSCs appear to directly affect the dermal subcutaneous junction by producing greater levels of hyaluronic acid, collagen and other skin rejuvenation factors. This results in a long term improvement in the quality of the overlying skin cells.



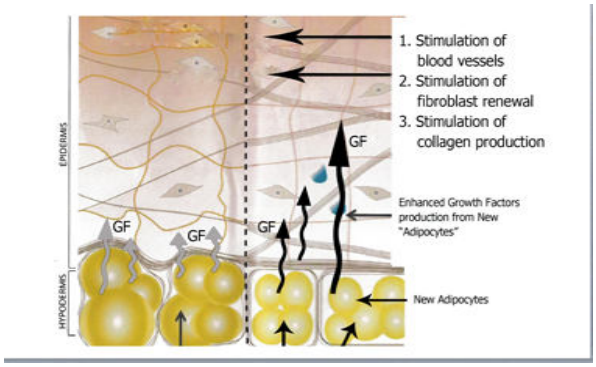


Figure 8. Paracrine factors produced by young adipose derived mesenchymal stem cells (above). Effects of new stimulated adipocytes on skin.

In the initial research publication (D. Rodgerson, V. Giampapa et al. 2013), it is shown that the same growth factors can rapidly induce differentiation of over 95% of purified human bone marrow derived MSCs as well as adipose derived MSCs into adipocytes. Morphologically, the cells exposed to growth factor mixtures showed adipogenesis as indicated by the appearance of fat globules in the cells. No adipocytes could be seen in cells cultured in control (sterile water) medium. This rapid formation of adipocytes within a 16-hour period is highly unusual since differentiation can take over a month to occur with most other established differentiation mediums. The treatment did not cause fibroblast formation, nor was there any appearance of abnormal cell formation. Thus, the growth factor combination is able to enhance survival and dynamics of adipocyte grafts and rapidly induce stem cell differentiation to adipocytes without increased risk of malignant transformation. This is most likely the basis of the enhanced facial volume and contour seen in the patients.

CONCLUSIONS

This non-surgical rejuvenation of the face improves long term facial volume, facial contour and skin quality through filling and lifting, unlike the pulling and stretching of a surgical "face lift". A standard facelift only improves facial contour. With this procedure Skin quality improvement also occurs without laser or energy modality interventions. Additionally, the procedure meets the new market demand for non-invasive office-based procedures now in vogue, with the hallmarks of local anesthesia, shorter recovery period, fewer postoperative complications and a more natural looking result.

The use of the specific growth factors combined in specific amounts, stimulates on a long-term basis both the local latent stem cells and the stimulated and transplanted MSCs obtained from the liposuction aspirate. It is our theory that these autologous stimulated MSCs have been documented to produce young adipocytes (D. Rodgerson, V. Giampapa et al. 2013) that are responsible for the permanent volume enhancements and the enhanced collagen and elastin content of the treated skin, which otherwise declines with the natural aging process. More generally, adipose tissue and its Mesenchymal Stem Cell content, has been shown to have expanded utility in treating other medical indications within reconstructive surgery, including soft tissue repair after trauma (Bauer-Kreisel et al. 2010), wound healing, and scar remodeling due to burns and acne scarring as well as restoration of midface soft tissue deformities, Ebrahimian et al. 2009. Adipose derived MSCs have also successfully been used in treating cartilage and bone defects (Estes et al 2010;) and cardiac repair (Madonna et al. 2009).

Appendex

The Procedure-video

https://youtu.be/diSG-DPjfJY

FIG 1- 55 Year old female before and one year post treatment. No other procedures done.

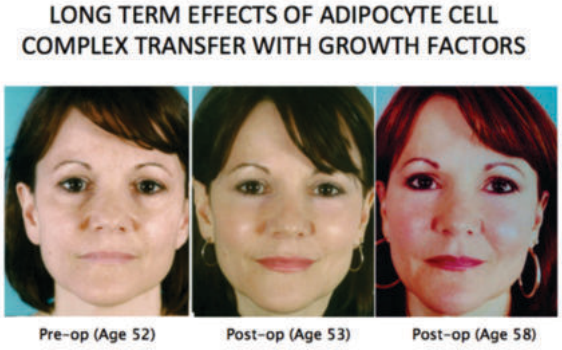


FIG -2 Six years post treatment with one grafting procedure and no additional procedures or fillers given during this time.

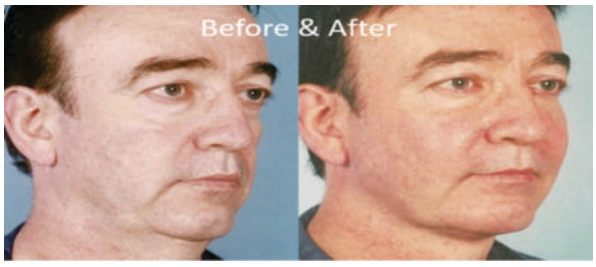


FIG-3

FIG-4

FIG-5 Acne scar treatment

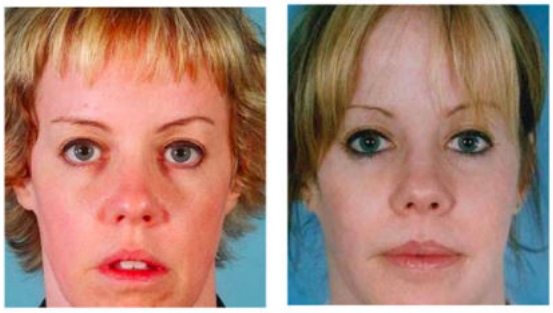


FIG-6 In place of Orthognathic and Implant Surgery

Midface volume was replaced with the growth factor enhanced fat grafts as well as the maxillary area and lips.

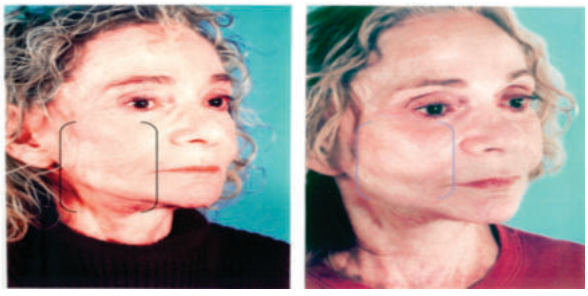


FIG-7 Burn treatment

Improvement in skin color and texture was seen in the patients with old burn scars post grafting Long term effects of adipose complex cell transplant with stem cell specific growth factors and face and neck lift procedure

The growth factor enhanced grafts were noted to maintain their volume as well as appear to enhance skin quality over lying the graft sites, for extended periods of time.

FIG-7 6 years evolution reference

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