Original Article

Periorbital wrinkles treatment using collagen induction therapy

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ABSTRACT

Introduction: Periorbital wrinkles are very common and permanent cutaneous folds that develop on the periocular area. The treatment for wrinkles varies with the degree of severity. A relatively new treatment, called collagen induction therapy (CIT), seems to be appropriate for the treatment of periorbital wrinkles. **Objective**: To confirm the usefulness of CIT in periorbital wrinkles treatment. **Patients and methods**: In total, 20 patients (12 females, 8 males; between 50-65 years of age) with periorbital wrinkles were included. Each patient was treated with a specific tool in two sessions. Using digital cameras, photos of all patients were taken to evaluate wrinkles' depth, and silicone rubber was used to make a microrelief impression of the wrinkles. The photographic data were analysed by using the sign test statistic ($\alpha < 0.05$). Skin layer data were analysed by computerized image analysis. **Results**: Analysis of the patient photographs, supported by the sign test, and the degree of irregularity of the surface microrelief, supported by Fast Fourier Transform and by wrinkle's image processing, showed that, after only two sessions, the wrinkles' severity grade in most patients was greatly reduced. **Conclusion**: The present study suggests that CIT can be a suitable technique to improve periorbital wrinkles.
Keywords: collagen, skin aging, ambulatory surgery procedures.

INTRODUCTION

Periorbital wrinkles, also known as "crow's feet", are very common and permanent cutaneous folds, generally symmetrical, that develop on the periocular area. They are both part of the normal aging process as well as result of sun damage and of the excessive activity of mimic muscles. Wrinkles are caused by a loss of the elastic fibers, collagen and fat within the skin. The normal aging process produces wrinkles that are usually fine lines that disappear when the skin is stretched. The sun-caused wrinkles are coarser and deeper and do not disappear when the skin is stretched. Nowadays, wrinkles have a greater social impact and, obviously, science and hedonism overlap in the search for causes, treatments and prevention of wrinkles. The treatment for wrinkles varies with the degree of severity. The treatments can be based on the prescription of topical medications, chemical peels, dermoabrasion, muscle-relaxing injections, lasers resurfacing, cosmetic filler injections, and, most recently, collagen induction therapy (CIT). CIT is an effective method of treating wrinkles and other dematological lesions that involves puncturing the skin multiple times with a small needle to induce collagen growth. It has been used since 1995 to achieve percutaneous collagen induction (PCI). In 1995, Orentreich and Orentreich[1] described "subcision" as a way of building up connective tissue beneath retracted scars and wrinkles. Fernandes,[2] simultaneously and independently, used a similar technique to treat the upper lip by sticking a 15-gauge needle into the skin and then tunneling under the wrinkles in various directions, parallel to the skin surface. Camirand and Doucet treated scars with a tattoo gun to "needle abrade" them. Although this technique can be used on extensive areas, it was laboriously slow and the holes in the epidermis were too close and too shallow. All these techniques worked because needles break old collagen strands in the most superficial layer of the dermis that limit scars or wrinkles. It is presumed that this process promotes removal of damaged collagen and induces more collagen immediately under the epidermis. Fernandes believed that the standard technique of tattooing was too superficial to give good effects for thicker scars or for stimulating neo-collagen synthesis in the

reticular dermis. Needles need to penetrate relatively deeply to stimulate the production of elastin fibers oriented from the deep layers of the dermis to the surface. Based on these principles, Fernandes designed a special tool for CIT, consisting of a rolling barrel with microneedles at regular intervals. Skin needling procedure is done under topical anesthesia, by rolling a special tool on the skin area backward and forward, applying some pressure, in various directions to achieve an even distribution of the holes. The microneedles penetrate through the epidermis but do not remove it; the epidermis is only punctured and rapidly heals. The needles seem to divide cells from each other rather than cut through them, therefore, many cells are spared. Because the needles are set in a roller, every needle initially penetrates at an angle and then goes deeper as the roller turns. Finally the needle is extracted at the converse angle therefore curving the tracts, reflecting the path of the needle as it rolls into and then out of the skin for about 1.5 to 2 mm into the dermis. The epidermis, and particularly the stratum corneum, remains "intact" except for the minute holes that were made, each about four cells in diameter. When a needle penetrates into the skin, the injury causes localized damage and minor bleeding by rupturing fine blood vessels. A completely different picture emerges when thousands of fine pricks are placed close to each other. Normal wound healing is promoted, which develops in three phases (inflammation, proliferation and remodeling) and culminates in laying down new collagen in the upper dermis just below the basal layer of the epidermis.

Recently, a new hypothesis has been proposed to explain the CIT mechanism of action:[3] when CIT is correctly performed, using a high quality device, the fine microneedles - not longer than 1,5 mm - do not set a wound in the classical sense. The wound healing process is somewhat cut short, as the body is only somehow "fooled" into believing that an injury has occurred. According to this new theory, bioelectricity (also called "demarcation current") triggers a cascade of growth factors that stimulate the healing phase.

OBJECTIVE

In order to confirm the usefulness of CIT in periorbital wrinkles treatment, we applied this procedure to treat a group of 20 patients affected with different grades of periorbital wrinkles.

PATIENTS AND METHODS

The present study was conducted from January 2008 to February 2009 at the University of Naples "Federico II", Department of Systematic Pathology, Division of Clinical Dermatology. In total, 20 patients (12 females, 8 males; between 50-65 years of age) with periorbital wrinkles were recruited and signed an informed consent for this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Exclusion criteria are listed in Table I.

Before the treatment (baseline, T_0), the severity of lesions in each patient was scored by an experienced dermatologist involved in the study. Periorbital fold severity was evaluated using the Wrinkle Severity Rating Scale[4] (WSRS: 1 = absent to 5 = extreme), a 5-point scale with the right end (5) indicating maximum severity and the left end (1) absolutely no lesions. In accordance to severity score, 3 groups of patients were identified: 1st group (A) comprised 9 patients with a severity score of 3; 2nd group (B) comprised 7 patients with a severity score of 4; 3rd group (C) comprised 4 patients with a severity score of 5. Facial fine lines and wrinkles depth were faithfully captured by photographic digital technology: 2 photographs of each periorbital area were taken of every patient by another dermatologist not involved in the study and filed in a database. In addition, to achieve reliable evidence, we made, for each patient, a periorbital cutaneous cast, at identical sites bilaterally, using silicone rubber to make a wrinkles' impression (silicone replicas). Computerized digital image processing of such specimens provides objective measurement of skin's topography, which has a significant degree of correlation with clinical grading. Profilometric data were obtained from skin replica analysis at baseline and at the end of the study.

As percutaneous CIT works better when combined with a scientific skin-care programme to restore a youthful appearance, the skin of each patient was treated with a topical product containing alpha–omega hydroxyl-acids, omega hydroxyl-acids, enoxolone and zinc for at least 3 weeks (preparation phase) before the skin needling began.

Three weeks later, before the first session of treatment began (T_1), 2 standardized digital photographs were obtained of each patient's periorbital areas (right and left) and filed in a database. During the first session, each patient was prepared in a manner similar to a surgical procedure: facial skin was

Tabele I – Exclusion Criteria
- Extremely rare but severe form of keloid scarring
- Diabetes
- Neuromuscular disease
- Bleeding disorder
- Collagen vascular disease
- Acute or chronic corticosteroid therapy
- Acute or chronic anticoagulant therapy
- Presence of skin cancers
- Warts
- Solar keratoses
- Skin infection
- Pregnancy

disinfected, then a topical anesthetic (EMLA) was applied which was left for 60 minutes. Each patient was treated with a highly specific tool: a rolling barrel 20 mm wide, equipped with 192 needles in 8 rows. The needles used had a length of 1,5 mm and a diameter of 0,25 mm. According to the applied pressure, they penetrate the skin between 0,1 and 1,3 mm. The diameter at maximum penetration level was only 0,07 mm. The special tool was rolled over the areas affected by wrinkles. Rolling consisted in moving four times in four directions (where possible): horizontally, vertically and diagonally right and left (Figure 1). This ensured an even pricking pattern, resulting in about 250 to 300 pricks/cm^2. Special attention was given when we rolled around the eyes because this tissue is very thin and sensitive. As expected, after the treatment, the skin bled for a short time. When bleeding stopped, a serous ooze formed and was removed from the surface of the skin with the use of sterile saline solution (Figure 2). Further wound treatment was not necessary. A week later, each patient was seen to estimate the response to CIT and any side effects that may have occurred.

The second session of treatment was conducted 8 weeks after the first one (T_2). The purpose was to evaluate the clinical improvement of wrinkles using CIT. Before this second intervention, for each patient, new digital photographs were obtained using identical patient positioning, lighting, and camera settings, filed in a database and compared to the previous one taken before the first treatment. To estimate the improvement of wrinkles and the efficacy of CIT, each patient was also examined and, according to severity lesions, a new score was given using the WSRS. Furthermore, all patients were rated as improved or better on the Global Aesthetic Improvement Scale (GAIS) (Table II). The procedure of the second treatment was the same as the first treatment.

Figure 1 – The special device is rolled on periorbital skin of a 60 year-old-woman.

Figure 2 – An image of a 60 year-old-woman treated using CIT: note the bleeding induced by CIT.

The last check-up was conducted 32 weeks after the second treatment (T_3): patients' photographs were taken and compared to the photographs taken before the first treatment. Each patient was conferred a new WSRS score and GAIS rating. We assessed the real immediate improvement induced by CIT on periorbital wrinkles after two session of skin needling. Moreover, during the last check-up, we made other cutaneous casts that were compared with the ones made before beginning the first treatment and assessed the degree of irregularity in these casts by a computerized image analysis.[5] The use of skin replica analysis in conjunction with photographic digital technology provided an instrument to evaluate the changes in the photodamaged skin during the study. Studies[6-7] have confirmed the value of optical profilometry as an objective technique that could reproducibly measure changes in skin topography with minimal variability or potential for bias.

STATISTICAL ANALYSIS

The digital photographic data were analysed using a test for nonparametric data (sign test for paired data). The null (H_0) is that the median of the difference is zero ($P_+ = P–$) and the alternative hypothesis (H_A) is that the median of the differences is negative ($P+ < P–$), = 0.05. The result is given by computing the binomial probability.

Computerized image analysis of skin casts

Acquisition of images: The acquisition of skin casts was carried out using a stereomicroscope connected to an analog video camera.

Evaluation of surface's microrelief: The morphometric study of skin surface allows to evaluate the surface's irregularity (skin surface texture) and to determine the possible variation caused

Tabele II - Global Aesthetic Improvement Scale (GAIS)

Rating	Description
Very much improved	Optimal cosmetic result for CIT in this patient
Much improved	Marked improvement in appearance from initial condition but not completely optimal for this patient
Improved	Obvious improvement in appearance from the initial condition, but retreatment indicated
No change	Appearance essentially the same as the original condition
Worse	Appearance worse than the original condition

by the treatment. Determination of microrelief's irregularity degree was made by the study of Fourier spectrum (FFT = Fast Fourier Transform) on skin casts images. In detail, using a special software, on skin texture's images, the average values of grey obtained along the X axis and Y axis were evaluated; the estimated indexes, ISIωx and ISIωy (Irregular Skin Index of ωx-axis e ωy-axis), are the integrals of areas bounded by the curves resulting from pixels' distribution along X and Y axes.

Wrinkles' images processing: Images processing was carried out by a method of computerized image analysis. Skin cast's were shot with a light incident at 45° creating shadows at the ridges (= negative image of wrinkles). The shadows were converted into a grayscale, whose intensity was directly proportional to the shadows' intensity and then to the wrinkles' depth. Once the image was displayed on the screen and the area to be studied was identified for each patient, the definition pixel per pixel of a lines' series (scanning), that pass through this area perpendicularly, was started. Thus, the average intensity of gray for each pixel in the intercepted area was obtained. To obtain reproducible scanning (uncertainty level < 13), particularly care was given to skin casts carrying out and direction. The uncertainty was calculated in accordance with the rules EN45001. The following profilometric parameters were calculated: Ra (average roughness), which is the arithmetic mean in absolute value of all variation of the mean; Rt, which is the maximum depth of the wrinkles in the considered area; Rz, which is the average depth of wrinkles; Rmax, which is the maximum height of the filtered profile; Rmin, which is the minimum height of the filtered profile.

RESULTS

The results achieved after two sessions of treatment, preceded by the preparation phase, were assessed. After each session of treatment, the facial skin appeared reddened and swollen but patients stated that the redness and swelling disappeared in 2–3 days. No side-effect was reported or observed.

Eight weeks after the first session of CIT, all patients had smoother facial skin, a slight reduction in lesions severity and a minimal aesthetic improvement.

Thirty-two weeks after the second session of CIT, the improvement of periorbital wrinkles was evident: the photographic comparison highlighted that, independently of lesions grading, in every group of patients, wrinkles relative depth was significantly reduced. In fact, the sign test for paired data ($P < 0.05$) highlights that the median of the differences is negative, showing that the reduction in severity grade of periorbital wrinkles, before and after CIT, should be considered significant. According to WSRS, 45% patients at T_3 had a score of 1 compared to a score of 3 at the baseline, and 35% patients at T_3 had a score of 1 compared to a score of 4 at the baseline. The global aesthetic improvement was significant: specifically at T_3, 5% patients had GAIS ratings of very much improved, 40% were rated as much improved and 55% as improved.

In view of the small sample considered, the results of computerized image analysis of skin casts were not subjected to inferential statistical analysis but expressed in percent rate and they are only indicative of the trend of considered parameters. The results of surface microreliefs' evaluation (Figures 3-4) showed, after the treatment, a reduction (average) of irregularity degree of skin texture compared with the basal, corresponding to 25% for both axes considered (X-axis and Y-axis) (Figures 5-6). The results of wrinkles' profilometric evaluation showed an important reduction of Ra and Rt parameters compared to the basal corresponding to 33% for R_a (from an average at T_0 equal to 13.26 to 8.84 at T_1) (Figure7) and to 31% for R_t (Figure 8). In addition, it was estimated a clinically relevant increase in R_{min} parameter at T_0 equal to 23% (from an average equal to 133.17 to an average equal to 164,13 at T_1) (Figure 9). The R_z and R_{max} parameters did not show important variations compared to the baseline in the profilometric evaluation .

DISCUSSION

Most authors consider that CIT induces a normal wound healing process that occurs in three phases. The

Figure 3 – Stereomicroscopic image of periorbital wrinkles' cast at baseline (T_0).

Figure 4 – Stereomicroscopic image of periorbital wrinkles' cast after two sessions of CIT (T_3): note the improvement of irregularity degree of skin texture with respect to the basal.

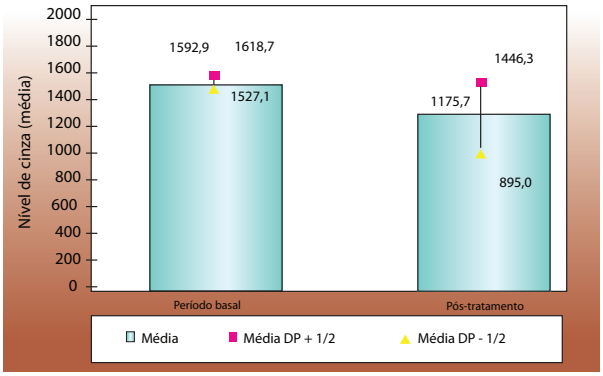

Figure 5 – Surface microrelief FFT (y-axis) .

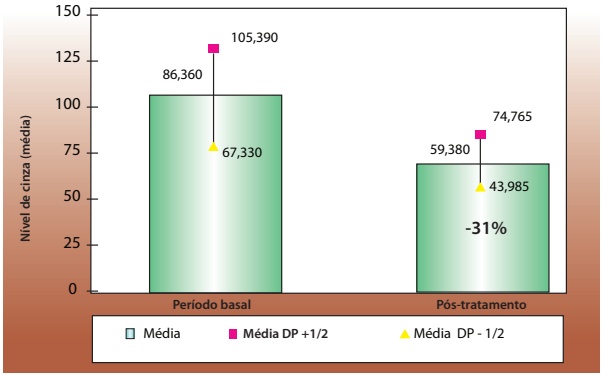

Figure 8 Image analysis - wrinkles profilometry Rt (maximum roughness).

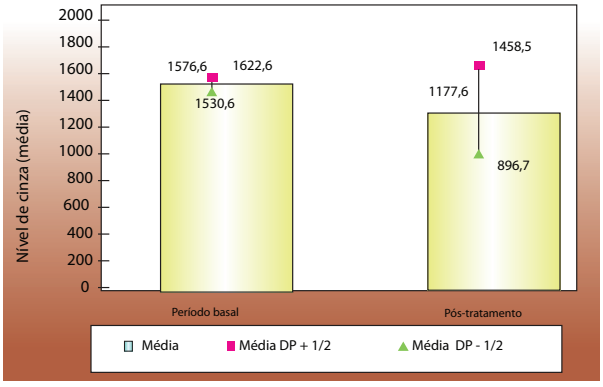

Figure 6 – Surface microrelief FFT (x-axis).

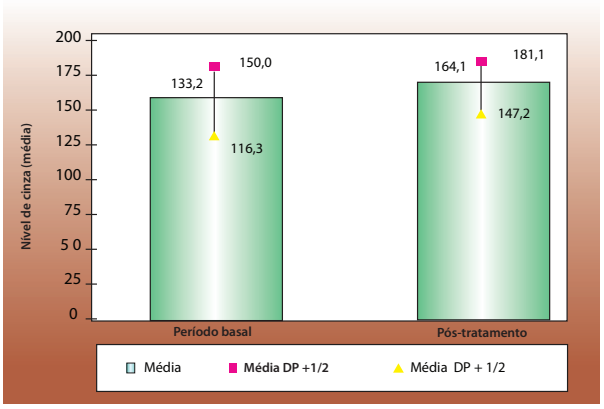

Figure 9 – Image analysis - wrinkles profilometry Rmin (minimum height).

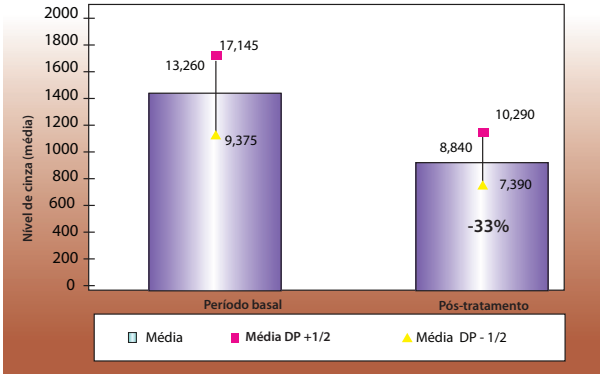

Figure 7 – Image analysis - wrinkles profilometry Ra (average roughness).

inflammation phase (phase 1) starts soon after the injury: platelets, once activated, release chemotactic factors, which cause an invasion of other platelets, neutrophils, and fibroblasts. During the proliferation phase (phase 2), neutrophils are replaced by monocytes that change into macrophages and release several growth factors including PDGF, FGF, TGF-b, and TGF-a, which stimulate the migration and proliferation of fibroblasts. Keratinocytes then become mobile to cover the gap in the basement membrane. They start producing all the components to re-establish the basement membrane with laminin and collagen types IV and VII. A day or two after CIT, the keratinocytes begin to proliferate and release growth factors to promote collagen deposition by the fibroblasts: collagen type III is the dominant form of collagen in the early wound-healing phase. New blood vessels are also created. Tissue remodeling (phase 3) continues for months after the injury and is mainly done by the fibroblasts: collagen type III is laid down in the upper dermis, just below the basal layer of the epidermis, and is gradually replaced by collagen type I. The matrix metalloproteinases (MMPs 1-2-3) are essential for the conversion process.[3] Blood supply is normalized, so the skin becomes smoother and presents a natural color.

Recently, a new hypothesis has been proposed to explain the CIT mechanism of action: when CIT is performed using a high-quality device, the fine microneedles do not create a wound in the classic sense. The wound healing process is shortened, as the body is somehow "fooled" into believing that an injury has occurred. According to this new theory, bioelectricity (also called "demarcation current") triggers a

cascade of growth factors that stimulate the healing phase. When microneedles penetrate the skin, they cause fine wounds. Cells react to this intrusion with a demarcation current that is additionally increased by the needles' own electrical potential. In some findings by Jaffe *et al.*,[8] the membrane of a living cell has been shown to have a resting electrical potential of -70 mV. The electrical potential depends greatly on the transport mechanisms. If a single acupuncture needle comes close to a cell, the inner electrical potential quickly increases to -100 mV. Cell membranes react to the local change with an electrical potential that creates increased cell activity and a release of potassium ions, proteins and growth factors.

CONCLUSION

The results of this study show and confirm that CIT is a simple technique and, with a specialized tool, can have an "immediate effect" on the improvement of periorbital wrinkles. The results of computerized image analysis of skin casts, reflecting a smoothening of the skin surface in CIT-treated subjects, were consistent with clinical data showing greater improvement in fine wrinkling and roughness after collagen induction therapy. This means that in all patients severity grade of periorbital wrinkles, after only two sessions, was highly reduced too and the global aesthetic improvement was important as well no patient showed visible signs of the procedure or hyperpigmentation.

The formation of new collagen reaches its peak in about 10 to 12 weeks after treatment, so, in accordance to the literature, a complete result after CIT may be observed after 8-12 months of treatment. As shown by Fernandes and Signorini,[9] compared with the conventional peeling methods, CIT has undisputable advantages. The most important one is that the epidermis remains intact because it is not removed or otherwise damaged, eliminating most of the risks and negative side effects of chemical peeling or laser resurfacing. Compared to surgical procedures and dermoabrasion, CIT has lower risks and side effects, and is a less invasive method. An added benefit is also the short healing phase that follows after each treatment and, when the result is not sufficient, the treatment can be repeated.

CIT and all its therapeutic possibilities are still under initial research. There are scientific proofs that the needling procedure also stimulates revascularization, repigments stretch marks and fills rolling acne scars: under this point of view, the CIT is a suitable procedure for different dermatologic pathologies.

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REFERENCES

1. Orentreich DS, Orentreich N. Subcutaneous incisionless (subcision) surgery for the correction of depressed scars and wrinkles. Dermatol Surg 1995;21:543-549.
2. Fernandes D. Minimally Invasive Percutaneous Collagen Induction. Oral and Maxillofacial Surg Clin N Am 2005;17:51-63.
3. Liebl, H. Abstract reflections about Collagen-Induction-Therapy (CIT). A hypothesis for the mechanism of action of collagen induction therapy (cit) using micro-needles, January 2-7. http://www.dermaroller.de/us/science/abstract-reflections-26.html February 2006.
4. Day DJ, Littler CM, Swift RW, Gottlieb S. The wrinkle severity rating scale: a validation study. Am J Clin Dermatol 2004:49-52.
5. Grove GL, Grove MJ, Leyden JJ., Optical profilometry: an objective method for quantification of facial wrinkles, J Am Acad Dermatol. 1989;21:631-637.
6. John D Rachel, Jasmine J Jamora. Skin rejuvenation Regimens: a profilometry and histopatologic study, Arch Facial Plast Surg 2003;5:145-149.
7. Creidi P, Vienne MP, Ochonisky S *et al.* Profilometric evaluation of photodamage after topical retinaldehyde and retinoic acid treatment. J Am Acad Dermatol 1998;39:960-965.
8. Jaffe L. Control of development by steady ionic currents. Federation Proceedings 1981;40:125-127.
9. Fernandes D, Signorini M. Combating photoaging with percutaneous collagen induction. Clin Dermatol 2008;26:192-199.