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Anti-Age Activity and Tolerance Evaluation of Collagen Micro-Injection Treatment Associated to Topical Application of a Cosmetic Formulation (Investigator-Initiated Multicentre Trial)

Adele Sparavigna[*], Antonello Tateo, Elena Inselvini, Massimiliano Tocchio, Maria Cristina Orlandini and Giorgio Botali

DermIng srl, Clinical Research and Bioengineering Institute, Milan, Italy

[*]**Corresponding author:** Adele Sparavigna, DermIng srl, Clinical Research and Bioengineering Institute, Milan, Italy, Tel: +39 3420399117; E-mail: adele.sparavigna@derming.com

Abstract

Objective: A novel equine type I collagen treatment consisting of an intradermal injection associated with the use of a topical mousse was developed with characteristics especially suited for global face rejuvenation. A multicenter, investigator-initiated, open clinical trial, conducted by 6 italian centers, was carried out to evaluate the anti-age performance of this combined treatment.

Methods: The study was conducted on 72 female healthy subjects, age range 40-65 years, asking for midface volume restoration and presenting cutaneous aging/photoaging signs. The 1st injection treatment with the injectable product was performed immediately after the basal evaluation (T0). Two touch-up treatments were performed after 2 (T1) and 4 weeks (T2). Collagen mousse was to be applied by the volunteers on the face (including the submental area) twice a day, with a mild massage. Subject returned 2 (T3), 3 (T4) and 6 months (T5) after the 1st injection treatment for the follow-up evaluation. The aesthetics results were established through the use of clinical evaluations (Wrinkle Severity Rating Scale (WSRS), Facial Volume Loss Scale (FVLS), and wrinkles grade of the area around the eyes (Glogau's reference photographic scale)) and instrumental assessments (profilometry on skin replicas) supported by photographic documentation and face volume image analysis. Secondary endpoints were tolerance evaluation, performance duration and subjects' efficacy judgement.

Results: The study treatment determined a very significant reduction of all clinical parameters considered (Crows' feet grade, FVLS and WSRS) at every study time. Profilometry on crows' feet skin replicas and face volume image analysis confirmed the clinical evaluation, showing a statistical/clinical significant reduction of all the profilometric parameters (anti-wrinkle efficacy) and a consistent improvement of cheek volume (bio-revolumetric effect).

Conclusion: Obtained results confirm the anti-aging activity of the associated collagen treatment (injectable dermal filler and topical mousse). The aesthetic performance resulted persistent up to the final follow-up visit, sign of a long-lasting stimulating activity on cellular functionality of the associated treatment. The majority of volunteers underlined a very marked reshaping of face contour as well as an important improvement of skin smoothness, brightness and hydration. The study treatment was well tolerated, no unexpected adverse reaction related to the tested products/injection procedure occurred during the trial.

Introduction

Background

The physiological aging process, adversely affected by ultraviolet radiation, environmental factors, unbalanced diet, pollution and hormonal factors, causes major changes to the skin [1,2].The loss of the normal level of epidermis hydration is followed by major structural alterations of the extracellular matrix (ECM), such as degradation of collagen [1-3]. The result is severe structural and functional impairment of the dermis, with inevitable aesthetic consequences: sagging skin, loss of volume and deep wrinkles [4]. Collagen, a fibrous protein of the ECM, is the predominant component of most connective tissues within mammalian body. The collagen superfamily comprises 28 known types, type I is the most abundant in the dermis, which constitutes at least 80-90% of the entire collagen [5].

The complex three-dimensional structure of this protein, allows the formation of a scaffold that guarantees a structural and functional support to the dermis and to the overlying epidermis.

Fibroblasts are the main cell population responsible for the production of collagen fibers, which is continuous, and is balanced by the degradation of old collagen by metalloproteinases [6,7]. As we age the collagen fibrils tend to "age" too, due to the formation of covalent bonds (crosslinking) [8] within the structure that destabilize the molecule, making it less elastic and more fragile. This process increases also with the intense exposure to sunlight [9]. The visible results of collagen aging is sagging skin and wrinkles, especially on the face.

Collagen has been being for decades one of the most widely used biomaterials for treatment of the skin [10,11]. It is used as filler, such as dressing in the repair process of wounds and as an ingredient in cosmetic products. Collagen is the most abundant protein in mammals, obtained from different sources animals (cattle, horses, sheep, swine, but also fish, eg. shark) and vegetable (leguminous plants, soy, seaweed and wheat seeds). The use of collagen as a filler has experienced alternating phases, due to the known ability of this protein to cause allergic reactions and immune dysregulation and the fear that the protein extracted from the tissues of some animals can function as a vector for prion infection, such as in case of 'spongiform encephalopathy (such as Creutzfeld-Jacob disease, known with the name of "mad cow disease"), which precludes, in practice, the possibility of using collagen of bovine and ovine origin for injections and cosmetic applications. New preparations of collagen appeared currently on the market have been studied to prevent allergic sensitization and to make the molecule free from any risk of infection. In addition, molecular biology and medical research have made considerable progress in identifying the role of collagen in the maintenance of homeostasis and trophic level of extracellular matrix (ECM) of the skin and connective tissues in general.

Objective

Based on the considerations above, aim of this study was to evaluate, clinically [12-14] and by non-invasive instrumental evaluations [15,16], the anti-age performance of an intradermal filler (NITHYA injectable product, manufactured and distributed by Euroresearch S.r.l. Milan - Italy) containing equine type I collagen, associated to a topical treatment consisting of equine type I collagen in form of mousse (NITHYA mousse, manufactured and distributed by Euroresearch S.r.l. Milan - Italy). Primary endpoint was to assess the treatment performance on face volume and contour restoration, as well as the filler activity at level of nasolabial folds and wrinkles around the eyes. Secondary endpoints were tolerance evaluation, and subjects' efficacy judgement.

Methods

Procedure

This was a multicenter, investigator-initiated, open clinical trial, conducted in 6 Italian centers (named A, B, D, E, F, G) by specialized physicians (one for each center) (Table 1).

Center A	Dr. Antonello Tateo, Milano (MI) – Italy
Center B	Dr. Elena Inselvini, Brescia (BS) – Italy
Center D	Dr. Massimiliano Tocchio, Osio sotto (BG) – Italy
Center E	Dr. Maria Cristina Orlandini, Rodengo Saiano (BS) – Italy
Center F	Dr. Giorgio Botali, Desenzano del garda (BS) - Italy
Center G	Dr. Adele Sparavigna, DermIng S.r.l., Clinical Research and Bioengineering Institute - Monza (MB) – Italy

Table 1: List of the centers involved in the study with their relative Investigators.

The main investigator at the head of the study coordinating center (center G) was at DermIng, Clinical Research and Bioengineering Institute, Milan - Italy.

7 visits were performed during the study: T-2 (2 weeks before the treatment start) screening and predictive allergy test; T0 (treatment start) clinical and instrumental evaluations, followed by 1st treatment injection; T1, T2 (respectively 2 and 4 weeks after the treatment start) clinical and instrumental evaluations, followed by 1st and 2nd touch-up injection treatment in order to treat possible asymmetry; T3, T4 (respectively 2 and 3 months after the treatment start) clinical and instrumental evaluations; T5 (final visit, 6 months after the treatment start), clinical and instrumental evaluations and subjects' efficacy and tolerance judgement questionnaire.

Study products and injection technique

NITHYA injectable product (manufactured and distributed by Euroresearch S.r.l. Milan - Italy) is a type I medical device containing type I horse collagen, dried, in form of fine powder, used diluted in 5 ml of saline, as a filler for the correction of deep facial cutaneous sagging and to increase facial volume. The injections (1st treatment and 2 touch-up) with NITHYA injectable product were performed by the physicians bi-laterally on the face by serial puncture technique using a 30-gauge needle (13 mm). The amount of product to be injected was determined for each subject between 2 ml and a maximum of 5 ml.

NITHYA mousse (manufactured and distributed by Euroresearch S.r.l. Milan - Italy) is a medical device contains heterologous equine collagen at concentration of 0.28%; this device is in form of mousse, in aluminum package of 50 mg. NITHYA mousse was applied by the volunteers, for the whole duration of the study, on the face (including the submental area) twice a day, in the morning and in the evening (preferentially always at the same hour), with a mild massage according to the instructions received by the investigator during the basal visit (T0).

Study population

The study was conducted on 72 healthy volunteers from 10 to 15 cases for each center. In particular were treated and evaluated 11 subjects in the center A, 10 subjects in the center B, 12 subjects in the center D, 15 subjects in the center E, 12 subjects in the center F and 12 subjects in the center G. Subjects were selected and enrolled with the following characteristics: Caucasian female, aged 40-65 years, asking for midface volume restoration (Facial Volume Loss Scale (FVLS) score >2), presenting cutaneous aging/photoaging signs, available to return to the study site for the post-procedural follow-up examinations, accepting not to expose their face to strong UV irradiation (UV session, or sun bathes) without appropriate sun protection. Exclusion criteria were: pregnancy; lactation; subjects not in menopause who do not use adequate contraceptive precautions or do not accept to perform the pregnancy test; Body Mass Index (BMI) variation during the study period ± 1; subjects who performed in the 6 month before the trial beginning HA injections, radiofrequency treatment, toxin treatment; use of permanent filler in the past; sensitivity or know allergy to the test products or theirs ingredients, confirmed by a predictive allergy testing performed 2 weeks before the collagen injection; participation in a similar study actually or during the previous 6 months. Subjects were also excluded if affected with dermatological diseases of the face, as well as scars of malformations,

with general significant diseases (diabetes, endocrine, hepatic, renal, cardiac, pulmonary disorders, cancer, neurological of psychological diseases, inflammatory/immunosuppressive disease, drug allergy), undergoing some pharmacological treatments (anti-inflammatory, anti-histaminic, topic and systemic corticosteroids, narcotics, antidepressants, immunosuppressive drugs, and any drug able to influence results in the investigator's opinion). Subjects were removed from the trial if they withdrew from the study for personal reasons, developed any of the conditions specified in the original exclusion criteria or contracted a serious illness preventing continuation of the study. Subjects removed from the study were not replaced.

Efficacy evaluation

The aesthetics results were established through the use of qualitative (clinical evaluations) and quantitative assessments (profilometry on skin replicas) supported by photographic documentation and face volume image analysis (only for the study coordinating center). All evaluations were performed directly on the subjects at each study visit, under standard environmental conditions: temperature=22 ± 2°C, relative humidity<60%. Before each visit the volunteers get acclimatized under controlled and relax conditions for at least 15 minutes.

The evaluations were carried out mono-laterally on the right or left side according to a subjects' randomization list defined by the each investigator before the subjects' inclusion.

Clinical assessment was performed at each study time; in particular nasolabial folds severity was rated according to the Wrinkle Severity Rating Scale (WSRS), cheek ptosis according to the Facial Volume Loss Scale (FVLS) photographic scale, while wrinkles grade around the eyes (Crow's feet) was performed according to the Glogau's reference photographic scale.

Replicas of area around the eyes were performed at T0 and T3 using silicone rubber (Silicon liquid Optosil – Heraeus Kulzer, Germany and Catalyst Universal Activator – Optosil˚ –Xantopren˚, Germany). A picture of the skin replicas was taken thanks to Primos compact portable device (GFMesstechnik, Teltow - Germany). Primos software is able to elaborate 3D representations of skin wrinkles as well as to measure skin principal profilometric parameters: Ra (average roughness of the analysed profile), Rt (wrinkles total high), Rv (wrinkles maximum depth).

At each study time for the volunteers included by the center G, 3D pictures of the face were taken by VECTRA H1 (Canfield, Parsippany - USA) handheld imaging system.

Face volume image analysis was carried on the 3D pictures of the volunteers included by the center G taken at T0, T3 and T5 by Vectra H1.

In particular Vectra analysis module (VAM) (Canfield, Parsippany - USA) merges and compares the images taken at two different times and automatically calculates the volume difference using a colour distance map. In order to calculate the volume variation induced by the study treatment, the volume measurement was performed for each subject mono-laterally on a selected area of the cheek and then analyzed by the software.

Tolerance evaluation

Treatment tolerance was evaluated considering: local expected events/reactions induced by the injection procedure (tardive swelling, pain, erythema, bruise); any other adverse event/reaction, also of systemic source occurred during the study.

Data analysis

The statistical analysis of clinical data was carried out with not parametric test. The analysis of all numeric parameters (arithmetic mean, standard deviation) were carried out: by non-parametric test when the normality hypothesis is rejected by the Shapiro-Wilk test at the threshold inferior to 5%; by parametric test, when the normality hypothesis is confirmed by Shapiro-Wilk test. The activity of the test treatment is expressed in absolute values versus baseline (T0).

Ethical and regulatory aspects

A final version of the study protocol and appendices were submitted to the Local Ethic Committee at DermIng s.r.l. (Milan - Italy); on 28th April 2016 the study obtained the approval and started on 23rd May 2016. This study was performed in agreement with the Declaration of Helsinki. Before the screening, all subjects gave the written informed consent.

Results

Five "drop-outs" occurred during the study; in particular subjects with randomization n. 13, 49, 62, 65 and 82 stopped prematurely the trial, due to personal reason not related with the study treatment. No other important event, which may have interfered to the test results occurred during the study period.

The statistical analysis was performed on a total of: 67 cases for the clinical evaluations up to T3; 57 cases for the clinical evaluations at T4 and T5 (follow-up data of F centre are not available); 65 cases for the crow's feet profilometry; 11 cases for the 3D-photographic documentation and face volume image analysis (subjects of G center).

Clinical assessment

The study treatment determined starting from T1 (2 weeks after the 1st injection procedure) and more markedly from T3 (2 months after the 1st injection procedure) a very significant reduction of facial volume loss scale (-5.9% at T1, -20.6% at T2, -32.4 at T3, -35.3% at T4 and -32.4% at T5; P <0.05 all study time *vs.* T0) corresponding to a reduction of the clinical score of at least 1 grade (FVLS reference photographic scale) respectively on 21%, 67%, 88%, 89% and 88% of volunteers (Figure 1).
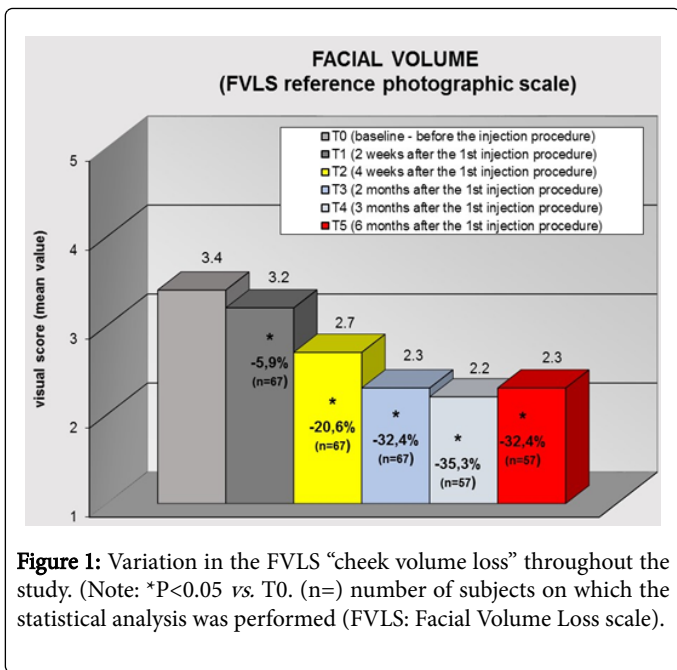
Figure 1: Variation in the FVLS "cheek volume loss" throughout the study. (Note: *P<0.05 *vs.* T0. (n=) number of subjects on which the statistical analysis was performed (FVLS: Facial Volume Loss scale).

The results were also significant for the rating of wrinkles severity and the aspect of crows' feet wrinkles. WSRS shows a decrease of 6.1% at T1, 21.2% at T2, 33.3 at T3, 33.3% at T4 and 30.3% at T5 (P<0.05 all study times *vs.* T0), corresponding to a decrease of the clinical score of at least 1 grade (WSRS reference photographic scale) respectively on 18%, 61%, 90%, 84% and 75% of included subjects (Figure 2). Crows' feet severity shows a reduction of 6.5% at T1, 22.6% at T2, 32.3 at T3, 29% at T4 and 25.8% at T5 (P<0.05 all study time *vs.* T0) corresponding to a decrease of the clinical score of at least 1 grade (Glogau's photographic scale) respectively on 18%, 58%, 94%, 84% and 77% of included subjects (Figure 3).
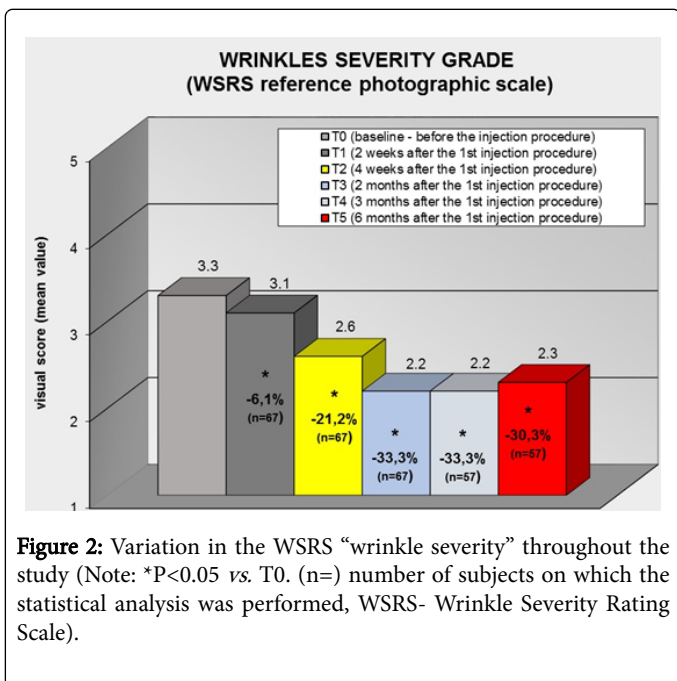

Figure 2: Variation in the WSRS "wrinkle severity" throughout the study (Note: *P<0.05 *vs.* T0. (n=) number of subjects on which the statistical analysis was performed, WSRS- Wrinkle Severity Rating Scale).
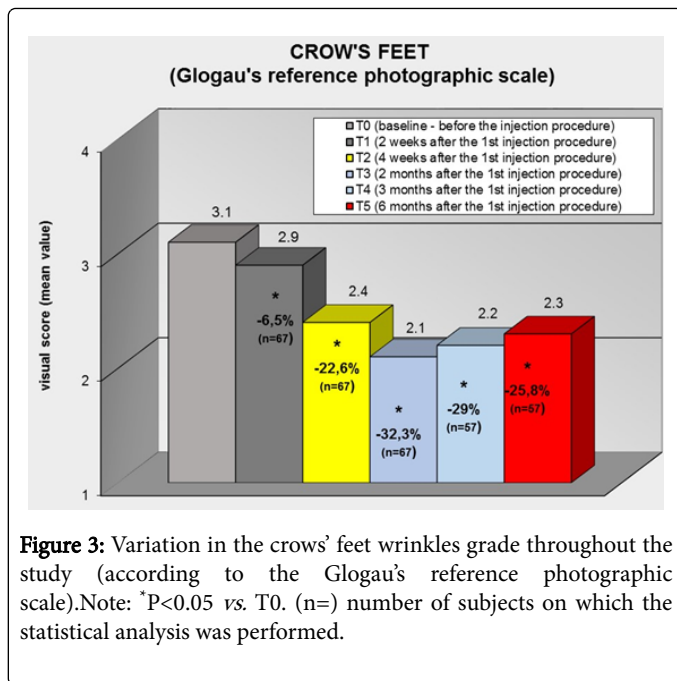

Figure 3: Variation in the crows' feet wrinkles grade throughout the study (according to the Glogau's reference photographic scale).Note: *P<0.05 *vs.* T0. (n=) number of subjects on which the statistical analysis was performed.
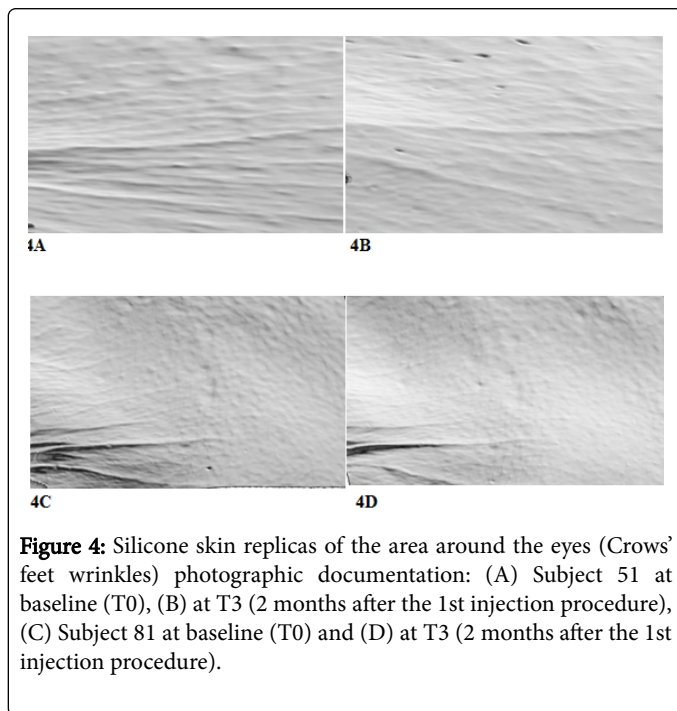

Figure 4: Silicone skin replicas of the area around the eyes (Crows' feet wrinkles) photographic documentation: (A) Subject 51 at baseline (T0), (B) at T3 (2 months after the 1st injection procedure), (C) Subject 81 at baseline (T0) and (D) at T3 (2 months after the 1st injection procedure).

Instrumental assessment

The skin profilometry of wrinkles (Primos compact portable device - GFMesstechnik, Teltow, Germany) performed on crows' feet skin replicas underlined 2 months after the first injection procedure (T3) an important and significant "filling" and "anti-wrinkles" activity of the tested product (Figure 4). More precisely was highlighted a statistically/clinically significant reduction of all the profilometric parameters considered. Ra (average roughness of the analyzed profile) shows a reduction of 21.7% (P<0.01 T3 *vs.* T0), index that the periocular area is generally less wrinkled; Rt (wrinkles total high)

shows a reduction of 22.9% (P<0.05 T3 *vs.* T0), index that wrinkles are less marked; Rv (wrinkles maximum depth) shows a reduction of 17.5%, sign that wrinkles are less deep (Figure 5).
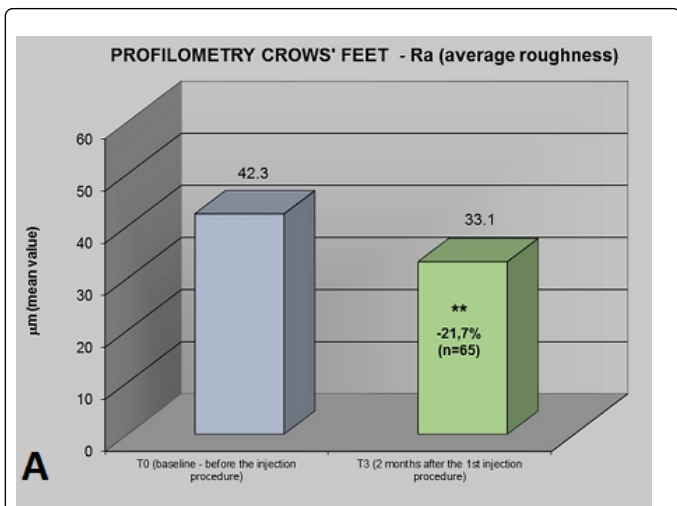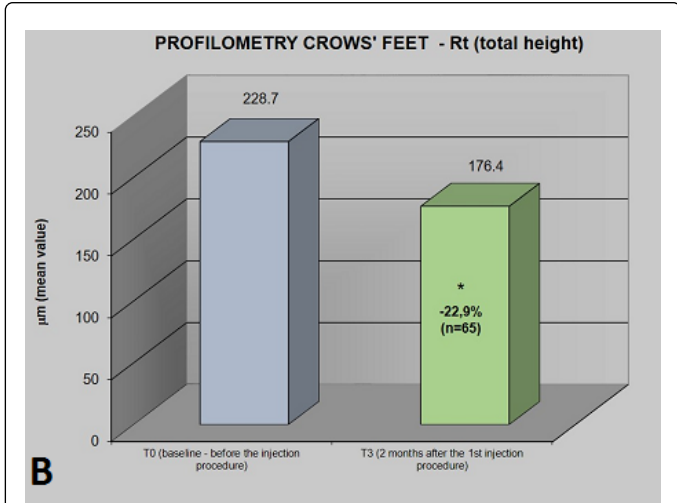

Figure 5A: Reduction of profilometric parameters (crows' feet wrinkles) at baseline (T0) and at T3 (2 months after the 1st injection procedure). Ra: avarage roughness. (Note: *P<0.05 vs T0. (n=) number of subjects on which the statistical analysis was performed).

Figure 5B: Reduction of profilometric parameters (crows' feet wrinkles) at baseline (T0) and at T3 (2 months after the 1st injection procedure). Rt: total height. (Note: **P<0.01 vs T0. (n=) number of subjects on which the statistical analysis was performed).
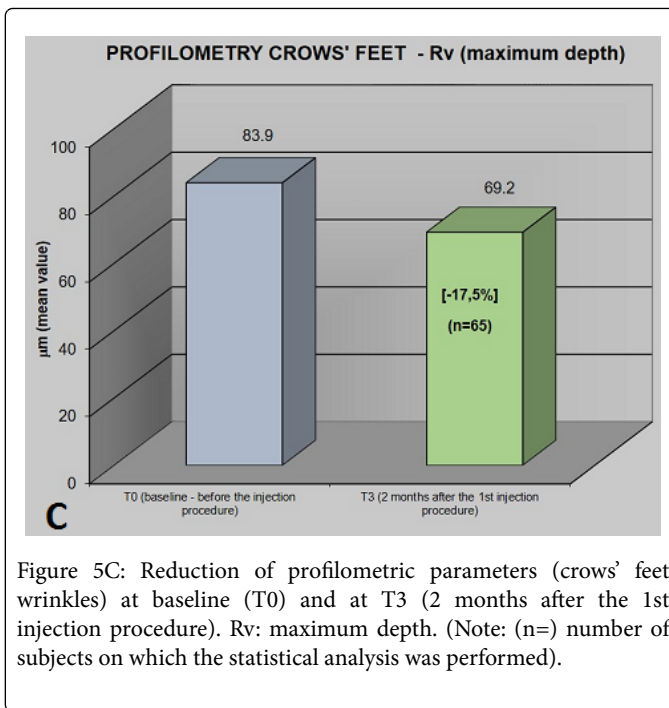

Figure 5C: Reduction of profilometric parameters (crows' feet wrinkles) at baseline (T0) and at T3 (2 months after the 1st injection procedure). Rv: maximum depth. (Note: (n=) number of subjects on which the statistical analysis was performed).

Face volume image analysis was carried on the 3D pictures of the volunteers included by the study coordinating center (G) taken at T0, T3 and T5 by Vectra H1 (Canfield, Parsippany - USA). Image analysis highlighted an average improvement of face volume cheek versus T0 of 0.812 cc at T3 and of 0.939 cc at T5, with an increasing percentage T5-T3 of 15.6% (+0.127 cc) (Figure 6); these results show a clinically important and long lasting bio-revolumetric effect of the tested treatment (Figure 7).
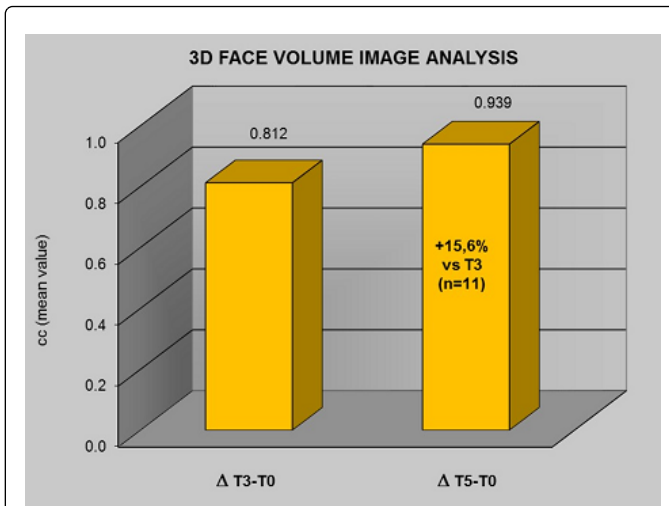

Figure 6: Three-dimensional face volume analysis: increase of volume (cc) T3 (2 months after the 1st injection procedure) versus T0 (baseline) and T5 (6 months after the 1st injection procedure) versus T0 (baseline). (Note: (n=) number of subjects on which the statistical analysis was performed).
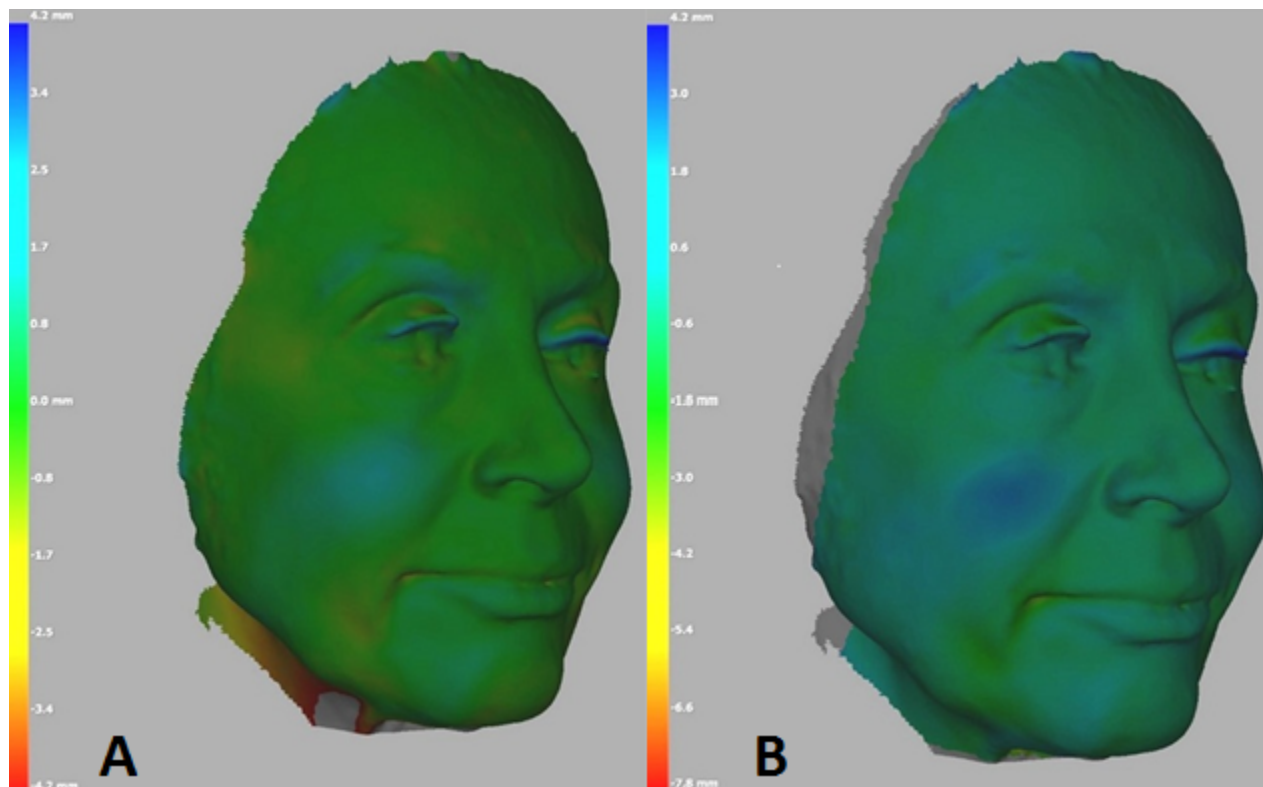
Figure 7: Face volume image analysis (volume difference using a colour distance map). (A) Subject 76 T0 (baseline) *vs.* T3 (2 months after the 1st injection procedure) and (B) T0 (baseline) *vs.* T5 (6 months after the 1st injection procedure).

	Sum of Medium, Marked and very Marked Judgements (Subject %)
Improvement of cheeks volume	95%
Reshaping of face silhouette	88%
Improvement of deep wrinkles	87%
Improvement of superficial wrinkles	95%
Lifting effect	89%
Improvement of skin suppleness	94%
Improvement of skin smoothness	95%
Improvement of skin brightness	93%
Improvement of skin hydration	89%

Table 2: Summary table: Subject's judgements on the treatment efficacy (sum of subject % who expressed a medium, marked and very marked judgements).

Efficacy evaluation by the volunteers

At the end of the trial (6 months after the first injection treatment), each volunteer filled in a questionnaire regarding treatment efficacy. Obtained results are summarized in the following table (Table 2). In particular a very high percentage of subjects confirmed the re-densifying, lifting and anti-wrinkles efficacy of the tested treatment and referred a very marked reshaping of face silhouette as well an important improvement of skin smoothness, brightness and hydration.

Safety results

Some subjects referred burning sensation during the injection procedure and the appearance of light/moderate bruises on the injection points that totally disappeared within 5-10 days. All these reactions represent expected events imputable to the typology of the injected product and/or to the injection procedure, then the investigators judged the final treatment tolerance generally good-excellent.

Conclusion

Currently the greatest efforts of aesthetic medicine research are oriented to the dermis biorestructuration, through specific treatments aim at improving the dermis quality, in order to counter from the inside the main imperfections due to skin aging. The main goal is the identification of the best possible chemical composition, capable of stimulating the fibroblasts, providing them the precursors of collagen and inducing the activity directly. Among the biomaterials available for use intradermal, collagen is always a very popular choice by physicians, both for its biocompatibility and ease of handling both for the particularly natural aesthetic result.

The type I collagen mechanism of action is currently living a revived interest, thanks to the knowledge of new molecular mechanisms of ECM homeostasis. It has been discovered that type I collagen operates a traction on the type VI collagen fibrils, which form a network of fibrils in the immediate vicinity of the cell membranes [11]. The mechanical stress that results on the cells is able to stimulate the production of new extracellular matrix (mechanotransduction process).

Obtained results confirm the anti-aging activity of the associated treatment "NITHYA injectable product" and "NITHYA mousse" (manufactured and distributed by Euroresearch S.r.l. Milan - Italy). In fact the synergic activity of two formulations determined already at T1 (2 weeks after the 1st injection procedure) and more markedly at T3 (one month after the last injection procedure): a statistically significant improvement of FVLS score (bio-revolumetric effect), confirmed by the analysis of cheek volumes and supported by the 3D-photographic documentation (Figures 8 and 9); a statistically significant reduction of Glogau's and WSRS scores (filler efficacy and anti-wrinkles/lifting activity); the lifting performance was also highlighted by the skin replicas image analysis (crows' feet appeared generally less marked and visible).

Figure 8: Subject 76: (A) T0 (baseline), (B) T3 (2 months after the 1st injection procedure) and (C) T5 (6 months after the 1st injection procedure).

Figure 9: Subject 81: (A) T0 baseline, (B) T3 (2 months after the 1st injection procedure) and (C) T5 (6 months after the 1st injection procedure).

The aesthetic performance resulted persistent up to T5, as confirmed by the 3D-face volume image analysis results (Figure 7), sign of a long-lasting stimulating activity on cellular functionality of the associated treatment.

The majority of volunteers noticed the treatment efficacy and underlined a very marked reshaping of face silhouette as well an important improvement of skin smoothness, brightness and hydration.

The final treatment tolerance was judged good/excellent; in fact no unexpected adverse reaction related to the tested products/injection procedure occurred during the trial.

Disclosures

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