

Efficacy and Tolerance of an Injectable Medical Device Containing Hyaluronic Acid and Amino acids: A Monocentric Six-Month Open-Label Evaluation

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Abstract

Background: An innovative injectable solution containing low molecular hyaluronic acid (HA) and a specific Amino acids mixture was formulated to physiologically promote local neo-collagenesis and elastogenesis through fibroblasts chemotaxis migration into the injected area.

Objective: Aim of this open clinical trial is to evaluate efficacy and tolerance of the injectable product under study, on main sign of face skin photoaging.

Methods: A single Italian center treated 25 female subjects aged 48-65 years with 4 micro-injection sessions with 10-day time laps between each product administration. Subjects were evaluated in basal conditions and after 4, 8, 12 and 24 weeks, using validated clinical scales, subjective evaluations and objective quantitative outcome measures. Assessment of aesthetic results included photographic documentation.

Results: Obtained results showed an improvement of all the clinical and subjective assessments and on the majority of objective instrumental parameters. These were already significant 10 days after the first injection procedure and were still significant and still improving after 6 months (at follow up). Global judgment on tolerability was good/excellent, both in the investigators' opinion and volunteers' self-evaluation.

Conclusions: Obtained results confirm the aesthetic performance of the injectable product on main signs of face skin photoaging. In particular, it was demonstrated the biovolumetric effect, the antiwrinkle efficacy, a superficial and deep moisturising activity and elasticizing properties. This study supports the definition of extracellular matrix targeting (ECM-targeting) for this product.

Keywords: Hyaluronic acid; Amino acids; ECM-targeting; Facial rejuvenation

Introduction

Photoaging is premature skin aging resulting from prolonged and repeated exposure to solar radiation [1]. The changes of photo-damage are superimposed on the changes caused by chronological aging and are responsible for most of the age-associated features of skin appearance [2,3].

Main signs of photoaging include fine and deep wrinkles, dyspigmentation, loss of skin tone and elasticity [2,4]. Several studies have demonstrated that the skin can be stimulated to correct aging/photoaging alterations by the intradermal injection of biological substances able to induce a revitalization of the dermis. The aesthetic medicine frequently uses HA as Biorevitalizer product to decrease the skin aging [5,6]; thanks to its natural hydrating and stimulating properties, the HA reduces the signs of age improving skin turgor and elasticity [7]. Hyaluronic acid is also involved in tissue repair, promotes the proliferation of fibroblasts, stimulates the neo-synthesis of collagen and other constituents of the extracellular matrix, moreover thanks to

its scavenger action on free radicals the HA is used to accelerate the wound healing [8].

There are several HA preparations for bio-revitalization with different molecular weight, concentration and viscosity.

The product studied (Sunekos[®] 200, Professional Dietetics SpA, Milano, Italy) is a medical device containing low molecular HA and a specific Amino acids mixture.. Sunekos[®] 200 is able to physiologically promote local neo-collagenesis and elastogenesis through fibroblasts chemotaxis migration into the injected area. A recent *in vitro* study conducted on human dermal fibroblast has shown the efficacy of Sunekos[®] 200 on the biosynthesis of extracellular matrix proteins, in particular of elastin; it has been demonstrated that varying the quality and quantity of Amino acids in the mixtures is it possible to increase the expression, at gene and protein level, of elastin though maintaining a stimulation of collagen [9]. The structural function of collagen in the dermis is known. However, the integrity of matrix is not restricted to collagen but it is linked to the production and physiological interaction of all structural proteins produced by fibroblasts. Today more and more important is the role attributed to elastin because the interaction of elastin with collagen keeps the anisotropy (which is the ability of fibres to propagate tensile forces, characteristics that is lost with age) of the matrix. In order to prevent free radical damage of dermis structure,

fibroblasts require synthetic, physiological and controlled input to produce constitutive proteins of the matrix [10]. In the *in vitro* studies performed we demonstrated that in absence of constitutive Amino acids such as L-alanine and L-valine there is no production of elastin [9]. The same studies demonstrated that an optimal ratio between the cluster of Amino acids, of both collagen and elastin, is capable of stimulating fibroblasts to produce both proteins. Primary end point of the study was to evaluate clinically [10-14] and by non-invasive instrumental evaluations [15-19] tolerance and efficacy of Sunekos® 200 injectable treatment on main sign of face skin photoaging; the micro-injection of the study product were performed by a specialized dermatologist, bilaterally on the face (zygomatic protuberance, nostril's angle, inferior margin of tragus, lip marionette lines, mandibular angle) of female volunteers aged 45-65 years, with photoaging of mild/moderate grade, who meet the inclusion and exclusion criteria required by the study procedure. It is also aim of this study to evaluate tolerance both by investigator and volunteers and efficacy by the volunteers.

Methods

Study product

Sunekos® 200 is a medical device (class III) composed of small bottles containing 100 mg of sterile and apyrogenous lyophilized of Glycine, L-Proline, L-leucine, L-Lysine HCl, L- Valine, L-Alanine and sterile vials containing sodium hyaluronate (30 mg in 3 ml of distilled water), manufactured and distributed by Professional Dietetics SpA, used for the correction of photoaging/aging face and body signs (light and medium degree).

Materials

The list of materials employed in the study, including those for instrumental assessment is displayed in Table 1.

Materials	Model/Producer/Country
Sunekos® 200	Professional Dietetics SpA, Milano, Italy
30G, 13 mm needle	
Camera 3D	Vectra H1 - Canfield, USA
Corneometer	CM825 - Courage-Khazaka, Köln, Germany
MoistureMeterD	Delfin Technologies, Kuopio, Finland
Primos compact portable	GF Messtechnik, Germany
Dermal Torque Meter	Dia-Stron LTD, UK

Table 1: Materials and instruments employed in the study.

Study design

This was an open single-center conducted by Derming Srl., Clinical Research and Bioengineering Institute, Milano, Italy, under dermatological control.

The study foresaw 4 micro-injection sessions with an interval of 10 days: at basal visit (T0), and at 10, 20 and 30 days (T2i, T3i, T4i) after the 1st injection procedure. It included 7 observation visits, performed at T0, after 10, 20 and 30 days after basal evaluations and 3 during the

follow-up 2, 3 and 6 months from baseline (T2M, T3M, T6M) (Figure 1).

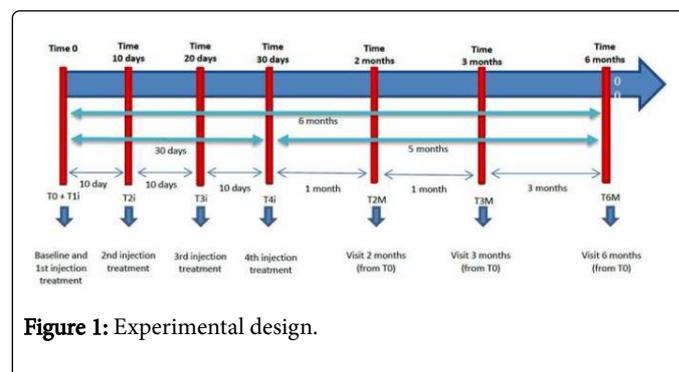


Figure 1: Experimental design.

Study population

The study included 25 healthy female subjects, age range 48-65 years (mean=57), whose informed consent had been obtained.

Subjects were selected fulfilling the following criteria:

- Female sex,
- Age 45-65 years,
- Mild/moderate cutaneous photoaging according to a reference photographic scale,
- Skin phototype I, II and III according to Fitzpatrick's classification, with a preference to grade II-III,
- Accepted to avoid during the study strong UV irradiation on the face (UV session, or sun baths),
- Accepted to present at each study visit without make-up and not to change habits regarding food, physical activity, make-up use, face cosmetic and cleansing products.

Subjects were excluded in presence of these criteria:

- Pregnancy
- Lactation
- Subjects not in menopause who did not use adequate contraceptive precautions
- Body Mass Index (BMI) variation more than ± 1 during the study period
- Subjects not in menopause who do not accept to perform the pregnancy test during the basal visit, 30 days (T4i) and 2 months (T2M) after the first treatment execution
- Subjects who performed aesthetic correction treatments (face lifting, biomaterials implants, botox injections, chemical peeling, and laser) in the 12 months prior to the study start, or performed permanent filler in the past
- Sensitivity to the test product or its ingredients.

Injection technique

The injectable solution was prepared *ex tempore*, mixing the Amino acids powder with the HA solution. The 1st intradermal treatment (T1i) was performed during the first visit (T0), after basal evaluations and repeated after 10 (T2i), 20 (T3i) and 30 (T4i) days. Treatments

were performed with a 30G, 13 mm needle, with microinjections technique by a specialized dermatologist, bilaterally on the face (zygomatic protuberance, nostril's angle, inferior margin of tragus, lip marionette lines, mandibular angle). The injected volume selected for the 4 implants was 3 ml (1.5 ml for emi-face side) for each session performed.

Efficacy assessments

The assessment of the aesthetic results was established through the use of clinical evaluations, instrumental measurements and three-dimensional photographic documentation with face volume image analysis.

A randomization list was defined by the investigator before the inclusion and all evaluations were carried out at each observational time mono-laterally on the right or left side according to this subjects' list.

All evaluations were performed under standard environmental conditions: temperature=22+/-2°C, relative humidity <60% and after at least 15 mins of subject's acclimation under controlled and relaxed conditions. The possible events which could have interfered to the test results were assessed at the end of the study.

Clinical evaluations included: wrinkles grade around the eyes (Crow's feet) and photoaging grade through the use of Glogau's reference photographic scale; nasolabial folds severity grade were rated through the use of Wrinkle Severity Rating Scale (WSRS) while cheek ptosis was rated according to the Facial Volume Loss Scale photographic scale (FVLS).

Superficial (electrical capacitance) and deep (tissue dielectric constant) skin hydration were measured at each study time at level of the cheek respectively with Corneometer (CM825, Courage-Khazaka, Köln, Germany) and MoistureMeterD (Delfin Technologies, Kuopio, Finland). Skin plasto-elasticity of superficial and deep skin layers (immediate and maximum extensibility, viscoelasticity, immediate elastic recovery) was measured at each study time at level of the cheek, with Dermal Torque Meter (Dia-Stron Ltd, Andover, UK). The profilometric analysis of crow's feet area roughness (Ra: average roughness of the analysed profile, Rt: wrinkles total high, Rv: wrinkles maximum depth) was performed at T0, T2M, T3M and T6M with Primos compact portable device (GFMeasstechnik, Teltow-Germany).

Three-dimensional photographic documentation and face volume analysis was performed with Vectra H1 (Canfield, Parsippany, NJ, USA) at T0, T2M, T3M and T6M. Vectra analysis modules (VAM) overlap and compare two pictures taken at different times and calculate the volume difference.

Tolerance evaluation

Treatment tolerance evaluation was performed considering:

-Local expected events/reactions (bruise, pain, erythema, tardive swelling).

-Any other adverse event/reaction, also of systemic source occurred during the study.

Ethical and regulatory aspects

The study was performed in agreement with the Declaration of Helsinki [20]. The study protocol was submitted to an Independent

Ethic Committee (I.E.C.). The clinical trial was approved by the I.E.C. on July 18th 2016 and started on October 27th 2016.

Before the screening, all subjects gave written informed consent.

Statistical methodology

23 cases were included in the the statistical analysis for the treatment phase and 21 of which were included in the follow-up phase. The data processing was performed as follows:

Clinical data: Friedman test followed, in case of statistically significant result, by Holm-Sidak Adjusted test.

Instrumental data: non-parametric test (Friedman test) when the normality hypothesis was rejected by the Shapiro-Wilk normality test (threshold at 5%) or parametric test (ANOVA test for repeated measures), when the normality hypothesis was confirmed, followed in case of statistically significant result by Holm-Sidak Adjusted test.

Results

Four "drop-outs" occurred during the study due to personal decisions. No other important event which may have interfered to the test results occurred during the study period.

Clinical efficacy evaluation

During the study it was observed:

Starting from T2i (10 days after the 1st injection procedure) a statistically important improvement of cheek volume (-13.2% at T2i, -26.3% at T3i, -36.8% at T4i, -47.4% at T2M and T3M, -42.1% at T6M - Holm-Sidak Adjusted test $p < 0.05$ vs. T0) corresponding to a reduction of the clinical score of at least 1 grade (FVLS photographic scale) on 57% of subjects at T2i, on 91% at T3i and on 100% at the other times (Figure 2).

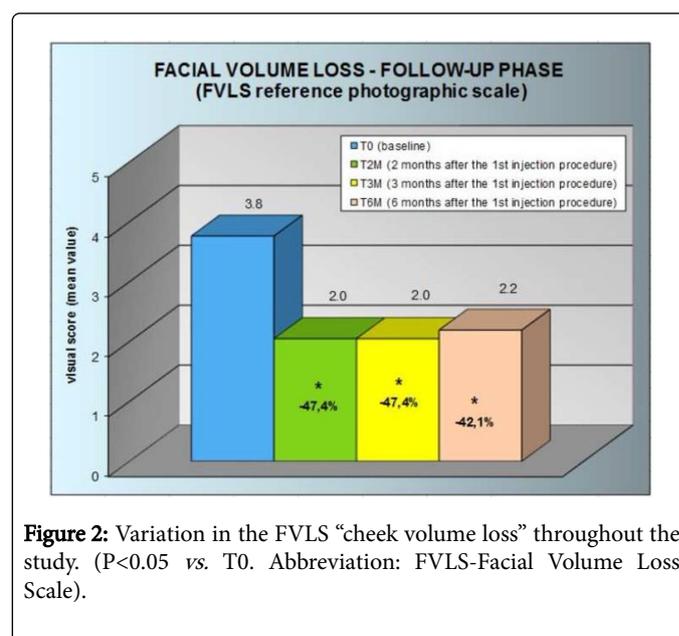


Figure 2: Variation in the FVLS "cheek volume loss" throughout the study. ($P < 0.05$ vs. T0. Abbreviation: FVLS-Facial Volume Loss Scale).

Starting from T2i (10 days after the 1st injection procedure) a statistically significant improvement of wrinkles severity (-7.5% at T2i, -22.5% at T3i, -30% at T4i, -35% at T2M, -32.5% T3M, -22.5% at T6M

Holm-Sidak Adjusted test $p < 0.05$ vs. T0) corresponding to a reduction of the clinical score (WSRS reference photographic scale) of at least 1 grade respectively on 39% of subjects at T2i, on 87% at T3i, on 100% at T4i, T2M and T3M, on 76% at T6M (Figure 3).

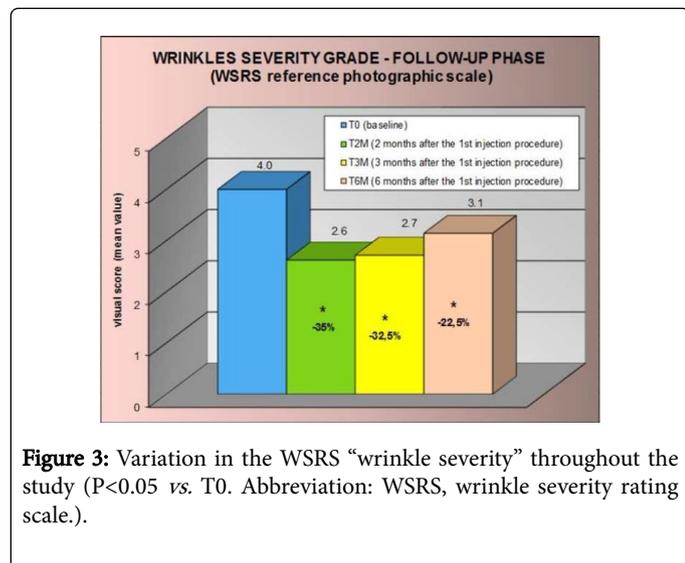


Figure 3: Variation in the WSRS “wrinkle severity” throughout the study ($P < 0.05$ vs. T0. Abbreviation: WSRS, wrinkle severity rating scale.).

Starting from T4i (10 days after the 3rd injection procedure) a very significant reduction of crow’s feet (-21.4% at T4i, -25% at T2M, T3M and T6M-Holm-Sidak Adjusted test $p < 0.05$ vs. T0) corresponding to a decrease of the clinical score of at least 1 grade (GLOGAU’S reference photographic scale) on 61% of subjects at T4i, on 57% at T2M, on 52% at T3M and T6M (Figure 4).

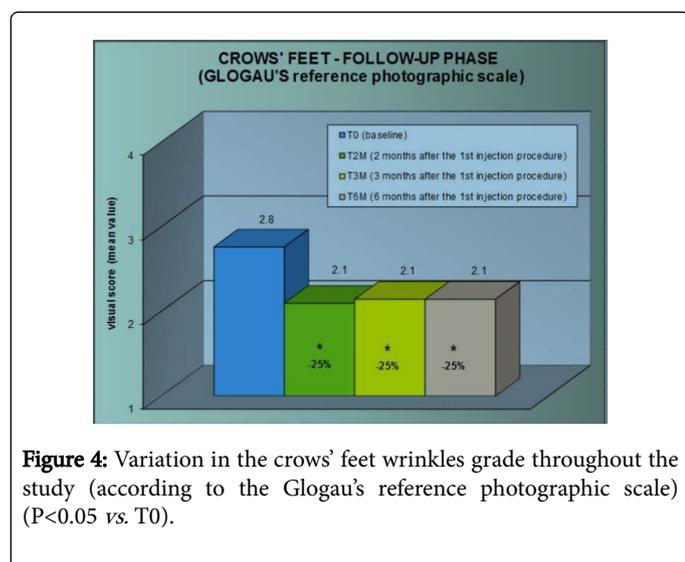


Figure 4: Variation in the crows’ feet wrinkles grade throughout the study (according to the Glogau’s reference photographic scale) ($P < 0.05$ vs. T0).

Instrumental evaluation

Obtained results highlighted starting yet 10 days after the first injection procedure a statistically significant increase of skin electrical capacitance equal to: +19% at T2i, 24% at T3i, 27.5% at T4i, 22.3% at T2M, 13.1% at T3M and 24.5% at T6M (Holm-Sidak Adjusted test $p < 0.05$ vs. T0) (Figure 5), sign of a rapid and persistent improvement of stratum corneum moisturize.

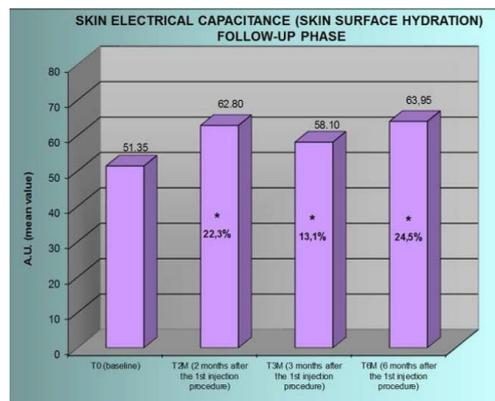


Figure 5: Variation from baseline in the skin electrical capacitance (superficial hydration) ($P < 0.05$ vs. T0).

Moreover starting from T4i (30 days after the 1st injection procedure) a clinically/statistically significant increase of deep skin layers hydration measured at 0.5 mm of depth was observed, corresponding to 9.8 at T4i, 9.5% at T2M, 4.8% at T3M and 9.9% at T6M (Holm-Sidak Adjusted test $p < 0.05$ vs. T0) (Figure 6).

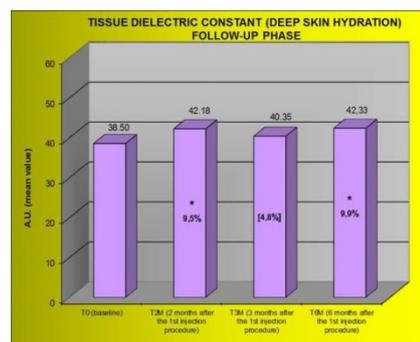


Figure 6: Variation from baseline in the tissue dielectric constant of deep skin layers (deep hydration) ($P < 0.05$ vs. T0).

Superficial and deep skin plastoelasticity measurements were performed monolaterally at level of the cheek (malar region); the following torsionometric parameters were analysed:

- Ue=Immediate extensibility,
- Uf=Maximum extensibility,
- Uv=Viscoelasticity,
- Ur=Immediate elastic recovery.

Although the evaluation of the deep plastoelasticity during the treatment phase did not showed any statistically significant variation of the considered parameters, it is important to note starting from T2i a clinically relevant increase of Uv parameter (26.2% at T2i, 28.6% at T3i, 28.1% at T4i), index of the initial re-densifying activity of the injective treatment confirmed by the follow-up data (statistically significant improvement of Uv parameter of 39.5% at T2M, 46.8% at T3M and 37.9% at T6M - Holm-Sidak Adjusted test $p < 0.05$ vs. T0).

Regarding the superficial skin plastoelasticity starting from T3i/T4i a clinically/statistically significant reduction of all torsionometric parameters, index of a progressive skin firming improvement was highlighted.

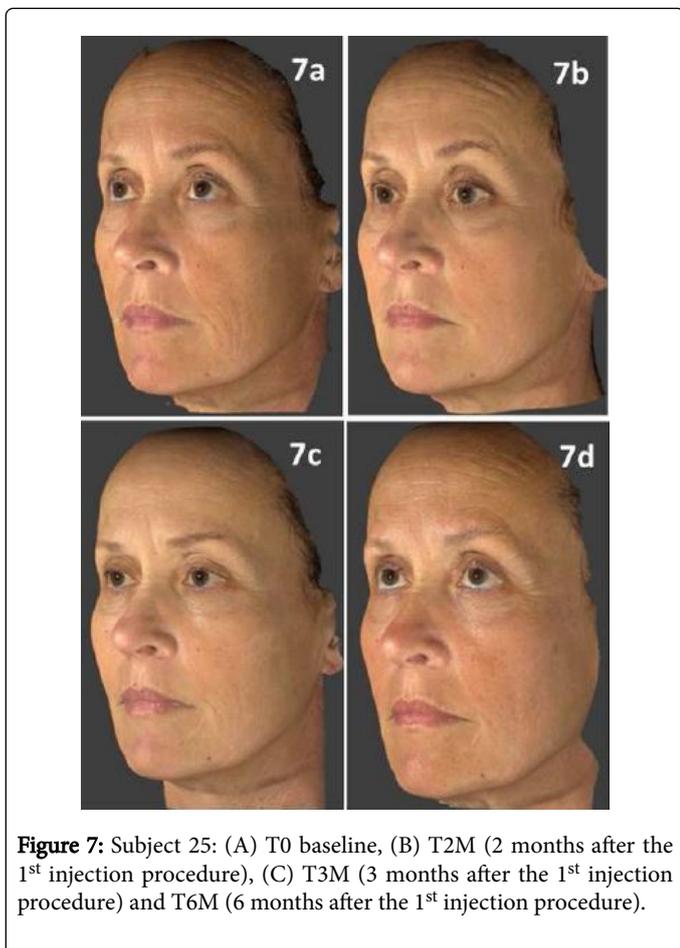


Figure 7: Subject 25: (A) T0 baseline, (B) T2M (2 months after the 1st injection procedure), (C) T3M (3 months after the 1st injection procedure) and T6M (6 months after the 1st injection procedure).

Image analysis of the area around the eyes (“Crow’s Feet”) showed starting from T2M an important and long lasting (still present at T6M) “anti-wrinkles” activity of the injective treatment also identifiable on three-dimensional photographic documentation (Figure 7a-d). More precisely a clinically/statistically significant reduction of the following parameters *versus* baseline was highlighted:

Ra (average roughness of the analysed profile) reduction of 21.9% at T2M, 18.6% at T3M and 14.7% at T6M (Holm-Sidak Adjusted test $p < 0.05$ T2M and T3M vs. T0), index that the area around the eyes is generally less wrinkled;

Rt (wrinkles total high) reduction of 16% at T2M, 15.5% at T3M and 12.2% at T6M, index that wrinkles are less marked;

Rv (wrinkles maximum depth) reduction of 18.5% at T2M, 19.3% at T3M and 17.5% at T6M, sign that wrinkles are less deep.

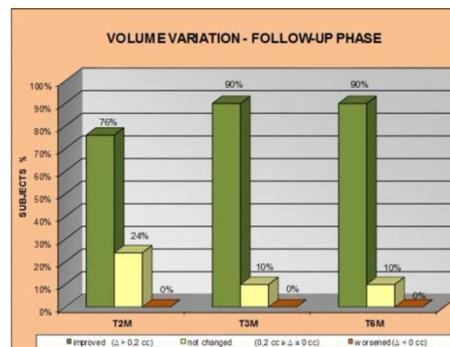


Figure 8: Three-dimensional face volume analysis: Variation % of improved subjects throughout the study (volume increase >0.2 cc).

Face volume image analysis was carried on the 3D pictures taken at T0, T2M, T3M and T6M by Vectra H1. Obtained results highlighted an average increase of volume *versus* T0 of 0.902 cc at T2M, of 0.9 cc at T3M and of 0.735 cc at T6M with a reduction percentage ΔT_{6M-T0} vs. ΔT_{2M-T0} of 18.5%, indicating a clinically important and long lasting bio-volumetric effect of the tested treatment. In particular 76% of subjects at T2M and 90% at T3M and at T6M presented an improvement of face volume cheek >0.2 cc (0.2 cc is the threshold value of the Vectra H1 software analysis) (Figure 8).

Efficacy evaluation by the volunteers

At the end of the trial (T6M) each volunteer filled in a questionnaire regarding treatment efficacy and tolerance.

It's evident the subjects' positive judgement; in particular the anti-wrinkles/filling efficacy, the improvement of skin smoothness, suppleness, brightness and hydration reach the most positive scores at the end of the study. Moreover the most part of volunteers noticed the lifting effect as well as the reshaping of face silhouette (Table 2).

	Sum of medium, marked and very marked judgements
Improvement of cheeks volume	57%
Reshaping of face silhouette	62%
Reduction of deep wrinkles	57%
Reduction of superficial wrinkles	76.2%
Lifting effect	76%
Improvement of skin suppleness	67%
Improvement of skin smoothness	71.4%
Improvement of skin brightness	81%
Improvement of skin hydration	71.4%

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

Safety results

A total of nine light/moderate bruises on some injection points occurred during the protocol, after injections. They totally disappeared within 5-10 days.

One subject complained 48 h after the second treatment the appearance of moderate bruise associated to a light oedema at level of the left periocular area; the event was treated with lactoferrin cosmetic cream (2/die for 5 days) and followed until complete resolution.

Since all these reactions represent expected events imputable to the injection procedure, the investigator judged the product tolerance good-excellent in 100% of subjects as confirmed also by the subjects' self-assessment (38% as good and 62% as excellent).

Discussion

Photoaging is characterized especially by profound alterations in the extracellular matrix cell of the dermis.

Today any intervention on anti-aging phenomena affects the matrix and it is correct to speak of ECM-Targeting (Extra Cellular Matrix-Targeting) when it comes to the dermis.

The classic biorevitalization is based on concepts and potential of HA, but in fact, although it is true that hyaluronic acid stimulates the fibroblasts even alone, for physiological and specific stimulation of new components of the ECM matrix fibroblasts it is useful to find an appropriate amount of Amino acids locally to induce the expression of mRNAs for the structural proteins and for their production.

The experimental work confirms that all objective and subjective outcomes achieved in the clinical study over time are comparable to those obtained with filler, despite the fact that this medical device is based on a non-cross-linked hyaluronic acid.

This is the first time that and hypotheses tested *in vitro* is confirmed in clinical practice. It has been demonstrated that a simultaneous and ordered activation of collagen and elastin in the matrix is possible. This activation has proved to be capable of counteracting elastosis.

The efficacy of the product is not limited to the period of treatment but has been verified in "follow up", a significant increase of the parameters of "rejuvenation" of the extracellular matrix are still present after 6 months.

Conclusions

Obtained results confirm the aesthetic performance of "Sunekos® 200" injectable treatment on main signs of face skin photoaging.

In particular, it was demonstrated the biorevolumetric effect, the anti-wrinkle efficacy, a superficial and deep moisturising activity and elasticizing properties.

The aesthetic performance of the tested product resulted generally more marked during the follow-up phase starting from T2M, sign of a stimulating activity on cellular functionality, and persistent up to T6M, as confirmed by the 3D-face volume image analysis results.

The majority of volunteers noticed the treatment efficacy and underlined in particular the study product anti-wrinkles, filling and

biorevitalizing activity as well as the lifting effect and the reshaping of face silhouette.

The final product tolerance was judged good/excellent, in fact no unexpected adverse reaction related to the injection procedure occurred during the trial.

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