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Original Research

A pilot study testing efficacy and tolerability of hybrid cooperative complexes of hyaluronic acid intradermal injections in Chinese women

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Abstract:
Objective: To assess the efficacy and tolerability of a hyaluronic acid (HA)-based injectable formulation of stable hybrid cooperative complexes of high (H-HA) and low (L-HA) molecular weight HA (32 mg of H-HA and 32 mg of L-HA) produced by NAHYCO® Hybrid Technology (Profhilo®, hereinafter referred to as "The product"), for the treatment of wrinkles, roughness and laxity of the skin of the face and neck areas in women of Chinese ethnicity resident in Italy.
Methods: 28 women from 30 to 60 years of age were enrolled, 18 for the neck area and 10 for the face. The self-isolation of the included subjects due to the COVID-19 pandemic outbreak prevented the completion of the trial as to the approved protocol, which scheduled a follow-up until week 16 after baseline, and amendments to original plan were necessary. The product was intradermally injected in 2 sessions, 4 weeks apart, assessments were performed at 4, and 8 weeks after the first injection as to the neck, while 3 subjects were evaluated until week 12 for the face. Wrinkle Severity Rating Scale (WSRS), Facial Volume Loss Score (FVLS) were used to clinically assess results for the face, while the IBSA Neck Laxity Scale was used for neck evaluation; superficial and deep hydration were assessed by a corneometer and a moisturemeter while skin color changes of the face were measured with a spectrophotometer; all these evaluations were supported by 3D photographic documentation.
Results: Data showed overall improvement of the evaluated parameters already after the first round of treatment both at the facial and neck level, with a benefit that was kept or increased after the second session, with a very high tolerability profile. Although the amelioration trend was clearly visible, the obtained data are not statistically significant, probably due to the COVID-19 pandemic outbreak that prevented to analyze results from a larger population.
Conclusions: Despite further analysis are required, the product injections could represent a promising treatment to induce skin amelioration in both face and neck areas in Chinese women, and it was well-tolerated.

Keywords: Hybrid cooperative complexes, Hyaluronic acid, Ageing, Skin laxity, Facial volume loss

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Introduction

Modern society presents a chronological contradiction: on one hand, the population is growing older and better thanks to medical advances, while on the other hand, science is being asked to find not only solutions that help live longer, but also that hide the signs of aging by stopping the relentless ticking biological clock. The face and neck are two areas of special concern, now more than ever, at a time when the popularity of video conferencing emphasizes specifically these body areas, and it is certainly not a coincidence that cosmetic procedures targeting these anatomical areas have increased exponentially over the past 3 years (i.e., Zoom Effect) [1]. Today's media impose aesthetic models with firm, rosy and radiant skin, full cheeks, and plump lips. Some lucky people are bound to maintain a youthful appearance longer than others, but the passing of time spares no one, not even those blessed by their genetics. In both the face and neck the unpleasant intrinsic and extrinsic signs of ageing sooner or later will make their appearance, with formation of wrinkles, folds, and dramatic and unstoppable worsening of skin texture and color [2-5].

There are several approaches that can be used to try to improve skin appearance of these body areas, and non-invasive methods (e.g., fillers and/or skin-boosters) are undoubtedly very popular, mainly because they allow an almost immediate return to daily and work activities by quickly restoring a fresh, and youthful appearance [6-12]. Whereas in the past cosmetic treatments were predominantly for the senior age and upper social classes, current users of aesthetic medicine are increasingly younger, better informed, and socially and economically diversified. Hence the success of techniques that are affordable and yield rapid results with minimum recovery-time. Hyaluronic acid (HA) is one of the compounds mostly used to restore skin appearance, and injections with this molecule have been proven to be effective and with a good safety profile [13-21]. The product is a medical device containing a hybrid mix of low and high molecular weight HA, which has shown its efficacy and high tolerability in the treatment of skin laxity of face and neck [22-24]. So far, these studies have been performed mainly in women of Caucasian ethnicity; however, investigators now want to evaluate efficacy and safety outcomes of this medical device by assessing clinical and instrumental parameters in subjects of different ethnicities. A physical appearance in which skin structures retain their tone and freshness over the years, defying the rules of time passing by, is globally recognized as a winner, therefore it makes sense that the efficacy and safety of an aesthetic treatment with such a power would be tested in subjects with different anthropometric and phenotypic features, as well as different phototypes.

In this study, the selected population is composed of Chinese women living in Italy, and future goal of the authors is to carry out a comparison between the outcomes presented in this paper and those previously published in a similar sample size of subjects of Caucasian ethnicity treated in the same body areas with the same medical device.

Materials and Methods

Study Population

This was a single-centre clinical trial (CT) approved by an independent Ethical Committee. Enrolled subjects were females, between 30 and 60 years of age and they were selected among the Chinese population living in Italy. In order to be included in the CT the subjects agreed not to change their habits as to food, physical activity, make-up or beauty routine. Moreover, they were asked to avoid any strong UV exposure (i.e., natural suntan, or sun-bathes) during the entire duration of the study, without any appropriate sun protection. Pregnant or breast-feeding women were excluded from the CT, as well as smokers, alcohol and drug abusers, subjects whose Body Mass Index (BMI) showed a variation (i.e., ± 1) during the study period, who had performed other skin aesthetic treatments (e.g., biomaterials implants, face lifting, Botulinum toxin injections, laser, chemical peeling) in the 6 months prior to the study start, or who had performed any permanent filler treatment in the past, or who had already being enrolled in similar studies in the previous 9 months. Additional exclusion criteria were sensitivity to the test product or any of its ingredients, and any concomitant pharmacological treatments which could affect the outcomes in the investigator's opinion (i.e., anticoagulants and antiplatelet drugs, anti-histaminic, topic and systemic corticosteroids, narcotic, antidepressant, immunosuppressive drugs), with the exception of contraceptive or hormonal treatment which was started more than 1 year before enrolment. Initial study population included 28 subjects who fulfilled the above-mentioned criteria.

Study Design

The protocol consisted in two intradermal injections of the product (IBSA Farmaceutici Italia S.r.l.), with a prefilled syringe containing 2 ml of 3.2% HA (i.e., 32 mg H-HA, and 32 mg L-HA in 2 ml of saline solution). The two injections were performed at a 4-week interval and 5 assessment visits were scheduled as follows:

-T0, baseline visit, first injective procedure;

-T4W, second visit, followed by second injective procedure, performed 4 weeks after the first injection;

-T8W, T12W and T16W performed 8, 12 and 16 weeks after the first injection, respectively.

Study layout for the face and the neck area with number or subjects involved, clinical and instrumental assessments, and procedures performed at each visit is shown in Figure 1 and Figure 2. Study was conducted by two different specialized dermatologists; first one only

performed the injection procedure, while the other one only performed the clinical and instrumental evaluations.

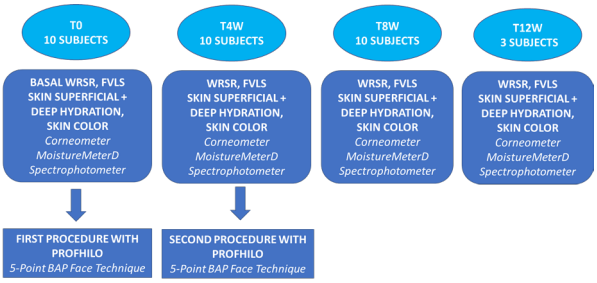

Figure 1. Study layout, face area

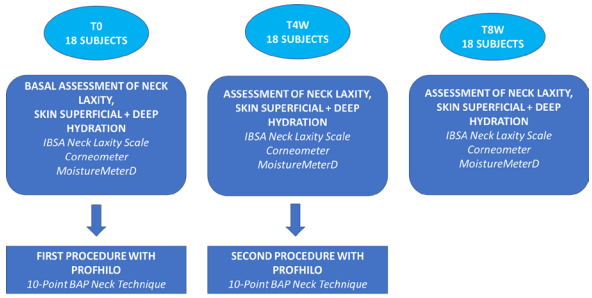

Figure 2. Study layout, neck area

Injection technique

The injections were performed by a specialized dermatologist, following a validated procedure according to the treated area:

-The 5-Point Bio Aesthetic Points (BAP) face technique bilaterally, consisting in 5 intradermal injections (0,2 ml for each bolus) at the level of the zygomatic protuberance, nostril's angle, inferior margin of tragus, lip marionette lines, mandibular angle with 29G needle (Figure 3A) [25];

-The 10-Point BAP Neck Technique, consisting in 10 intradermal injections (0,2 ml for each bolus) on 3 vertical-lines following a V-shape with 29G needle (Figure 3B) [26].

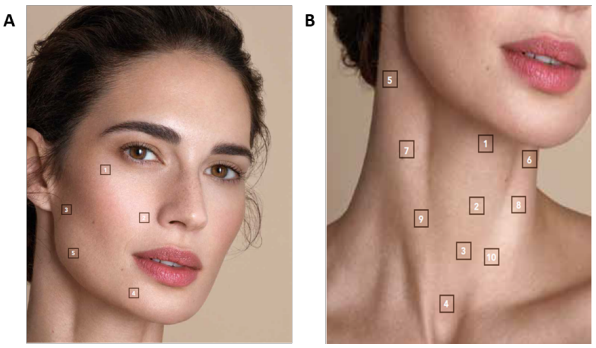

Figure 3. The 5-Point BAP Face Technique (A) and of the 10-Point BAP Neck Technique (B) with injection sites

Efficacy Assessment

For the face, efficacy was clinically determined by means of the Wrinkle Severity Rating Scale (WSRS) and the Facial Volume Loss Score (FVLS), which ranges from 1 (i.e., absence of signs) to 5 (i.e., very severe signs), with evaluations at baseline, and at each scheduled visit to assess any changes [24, 27].

To clinically grade neck laxity, the IBSA Neck Laxity Scale was used with evaluations at baseline, and at each scheduled visit to assess any changes. This photographic assessment tool consists of 5 different degrees of increasing severity of skin laxity of the neck region, ranging from 1 (i.e., normal trophism of the tissues of the neck), to 5 (i.e., severe laxity of the tissues of the neck) (Figure 4) [28].

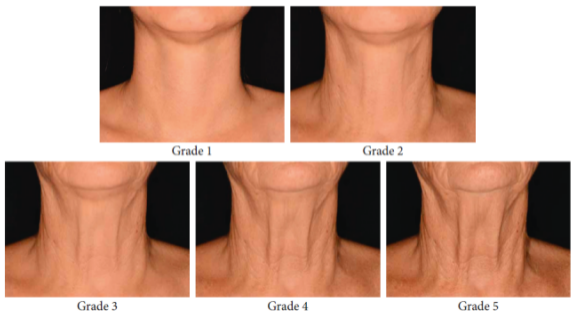

Figure 4. IBSA Neck Laxity Scale

Face and neck evaluations were supported by 3D photographic records (Vectra H1, Canfield, USA) taken using the same parameters for all the subjects. To standardize photographic records of face and neck, subjects were positioned in front of a white background, directly in front of the camera. Subjects remained in standing position with face and shoulders relaxed and avoiding facial expression. Pictures were taken according to Canfield manufacturer instructions avoiding light sources directly on subjects' face and neck.

Instrumental evaluations were performed for skin hydration and to evaluate skin redness and pigmentation variation. In order to standardize instrumental measurements, the different parameters were evaluated using the BAP technique injection point number 2 as landmark for both face and neck. Instrumental evaluations were then performed as follows:

-Superficial skin hydration (i.e., 0.5 mm) was assessed through electrical capacitance of the skin using a Corneometer CM825 (Courage-Khazaka, Koln, Germany), by carrying out 3 measures on the same skin area, and a mean value was calculated accordingly;

-Deep skin hydration (i.e., 1.5 mm) was determined by tissue dielectric constant of superficial and deep skin layers' measurements using a MoistureMeterD (Delfin Technologies, Kuopio, Finland), which generates high frequency, low power electromagnetic waves, and measures changes in the total water content of the tissue. Evaluations were performed at 0.5 mm and 1.5 mm depth by means of different probes;

-Changes in skin color were evaluated using a spectrophotometer/colorimeter CM-700D (Konica Minolta), which assesses 3 parameters, i.e., a* and b* which define redness and pigmentation, and L* which indicates brightness, under standard illumination conditions and 10° observer angle. The instrument emits a white light that is re-emitted by the object examined, collected by 36 photodiodes with spectral sensitivity from 400 nm to 700 nm, and data are then elaborated by a microprocessor.

Safety Assessment

The presence or absence of local reactions (i.e., tardive swelling, pain, erythema, or bruising) as well as any other adverse event or reaction occurring locally or systemically were monitored at each visit and after both procedures to assess the safety of the product.

Results

The study had to be prematurely stopped because of the COVID-19 outbreak and the subsequent decision of the Italian Chinese community to carry out a strict self-quarantine. Therefore, none of the 28 subjects could be evaluated at T16W as planned and amendments to original plan were made necessary. For the face area, data were available for 10 subjects at T0, T4W and T8W, and 3 of these individuals also performed the T12W visit, for the neck area data were available for 18 women at T0, T4W and T8W. A complete statistical analysis was not possible due to these circumstances; however, both clinical and instrumental data following the injection of the studied medical device were collected and analyzed. Mean age of evaluated subjects was 51 (range: 38-60) and mean BMI was 22 (range 20-27). BMI remains stable for the entire duration of the study.

Clinical Evaluation of Efficacy

Face

For the face area, all 10 subjects showed better rates as to WSRS and FVLS (i.e., -22% and -35%, respectively), already at T4W versus baseline. These outcomes were maintained or improved at T8W (i.e., -29% and -48% as to WSRS and FVLS, respectively). Three subjects were evaluated at T12W and kept the amelioration previously detected, but they were not included in the graphs due to lack of data. (Figure 5, Figure 6).

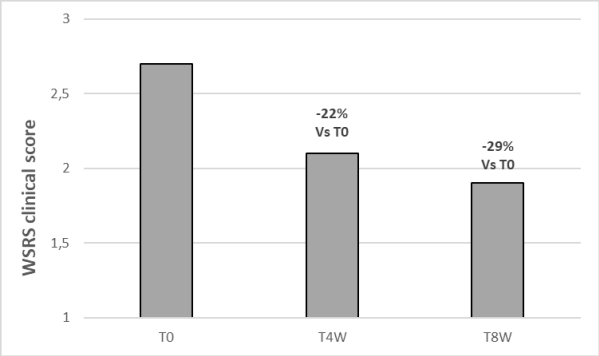

Figure 5. WSRS in the 10 subjects analyzed, face area

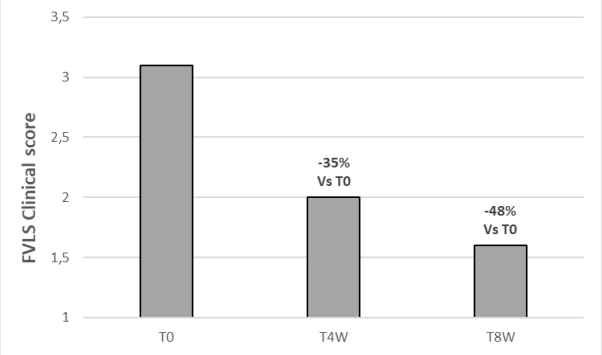

Figure 6. FVLS in the 10 subjects analyzed, face area

Neck

For the neck area, evaluations at T4W and T8W were possible for 18 subjects, of whom 3 already had the best possible score (i.e., 1) at baseline, while the other 15 women experienced a clear improvement of their skin laxity already at T4W (i.e., -33% versus baseline), which was more pronounced at T8W (i.e., -45% versus baseline), when 12 out of 18 subjects scored 1 (Figure 7).

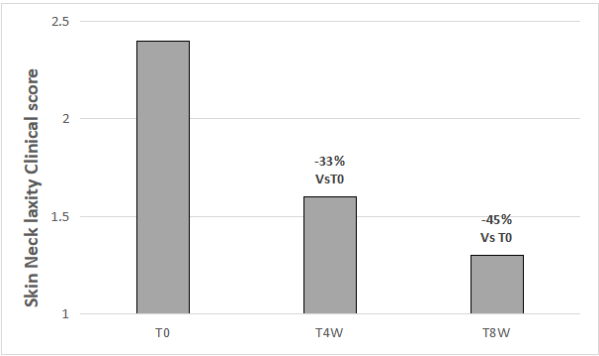

Figure 7. Clinical evaluation of skin roughness and laxity of the neck

Instrumental Evaluation of the Efficacy

Clinical improvement of data was mirrored by an overall amelioration of all instrumental parameters examined:

-As to superficial skin hydration, at T4W the increase was 2.6% and 11% for the face and neck, respectively; at T8W, the rise was 11% for the face, and 22% for the neck (Figure 8).

-A meaningful increased hydration was observed also at deeper levels. For the face, at 0.5mm depth, there was an increase of 15% and 25% at T4W and T8W, respectively; at 1.5 mm these values were 6% and 10%. Similarly, for the neck there was an increase of 6% (T4W) and 13% (T8W) at 0.5 mm, while at 1.5 mm the improvement was 2% and 7% at T4W and T8W, respectively (Figure 8).

-The assessment of skin color recorded an increase as to the L* parameter (i.e., +3% at T4W, and +2% at T8W) associated with skin brightness; a decrease as to the a* parameter indicating skin redness (i.e., -8% at T4W, and -10% at T8W) was detected; while skin pigmentation, determined by the b* parameter doubled (i.e., +3% at T4W, and +6% at T8W) (Figure 9).

The overall visual improvement was evident with an easily noticeable improvement in skin tone and texture (Figure 10).

Tolerance was evaluated good/excellent by both injector and assessor. No serious adverse events were registrated during study.

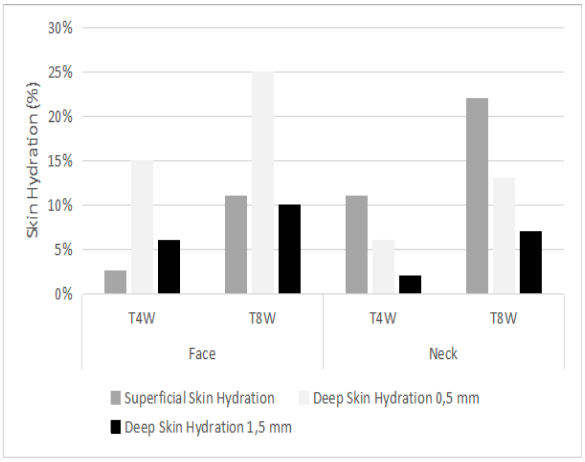

Figure 8. Increase over basal superficial (i.e., 0.5 mm) and deep (i.e., 1.5 mm) skin layers hydration of the face and the neck at T4W and T8W

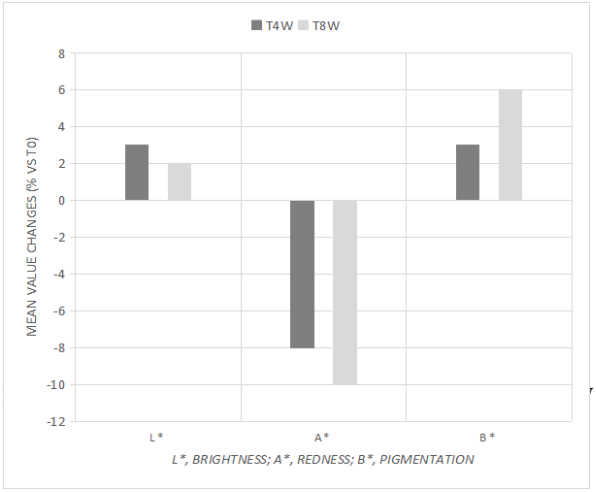

Figure 9. Mean Changes in Face skin color at T4W and T8W compared to T0

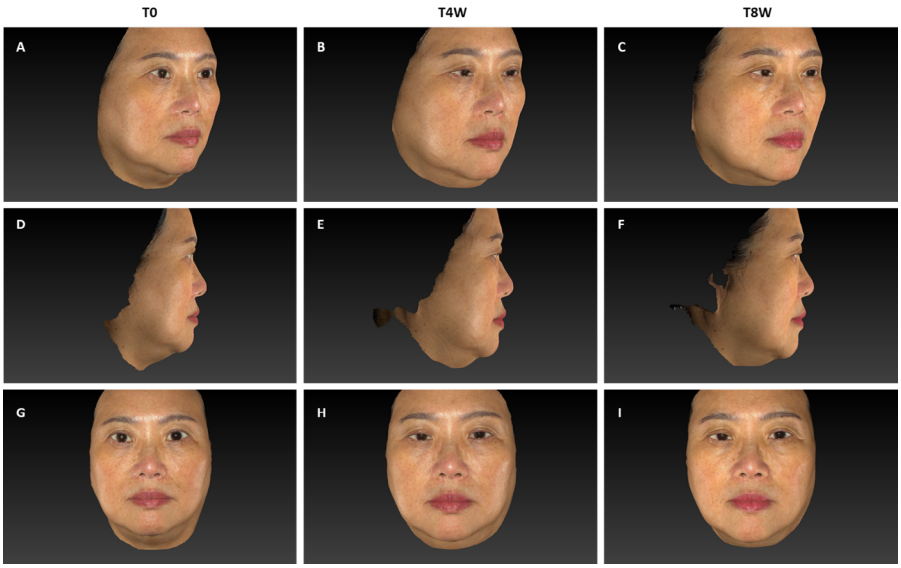

Figure 10. Photographic documentation of 45 degrees, lateral and frontal views. A, D, G: baseline visit before treatment (T0); B, E, H: 4 weeks after 1st treatment (T4W); C,F,I: 8 weeks after 1st treatment (T8W)

Discussion

Hyaluronic acid is a molecule that has proven to be capable of restoring tone and volume in various body areas, with even regenerative capabilities with respect to certain tissues, and therefore its use in aesthetic medicine is increasingly popular. An unquestionable advantage of aesthetic treatments based on hyaluronic acid fillers is its extreme biocompatibility since it is a molecule naturally present in our body hence very fast recovery time and few side effects. In addition, hyaluronic acid allows the achievement of aesthetic goals quickly but not permanently, allowing the subjects who use it to appreciate its benefits and possibly carry out consolidation sessions of the results, if and when they feel the need [14,16,19, 29-31]. As a well-characterized formulation of low and high molecular weight HA, the product has already shown efficacy and tolerability to treat the signs of skin ageing in areas such as the face and the neck, which are increasingly exposed and therefore judged in working as well as in personal life, hence the growing demand of procedures to restore the lost freshness and youthfulness to these anatomical regions quickly and with short recovery times [21-23].

Since the product has so far been studied in populations of Caucasian origin, it was planned to test its efficacy and safety in subjects of different ethnicities and therefore to carry out subsequent direct comparisons. Specifically, a cohort of Chinese women residing in Italy was analyzed in this study [32-35].

The authors believed it was worthwhile to analyze the data obtained although on a smaller population sample than in the initial design and despite few protocol amendments that were a consequence of the self-isolation of the Chinese population during the first lockdown in the spring of 2020, which involved the enrolled subjects. Outcomes obtained showed a clear effect of the product in this cohort at very early time as to all the clinical and instrumental evaluation endpoints considered, with beneficial effects which were evident already after the first round of treatment. Furthermore, through the collection of 3D documentation obtained at different projections, a rapid and consistent effect of the product on midface volume loss was demonstrated. However, considering the limitations of this pilot study due to the SARS-CoV-2 pandemic restrictions, further studies will be necessary to evaluate subjects at longer timepoints and to increase sample size, therefore performing a statistical analysis on the obtained data and confirming the effectiveness of the treatment. Although the limitations of this pilot study do not allow to perform a complete analysis, the available data suggest that the product appears to be an effective treatment for both Face and Neck areas of Chinese subjects with a good tolerability profile and no immediate or delayed adverse reaction reported.

Authors' contributions

A.S. designed the research study, collected the data, and performed the analysis for this study. All authors contributed to data interpretation and revised and approved the final version of the manuscript.

Funding information

This project has been sponsored by IBSA Farmaceutici Italia Srl.

Conflict of interest

A.S. and R.L. declare no conflict of interest. M.C., A.M.G. and G.B. are employee of IBSA Farmaceutici Italia Srl.

Ethics and Informed Consent statement

Approval for the study was previously obtained from a local ethics committee (Protocol number, E0219). Informed consent was obtained from all subjects involved before they participated at the evaluations.

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