

REVIEW

Hybrid cooperative complexes of high and low molecular weight hyaluronans (Profilo[®]): review of the literature and presentation of the VisionHA project

Complessi ibridi cooperativi di acido ialuronico ad alto e basso peso molecolare (Profilo[®]): review della letteratura e presentazione del Progetto VisionHA

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Abstract - Riassunto

During life, skin undergoes chrono-aging, a process characterized by the slowing of metabolic processes and normal cellular activities. Laxity, dermal thinning and wrinkles formation, major aspects of skin aging, are strictly related with a marked reduction in hyaluronic acid (HA) content in the extracellular matrix. HA has therefore become a well-known dermal agent, shown to promote collagen synthesis, stimulate fibroblast proliferation, and correct skin defects. Most HA-based products used in aesthetic medicine are based on chemical cross-linking that, while improving stability, rigidity, and elasticity, substantially modifies the natural structure of HA. Profilo[®] is a novel HA preparation, based on stabilized, cooperative, hybrid complexes, produced with NAHYCO[®] Hybrid Technology, an innovative patented thermal production process to stabilize the HA without the use of cross-linking agents. Profilo[®] has high HA concentration, low viscosity, optimal tissue diffusion, and high manageability, favoring a multilevel dynamic tissue remodeling. The effectiveness of Profilo[®] in improving the extracellular environment was demonstrated in *in vitro* studies, and its use in aesthetic medicine by a specifically developed 5-injection points technique, has been object of publications, showing positive results in terms of skin vitality and turgidity with a good tolerability profile. In order to explore new, advanced procedures for HA use in aesthetic medicine, the VisionHA project was launched, including educational activities and experience-sharing workshops. During the VisionHA project, different combination protocols and injection techniques have been tested for different facial and body areas. A few exemplary clinical experiences are here described.

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KEY WORDS: Hyaluronic acid - Skin aging - Treatment.

Nel tempo, la pelle subisce un invecchiamento cronologico, questo evento è caratterizzato dal rallentamento fisiologico delle cellule. Il rilassamento, l'assottigliamento dermico e la formazione di rughe, cioè gli aspetti rilevanti dell'invecchiamento cutaneo, sono direttamente correlati a una marcata riduzione di acido ialuronico (HA) nella matrice extra-cellulare. Pertanto HA è considerato un agente dermico ben noto, che

promuove la sintesi di collagene, stimola la proliferazione dei fibroblasti e corregge i difetti cutanei. Molti dei prodotti a base di HA, usati nella medicina estetica, sono realizzati mediante reticolazione chimica che, se da un lato ne migliora la stabilità, la rigidità e l'elasticità, dall'altro ne modifica sostanzialmente la natura strutturale. Profhilo® è un nuovo preparato, basato su complessi stabili, ibridi e cooperativi, sviluppato grazie alla NAHYCO® Hybrid Technology, basata su di un processo termico innovativo, brevettato, che esclude l'utilizzo di reagenti chimici. Profhilo® ha un'alta concentrazione di HA, una bassa viscosità e una diffusione ottimale a livello tissutale, favorisce quindi il biorimodellamento a più livelli del tessuto. L'efficacia di Profhilo® nel migliorare l'ambiente extra-cellulare è stata determinata mediante studi in vitro e il suo uso in medicina estetica, tramite una tecnica sviluppata in modo specifico, con 5 punti d'iniezione, è stato oggetto di pubblicazioni, dimostrando buoni risultati in termini di vitalità cutanea e turgore, con un buon profilo di tollerabilità. Il progetto VisionHA è stato promosso per esplorare procedure nuove e avanzate per l'uso di Profhilo® nella medicina estetica, includendo attività formative e workshop per la condivisione delle esperienze personali. Durante il progetto VisionHA, sono stati testati diversi regimi di combinazione e tecniche d'iniezione per differenti aree del volto e del corpo. Sono inoltre riportate alcune esperienze cliniche.

In recent years, body appearance has gained great social importance, and new cultural factors impact the modern perception of attractiveness making beauty an important factor that affects self-esteem and quality of life.¹ This contributes to the increased demand for aesthetic treatments and rejuvenating procedures, with the aim of recovering a young and healthy appearance. Aesthetic medicine is a relatively new and intensively evolving branch of medicine, with new products and techniques under continuous development, and the growing demand for simple procedures, involving minimal risks and warranting a rapid recovery, has particularly prompted the development of non-invasive techniques.

Skin aging and hyaluronic acid

During the aging process, skin physiology undergoes several changes which eventually slow down metabolic processes and normal cellular activities, a process called chrono-aging.² One of the first skin aging phenomena is skin laxity (loss of elasticity), which first begins as early as the age of 35, though not becoming evident until later. Laxity is strictly related to a reduction in the production of collagen and elastin and associated with alterations of the extracellular matrix, particularly with a decrease in hyaluronic acid (HA) concentration.³

The high percentage of HA in the dermis, thanks to its viscoelasticity and capacity to retain water, is crucial in the control of tissue hydration and to maintain an appropriate tissue volume capable to protect skin cells from mechanical damage. HA has excellent biocompatibility and, either alone or combined with other molecules, accelerates wound healing⁴ and *in vivo* tissue regeneration.⁵ Furthermore, according to its molecular weights, it has anti-inflammatory and bio-stimulating properties, and may interact with receptors on cell membranes to activate other signaling pathways.⁶ HA is also effective in preventing oxidative stress.⁷ More than half of the HA in our body is localized in the skin where it is synthesized by fibroblasts, keratinocytes, and endothelial cells of the dermal microcirculation in many variants of different molecu-

lar weight.⁸ In the epidermis, it plays a trophometabolic role, contributing to the maintenance of the cutaneous homeostasis,⁸ whereas in the dermis it plays a pivotal role in the maintenance of structural stability.⁹ HA is markedly reduced in the aged skin, and this is a major factor in skin aging, since an HA decrease contributes to dermal thinning and the formation of wrinkles.¹⁰⁻¹²

HA as a dermal agent

For all its properties, HA is a useful dermal agent for correcting soft tissue defects,¹³ and it was widely shown to stimulate fibroblast proliferation and increase collagen production, which are the main components of the dermal matrix.^{14, 15} The procedure of HA injections in the dermis restores tone, fullness, and elasticity of the skin; however, native HA is rapidly degraded by hyaluronidase and thus remains stable in the dermis only for few days.¹⁶ Therefore, chemically stabilized cross-linked HA preparations have been developed to prolong dermal stability.¹² Currently, a lot of dermal HA-based medical devices are used in aesthetic medicine, most of which are based on chemical cross-linking that improves stability, rigidity, and elasticity, but substantially modifies the natural molecular structure. Topical or injected HA products have shown variable effects in restoring the physiological and hydrated microenvironment typical of youthful skin.^{17, 18} The different HA dermal fillers vary widely in their physical and chemical characteristics, and many variables contribute to their overall performance. In general, they improve skin turgor and elasticity with injections whose depth varies depending on the type of product used and the depth of the wrinkles.

Profhilo®

Launched in 2015, Profhilo® is a novel HA preparation by IBSA, based on stable, cooperative, hybrid complexes, which is the first product developed by NAHYCO® Hybrid Technology, an innovative thermal production process

patented by IBSA. The production process starts with a simple mixture of 32 mg of high molecular weight HA (1100-1400 kDa) and 32 mg of low molecular weight HA (80-100 kDa). The mixture is then stabilized by the above-mentioned thermal process, which does not use cross-linking agents and consists first of a high-temperature step followed by a low-temperature step. The result is Profhilo®, a product with unique characteristics, such as:

- high HA concentration (64 mg/2 mL);¹⁹
- high manageability;⁴
- optimal tissue diffusion;²⁰
- low viscosity;⁴
- no BDDE¹⁹ or other chemical agents;
- low inflammatory response;⁴

• thermally stabilized natural HA with duration similar to a weakly cross-linked gel.^{20, 21}

Profhilo® favors a multilevel dynamic remodeling and is indicated for tissue remodeling and improvement of skin laxity of the face, neck, and body.

The effectiveness of Profhilo® in improving the extracellular environment was demonstrated in *in vitro* studies, where the product was shown to:

- maintain optimal conditions for fibroblast, keratinocyte, and adipocyte vitality (Figure 1);
- favor a remodeling of the extracellular matrix in terms of elasticity and support.

An important characteristic of Profhilo® is its capability to integrate into tissue, as expressed by its high cohesion coefficient.²² Profhilo® diffuses uniformly through anatomic units, thus allowing to homogeneously expand adipose compartments also in different tissues and layers.²² Its behavior in skin reflects its unique biophysical profile, especially in terms of predominance of fluidity over elasticity, which is lacking in cross-linked gels.²²

The favorable rheologic profile of Profhilo® allows it to obtain tissue remodeling with only 2 treatments performed 4 weeks apart. All injection techniques are suitable, provided injections are performed within the superficial subcutaneous tissue. However, the most widely used and recommended technique is the Bio Aesthetic Points (BAP) technique, specifically developed by IBSA for Profhilo®.²²⁻²⁵ The BAP technique, especially designed for the malar and sub-malar areas that are particularly prone to dermal atrophy due to skin aging, implies:

1. The identification of 5 injection points on each hemisphere (Figure 2); these points represent 5 anatomically receptive facial areas devoid of large vessels and nerve branches, which minimizes risks and maximizes product diffusion.

2. The injection of a 0.2 mL bolus in each point at the level of the superficial subcutaneous tissue.

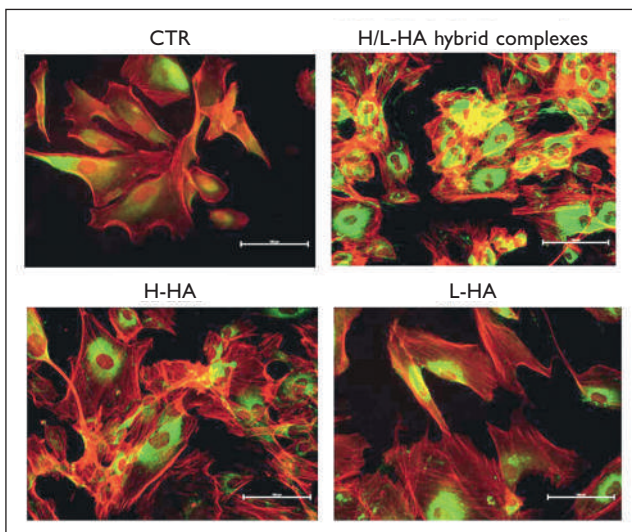


Figure 1.—Keratinocytes-fibroblasts immunofluorescence pictures relative to COL1A1 expression in presence of H-HA, L-HA and H/L-HA complexes 0.16% (w/w) at 24 h. Green: type I collagen, red: Cytoskeleton (Phalloidin). Modified from Stellavato *et al.*²¹

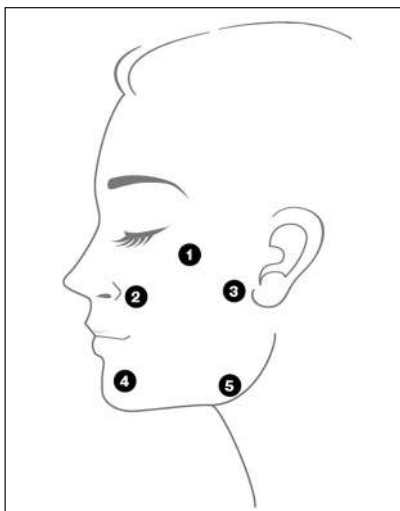


Figure 2.—Bio Aesthetic Points (BAP).

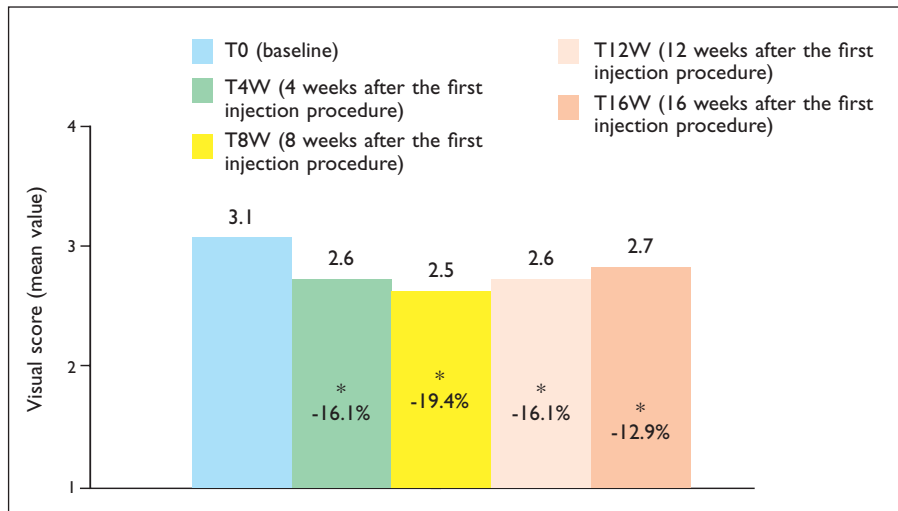


Figure 3.—Reduction in the surface microrelief throughout the study (*P<0.05 vs. T0). Modified from Sparavigna *et al.*²²

Agoli

Profilo® may be used in combination with cross-linked fillers, thus maximizing the outcome in terms of volume.²⁰

In vitro data

A role for HA chains of different lengths has been reported in wound repair, especially considering the simultaneous occurrence *in vivo* of both high-(H-HA) and low-(L-HA) molecular weight hyaluronan at an injury site. It has been shown that HA fragments ($5 \leq MW \leq 20$ kDa) trigger a mild inflammatory response, activating a repair mechanism. The study by D'Agostino *et al.*⁴ aimed at investigating the capability of H-HA, L-HA, and the newly developed hybrid cooperative complex of the two (Profilo®) to promote wound healing of human keratinocytes *in vitro* and at assessing its effect on cellular biomarker expression trends. The effect of H-HA, L-HA and the H-HA/L-HA hybrid cooperative complex on wound closure was tested in keratinocyte cell monolayers (HaCaT). The H-HA/L-HA hybrid cooperative complex (0.1% and 1% w/v) proved faster in regeneration also in a co-culture scratch test, where wound closure was achieved in half the time of H-HA stimulated cells and 2.5-fold faster than the control. The outcomes of this research showed that, both at high and low concentrations, hybrid complexes proved to perform better than HA alone, thus suggesting their potential as medical devices in aesthetic and regenerative medicine.

The same group²¹ investigated the biological activity of Profilo® in comparison with H-HA or L-HA alone. Using *in vitro* assays, based on keratinocytes, fibroblasts and on the Phenion® Full Thickness Skin Model 3D (Henkel, Düsseldorf, Germany, diameter 1.3 cm), hybrid cooperative complexes were compared to H-HA and L-HA. Quantitative real-time PCR analyses were used for the transcript quantification of collagens and elastin, whereas immunofluorescence staining permitted to evaluate the complete biosynthesis of all the investigational molecules investigated. An increase in the expression levels of type I and type III collagen in fibroblasts and of type IV and type VII collagen in keratinocytes were found with the hybrid cooperative complexes, compared to untreated cells and to the H-HA and L-HA treatments. An increase in elastin expression was also found, supporting a potential clinical improvement of skin elasticity. The biomarkers analyzed suggested an increase of tissue remodeling in the presence of Profilo®, probably due to the long-lasting release and the concurrent action of the two HA components. Profilo® was shown to deliver a higher HA amount than the unmodified HA products. Moreover, Profilo® is characterized by a reduced dynamic viscosity that, in clinical practice, allows it to deliver and inject very high concentrations of HA, and it is more stable than the linear H-HA, even without crosslinking. In conclusion, the results of this study showed a notable difference in biological response, in terms of collagen and elastin expression and synthesis,

of Profilo® compared to native HA formulations, with the potential for a better global bioremodeling performance.

Adipose-derived stem cells (ASCs) are used in regenerative medicine for fat grafting, or recovery from local tissue ischemia and scar remodeling. Stellavato *et al.*²⁶ evaluated the effect of Profilo® on differentiation and proliferation of ASCs. ASCs were treated for 7, 14 and 21 days with different types of hyaluronans. Profilo® induced high differentiation of ASCs by up-regulating adipogenic genes and related proteins, while H-HA and L-HA induced a lower differentiation and cross-linked HAs were not able to induce adipogenic differentiation. The authors concluded that Profilo® is superior to both linear HA and cross-linked hyaluronans in enhancing adipogenic differentiation and proliferation and has the potential to considerably improve fat tissue renewal. These effects are of great importance in aesthetic medicine, since the resident ASCs may be stimulated to differentiate into adipocytes, leading to a multi-level remodeling approach. It can thus be assumed that Profilo® may affect the differentiation of resident fat cells in the dermis and the hypodermis, counteracting the characteristic age-related "resorption" of the fat compartment.

Published clinical experience

Laurino *et al.*²³ investigated, in a monocentric, retrospective, observational study, the efficacy and tolerability of Profilo® for facial skin rejuvenation in 11 women aged 48 to 67 years (mean=56 years), treated with 2 injections (once a month for 2 months) of 2 mL of the product, injected in the subcutaneous layer of the right and left malar/submalar areas. Skin echography was performed, and facial skin hydration, elasticity, and trans-epidermal water loss (TEWL) were assessed at baseline, and after 1 and 3 months. Physicians' and patients' respective satisfaction were also recorded. The treatments were performed by BAP technique, identifying the 5 injection sites for each hemiface.²⁷ Visual pre- and post-treatment comparisons showed an improvement in skin wrinkles with facial skin appearing brighter and more turgescient at the final evaluation. Echographic signals were consistent, showing substantial widening of the subdermal area with increased echoes and well-arranged strands of newly formed collagen bundles after 3 months. Skin hydration, elasticity and TEWL were significantly improved ($P < 0.01$). Profilo® was judged by the physician as easy to inject in more than 70% of cases. Effectiveness was judged by the physician as optimal in 52% and good in 46% of cases, while 88% of patients were very satisfied and 12% were satisfied. Only mild adverse events, such as localized hematomas following 12.1% of injections (4/33), were reported and these events disappeared after 2-3 days. The authors concluded that Profilo® administered by BAP technique represents a good treatment option to restore vitality and turgidity of aged skin with no relevant side effects.

Rodríguez Abascal and Saldaña Fernández treated 30

women aged 40-68 years with Profhilo®.²⁴ Overall, two 64 mg/2 mL doses were administered 30 days apart from each other and visual subjective evaluations were performed 1 and 2 months after the first treatment. Patients were also asked to fill out a satisfaction questionnaire at both visits. Table I summarizes the level of satisfaction of the patients. The treatment was also well tolerated, with only 3 cases of ecchymosis and 2 of pain of mild intensity.

Beatini *et al.*²⁵ evaluated 15 female patients aged 39-65 (mean=53) undergoing two Profhilo® treatments 4 weeks apart. The product was injected according to the BAP technique. At the follow-up visits (4 weeks and 8 weeks after the first treatment), viscoelasticity (viscoelastic/elastic resistance ratio, Uv/Ue) and skin hydration were measured for each patient with the Dermotricos Micro-CAMERA®. At each follow-up visit, the patients' satisfaction was also assessed, and photographic documentation was collected. After one treatment, a significant increase (Student's Test <0.05) in skin hydration was observed, while viscoelasticity improved significantly (Student's Test <0.05) after the second treatment. Mean skin hydration increased from 30% at baseline to 55% at 8 weeks; mean viscoelasticity (Uv/Ue ratio) increased from approximately 82 to 87. Only 2 patients were "unsatisfied" after the first treatment, whereas 10 were "satisfied" and 3 "very satisfied." After the second treatment 9 patients were "satisfied" and 6 were "very satisfied". In 2 cases bruising and in one case swelling at the injection site were reported. These side effects disappeared within two days.

Another assessment of the efficacy and tolerability of Profhilo® was conducted by Sparavigna and Tenconi²² in 64 women who were followed up for a longer period of time, i.e. 16 weeks. The women, aged 38-60 (mean=53), were administered two doses of the product by BAP technique 4 weeks apart and were evaluated at 4, 8, 12, and 16 weeks by subjective evaluation, validated clinical scales (WSRS, Facial Volume Loss Scale [FVLS], and Beagley and Gibson Scale) and objective quantitative outcome scales. From week 8, volunteers were asked to fill out a self-evaluation questionnaire, both on efficacy and tolerability. Instrumental, non-invasive evaluations included optical colorimetry, electrical capacitance of the skin (indicative of skin surface hydration), tissue dielectric constant of superficial and deep skin layers (another measure of hydration taken with the MoistureMe-

terD, measuring skin layer up to 5 mm deep), torsiometry (measures of skin plasto-elasticity: immediate and maximum extensibility, viscoelasticity, immediate elastic recovery), profilometry (replicas of nasolabial folds and marionette lines obtained with silicone rubber coupled with the Primos software elaboration), and photographic documentation. The subjective assessments indicated improvements throughout the study that were already significant at week 4 or 8 and were still significant at week 16 (3 months after the second treatment). Results from clinical assessments were significant (P<0.05, after testing for multiplicity) from week 4 for the skin surface microrelief (Figure 3). Results from instrumental assessments were statistically significant on the majority of parameters: starting from week 4 for the superficial skin hydration and from week 8 for the skin deep layers hydration; the effect size was more pronounced on the skin electrical capacitance.

The face volume analysis showed an increase of volume versus T0 of 0.549 cm³ at T8W and of 0.476 cm³ at T16W (Figure 4); an increase of at least 0.2 cm³ compared to the baseline was obtained in 73% and 65% of the analyzed subjects, respectively.

Minor and temporary local skin reactions were observed in 23% of subjects at the injection sites and the global judgment on tolerability was good or excellent, both in the investigators' opinion and volunteers' self-evaluation.

The VisionHA® Project

The VisionHA project was designed by IBSA with the aim of exploring what more can be done with Profhilo®. Aesthetic medicine is a rapidly evolving branch of medicine, where not only new products but new approaches and techniques are being continuously developed. IBSA, a pharmaceutical company with a deep knowledge in the de-

TABLE I.—Level of satisfaction of 30 women aged 40-68 years at 1 and 2 months after the first treatment with Profhilo®.²⁴

Subjective evaluation	At 1 month	At 2 months
1=0-25% improvement	1	0
2=26-50% improvement	3	0
3=51-75% improvement	13	8
4=76-100% improvement	13	22

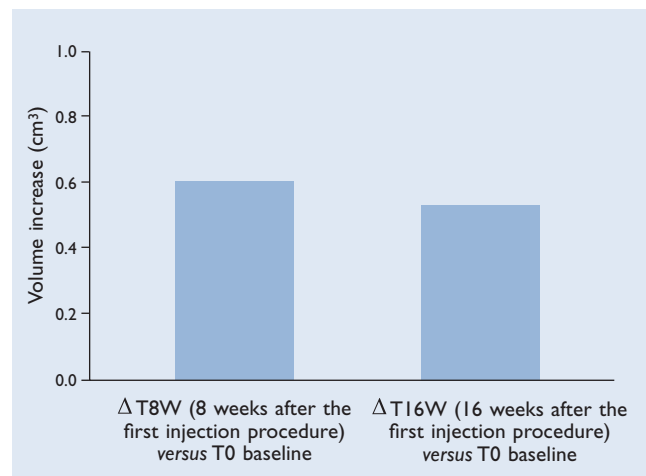


Figure 4.—Three-dimensional face volume analysis: increase of volume (cm³) T0 versus T8W and T16W versus T0. Modified from Sparavigna *et al.*²²

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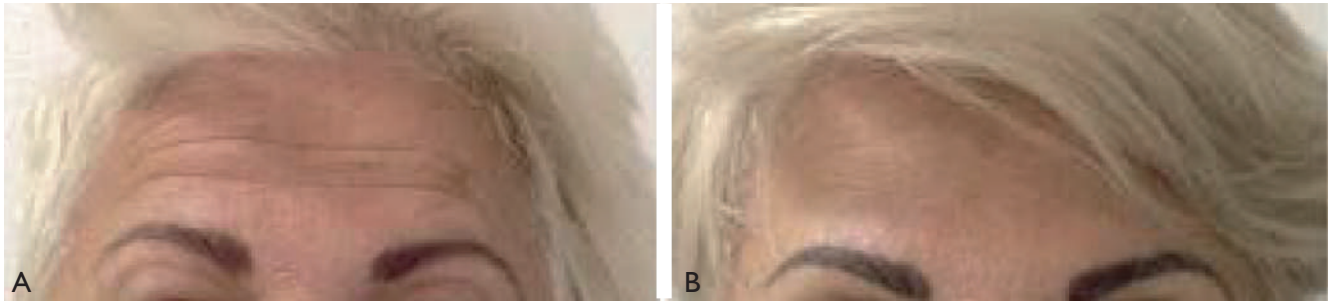


Figure 5.—A 64-year-old woman treated with Profhilo® and abobotulinum toxin: A) before the procedure; B) after 30 days from last procedure (day 120).

velopment technologies of HA, wished to create, by the VisionHA project, a partnership with aesthetic physicians aimed at defining, developing, testing, and sharing new, advanced procedures for HA use in aesthetic medicine. The project includes educational activities and experience-sharing workshops.

The first phase of the VisionHA project consisted in the development and testing, by the participating Italian physicians divided into four groups, of different injection techniques for different facial and body areas: 1) the upper third of the face; 2) the neck; 3) the perioral area; and 4) the body. During the second phase of the project, the proposed injection techniques were applied in a few exemplary clinical cases that are briefly reported.

Selected case reports

Case 1: Profhilo® and botulinum in the forehead

The aging process at the face upper third level starts rather soon, with the occurrence of dynamic wrinkles on the forehead and the glabella. Subsequently, such wrinkles may become deeper, showing initial signs of skin laxity and of skeletonization of the fronto-temporal area. The gold-standard treatment for such area may be the botulin toxin.²⁷⁻³⁰ However, botulin toxin usually improves wrinkle number and deepness, but may not modify skin quality. Among HA preparations, HA-based hybrid complexes, such as Profhilo®, have shown a better biological response, regarding collagen and elastin expression and synthesis, compared to native HA formulations.²¹ This type of HA complex may be very useful for the treatment of this face area to reduce skin laxity and making superficial soft tissues more elastic and firm, thanks to its capability to integrate and diffuse uniformly into forehead skin tissues that are strictly adherent to the underlying facial muscles.^{4, 21}

For these reasons, a treatment with botulin toxin injections alternated with Profhilo® applications was tested in a 64-year-old, Glogau stage 3, non-smoking woman in good health condition, assuming no medications nor following any diet. She had previously undergone upper and lower blepharoplasty surgery with removal of eye bags. Subse-

quently she underwent 3 sessions of Profhilo® administration 30 days apart. Profhilo® was injected by means of a cannula, with bilateral access, in the upper fronto-lateral area of the face for the forehead and near the upper portion of the temporal crest for the temples. Two mL of product was injected in each session. Thirty days after the last Profhilo® session, 100 units of botulin toxin (abobotulinum toxin) were injected (Table II).

During the procedures, the patient complained of mild pain, but no other adverse effects or complications occurred, either immediately after the procedure or in the following days. At each new session, an improvement of the skin texture and a distension of the concavities both in the forehead and in the temporal area were observed (Figure 5). The patient declared herself very satisfied and was prone to repeat the treatment.

The combination of Profhilo® and botulinum toxin may be particularly suitable in women with moderate-to-severe aging. In this setting, the combination and the proposed administration technique can be recommended. Conversely, it is not recommended in subjects with periorcular bags or ptosis of the eyebrow tail, and in those with a high degree of skeletonization. The treatment should be repeated every 4 to 6 months.

Case 2: Profhilo® and botulinum in the neck

There is an increasing downward trend in the aesthetic focus from the face to the aging neck and below. Whereas the characteristics of neck youth include clear skin texture and tone without laxity or fat, the aging process implies loss of volume, increased skin laxity, worsening texture, and wrinkling. The aesthetic treatment of neck skin is of crucial importance in the overall patient's rejuvenation approach. Profhilo® may be of great value in improving the

TABLE II.—Time-schedule for product administrations and patients' assessment.

T0	T1 (after 30 days)	T2 (after 60 days)	T3 (after 90 days)
Profhilo®	Profhilo®	Profhilo®	Botulin toxin

aged skin of the neck, having shown to reduce skin laxity and increasing elasticity and consistency of superficial soft tissues.^{20, 22} It may be used alone in case of mild aging, while it is preferably combined with botulin toxin in case of moderate-to-severe aging. Two cases of neck treatment with a combination of Profillilo® and botulin toxin are here reported.

The two patients were a 63-year-old woman (A) reporting multiple allergies, in good health condition, and a 56-year-old woman (B) with no known allergies, undergoing hormonal anti-tumor therapy, both with moderate skin aging. They had never undergone aesthetic procedures on their neck. The two women were treated with a combination of Profillilo® and toxin botulin. At the first session, 50 IU of Microbotox were injected, then two 2 mL doses of Profillilo® was injected at day 15 and at day 45, respectively (Table III). Profillilo® was injected according to a multi-point technique, selecting about 20 points according to consistency of skin laxity of superficial soft tissues.

As expected, small wheals appeared at the sites of injection, which disappeared, according to the patients, in 24 hours. No complications or undesirable effects emerged. Both patients found the procedure acceptable. On a ten-point scale, the level of satisfaction was rated 8 by both patients and by the physician. In particular, the physician noted a reduction in skin laxity and an increase in elasticity. Pictures taken before and at the end of the procedure are shown in Figure 6.

This combined Microbotox-Profillilo® protocol showed to be simple and safe to perform, provided the administering physician has good expertise in botulin toxin use. In our opinion it is particularly suitable for patients with moderate to severe skin laxity. In case of more marked skin aging, a cannula and a double dose of Profillilo® can be used (4 mL). In the presence of hypertone of the neck platismatic bands, traditional botulin toxin should be preferred to Microbotox.

TABLE III.—Time schedule for administration of the products.

T0	T1 (after 15 days)	T2 (after 45 days)
Botulin toxin	Profillilo®	Profillilo®

In conclusion, the combination of Profillilo® with Microbotox showed to be effective in treating moderate cutaneous aging of the neck, prolonging visible aesthetic results up to 3 months after treatment completion.

Case 3: Profillilo® and cross-linked fillers

The perilabial zone is particularly dynamic given the continuous stress induced by speaking, eating, smoking, etc. In the first aging phases, lips start showing vertical wrinkles along their edges. Later lips may also become thinner, losing turgidity, consistency, and hydration. In the most advanced phase, the reduced consistency tends to modify the shape of the lips, with elongation of the upper portion and introflexion of the red portion. Then, the whole area progressively undergoes significant changes: the naso-labial folds become deeper, while the first wrinkles appear on both sides of the mouth and prolong downwards.³¹

Profillilo®, with its hydrating and bioremodeling properties,^{4, 21} may represent an appropriate option to improve perioral area turgidity and elasticity in the initial phases of the aging process, without necessarily increasing their volume. In more advanced phases, Profillilo® should be combined with fillers. In order to test this latter approach, a protocol combining repeated Profillilo® sessions with fillers injections ≥30 days apart from each other was developed and a case treated accordingly is here described.

A 70-year-old non-smoking woman in good health condition, not undergoing any pharmacologic therapy or diet, was selected. At the first visit, she was diagnosed with a medium-high degree of chrono-aging, characterized by skin laxity and micro-roughness located in particular in



Figure 6.—Two patients treated with botulin toxin (Microbotox) and Profillilo® in the neck area. Patient A: 63-year-old; left, before the treatment, right, at the end of the treatment (day 45); patient B: 56-year-old; left, before the treatment, right, at the end of the treatment (day 45).

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the mid-lower part of the face, including the perioral area. The patient initially underwent two consecutive sessions of Profhilo® 30 days apart with 2 mL of product injected according to bio aesthetic points (BAP) technique (Figure 2). One month later, 1 mL of filler (Aliaxin FL®) was injected in the areas of the naso-labial folds and of the labial commissures. The patient was then administered two more 2 mL doses of Profhilo® in the perioral area with a 0.2 mL bolus technique one and three months after the filler injections (Figure 7). Two months later, one more 1 mL filler (Aliaxin FL®) injection in the lips was performed. After 2 more months, a final 2 mL Profhilo® was performed (Figure 7, Table IV).

No complications or undesirable effects were observed, either immediately after the injections or after a few days. Only a mild discomfort was reported by the patient, due to the swelling caused by the injections immediately after the sessions, which disappeared in a few hours, and rare bruising. The patient reported a high degree of satisfaction and the aesthetic result was still judged very positively by both patient and physician after 4 months from the end of the treatment (Figure 8). An overall improvement of the perioral area was observed, involving the naso-labial folds, with an increase in lips turgidity.

This treatment method may be of particular interest in medium-advanced skin aging, providing a visible improvement without changing the patient's connotations. Furthermore, the whole procedure is generally well accepted by the patients. An initial attack phase is required in order to achieve a visible improvement, whereas subsequent maintenance sessions can be spaced even by 3 to 6 months. The combination of the two products is favorable in that Profhilo® increases tissues hydration and elasticity and reduces micro-roughness, while the filler allows the redefinition of the lip contours. Special warning concerns patients with permanent filler in the perioral area. Furthermore, it should be remembered that efficacy can be reduced in heavy smokers.

Profhilo® injections in the body

The increasing popularity of aesthetic procedures in the face may lead to a great disparity between treated facial areas and body areas that still show evidence of true age. Therefore, injectable soft-tissue augmentation agents have become popular also for the aging body. Although many areas of the body may require multiple treatment procedures for optimal rejuvenation, protocols for specific areas are not yet well defined. Profhilo®, with its high HA concentration,¹⁹ was shown to favor a multilevel dy-

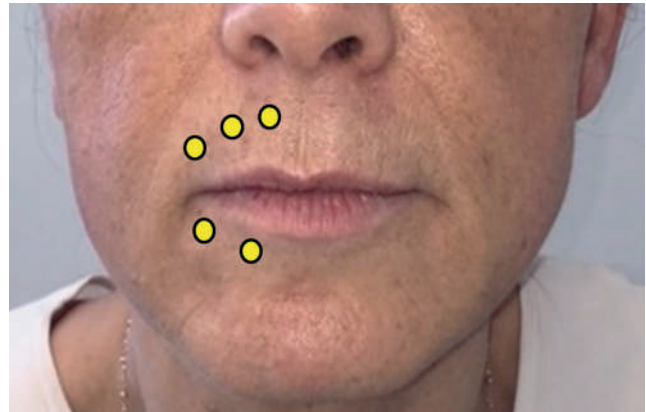


Figure 7.—Five-point injection technique in the perioral area of each hemiface.

namic tissue remodeling, improving face, neck, and body skin laxity. In a clinical study, the treatment of the inner arm with Profhilo® administered by an innovative 7-point technique induced a visible improvement of tone and texture.³² Profhilo® was tested in the aesthetic treatment of the arms of an elderly woman, according to a simple and rather short procedure.

A 72-year-old woman with diabetes and cardiopathy asked for a rejuvenating treatment for the volar surface of her arms and axillary cords. Given the advanced grade of skin aging, the first-choice treatment would have been aesthetic surgery; however, the patient could not undergo surgery due to her comorbidities. Therefore, a bioremodeling approach with HA was agreed upon. The procedure consisted in 3 sessions 30 days apart; in each session, 2 mL of Profhilo® was injected in each arm, distributed in 10 injection points per arm (0.2 mL/point), 4 in the axillary cord and 6 along the volar surface.

The technique proposed by Bovani³² was slightly modified in order to distribute the product in the areas most affected by skin laxity.

The obtained results are shown in Figure 9. No adverse effects were complained by the patient or observed by the physician. Both patient and physician were satisfied of the results, given the severe baseline skin aging; the patient rated her satisfaction 6-7, the physician 7-8.

This Profhilo®-based protocol represents a simple, mini-invasive approach, and is particularly indicated in patients with a mild-moderate skin laxity, but can be considered, as in this case, in the presence of contraindications to surgery. It ensures good patient compliance and has a good safety and tolerability profile. Given the positive

TABLE IV.—Time-schedule for treatment sessions and administered products.

T0	T1 (after 30 days)	T2 (after 60 days)	T3 (after 90 days)	T4 (after 150 days)	T5 (after 210 days)	T6 (after 270 days)
Profhilo® 2 mL	Profhilo® 2 mL	Aliaxin FL® 1 mL	Profhilo® 2 mL	Profhilo® 2 mL	Aliaxin FL® 1 mL	Profhilo® 2 mL

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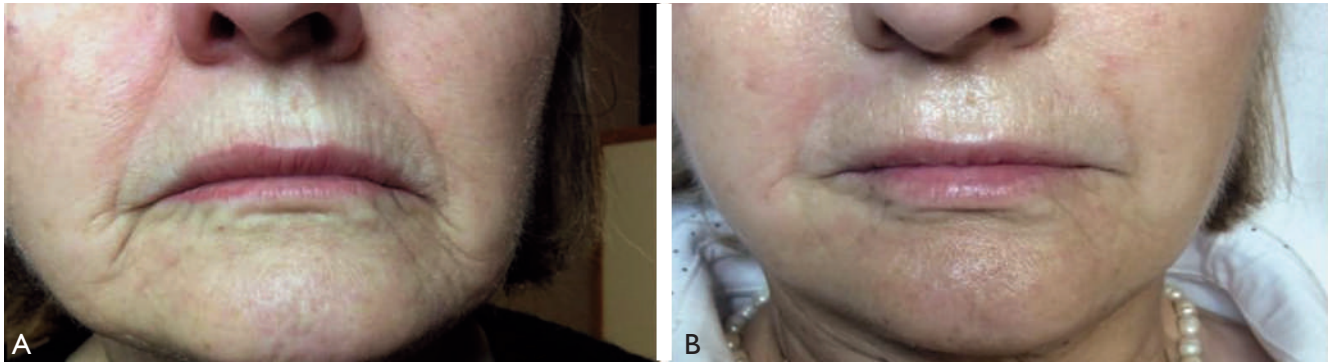


Figure 8.—A 70-year-old woman treated with a combined Profhilo® and Aliaxin FL® protocol in the perioral area: A) left, before the procedure; B) at the end of the whole treatment course (day 270).

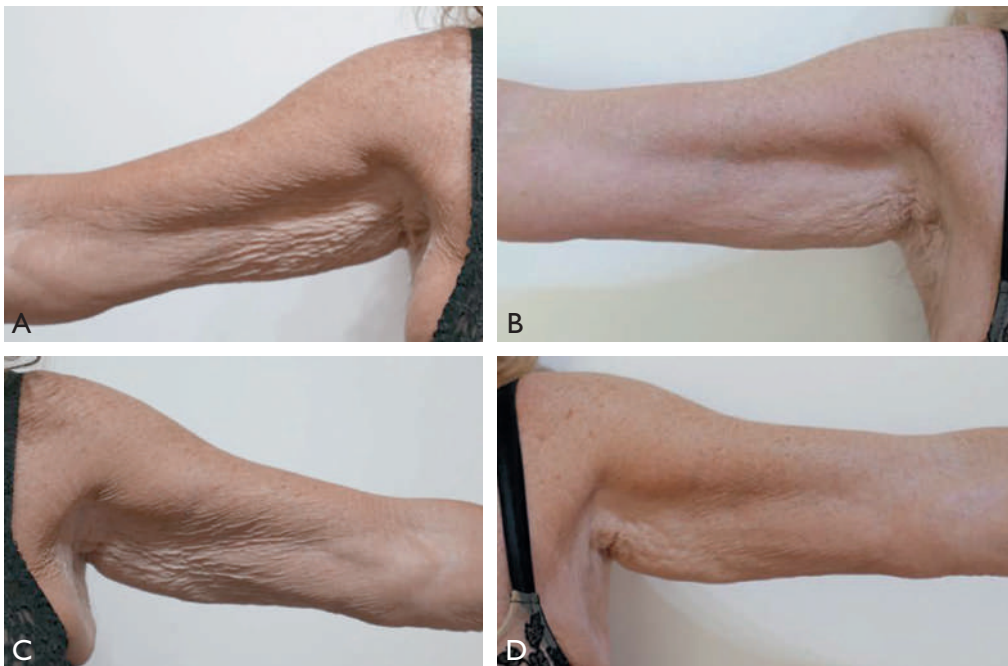


Figure 9.—A 72-year-old woman treated with Profhilo® in the volar surface of the arms and in the axillary cords. Right arm: A) before treatment; B) at treatment completion (60 days). Left arm: C) before treatment; D) at treatment completion (60 days).

result, this may be a suitable approach for patients looking for tissue remodeling and recompacting without surgery,

by virtue of the neofibrogenic and adipogenic properties of Profhilo®.^{21, 26}

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