

Clinical Trial

**A combination treatment protocol using PEGylated hyaluronic acid hydrogel,
non-ablative laser and hyaluronic acid skin boosters
for rejuvenation of the perioral area**

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ABSTRACT

Personalized treatment with a combination of aesthetic protocols targets the different root causes, contributing to the patient's aesthetic concerns and optimizes outcomes. This report presents the outcomes of a new protocol combining PEGylated hyaluronic acid (HA) hydrogel, non-ablative laser, and non-crosslinked HA skin booster to rejuvenate the perioral area within a single treatment session. This was a retrospective analysis of medical data from subjects who had undergone treatment with the perioral rejuvenation protocol at Kalea Clinic in the Netherlands. All subjects had been treated with the 3-step protocol involving: (i) injection with PEGylated HA hydrogel 24 mg/ml to contour and shape the lips; (ii) full-face treatment with a non-ablative, fractional diode laser with a wavelength of 1470 nm to smooth and tighten the skin; and (iii) full-face mesotherapy treatment with injection of non-crosslinked HA (18 mg/mL) with 0.01% calcium hydroxyapatite, glycine, and L-proline to improve skin quality. Follow-up visits took place at 6 (Visit 1) and 12 weeks (Visit 2), at which point physicians and patients assessed lip fullness and skin wrinkling on separate validated rating scales. Additional subjective assessment of treatment results was performed by both physicians and patients using the 5-point Global Aesthetic Improvement Scale (GAIS). Physician and patient satisfaction with treatment results was also assessed. Data were analyzed retrospectively for 14 female subjects with a mean age of 39.1 years. At baseline, lip fullness was rated as moderate by 71% of physicians and 64% of patients. At visit 2, 71% of lips were rated as thick and 14% as full by both physicians and patients. The mean skin wrinkle score improved from 1.30 (visible wrinkles with clear indentation) at baseline to 0.79 (fine wrinkle, slight indentation) at Visit 2. Mean GAIS scores for both physicians and patients indicated appearance was 'improved' at Visit 1 (mean scores of 3.0 and 2.7, respectively) and 'much improved' at Visit 2 (mean scores of 2.4 and 2.3, respectively). All treatments in the protocol were well tolerated. Both physicians and patients were satisfied with the combination protocol, and all patients reported they would be 'likely' or 'very likely' to recommend it. The combination of three different treatment modalities (PEGylated HA hydrogel, non-ablative laser, and HA mesotherapy) in a single treatment session demonstrated effectiveness in rejuvenating lips and perioral area. The complementary treatments were tailored to the individual for a natural-looking effect.

INTRODUCTION

Many factors, but in particular balanced facial proportions and good skin condition, affect facial attractiveness and to the perception of age (1). This is most evident in areas of facial expression, which become the center of attention during social interactions (2). In the lower face, this focus is on the mouth and surrounding perioral area. An attractive smile creates a social perception of happiness and youthfulness (3). An improvement in smile aesthetics is the main reason adult patients seek orthodontic care (4) and lip enhancement continues to be one of the most requested aesthetic procedures worldwide (5).

The mouth and surrounding soft tissues undergo significant physiological and structural changes with age as a result of extrinsic and intrinsic factors, including photodamage, cigarette smoking, loss of subcutaneous fat and elasticity, changes in bony structures of the mouth, and the repetitive action of the perioral muscles (6, 7). Common clinical presentations include fine wrinkling around the lips (bar code lines), marionette lines, downturned corners of the mouth, uneven pigmentation and poor skin texture, volume loss (fat atrophy), lengthening of the upper lip, and reduced visibility of the upper teeth (6, 7).

As the aging process is multifactorial, perioral rejuvenation has several aims other than just restoring lip volume. A combination of treatments, each addressing a relevant component of the aging process, will provide

synergistic benefits beyond that which can be achieved by a single procedure. To achieve a comprehensive, long-lasting, and natural improvement, it is important to balance facial features and harmonize treatments across the perioral area by targeting volume loss, asymmetries, and skin quality. A new perioral rejuvenation protocol has been developed which addresses these defects by combining three treatments from the Neauvia range in a single treatment session: poly (ethylene glycol) diglycidyl ether (PEGDE) crosslinked hyaluronic acid (HA) hydrogel (Neauvia Intense Lips, Matex Lab, Switzerland), 1470 nm diode laser (LaserME, Berger&Kraft Medical Sp. z o.o., Poland), and mesotherapy with a non-crosslinked HA (Neauvia Hydro Deluxe, Matex Lab, Switzerland).

All three treatments possess characteristics suitable for the treatment of the highly mobile perioral area. PEGDE-HA has the rheological characteristics to withstand dynamic movement at the same time as integrating seamlessly with the target tissue. The non-ablative skin resurfacing treatment can be used for all Fitzpatrick skin types and is able to focus on small areas such as those around the lips. The mesotherapy product complements the laser treatment, stimulating collagen production and skin hydration. This case series describes the use of all three treatments in a single session to rejuvenate the perioral area.

METHODS

This was a retrospective analysis of medical data for subjects who had undergone treatment with the perioral rejuvenation protocol at Kalea Clinic (Amsterdam, the Netherlands) over the period 2023-2024.

This protocol is offered to subjects with signs of aging and loss of volume in the perioral area and consists of three consecutive treatments. The first step is to address lip shape, definition and fullness with PEGDE-HA. This product has an HA concentration of 24 mg/ml. It can be injected into the vermilion and/or perioral area to address a range of indications, including perioral lines, contouring of the vermilion border, restructuring philtral columns, and lip volumizing. Injection volume was determined by the physician and tailored to the patient's treatment requirements, but the total volume did not exceed 1.0 ml. The product can be injected with a 27G needle or 23-25G cannula, depending on the indication. Pain during lip injections typically results from the procedure itself rather than the product and can be reduced by the use of a topical anesthetic or a nerve block. Post-procedure, all patients moisturized their lips with the manufacturer's lip balm (Neauvia Lip Bliss 3D HA volumizer).

The second step was full-face treatment with a non-ablative, fractional diode laser (LaserME). This is an FDA-cleared technology for skin resurfacing that has a wavelength of 1470 nm and a maximum power of 2 W. The device functions by punctuating the epidermis with micro-laser beams and delivering micro-columns of heat energy into the dermis. Within these micro-thermal zones, the focused light breaks up damaged tissue and stimulates neocollagenesis and elastogenesis thereby rejuvenating the skin. The points are evenly distributed with adjustable spacing from 1 mm to 4 mm. The energy delivered to a single point can be regulated from 5 mJ to 50 mJ. As the thermal zones occupy a relatively small percentage of the skin's surface, the treatment does not cause the extensive downtime associated with ablative procedures. The procedure can be used on skin of any color and type, including sensitive skin. An adjustable pulse width and multi-level scanner allows providers to choose the optimal distance between micro-columns for an individualized treatment.

The protocol was completed by performing a full-face mesotherapy treatment with non-crosslinked HA (18 mg/mL) containing 0.01% calcium hydroxyapatite microspheres, glycine, and L-proline (Neauvia Hydro Deluxe, Matex Lab, Switzerland). Mesotherapy treatment consisted of performing multiple intradermal or subcutaneous injections (depth from 0.5 to 4 mm) of small volumes of HA using a 30G needle and the pre-filled 2.5 ml syringe.

All three treatments comprising the protocol were performed on the same day. Two follow-up visits were scheduled post-treatment at 6 weeks and 12 weeks. At each visit, the treating physician and patient assessed lip fullness on a validated scale of 0 to 4, where 0 indicated very thin lips and 4 indicated full lips (8). The degree of skin wrinkling was assessed using the Fitzpatrick Wrinkle Assessment Scale. Using reference photographs, the scale was used to classify wrinkles into one of four classes (0, 1, 2 or 3), defined as absent, mild, moderate, or severe (9, 10). Three interclasses could be used to further assess wrinkle severity (i.e., 0.5, 1.5 and 2.5). Additional subjective assessment of treatment results was performed by physicians and patients using the 5-point Global Aesthetic Improvement Scale (GAIS) by comparing photographs of the patient’s face before treatment and at each post-treatment visit and asking the question: ‘How would you describe the results?’ The available choices were: (5) worse (the appearance is worse than at baseline), (4) no change, (3) improved, (2) much improved, and (1) very much improved. In addition, a physician questionnaire was completed at the 12-week visit with responses ranging from (1) very much improved to (5) worse. A patient satisfaction questionnaire rated responses on a 4-point scale from (1) happy/very likely to (4) dissatisfied/never. Patients were also asked to rate the improvement in their lips following PEGDE-HA treatment on a scale of 0 (no improvement) to 10 (maximum improvement).

As part of standard practice procedure and following the principles of the Declaration of Helsinki, all subjects received written information about the products and protocol and signed an informed consent form for the procedure and use of their data for scientific purposes.

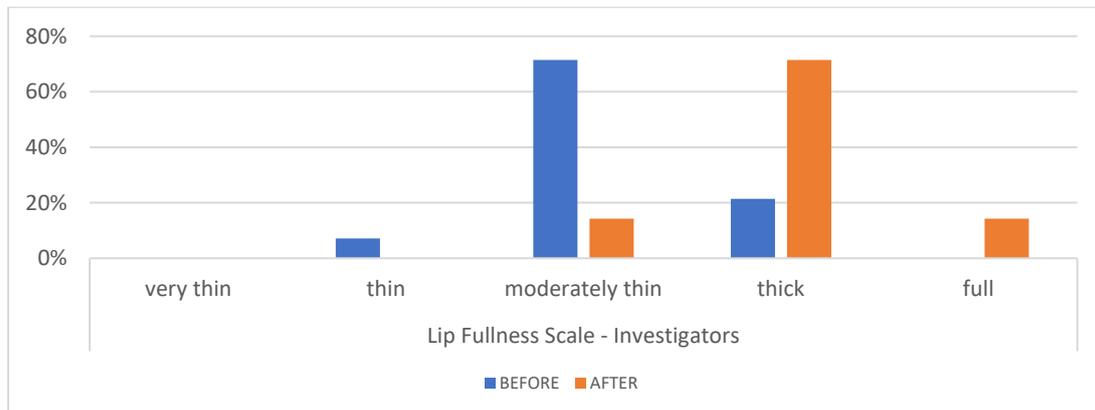
RESULTS

Data were analyzed retrospectively for 14 female subjects who had attended the center for rejuvenation of the perioral area between 2023 and 2024. Subjects had a mean age of 39.1 years (range 32 to 45 years). At baseline, both physician and patients provided a mean score for lip fullness of 2.1, indicating moderate lip fullness. Skin wrinkling rating was described as ‘visible wrinkles with clear indentation’ on the Fitzpatrick Wrinkle Assessment Scale with a mean score of 1.3.

A total volume of 1 ml was injected in the lips (0.5 ml in the upper lip and 0.5 ml in the lower lip).

Lip fullness improved post-treatment with mean physician and patient scores of 2.9 and 2.8, respectively, at Visit 1, and both provided a mean score of 3.0 at Visit 2 (thick). Scores were very similar between physicians and patients at each visit. At baseline, 71% of physicians and 64% of patients rated the lips as moderate on the lip fullness scale. At Visit 2, 71% of lips were rated as thick and 14% as full by both physicians and patients (Fig. 1).

(A)



(B)

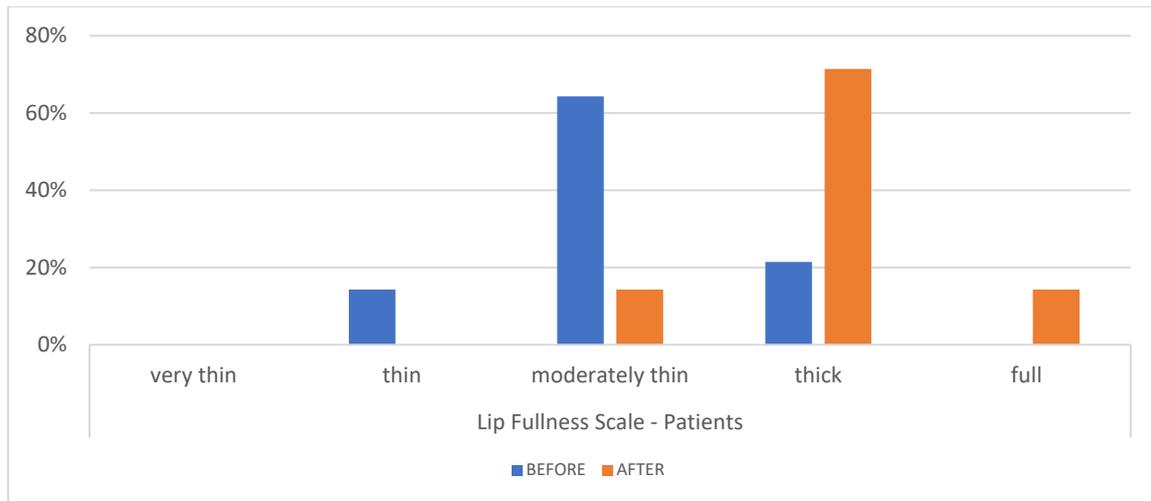
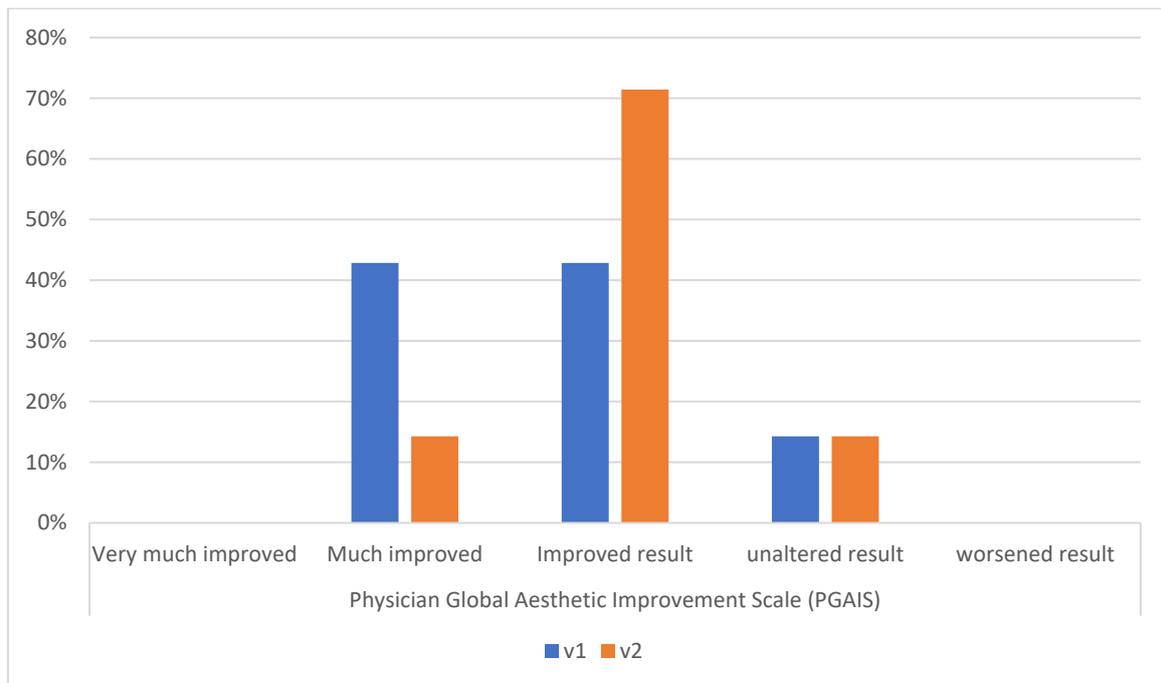


Fig. 1. Assessment of lips on the fullness rating scale at baseline and at Visit 2 (12 weeks) by (A) physicians and (B) patients.

Following the combined treatment protocol, mean skin wrinkle score had improved from 1.3 at baseline to 0.9 at Visit 1 (6 weeks) and to 0.8 at Visit 2 (12 weeks) (fine wrinkle, slight indentation).

Improvements in GAIS were noted by both physicians and patients at each post-treatment visit. Mean scores indicated appearance was ‘improved’ at Visit 1 (mean scores of 3.0 and 2.7, respectively) and ‘much improved’ at Visit 2 (mean scores of 2.4 and 2.3, respectively) (Fig. 2). Figure 3 illustrates before and after images of a patient treated with the protocol.

(A)



(B)

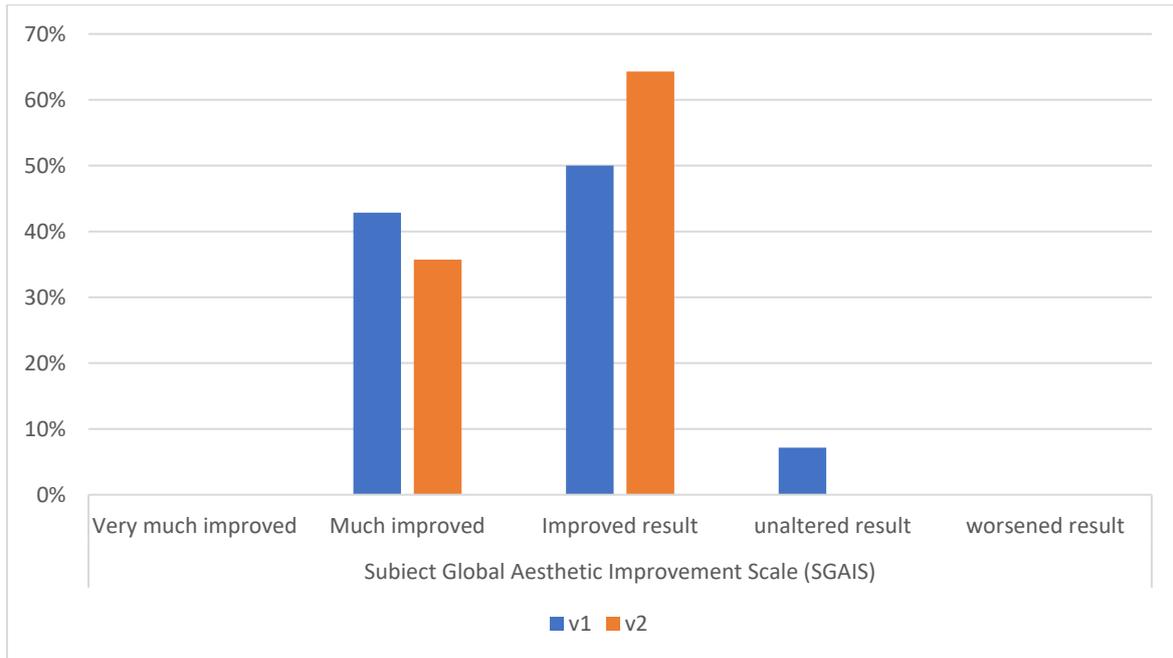


Fig. 2. Global Aesthetic Improvement Scale (GAIS) ratings at visits 1 (6 weeks) and 2 (12 weeks) by (A) physicians and (B) patients.



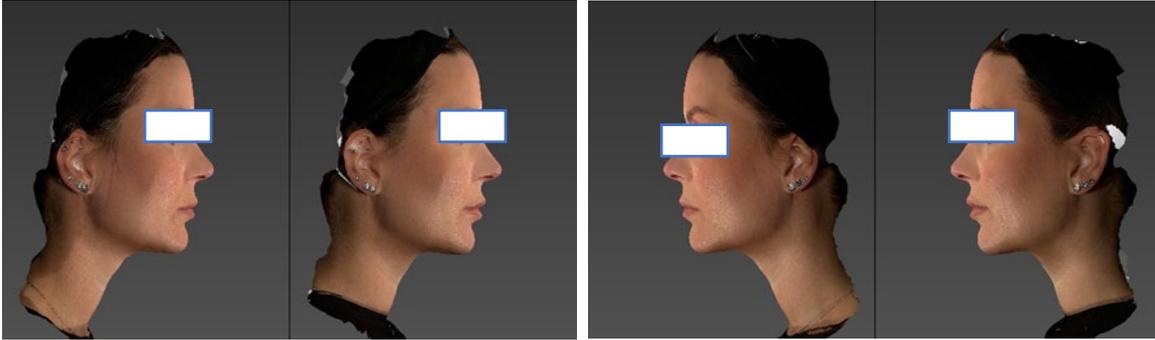


Fig. 3. Patient images before (left image in pair) and 12 weeks after (right image in pair) treatment with the protocol.

As part of the physician satisfaction questionnaire, investigators were asked how they rated improvements in the smoothness of the skin and how they rated improvements in the hydration of the skin after the laser and HA mesotherapy treatment. For both questions, the mean satisfaction score was 3.6, indicating skin smoothness and hydration were ‘improved’ to ‘much improved’. When asked how they would rate the rejuvenation effect after laser and HA mesotherapy treatment, the satisfaction score was 3.8, indicating the effect was ‘much improved’ with treatment.

When patients were asked how happy they were with the protocol, all replied they were ‘somewhat happy’ or ‘happy’. When asked how likely they would recommend the treatment protocol to friends and/or family, all replied that they would be ‘likely’ or ‘very likely’ to recommend it. When asked how much they thought their lips had improved after treatment with PEGDE-HA, the mean satisfaction score on a scale from 0 to 10 was 7.9, reflecting substantial improvement.

All patients tolerated the treatment well, and no adverse events or complications were reported throughout the course of the study. Participants did not experience any unexpected side effects, and there were no significant safety concerns observed during or after the treatment.

This study has certain limitations that should be acknowledged. One notable limitation is the relatively small sample size of 14 participants, which restricts the generalizability of the findings. Additionally, the study cohort did not include patients with Fitzpatrick skin types V and VI. While the results demonstrated excellent safety and efficacy in the treated population, further research involving a larger and more diverse group of patients is necessary to confirm the safety and efficacy of the procedure for all skin types, particularly those with higher Fitzpatrick classifications. These factors are crucial to consider, especially when evaluating the safety profile of laser treatments. The relatively short follow-up period of 12 weeks represents a limitation of this study, as it does not allow for the assessment of long-term durability or the potential gradual decline of treatment effects. Future research with extended follow-up periods is necessary to evaluate the longevity of the results and provide a more comprehensive comparison to both cross-linked and non-cross-linked HA products.

DISCUSSION

This retrospective analysis of subject data has demonstrated the effectiveness of a treatment protocol combining lip and perioral rejuvenation, laser resurfacing, and HA mesotherapy. For natural-looking results, treatment should not be restricted to enhancing one facial feature or correcting a single deficiency; instead, it should blend seamlessly into the surrounding areas. The 3-step protocol was developed to address this need, combining lip shaping, contouring, and volumizing with treatments applied to the whole face that tighten, smooth the skin, and enhance skin quality.

Both lip fullness and wrinkle scale ratings demonstrated clinically significant improvements compared with baseline, whether assessed by the treating physicians or patients themselves. These results translated into physician- and patient-assessed favorable changes in GAIS scores at both post-treatment visits.

The lips and perioral region are part of a very dynamic facial area, and products must be carefully selected to achieve a natural-looking outcome. PEGDE-HA 24 mg/ml has been specifically formulated to withstand movement in this area at the same time as integrating seamlessly into the surrounding tissue. Previous research has shown that the less rigid crosslinking in this product combined with the relatively high HA concentration provide it with viscoelastic properties that are compatible with lip tissue (11). Aesthetic results following injection of PEGDE-HA hydrogels are associated with minimal edema and provide a 1:1 correction. The inclusion of the amino acids glycine and L-proline is also thought to stabilize the structure of the hydrogel in terms of its viscoelastic properties and limit swelling in the post-implant phase (12).

In the protocol, PEGDE-HA was injected first, followed by the laser treatment. This is possible with PEGDE-HA products because they are thermostable and not denatured by lasers or other energy-based devices (13). A further feature that distinguishes PEGDE-HA hydrogels from BDDE-crosslinked HA fillers is their very low risk of immune-mediated adverse effects. Studies to date have shown no evidence of foreign body reactions or granulomas related to PEGDE-HA (14, 15). They have also been safely used in individuals with autoimmune diseases such as Hashimoto's thyroiditis and systemic lupus erythematosus (16, 17).

The non-ablative laser used in the protocol is not painful and is associated with no downtime. Controlled heat energy is delivered to the dermis, stimulating fibroblasts to increase collagen production and remodeling (18). The procedure is very effective for correcting mild-to-moderate skin laxity, tightening the skin, and improving its firmness, elasticity, and overall texture without damaging the epidermis. The laser treatment also prepares the skin for the final stage of the protocol: HA mesotherapy. The thermally-induced micro-columns created by the laser allow deep penetration of the high concentration of non-crosslinked HA, CaHA, and amino acids. In addition to replenishing HA, the product takes advantage of the collagen-stimulating properties of CaHA (19, 20). The inclusion of glycine and L-proline, essential for collagen production (21, 22), further enhances the skin rejuvenation properties of the treatment.

It is now widely recognized that combining complementary interventions for face rejuvenation leads to better and more natural-looking aesthetic results than use of single modalities alone (23–26). Yet, only a few protocols have been published focusing on combination rejuvenation of the perioral area (27, 28).

Experienced aesthetic practitioners often develop their own protocols, which may not be shared with a wider audience. With the increasing use of combination treatments, the need for such protocols is increasing. This is particularly the case for novel therapeutic agents such as the HA hydrogels using PEGDE crosslinking, which differ in several aspects from most HA fillers on the market. PEGDE-HA hydrogels typically show enhanced tissue integration and long-duration of aesthetic results with a small amount of product. They also exhibit reduced swelling, a low risk of immunogenicity, and thermostability. Neauvia is currently unique among manufacturers because it simultaneously provides innovative products with defined protocols for their optimal use in combination. The aim of such protocols is to simplify treatment for physicians so that they know which treatments to combine in which sequence and for which indications. The protocol was developed with experts for optimal rejuvenation of the perioral area in harmonization with the rest of the face. The protocol was not associated with any adverse events, and physician and patient satisfaction questionnaires confirmed the acceptability of the treatments and the results achieved.

Further research is now warranted to assess the applicability of the protocol for greater numbers of patients.

CONCLUSION

The sequence of PEGDE-HA lip rejuvenation followed by full face laser resurfacing and HA mesotherapy treatment demonstrated effectiveness in enhancing the perioral area's appearance within a single session. The combination of three complementary treatments enhanced skin quality as well as lip shape and volume for a natural-looking, anti-aging effect. All treatments were well-tolerated, and both physicians and patients were satisfied with the improvement in lip and skin appearance.

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