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Original article

Double-blind study comparing the *in vivo* efficacy of two hyaluronic acid products (high and very high molecular weight) in improving skin elasticity in cosmetic treatments. Are all hyaluronic acids the same? Points to bear in mind

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Abstract *Background:* Hyaluronic acid (HA) is in high demand in aesthetic medicine, leading to a wide variety of products differing in concentration, molecular weight, additives, and delivery methods. Patients often choose HA treatments based solely on the presence of HA, without understanding product-specific indications or effects. However, the formulation and application method significantly influence clinical outcomes. *Objective:* To compare the effectiveness of two injectable HA products—Restylane® Vital and TKN HA3®—commonly perceived as similar despite differences in their composition and manufacturing. *Methods:* A split-face study was conducted on 20 subjects. Restylane® Vital was injected on one side of the face, and TKN HA3® on the other. Objective skin elasticity was measured using the Cutometer® MPA 580. Subjective assessments included the Global Aesthetic Improvement Scale (GAIS), completed by both an independent investigator (GAIS-I) and the participants (GAIS-S), along with patient satisfaction questionnaires. *Results:* Objective measurements showed differences in skin elasticity between the two products, further supported by GAIS-I evaluations. Despite these findings, subjective assessments (GAIS-S and satisfaction questionnaires) indicated that participants perceived both treatments as equally effective, with no clear preference. *Conclusions:* Although both HA products produce similar visual outcomes and are often used interchangeably, their differing formulations result in distinct clinical effects. This study found that although patients perceived similar results from both products, objective assessments showed that VHMWHA was more effective than modified HA in enhancing skin elasticity.

Key words: stabilised hyaluronic acid, prevention, anti-ageing, elasticity, cross-linked hyaluronic acid, very high molecular weight hyaluronic acid

Introduction

Hyaluronic acid made its appearance on the aesthetic medicine market in 1996[1], quickly gaining recognition for its physicochemical characteristics as a powerful moisturiser with the capacity to retain a volume of water up to 1000 times its own weight, thus launching its use in cosmetic treatments[2]. Discovered in 1934 by Meyer and Palmer, who isolated it from bovine vitreous humour, this new polysaccharide was named hyaluronic acid[3]. Later, in the 1930s and 1950s, it was successfully isolated from human umbilical cords, cockscombs and streptococci[4,5].

In 1979, Balazs developed the first efficient method to extract and purify HA from human umbilical cord and cockscombs, laying the foundations for its industrial production[6,7]. From this point on, due to its characteristics, HA had the potential to be used as a medical product for a wide range of clinical, pharmaceutical, nutritional and cosmetic applications[8-10]. Since then, different lines of research have continued to be pursued for the extraction and optimisation of HA[7].

In aesthetic medicine specifically, HA is primarily obtained through microbial fermentation biotechnology. As its structure is highly similar between different species, it is extremely well tolerated by the human body[7,11]. The main strains of streptococci used in aesthetic medicine to obtain HA are types A and C. Currently, numerous companies in the aesthetic field that market HA-based products, such as Galderma (Restylane®) Allergan (Juvéderm®) and ToskaniMED (TKN HA3®), among others, use *Streptococcus equi* cultures to produce HA. The conditions necessary to grow this bacterial culture are 37°C, pH 7 and the presence of lactose and sucrose[12,13]. The HA yield achieved by optimising the process is between 6-7 g/L. This yield cannot be improved for the time being, due to the viscosity of the product[7]. Under these conditions, it is possible to obtain very high molecular weight HA (in the range of 3,500 to 3,900 kDa), similar to the molecular weight of the HA naturally produced in human skin[14]. However, this process is not free of harmful microorganisms and the HA must be sterilised in the production chain until reaching the final packaging stage to ensure it is free of microbial contamination and pyrogens[15,16], in compliance with European regulations[17].

Many lines of research and development are currently underway to improve this final sterilisation process, as this step in the production chain fractures and weakens HA chains due to the high temperatures used[15,18,19]. This reduces the molecular weight of HA and modifies its biological characteristics when it interacts with different skin cell types, potentially achieving different or even opposite effects[20-22].

Therefore, the industry is seeking alternatives to produce medical HA products with molecular weights close to those naturally made by the human body, by exploring new sterilisation methods or devising additional post-stabilisation processes, such as introducing particles that facilitate renewed cohesion between HA fragments, known as fillers. These processes can alter the final molecular structure, concentration, or biophysical and rheological characteristics of HA, potentially improving the product's effectiveness and expanding its clinical applications[23].

In aesthetic medicine, various HA-based products have modified physicochemical or rheological characteristics to address specific aesthetic challenges. The differences between products lie mainly in their concentration, molecular weight, and the degree and type of cross-linking. HA is presented as a moisturising agent that improves skin elasticity by restoring the physiological microenvironment typical of young skin[24-26]. The physiological properties of HA are highly dependent on its molecular weight, the stability of its molecular structure to the action of hyaluronidases and its concentration[26,27]. Although they possess different characteristics, many injectable products are credited with similar physiological benefits in the context of skin care and treatment.

The aim of this double-blind observational study was to compare the efficacy of two products, TKN HA3® and Restylane Skinbooster® Vital, which differ in terms of concentration, molecular weight and HA stabilisation, but deliver the same physiological benefits to the skin when injected into the dermis. To control for differences between patients, a comparative split-face study was performed. Objective data were obtained using the Cutometer®[28,29] to assess skin elasticity and firmness. Subjective data were assessed by an independent investigator and by the study subjects using the GAIS.

Materials and Methods

Ethical considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki (2013).

Study subjects

This prospective, observational efficacy study comparing a set of cases included a total of 20 patients of

both sexes, aged 34-72 years, with different degrees of ageing according to the Glogau scale and different phototypes. All subjects had before and after photos taken with a QuantifiCare 3D LifeViz® system. Skin elasticity and firmness were measured with a Cutometer MPA 580® at baseline and 21 days after the last session.

The baseline characteristics of the study subjects and the inclusion and exclusion criteria are listed in Table 1.

Physico-chemical characteristics and production methods of the study products

The products used for the efficacy comparison were Restylane Skinboosters® Vital with lidocaine from Galderma (Zug, Switzerland) and TKN HA3® from ToskaniMed (Barcelona, Spain). Their composition and clinical indications according to the package leaflet are detailed in Table 2.

Table 1. Inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
• Age range: 25–70 years • Skin type II to IV on the Glogau wrinkle scale • No acute disease or active infection • Prior signature of the informed consent form • Prior signature of a photo release form	• Pregnancy • Breast-feeding • Hypersensitivity to hyaluronic acid • History of cosmetic facial procedures in the last 3 months • Autoimmune disease • Topical or oral collagen use • Absolute contraindication to treatment • Existing permanent fillers in the face • Use of topical retinoids in the last month • Use of oral retinoids in the last 3 months • Lack of pre- and post-treatment probe measurements or photographs

Table 2. Comparison of the physico-chemical properties of the products

COMPARISON BETWEEN PRODUCTS	
TKN HA3®[30,31]	**RESTYLANE SKINBOOSTERS® VITAL[32-34]**
Concentration: 9 mg/mL 0.9% sodium chloride solution for injections **Molecular weight:** (2700–3500) kDa Very high molecular weight hyaluronic acid chains (VHMWHA) **Manufacturing technology:** HYAsep® **pH:** 7.0 **Sterility:** Sterile **Viscosity:** dynamic 16,000 cP **HA chain type:** Non-crosslinked **Degree of cross-linking:** Not applicable **Bacterial endotoxin:** < 0.25 EU/device **Inflammatory response:** low **Delivery method:** 30 G/4 mm hypodermic needle **Duration:** 4 months **Injection layer:** Deep dermis **Indication:** Deep hydration Filling of fine wrinkles in superficial dermis Improvement of skin quality	**Concentration:** 20 mg/mL Lidocaine hydrochloride: 3 mg/mL **Molecular weight:** *Large stabilised hyaluronic acid molecules* **Manufacturing technology:** NASHA® **pH:** 7.0 **Sterilisation method**: Moist heat **Viscosity:** Not known **HA chain type:** Stabilised HA **Degree of stabilisation:** 1% **Bacterial endotoxin:** Not known **Inflammatory response:** **Delivery method:** Fine hypodermic needles 29 G 1/2" **Duration:** 6 months **Injection layer:** Deep dermis recommended **Indication:** Restore the skin's moisture balance Improve the structure and the elasticity of the skin

Study product production technology

HYAsep® technology

TKN HA3® has its own industrial manufacturing process to avoid HA fragmentation during the final sterilisation phase. It is one of the few non-cross-linked VHMWHAs (2700-3200 kDa) with a low concentration of 9 mg/mL, highly purified and manufactured under aseptic conditions throughout the production chain. This avoids the need for a final sterilisation step and ensures that the molecular weight remains the same as the starting material and is highly similar to that most commonly found in human skin[14,30].

NASHA® technology

Restylane Vital® is composed of stabilized hyaluronic acid of non-animal origin, produced using NASHA® (Non-Animal Stabilized Hyaluronic Acid), a unique and patented technology. Only 1% of the highly purified natural hyaluronic acid is modified, ensuring the product lasts for months in the tissues[32].

Equipment for measuring objective and subjective variables

Objective variables

Skin firmness and elasticity were evaluated using a Cutometer®. The probe of the device employs suction and relaxation, coupled with an optical system, to measure skin penetration into its aperture, deformation, and the return to its pre-deformation state

The Cutometer® probe was set to work in the M1 mode.

The following variables were selected for this study to characterise the skin's capacity for stretching and recovery, as well as its viscoelastic and elastic properties: R0, R1, R2, R5, R6, R7 and R8.

Erythema was measured using a Mexameter®, which gauges the absorbance reflected by the skin.

Skin hydration was assessed with a Corneometer® (Courage+Khazaka Electronic GmbH, Cologne, Germany). This system consists of a probe which is placed on the skin to measure its water content based on the principle of capacitance of a dielectric medium.

Subjective variables

The photographs were taken with a high-resolution system (QuantifiCare®, Biot, France) at baseline and end of study.

Treatment protocol

No anaesthetic cream was applied before treatment. The skin was cleansed with Energizing Cleanser (Toskani, Spain) and the treatment area disinfected with 1% chlorhexidine digluconate (Cristalmina®, Laboratorios Salvat, Esplugues de Llobregat, Spain).

To avoid discrepancies in the efficacy evaluation, each subject was treated with both products simultaneously. Restylane® Vital was applied on the right side of the face, and TKN HA3® was applied on the left side.

The 5-point BAP (Bio Aesthetic Points) technique was used on both sides of the face due to its simplicity and safety, targeting anatomical points that lack large vessels and nerve endings[35].

This technique involved the injection of a total of 1 mL of each product per hemiface, divided into 5 points on each side (0.2 mL per point). The injection sites were:

- Point 1: zygomatic protrusion, 2 cm below the end of the eye.
- Point 2: tragus, 1 cm below the lower part of the tragus.
- Point 3: base of the nose, at the intersection between the line connecting the nostril and the tragus and the perpendicular line, starting vertically from the pupil.
- Point 4: chin, 1.5 cm from the intersection between the vertical line starting from the midpoint of the chin and the perpendicular line one-third from the top.
- Point 5: mandibular angle, 1 cm above the gonial angle.

Overcorrection was avoided to prevent the appearance of papules in the days following the procedure. For Restylane Skinboosters® Vital, the needle

used was the SMARTCLICK 29 G provided by the manufacturer, and for TKN HA3®, the 30 G/4 mm needle recommended in the specifications was used.

It was recommended to gently massage the area after the treatment session. Patients were instructed that no additional massage was necessary at home.

Treatment regimen

Three treatment sessions were performed at intervals of one month from January to June 2023. No immediate post-treatment measures were necessary after the procedure, with participants able to resume their daily activities.

Recording of objective parameters. Cutometer® and Corneometer®

The objective variables were recorded at two different time points: before the start of treatment (baseline) and 30 days after the third and last session (end). Parameters were recorded using the following systems:

1. Cutometer®:
   a. Parameter R0 as an indicator of skin firmness (mm)
   b. Parameter R1 as an indicator of recovery after stretching (mm)
   c. Parameter R2 as an indicator of gross elasticity (%)
   d. Parameter R5 as an indicator of intrinsic skin elasticity (%)
   e. Parameter R6 as an indicator of viscoelasticity (%)
   f. Parameter R7 as an indicator of recovery after stretching (%)
   g. Parameter R8 as an indicator of overall recovery of the skin (mm)
2. Corneometer®:
   a. Skin hydration, in arbitrary units (AU) (range 0-130), where < 40 AU indicates dehydration and > 40 AU indicates adequate hydration
3. Mexameter®:
   a. Assessment of erythema, in arbitrary units (AU) (range 0–500)
4. QuantifiCare 3D LifeViz®:
   a. Photographic monitoring of treatment progress over time

Recording of subjective parameters. Global Aesthetic Improvement Scale (GAIS) and satisfaction questionnaires

The investigator-rated Global Aesthetic Improvement Scale (GAIS-I) was used to assess aesthetic improvement according to the investigator and the subject-rated Global Aesthetic Improvement Scale (GAIS-S) was used to assess subjective aesthetic improvement according to the participant.

- Degree of satisfaction with radiance
- Degree of satisfaction with firmness
- Degree of satisfaction with hydration
- Degree of satisfaction with wrinkle reduction
- Degree of satisfaction with overall effect on the skin

The study subjects were asked to fill in satisfaction questionnaires rating the following aspects:

- Pain during the treatment (score of 0 to 10 on the VAS scale)
- Whether they would have the treatment again
- Likelihood of recommending the treatment to a third party (on a scale from 0, unlikely, to 10, highly recommended)

Statistical analysis

Objective parameters were reported as the mean ± standard error of the mean (SEM), while subjective parameters were reported as percentages. The normality of the data distribution was assessed using the Shapiro-Wilk test, which revealed a non-parametric distribution for all objective variables in the study. The difference between variables was analysed using the Wilcoxon *T*-test to compare means between the two time points of the study (baseline and end of study). Statistical significance was defined as $p < 0.05$. SPSS version 2.0 (IBM, Madrid, Spain) was used for statistical analysis.

Results

All subjects completed all three treatment sessions. No adverse effects were recorded during or after treatment. The side effects were those inherent to the procedure: bruising, transient erythema (lasting approximately one hour), and itching in some subjects, which subsided within 24 hours after treatment. On the side where Restylane Vital® was applied, 10% of the volunteers experienced bruising, 10% experienced rash, and 50% reported itching.

On the side where TKN HA3® was applied, 10% of participants experienced bruising, 20% experienced erythema, and 40% experienced itching. These data were extracted from the patient's medical records.

Objective variables

Firmness *(R0)*

Firmness (R0) is defined as the resistance of the skin to suction with the Cutometer®. The less skin enters the probe, in mm, the firmer the tissue.

Figure 1 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (0.29 ±0.01) mm at baseline and at three weeks it obtained an average of (0.31±0.01) mm. While TKN HA3 on average obtained a result of (0.26±0.01) mm at baseline and at three weeks obtained an average of (0.32±0.01) mm. On the Restylane® Vital side, firmness was found to decrease by 7.09% from baseline, with 35% of subjects showing improvement. On the TKN HA3® side, it decreased by 20.17% from baseline, with 25% of subjects showing improvement. The test showed no statistically significant differences between the two products with a with p-value of 0.476. There were also no differences between baseline and post-treatment for Restylane Skinboosters® Vital and TKN HA3® with with p-value of 0.14 and with p-value of 0.05 respectively.

Recovery capacity of the skin *(R1)*

The recovery capacity of the skin (R1) is defined as the mechanical capacity of the tissue to revert to its original shape after deformation, induced in this case by suction with a Cutometer®. It is quantified in millimetres and represents the vertical protrusion of the skin after suction is applied. Accordingly, the lower the number of millimetres of tissue elevation, the better its capacity for elastic recovery.

Figure 2 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital® on average obtained a result (0.15 ±0.01) mm at baseline and at three weeks obtained an average of (0.13±0.01) mm. While

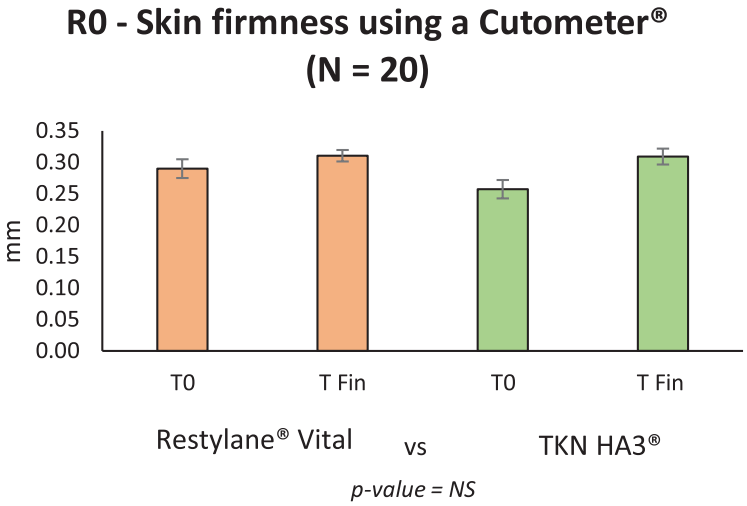

Figure 1. Firmness (mean ± SEM) at the two study time points, measured in the preauricular area.

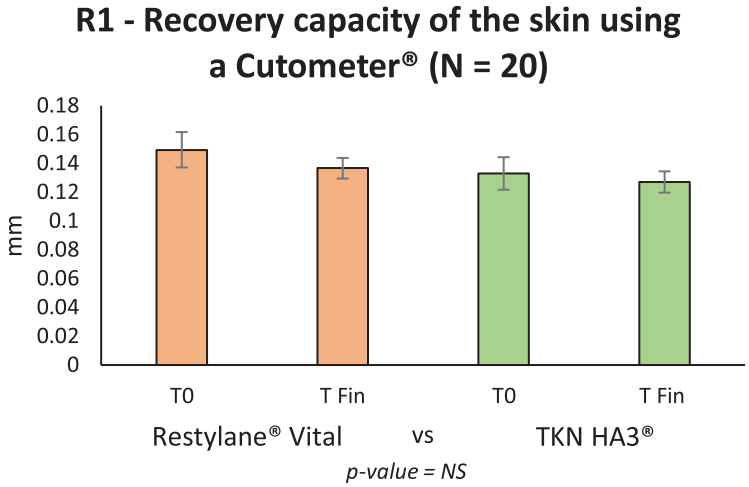

Figure 2. Recovery capacity of the skin (mean ± SEM) at the two study time points, measured in the preauricular area.

TKN HA3 on average obtained a result of (0.13±0.01) mm at baseline and at three weeks obtained an average of (0.12±0.007) mm.

On the Restylane Skinboosters® Vital side the improvement from baseline in the recovery capacity of the skin was 9.83%, with 50% of subjects showing improvement. On the TKN HA3® side, the improvement from baseline was 3.64%, with 60% of subjects showing improvement. The test showed no statistically significant differences between the two products with a p= 0.848. There were also no differences between baseline and post-treatment for Restylane Skinboosters® Vital and TKN HA3® with p-value of 0.626 and p=0.226 respectively.

Gross elasticity *(R2)*

Gross elasticity is defined as the skin's maximum extension when stretched by suction using a Cutometer®. This variable is related to the external factors to which the skin is exposed. The greater this value, the more elastic the tissue is.

Figure 3 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (49.09 ±2.5) % at baseline and at three weeks obtained an average of (55.9±2.0) %. While TKN HA3 on average obtained a result of (49.51±2.0) % at baseline and at three weeks obtained an average of (59.44±1.7) %. On the Restylane Skinboosters® Vital side, the improvement from baseline in gross elasticity was 14.01%, with 60% of subjects showing improvement. On the TKN HA3® side, the improvement from baseline was 20.05%, with 65% of subjects showing improvement. With a p-value of 0.602, the test showed no statistically significant difference between the two products. However, it did show differences between baseline and post-treatment for both Restylane Skinboosters® Vital and TKN HA3®, with p-value of 0.042 and p-value of 0.0041 respectively.

Net elasticity *(R5)*

Net elasticity is defined as the skin's ability to stretch and then return to its original position following deformation. This deformation is induced by suction using a Cutometer®.

Figure 4 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (44.51 ±3.1) % at baseline and at three weeks obtained an average of (47.40±2.6) %. While TKN HA3 on average obtained a result of (46.97±3.1) % at baseline and at three weeks obtained an average of (52.85±1.9) %. On the Restylane Skinboosters® Vital side, the improvement from baseline in net elasticity was 6.5%, with 55% of subjects showing improvement. On the TKN HA3® side, the improvement from baseline was 12.52%, with 55% of subjects

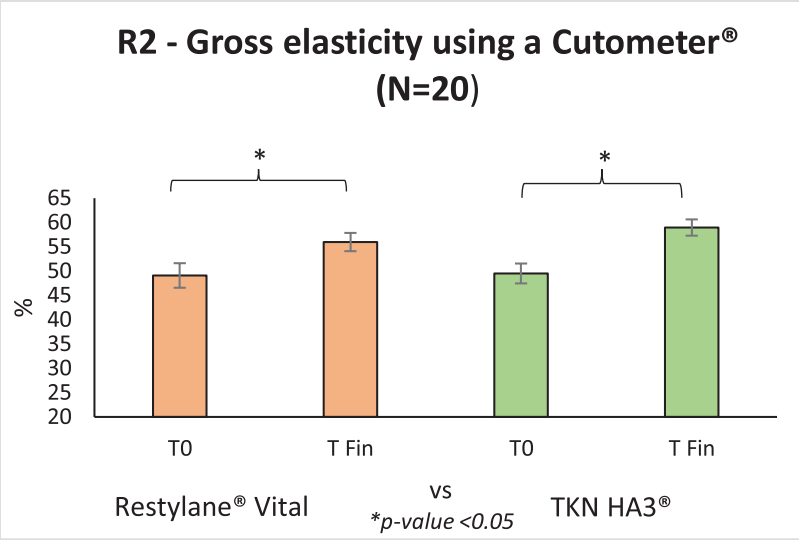

Figure 3. Gross elasticity (mean ± SEM) at the two study time points, measured in the preauricular area.

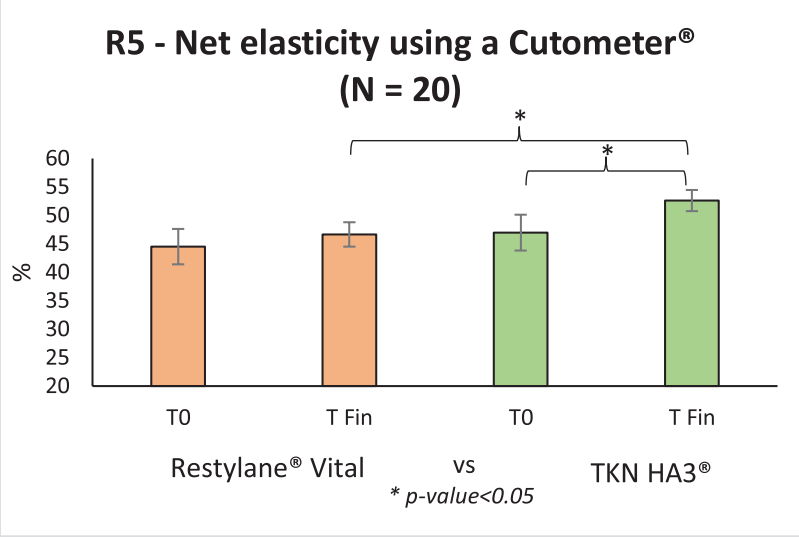

Figure 4. Net elasticity (mean ± SEM) at the two study time points, measured in the preauricular area.

showing improvement. With a p-value of 0.025, the test showed a statistically significant difference between the two products. However, it showed no differences between baseline and post-treatment for Restylane Skinboosters® Vital with a p=0.614. TKN HA3® showed differences between the baseline and post-treatment, with a with p-value of 0.00049.

Viscoelastic component *(R6)*

The viscoelasticity of the skin (R6) is defined as the ratio of viscoelastic deformation on application of force and elastic retraction following suction exerted by a Cutometer®. The lower the value, the greater the tissue elasticity.

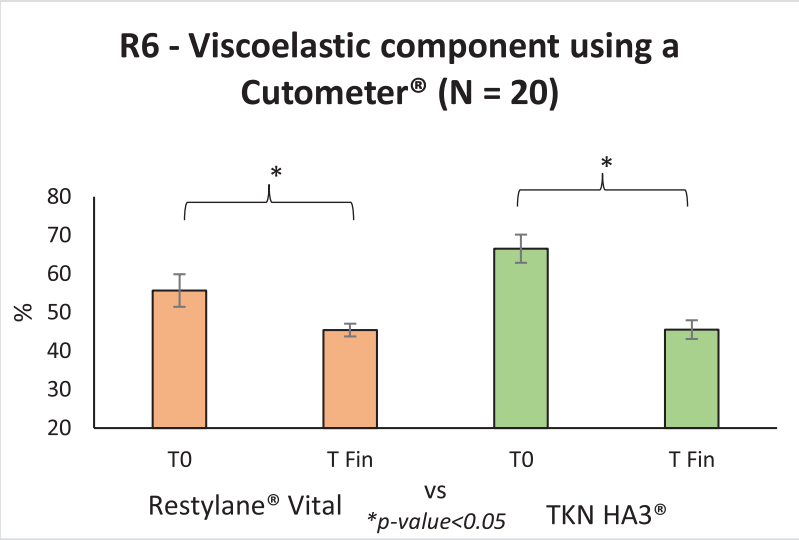

Figure 5. Viscoelastic component (mean ± SEM) at the two study time points, measured in the preauricular area.

Figure 5 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (55.68 ±4.2) % at baseline and at three weeks obtained an average of (45.43±1.6) %. While TKN HA3® on average obtained a result of (66.53±3.6) % at baseline and at three weeks obtained an average of (43.82±2.3) %. On the Restylane Skinboosters® Vital side, the improvement from baseline in the viscoelastic component was 18.43%, with 65% of subjects showing improvement. On the TKN HA3® side, the improvement from baseline was 34.04%, with 80% of subjects showing improvement. The test showed no statistically significant difference between the two products with p-value of 0.602. However, it did show differences between baseline and post-treatment for both Restylane Skinboosters® Vital and TKN HA3® with p-value of 0.047 and p-value of 0.00028, respectively.

Elastic recovery capacity *(R7)*

The skin's capacity for elastic recovery or retraction (R7) is defined as the ratio of immediate tissue retraction during the relaxation phase to the total tissue distension induced by suction, as measured by a Cutometer®. The higher the R7, the greater the elasticity; therefore, R7 can serve as a marker for skin elasticity.

Figure 6 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (28.37 ±1.6) % at baseline and at three weeks it obtained an average of (30.96±2.3) %. While TKN HA3® on average obtained a result of (27.81±1.4) % at baseline and at three weeks obtained an average of (36.90±1.4) %.

On the Restylane Skinboosters® Vital side, the improvement from baseline in elastic recovery capacity was 9.12%, with 60% of subjects showing improvement. On the TKN HA3® si with a p-value of de, the improvement from baseline was 32.68%, with 80% of subjects showing improvement. The test showed statistically significant differences between the two products with a p-value of 0.019. It showed no difference between baseline and post-treatment for Restylane Skinboosters® Vital with a p-value of 0.217, but did show statistically significant differences for TKN HA3® between baseline and post-treatment with a p-value of 0.000001.

Total recovery *(R8)*

Total recovery (R8) is defined as the ability of a tissue to return to its initial state after suction performed by a Cutometer® and subsequent relaxation. The ability of the skin to return to its initial state depends on its

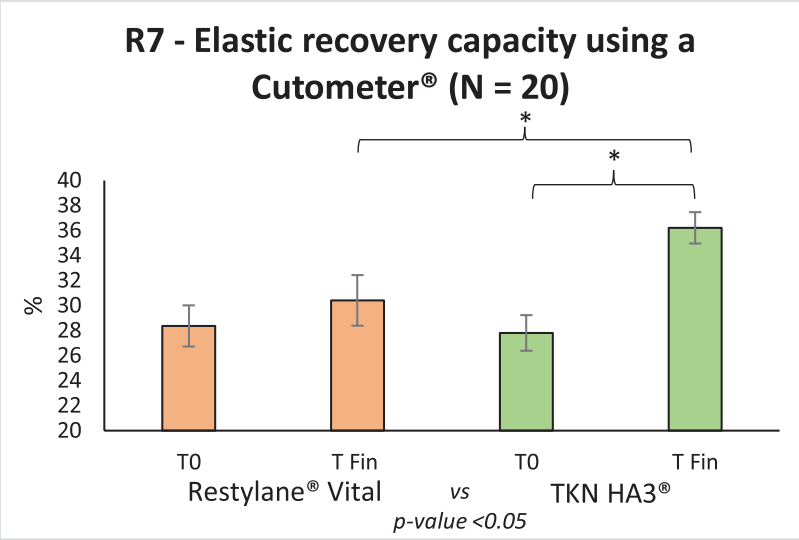

Figure 6. Elastic recovery capacity (mean ± SEM) at the two study time points, measured in the preauricular area.

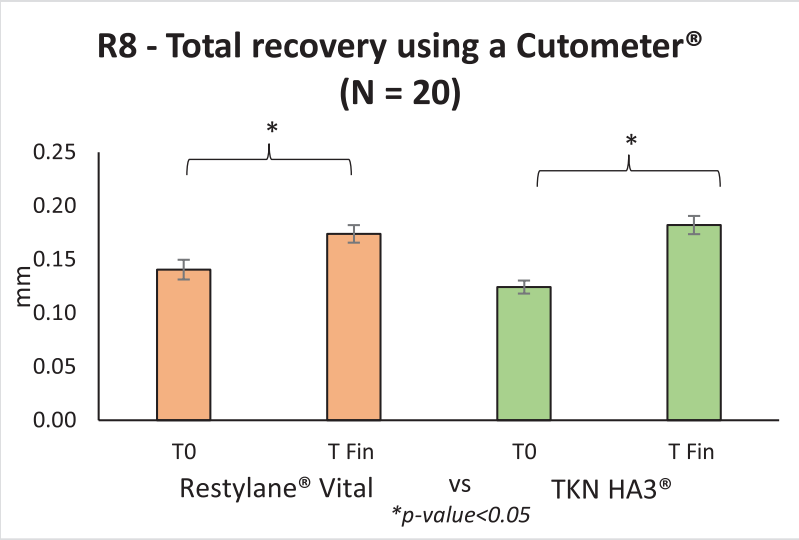

Figure 7. Total recovery (mean ± SEM) at the two study time points, measured in the preauricular area.

intrinsic properties in response to a mechanical action. The greater the R8 value, the greater the ability of the skin to return to its original position (in mm).

Figure 7 shows the results obtained (mean ± SEM). Restylane Skinboosters Vital® on average obtained a result (0.14 ±0.01) mm at baseline and at three weeks obtained an average of (0.18±0.01) mm. While TKN HA3® on average obtained a result of (0.12±0.01) mm at baseline and at three weeks obtained an average of (0.18±0.01) mm. On the Restylane Skinboosters® Vital side, the improvement from baseline in total recovery capacity was 26.83%, with 80% of subjects showing improvement. On the TKN HA3® side, the improvement from baseline was

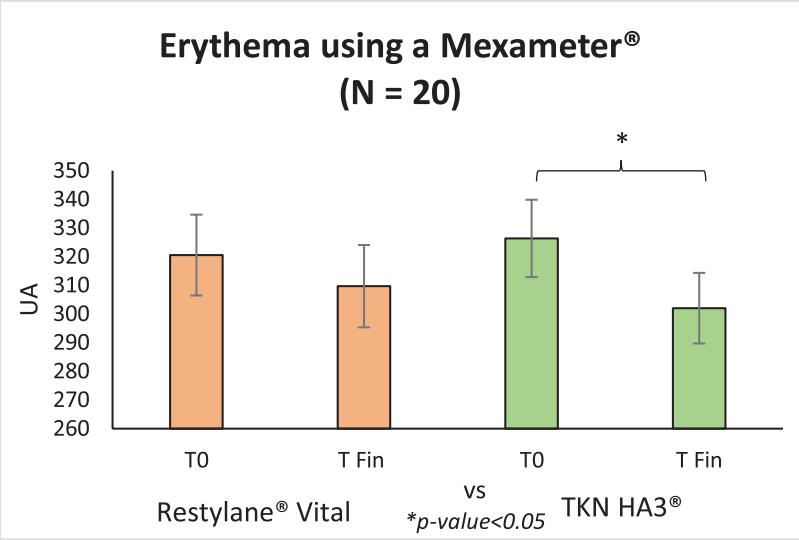

Figure 8. Erythema (mean ± SEM) at the two study time points, measured in the preauricular area.

51.63%, with 95% of subjects showing improvement. The test showed no statistically significant difference between the two products, with a p-value of 0.440. However, it did show differences between baseline and post-treatment for both Restylane Skinboosters® Vital and TKN HA3® with p-value of 0.01 and p-value of 0.0001, respectively.

Erythema

Erythema is defined as the ratio of absorption/reflection of light emitted on the skin by the Mexameter® using specific wavelengths corresponding to the spectral absorption peaks of haemoglobin. It is measured in arbitrary units (AU).

Figure 8 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (320.58 ±14.12) UA at baseline and at three weeks obtained an average of (311.61±14.18) UA. While TKN HA3® on average obtained a result of (326.39±13.49) UA at baseline and at three weeks obtained an average of (303.10±12.22) UA. On the Restylane Skinboosters® Vital side, the reduction from baseline in erythema was 2.80%, with 70% of subjects showing improvement. On the TKN HA3® side, the reduction from baseline in erythema was 7.13%, with 90% of subjects showing improvement. The test showed no statistically significant differences between the two products, nor between baseline and post-treatment for Restylane Skinboosters® Vital, with p-values of 0.17 and 0.205 respectively. However, it did show a difference between baseline and post-treatment for TKN HA3®, with a p-value of 0.0026.

Skin hydration

Figure 9 shows the results obtained (mean ± SEM). Restylane Skinboosters® Vital on average obtained a result (44.19 ±2.02) AU at baseline and at three weeks obtained an average of (41.14±1.81) AU. While TKN HA3® on average obtained a result of (44.03±2.2) AU at baseline and at three weeks obtained an average of (39.78±1.5) AU. On the Restylane Skinboosters® Vital side, the reduction from baseline in skin hydration was 6.70%, with 30% of subjects showing improvement. On the TKN HA3® side, the reduction from baseline in skin hydration was 9.46%, with 30% of subjects showing improvement. The test showed no statistically significant differences between the two products with a p-value of 0.476 and no differences between baseline and post-treatment for Restylane Skinboosters® Vital with a p-value of 0.052. While TKN HA3® showed differences between the initial and final state with a p-value 0.007.

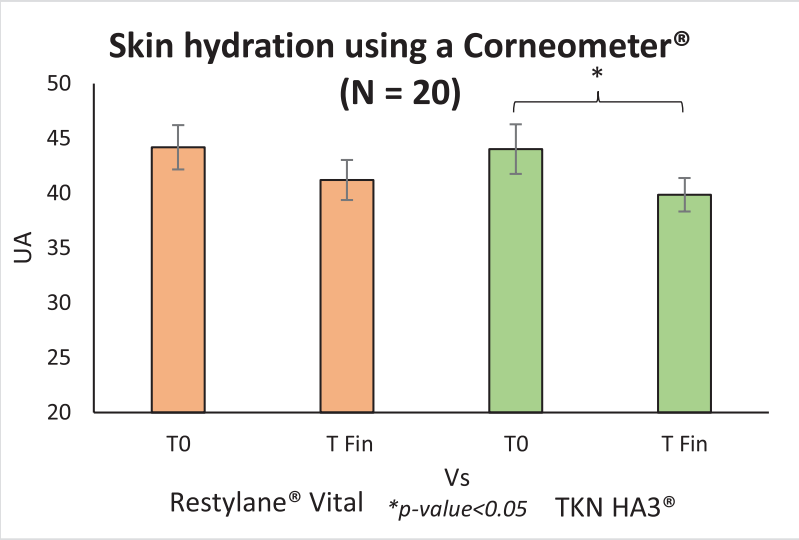

Figure 9. Skin hydration (mean ± SEM) at the two study time points, measured in the preauricular area.

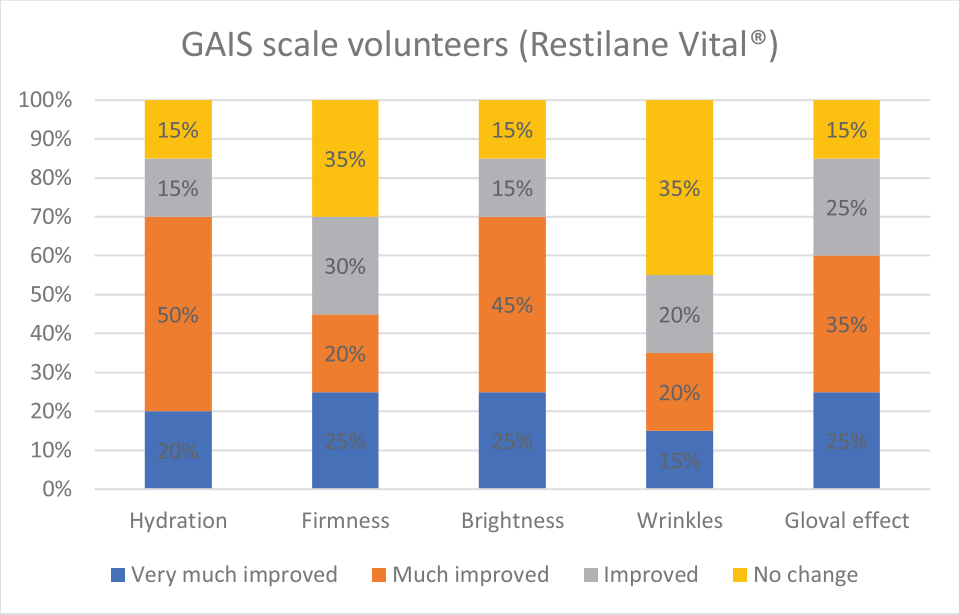

Figure 10. Subject-rated Global Aesthetic Improvement Scale, right side.

Subjective parameters

Data reported by patients based on the Subject Global Aesthetic Improvement Scale (GAIS-S)

A satisfaction survey was conducted where subjects, unaware of which product they were injected with, were asked about perceived improvements on each side of the face.

The results are shown in Figure 10 for Restylane Skinboosters® Vital and Figure 11 for TKN HA3®.

Subjects were asked about perceived improvements on each side of the face. For hydration, 85% of the study population reported an improvement on the

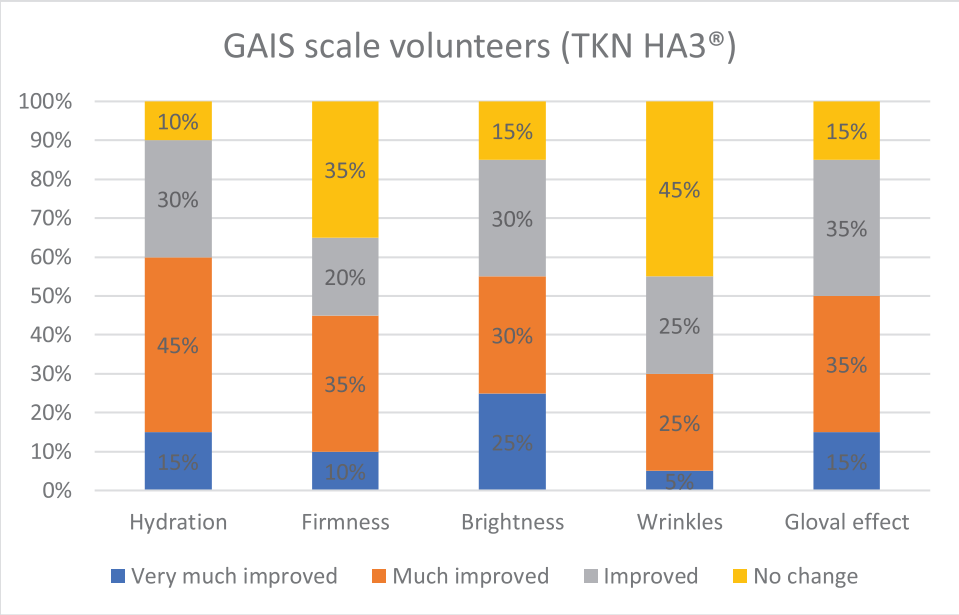

Figure 11. Subject-rated Global Aesthetic Improvement Scale, left side.

right side (Restylane Skinboosters® Vital) and 90% on the left side (TKN HA3®).

With regard to skin firmness, 70% of the study population reported an improvement on the right side (Restylane Skinboosters® Vital) and 65% on the left side (TKN HA3®).

With regard to radiance, 85% of the study population reported improvement on both the right side (Restylane Skinboosters® Vital) and the left side (TKN HA3®).

With regard to wrinkles, 55% of the study population found an improvement on both the right side (Restylane Skinboosters® Vital) and the left side (TKN HA3®).

When asked about overall improvement, 85% of the study population reported an improvement on the right side (Restylane Skinboosters® Vital) and 85% on the left side (TKN HA3®).

Participants were blinded to which product was applied to each side of their face. To determine their perception, they were asked about their perceived improvement using the GAIS scale. The results showed slight differences in hydration and firmness, but overall, the volunteers' perception was the same on both sides.

Data reported by an independent investigator using the Global Aesthetic Improvement Scale (GAIS-I)

The improvement data were assessed by an independent investigator who was unaware of which side the products had been administered on or whether the assignment of the product to each side was random, making them a fully blinded observer.

The results are shown in Figures 12 and 13.

The results, quantified by an independent investigator using photographs, were as follows: for hydration, the investigator reported 65% of improvement on the right side (Restylane Skinboosters® Vital), and 90% on the left side (TKN HA3®).

With regard to skin firmness, the investigator reported 70% of improvement on the right side (Restylane Skinboosters® Vital) and 80% on the left side (TKN HA3®).

With regard to radiance, the investigator reported 85% of improvement on the right side (Restylane Skinboosters® Vital), and 90% on the left side (TKN HA3®).

With regard to wrinkles, the investigator reported 60% of improvement on both the right side (Restylane Skinboosters® Vital) and the left side (TKN HA3®).

With regard to overall improvement, the investigator reported 70% of improvement on the right side

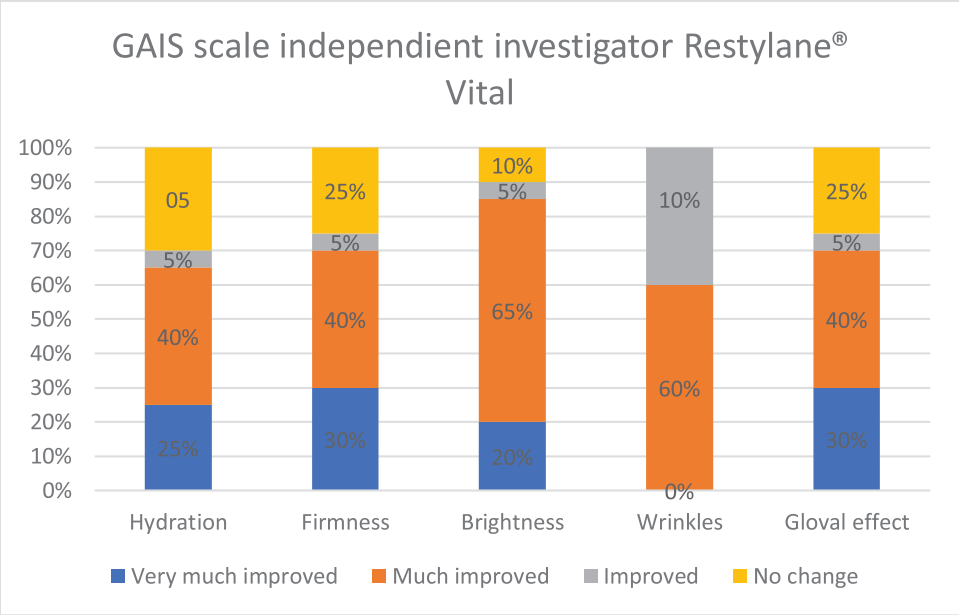

Figure 12. Investigator-rated Global Aesthetic Improvement Scale, right side.

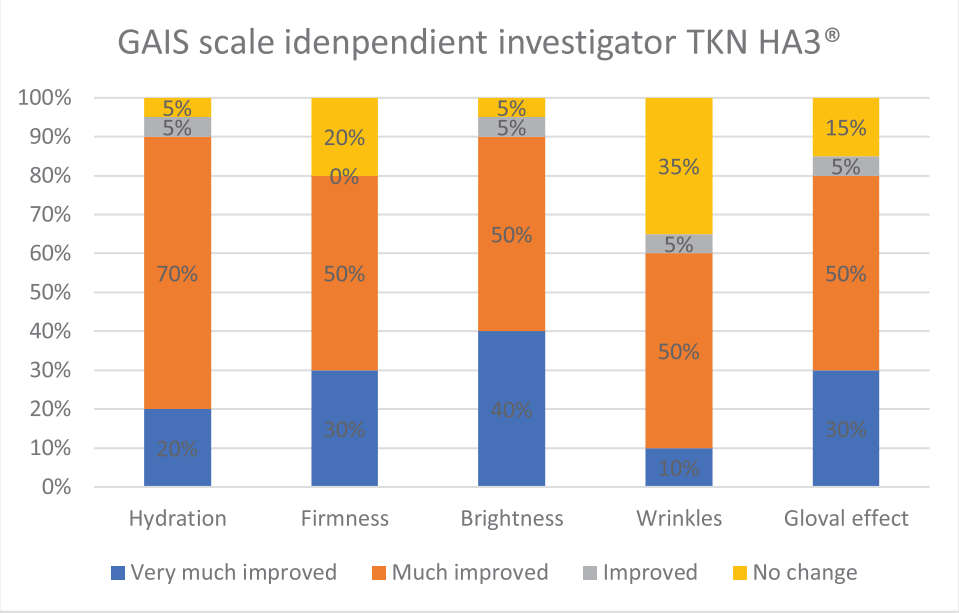

Figure 13. Investigator-rated Global Aesthetic Improvement Scale, left side.

(Restylane Skinboosters® Vital), and 80% on the left side (TKN HA3®).

Evaluation of satisfaction surveys

Subjects completed satisfaction questionnaires assessing pain during treatment, willingness to have the treatment again and likelihood of recommending the treatment.

Pain: the average pain score was 3.62±2.41 for the right side (Restylane Skinboosters® Vital) and 2.87±1.82 for the left side (TKN HA3®).

Willingness to repeat treatment: 55% of subjects responded they would repeat the treatment injected into the right side (Restylane Skinboosters® Vital); 70% said they would repeat the treatment administered to the left side (TKN HA3®).

Likelihood of recommending treatment: the average score was 6.43±2.74 for the right side (Restylane Skinboosters® Vital) and 7.12±5.47 for the left side (TKN HA3®).

Figures 14, 15, 16 and 17 show the clinical status before treatment (A) and 30 days after the last treatment session (B).

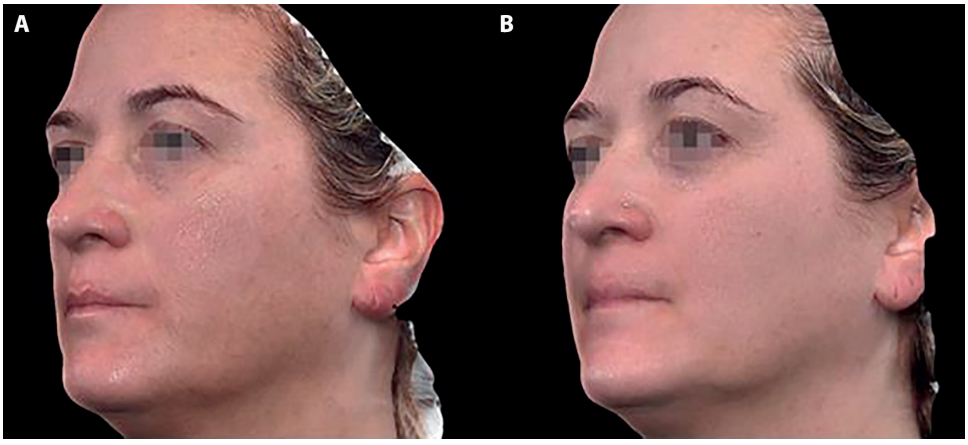

Figure 14. Left lateral view of a 45-year-old subject, TKN HA3® side. A: baseline B: 30 days after the last treatment session.

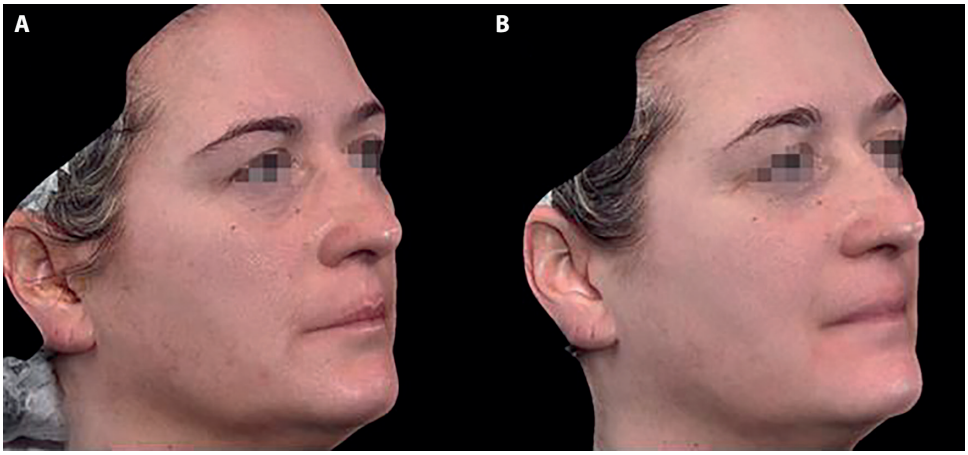

Figure 15. Right lateral view of a 45-year-old subject, Restylane Vital® side. A: baseline B: 30 days after the last treatment session.

Figure 16. Left lateral view of a 34-year-old subject, TKN HA3 Vital® side. A: baseline B: 30 days after the last treatment session.

Figure 17. Right lateral view of a 34-year-old subject, Restylane Vital® side. A: baseline B: 30 days after the last treatment session.

Discussion

The aim of this study was to demonstrate the *in vivo* efficacy of two HA products that differ completely in formulation, production method, and biological effect but share the same therapeutic purpose and indication: the prevention and treatment of skin ageing[30-34]. The results obtained show that both products fulfil the stated clinical indications. Objective efficacy measurements using the Cutometer MPA 580® demonstrated that TKN HA3®—a very high molecular weight hyaluronic acid (VHMWHA) that is neither cross-linked nor chemically modified—outperformed Restylane Skinboosters® Vital by nearly twofold in terms of elasticity parameters (R2, R5, R7, and R8) and the viscoelastic component (R6).

In contrast, despite this being one of their main clinical indications, neither product showed any improvement in hydration, as evidenced by the Corneometer®. This is likely due to both products being injected into the deep dermis, beyond the effective measurement range of the device, which can only measure up to a depth of 20 μm. Therefore, no conclusions can be drawn about the differences between the two products in terms of hydration. Regarding firmness, there was no difference between the two products. As for erythema, the reduction was greater on the side treated with TKN HA3®.

Previous *in vivo* and *in vitro* studies comparing cross-linked HA with non-cross-linked HA have shown clear differences in terms of longevity, hydration, elasticity, and roughness of the skin *in vivo*[36-41]. *In vitro*, NASHA® cross-linking induced the formation of type I collagen and increased procollagen synthesis, thereby restoring components of the dermal matrix[42].

However, until now, cross-linked HA had never been compared with a non-crosslinked VHMWHA such as TKN HA3®, as the manufacturing technology, HYAsep®, is relatively recent. Recent studies have highlighted the benefits of using VHMWHA *in vivo*, as it mainly improves the elastic properties of the skin due to its deep moisturising power and consequently the physiological properties of the extracellular matrix[30].

Photographic acquisition and subsequent subjective evaluation by an independent investigator using GAIS-I showed similar improvements on both sides, with a slightly greater improvement on the TKN HA3® side, as confirmed by objective results.

Subjects' subjective impressions using GAIS-S indicated that the perceived both products as equivalent. The only aspect worth noting was that greater discomfort was reported on the Restylane Skinboosters® Vital side compared to the TKN HA3® side, even though the former contains lidocaine.

Manufacturers must declare the HA concentrations (mg/mL) of their products, but this concentration does not provide information about the molecular weight, the degree of cross-linking or modification, or the soluble fraction of the biopolymer added to improve extrusion or prevent degradation of the polymer during the sterilisation process[43-45]. Clinicians are generally not informed about the amount of soluble HA in the products; therefore, the reported concentration of HA in commercially available products is only an estimate and not an exact value for assessing product performance[44].

Conclusions

This study comparing the results achieved using VHMWHA and slightly modified HA in a set of cases showed that while patients perceived both products to yield very similar results, assessments performed by an external investigator and measuring equipment revealed the VHMWHA to be superior to the modified HA in improving skin elasticity.

Study Limitations

Due to the scarcity of studies conducted using VHMWHA for the treatment and prevention of skin ageing, further *in vivo* and *in vitro* studies are needed. These studies should determine whether the observed superiority of VHMWHA is specific to its comparison with slightly modified HA or if similar responses are seen with other injectable HA products that combine different molecular weights and concentrations.

Long-term studies on the efficacy and duration of the effects of the TKN HA3 product are needed to consolidate the results obtained in this study.

Furthermore, further research with larger study samples and follow-up at 6 and 12 months is required to evaluate the clinical impact of the differences found.

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