Research Article

Annals of Dermatological Science (ISSN 2831-8129)

Retrospective controlled study of the efficacy of very high molecular weight hyaluronic acid (VHMWHA) non-cross-linked as a dermabooster for the improvement of elasticity in the skin.

Gabriel Buendía Bordera[1], Francisca Rubio Toral[2], Anna Serrabou[3], Ariadna Gamboa Roget[4], Maria Llanos Pérez González[5], Maria Begoña García Díaz[6], Sandra Expósito González[7].

1. Scientific Director, Instituto de Fotomedicina, Centro Médico Teknon, Barcelona. Spain
2. Aesthetic Doctor, Toral Clinic Barcelona, Barcelona. Spain
3. Aesthetic Doctor, Esthetic BCN, Barcelona. Spain
4. Dermatologic Doctor, Instituto de Fotomedicina, Centro Médico Teknon, Barcelona. Spain
5. Medical Scientific Liaison, Toskani, Santa Eulalia de Ronçana. Spain
6. Technical Director, Toskani, Santa Eulalia de Ronçana. Spain
7. Clinical Study Coordinator, Toskani, Santa Eulalia de Ronçana. Spain

† These authors contributed equally to this work

Corresponding author

Gabriel Buendía Bordera ,
Scientific Director, Instituto de Fotomedicina, Centro Médico Teknon, Barcelona. Spain.
Telephone: + 34 666 41 48 56 /
+ 34 93 434 37 37
Email : gbb@fotomedicina.com

Received Date : July 21, 2024
Accepted Date : July 22, 2024
Published Date : August 23, 2024

ABSTRACT

Hyaluronic acid (HA), regardless of its molecular weight, presentation and route of administration, is currently among the products most in-demand by our clients for the prevention and treatment of skin ageing in aesthetic medicine clinics.

Among the injectables marketed for use in mesotherapy, the very high molecular weight non-cross-linked HA (VHMWHA ≈3000 kDa), TKN HA3®, is classified as a dermabooster that is characterized by its revitalizing, stimulating and regenerative properties: acting directly on the dermis, stimulates fibroblasts, promoting de novo collagen and elastin synthesis and thus improving the properties of the components of the extracellular matrix. It is also known for its hygroscopic properties, which hydrate the collagen and elastin fibres present in the skin, thus improving its elasticity and turgor.

The molecular weight of non-cross-linked VHMWHA is very similar to that of the main type of HA produced by the skin, which explains why no side effects have been reported to date.

To demonstrate the efficacy and safety of VHMWHA for improvement the elasticity in the skin, patients who had received a four-point injection of VHMWHA were selected, specifically TKN HA3®, in the first half of 2023, and had three sessions at three-week intervals. A total of 119 male and female volunteers were recruited.

The results show an improvement in skin elasticity as measured by a Cutometer® in 86% of patients, and an improvement in Global Aesthetic Improvement Scale (GAIS) score assessed by the patients and researcher, showing an overall improvement of the skin of 95.7% and 83.7%, respectively.

Dermal injection of VHMWHA with molecular weight ≈3000 kDa was shown to be safe and effective in improving skin elasticity.

Keywords

Treatment, prevention, anti-ageing, ageing, elasticity, non-cross-linked hyaluronic acid, very high molecular weight hyaluronic acid.

INTRODUCTION

The changes that occur in the skin due to aging are the result of two biologically independent processes. The first process is the contribution of intrinsic or innate aging, which is an inevitable phenomenon that affects the skin in the same way that it affects all the internal organs of the body. Extrinsic aging is the result of exposure to external factors, mainly ultraviolet

(UV) radiation, a process also known as photoaging [1].

From the age of 30, neocollagenesis (the generation of new collagen fibres) begins to slow down, while the amount of hyaluronic acid decreases, causing wrinkles, loss of elasticity and loss of radiance [2-3].

Elderly skin (> 50 years) mainly expresses proteins related to ossification, bone mineralisation and connective tissue development, causing mechanical damage and loss of elasticity [3-4]. The number of adipocyte progenitors decreases as we age, leading to loss of subcutaneous tissue, which is particularly noticeable on the face [4].

The extracellular matrix (ECM) is responsible for the elasticity of the skin due to the union of fibroblasts and collagen fibrils, giving consistency, through the action of mechanical forces that contribute to morphological regulation and cellular function. The components of the ECM can be split into two blocks: the fibrillar components (collagen, reticular and elastic fibres), which lend support, strength, elasticity and mechanical cohesion to connective tissues; and the non-fibrillar ground substance (composed of proteoglycans and glycoproteins associated with long-chain hyaluronic acid), which also provides mechanical support, compressive strength and filter functions and takes the form of a gel-like substance that fills the spaces between fibrils and fibroblasts [5].

The aging process causes changes in the skin that result in less attachment of fibroblasts to

the ECM and, consequently, in reduced mechanical forces, leading to a loss of dermal thickness and ECM density. The number and biological functions of fibroblasts also decrease with age, due to a decrease in collagen and elastin production [5-6]. The aging of fibroblasts not only affects

production of collagen and elastin, but also, the expression of genes involved in the formation and secretion of other components of the ECM is decreased, losing its identity and acquiring adipogenic traits which, in turn, regulate the expression of genes involved in inflammation, lipid metabolism and the process by which multipotent cells differentiate into mature adipocytes [7-8].

Clinically, this results in dermal atrophy, wrinkles and fragile skin [7]. The decrease in water in the skin has been associated with aging [9]. Hyaluronic acid (HA) is the molecule found in the largest proportion in the extracellular matrix and is one of several compounds involved in skin hydration. It also provides firmness and smoothness to the skin by hydrating the collagen fibres and helps the defensive barrier by having the ability to interfere with the action of certain pathogens. HA is synthesized mainly by fibroblasts, in addition to keratinocytes and endothelial cells in the skin region, to regulate the different homeostatic processes of the skin [10]. The molecular weight of the HA present in the ECM and generated by fibroblasts varies from 0.8 kDa to 3000 kDa, and the size of the HA molecules is determined by the number of repeating disaccharide units that join in their formation [11-12].

There is not a unified criterion on the classification of HA based on its molecular weight, despite this, it has been considered by different authors that high molecular weight hyaluronic acid is one whose molecular weight is greater than 1000kDa. For the rest of HA that are below this value, they have been considered low molecular weight HA [13-14]. In the skin we have a molecular weight of approximately 3000kDa [15]. HA is produced in the membrane of different cell types [5,13]. Its synthesis is mediated by glycosyltransferases that bind to the membrane. These isoenzymes have three isoforms called hyaluronan synthases (HAS1, HAS2 and HAS3) [14-17]. Although each of its isoforms synthesizes the same product, they do so at different rates, and consequently synthesize hyaluronan chains of different lengths. Polymers of 100 to 1000 kDa are synthesized by HAS3. Polymers of 200 to 2000 kDa are synthesized by HAS1 and HAS2 - particularly HAS2, which produces chains of 6000 to 7000 kDa [18]. The structure of HA in solution is stabilised by the formation of hydrogen bonds, which explains its physical properties, in particular the good water solubility of HA and its salts, as well as its high hygroscopicity and viscoelasticity [19-20]. Hyaluronic acid must exhibit viscoelastic properties, allowing it to change shape while maintaining sufficient elasticity [21]. This is because HA forms a polymeric system with a large number of monomeric units. The bonds within this system contribute to the rigidity of its chemical structure [20]. In addition, the electrostatic charges generated by the carboxyl groups cause HA molecules to spread, occupying a large volume and forming a mesh structure [22]. There are numerous hyaluronic acid-based injectables on the market that differ mainly in their concentration, cross-linking degree or molecular weight, among other properties [23-24]. These differences result in variations in the rheological properties of each HA product and therefore in its behaviour and the clinical outcomes [19]. Non-cross-linked VHMWHA ≈3000 kDa is not designed for wrinkle filling or as a volumiser; instead, it serves as a biostimulator, optimising the ECM [25]. A biostimulator is a substance capable of inducing cellular effects or facilitating temporary tissue regeneration, along with restoring the mechanical properties of the treated area [26-27]. Various authors have reported that HA with a molecular weight >1000 kDa exhibits anti-inflammatory effects against UVB induced keratinocyte damage [28]. It also inhibits angiogenesis, the inflammatory immune response and macrophage phagocytosis. Furthermore, it interacts with CD44, a transmembrane glycoprotein involved in

Annals of Dermatological Science (ISSN 2831-8129)

cellular and ECM interactions, leading to keratinocyte differentiation and increased cell survival [19-33]. In addition, the very high molecular weight of the product administered in the study makes it similar to the main type of HA produced in the skin. This ensures a prolonged persistence of the injected VHMWHA compared to other hyaluronic acids on the market. In fact, the higher molecular weight contributes to greater resistance to degradation by hyaluronidases, thus extending the half-life of HA [34]. The degradation of VHMWHA is mediated by the action of the enzymes HYAL-2 and HYAL-1. HYAL-2 is more active than HYAL-1, hydrolysing larger HA chains than HYLA-1, producing fragments of about 20kDa, which will subsequently be hydrolysed by PH20 [35]. The involvement in the degradation of HYAL-3 is not clear, although it is believed that it could contribute to the degradation by improving the activity of HYAL-1. This retrospective study aimed to evaluate the efficacy and safety of TKN HA3®, a product composed mainly of very high molecular weight hyaluronic acid ≈3000kDa, in patients seeking cosmetic treatment to enhance facial appearance using the 4-point injection technique in the dermis [37]. Objective measurement of skin elasticity was conducted using a Cutometer® [38-39], while subjective data were analysed by both patients and three independent investigators using the GAIS scale [38-39].

MATERIALS AND METHODS

Ethical considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki (2013) and was approved by the Clinical Research Ethics Committee (CEIm).

Study subjects

A retrospective efficacy and safety study was conducted which involved the review of the clinical records of 131 male and female patients. In the first half of 2023, these patients had undergone TKN HA3® treatment, using the 4-point technique for various degrees of facial ageing according to the Glogau scale. At the end of the screening period, 119 volunteers met the inclusion criteria and completed the three treatment sessions. It was verified that all participants had their data collected using probes, and the existence of photographic records obtained with QuantifiCare LifeViz 3D® was confirmed. Twelve patients (9.16%) were excluded due to lack of data or due to not having completed 3 sessions 3 weeks apart. Four of them had only had 1 session and 8 had no follow-up data for the third session. The screening period for participants covered the period from January to June 2023.

Table I describes volunteer characteristics and inclusion/exclusion criteria for VHMWHA-treated patients.

Table 1

STUDY SUBJECTS	EXCLUSION CRITERIA
119 volunteers: 104 women 15 men Age: 25–68 years Study period: January to June 2023	• Pregnant women. • Breast-feeding women. • Hypersensitivity to hyaluronic acid. • Volunteers who had undergone other cosmetic facial procedures in the previous 3 months. • Baseline R5 ≥ 65%. (Net elasticity) • Autoimmune disorder. • Topical or oral collagen use. • Absolute contraindication to treatment. • Existing facial permanent fillers. • 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.
	INCLUSION CRITERIA
	• Age range: 25–70 years. • Ageing II to IV on the Glogau scale. • Baseline R5 < 65%. (Net elasticity) • No acute disease or active infection. • Prior signature of the informed consent form. • Prior signature of a photo release form.

Table I. The table shows the inclusion and exclusion criteria of the patients.

Materials used in the clinic

The materials employed in the clinic for treatment and analysis of objective variables are detailed in Table II.

Annals of Dermatological Science (ISSN 2831-8129)

Table II

MATERIALS USED	DESCRIPTION
Injection of non-cross-linked HMWHA (TKN HA3®) HA chains of very high molecular weight Total: 1.6 mL	Concentration: 9 mg/mL HA ≈3000 kDa 0.9% sodium chloride solution for injection Sterile pH: 7.0 Bacterial endotoxin < 0.25 EU/device Manufacturing technology: HYAsep®
Hypodermic needles	30 G/4 mm
QuantifiCare 3D LifeViz®	Photographs Camera for inspection of the skin
Cutometer® (firmness and elasticity) Corneometer® (hydration) Mexameter® (erythema)	Measurement probes

Table II. The table shows the material and their descriptions.

The hyaluronic acid selected for patient selection was non-crosslinked HA, with a molecular weight of ~3000 kDa (VHMWHA), the manufacture of which is carried out under aseptic conditions throughout the manufacturing process (HYAsep®).

Photographs were taken with a high-resolution system (QuantifiCare®, Biot, France) at the beginning and at the end of the study.

Skin firmness and elasticity were evaluated using the Cutometer® equipment (Courage+Khazaka Electronic GmbH, Cologne, Germany). This probe applies suction and relaxation on the skin, which together with an optical system, allows measuring the penetration of the skin in its aperture, the deformation and the return to its state prior to the deformation, providing information about its elasticity and firmness [12-13].

The main variable selected for this study was R5 (intrinsic elasticity of the skin), because in in-vitro studies in aged fibroblasts, it has been shown that TKN HA3® has the ability to generate large amounts of elastin (unpublished results). The variables R0, R1, R2, R6, R7 and R8 as secondary variables were selected to characterize the stretch and recovery capacity, as well as the viscoelastic properties of the skin.

Erythema was measured using a Mexameter® (Courage+Khazaka Electronic GmbH, Cologne, Germany), based on the principle of the absorbance of two specific wavelengths to measure the haemoglobin index present in the skin. Skin hydration was evaluated with a Corneometer® (Courage+Khazaka Electronic GmbH, Cologne, Germany). This system consists of a probe that is placed on the skin and the measurement method is based on the difference between the dielectric constant of water compared to other substances, in a dielectric medium.

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 solution (Cristalmina®).

The patients chosen were those who were injected by the 4-point technique with non-cross-linked VHMWHA (TKN HA3®, ToskaniMED, Spain) into the dermis to a depth of ≤4 mm. The needle used was 30 G/ 4mm.

The treatment was administered using the 4-point per hemiface technique [37], which consists of injecting the content of a syringe of no more than 1.6 mL into strategic areas of the face corresponding to the upper ligaments and areas of maximum projection (zygomatic cutaneous ligament, masseteric ligament, malar eminence, top of the nasolabial fold) using 0.2 mL of product per injection site.

A gentle massage was recommended after the treatment session, and there was no requirement for the patient to have any massage at home.

Objective study parameters using the Cutometer® and Corneometer® probes.

The data were extracted from the centre's clinical unit's own database. In total, 131 volunteers were found, but only 119 met the inclusion criteria. The objective variables considered were the records taken at two different time points: before the start of treatment (baseline) and 30 days after the last session (final).

The parameters analysed were:

1. Cutometer®:
   a. Parameter R0 as an indicator of the change in skin firmness (mm).
   b. Parameter R1 as an indicator of skin recovery after a temporary elongation (mm).
   c. Parameter R2 as an indicator of gross elasticity influenced by external factors in the skin (%).
   d. Parameter R5 as an indicator of intrinsic elasticity of the skin not associated with external factors (%).
   e. Parameter R6 as an indicator of viscoelastic changes in the skin (%)
   f. Parameter R7 as recovery indicator after the first elongation (%).
   g. Parameter R8 as an indicator of global skin recovery (mm).

Annals of Dermatological Science (ISSN 2831-8129)

2. Corneometer®:
   a. Skin hydration through capacitance, in arbitrary units (AU) The measurement range is 0-130 AU, where the manufacturer indicates that values <40 AU indicate dehydration and >40 AU indicate adequate hydration [38].
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.

Evaluation of subjective results: GAIS scale

The Investigator Global Aesthetic Improvement Scale (IGAIS) was used to evaluate aesthetic improvement according to the researcher and the Subject Global Aesthetic Improvement Scale (SGAIS) was used to evaluate subjective aesthetic improvement according to the participant. The following indicators were taken into account. of improvement:

- Degree of satisfaction with skin luminosity
- Degree of satisfaction with skin firmness
- Degree of satisfaction with skin hydration
- Degree of satisfaction with wrinkle reduction
- Degree of satisfaction with the general effect on the skin
- Degree of social impairment post-treatment

Statistical analysis

The objective parameters obtained by the probes were reported as the mean ± standard error of the mean (SEM), while the subjective parameters obtained from the scale IGAIS and SGAIS were reported as percentages. Normality of data distribution was assessed using the Shapiro-Wilk test, which indicated a normal distribution for all objective variables in the study. The difference between variables was analysed using Student's t test to compare means between the two time points of the study (baseline and end of the study). Statistical significance was defined as $p < 0.05$. SPSS version 2.0 (IBM, Madrid, Spain) was used for statistical analysis.

RESULTS

The 119 volunteers who were included retrospectively in a controlled manner verified that they had completed the three sessions of the study, that the application technique of the TKN HA3 was 4 points and that photographic records had been made, in addition to having collected the data of the initial state and finally through the probes.

The sample selected for the retrospective study was composed of male and female volunteers who met all inclusion criteria, with a mean age of 47.74 ± 9.08 years (range 25-68). Of the 119 participants, 8 (6.72%) were grade I, 40 (33.62%) were grade II, 54 (45.38%) were grade III and 17 (14.28%) were grade IV on the Glogau aging scale.

No adverse effects were recorded during or after treatment. Side effects were those typical of the procedure: bruising, transient erythema and itching in some subjects, which subsided within 24 hours after treatment. These data were extracted from the patient's medical records.

Objective parameters of the study

Main variable:

1. Net elasticity of the skin: parameter R5, measured with a Cutometer®

Secondary variables:

1. Firmness: parameter R0, measured with a Cutometer®
2. Recovery capacity of the skin: parameter R1, measured with a Cutometer®
3. Gross elasticity: parameter R2, measured with a Cutometer®
4. Viscoelasticity: parameter R6, measured with a Cutometer®
5. Elastic recovery capacity: parameter R7, measured with a Cutometer®
6. Overall recovery: parameter R8, measured with a Cutometer®
7. Erythema: measured with a Mexameter®
8. Hydration: measured with a Corneometer®

Subjective parameters of the study (GAIS scale)

In aesthetic medicine, it is standard practice to ask patients who have undergone aesthetic enhancements to complete a satisfaction survey. In this case, the questionnaire used was the Global Aesthetic Improvement Scale (GAIS).

Three external investigators with no knowledge of the results obtained with the various probes conducted a retrospective assessment of before-and-after photos with the GAIS scale in order to quantify the improvements achieved. The items assessed by the two groups (patients and investigators) were as follows:

1. Degree of satisfaction with skin luminosity
2. Degree of satisfaction with skin firmness
3. Degree of satisfaction with skin hydration
4. Degree of satisfaction with wrinkle reduction
5. Degree of satisfaction with the general effect on the skin.
6. Degree of social impairment post-treatment

Objective variables:

1. Net elasticity: R5

Net elasticity was defined as the primary study variable due to its direct relationship with the intrinsic properties of skin elasticity.

Net elasticity is defined as the skin's ability to stretch and then

Annals of Dermatological Science (ISSN 2831-8129)

return to its original position following deformation. This deformation is induced by suction using a Cutometer®.
Figure 1 shows the results obtained (mean ± SEM). Net elasticity was found to improve by 23.11% with respect to baseline. The average increase between the two study time points was 10.37 ± 1.2% and was statistically significant ($p=1.08.10^{-18}$),84.03% of participants showed an increase in elasticity.

Figure 1

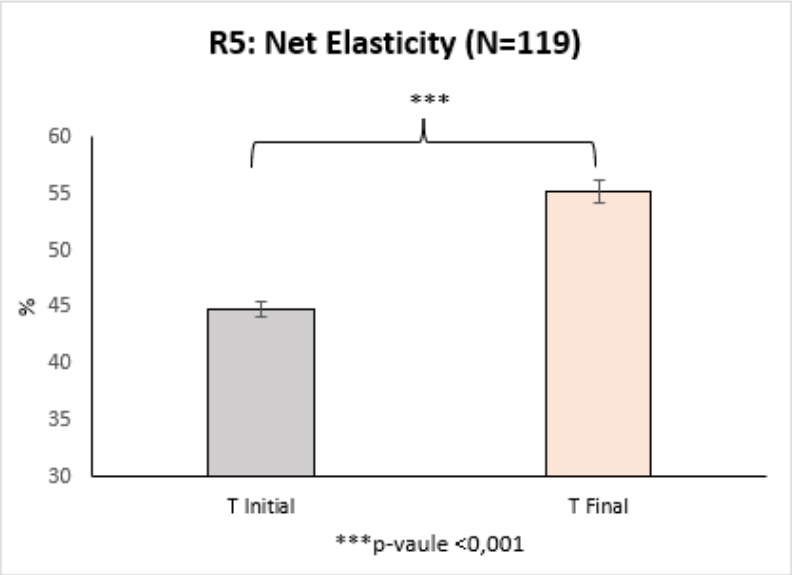

Figure 1. Parameter of study R5 (net elasticity). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

2. Firmness: R0

Firmness (R0) is defined as the skin's resistance to suction with a Cutometer®. The fewer the number of millimetres of skin that enter the probe, the firmer the tissue.
Figure 2 shows the results obtained (mean ± SEM). Firmness was found to decrease by 9.52% with respect to baseline. The average increase between the two study time points was −0.025 ± 0.008 mm and was statistically significant (p = 0.001). Only 34.45% of participants showed an increase in firmness.

Figure 2

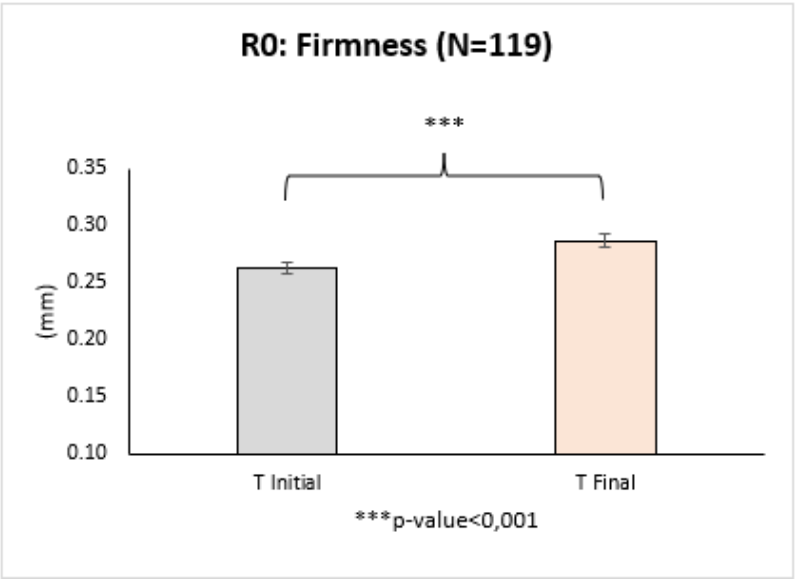

Figure 2. Parameter of study R2 (Firmness). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

Annals of Dermatological Science (ISSN 2831-8129)

3. 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 3 shows the results obtained (mean ± SEM). The recovery capacity of the skin was found to improve by 15.11% with respect to baseline. The average increase between the two study time points was 0.02 ± 0.004 mm and was statistically significant ($p = 1.13 .10^{-6}$). Overall, 68.91% of participants showed an increase in the recovery capacity of the skin in millimetres with respect to baseline.

Figure 3

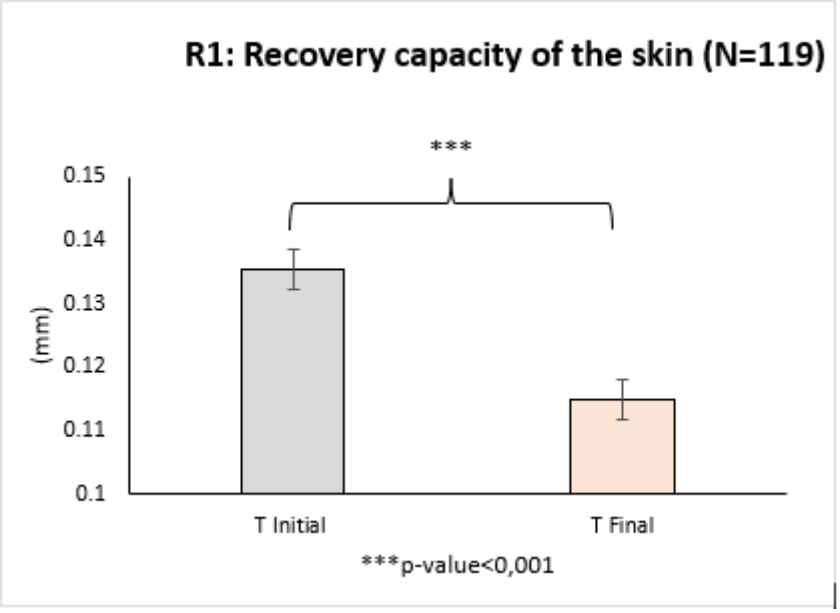

Figure 3. Parameter of study R1 (Recovery capacity). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

4. Gross elasticity: R2

Gross elasticity is defined as the skin's maximum extension on stretching and its ability to return to its original position following deformation. This deformation is induced 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 4 shows the results obtained (mean ± SEM). Gross elasticity was found to improve by 23.07% with respect to baseline. The average increase between the two study time points was 11.12 ± 1.2% and was statistically significant ($p = 1.13 .10^{-6}$), 86.55% of participants showed an increase in gross elasticity.

Annals of Dermatological Science (ISSN 2831-8129)

Figure 4

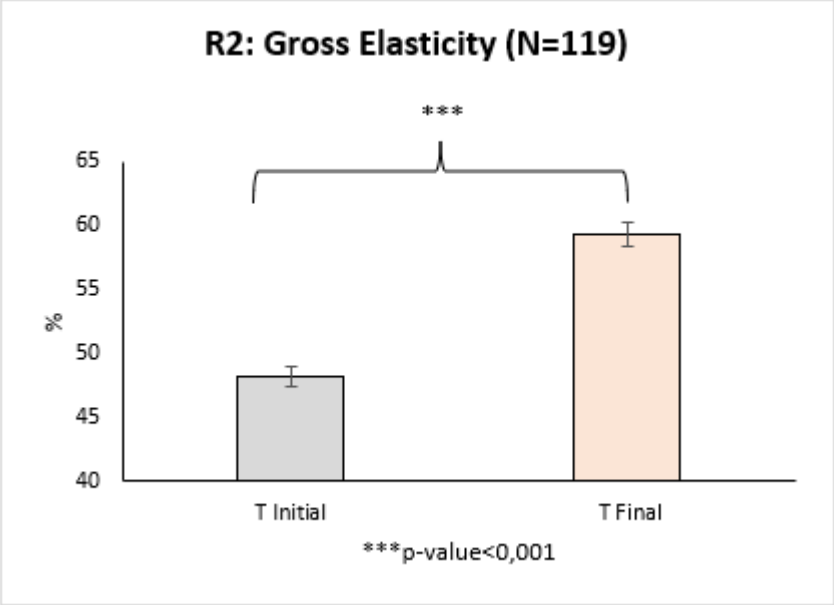

Figure 4. Parameter of study R2 (Gross elasticity). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

5. Viscoelasticity: 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 shows the results obtained (mean ± SEM). Viscoelasticity was found to improve by 8.11% with respect to baseline. The average increase between the two study time points was 4.79 ± 1.34% and was statistically significant ($p = 1.08 .10^{-18}$), 64.71% of participants showed an increase in viscoelasticity.

Figure 5

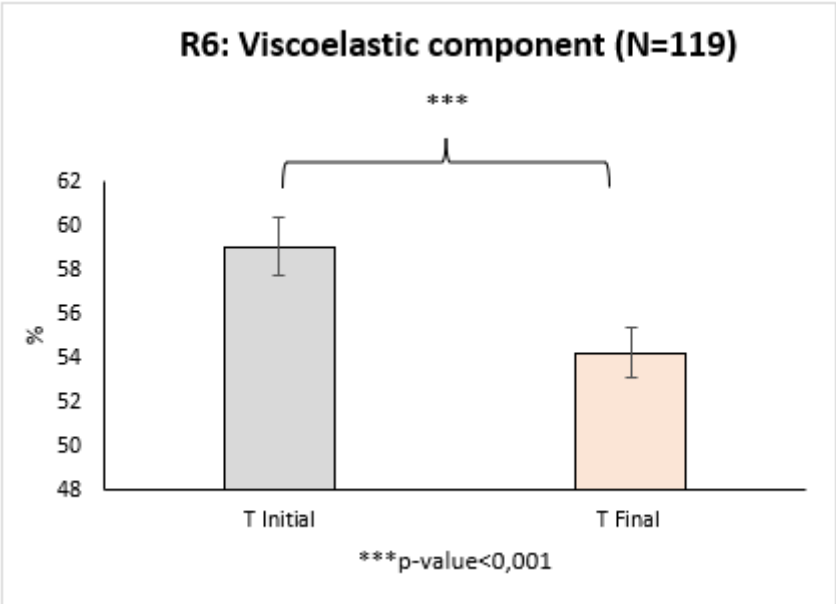

Figure 5. Parameter of study R6 (Viscoelastic component). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

6. Elastic recovery capacity: R7

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

Annals of Dermatological Science (ISSN 2831-8129)

can serve as a marker for skin elasticity.

Figure 6 shows the results obtained (mean ± SEM). The skin's capacity for elastic recovery was found to improve by 28.15% with respect to baseline. The average increase between the two study time points was 7.96 ± 0.7% and was statistically significant (p = 0.001), 89.92% of participants showed an improvement in capacity for elastic recovery.

Figure 6

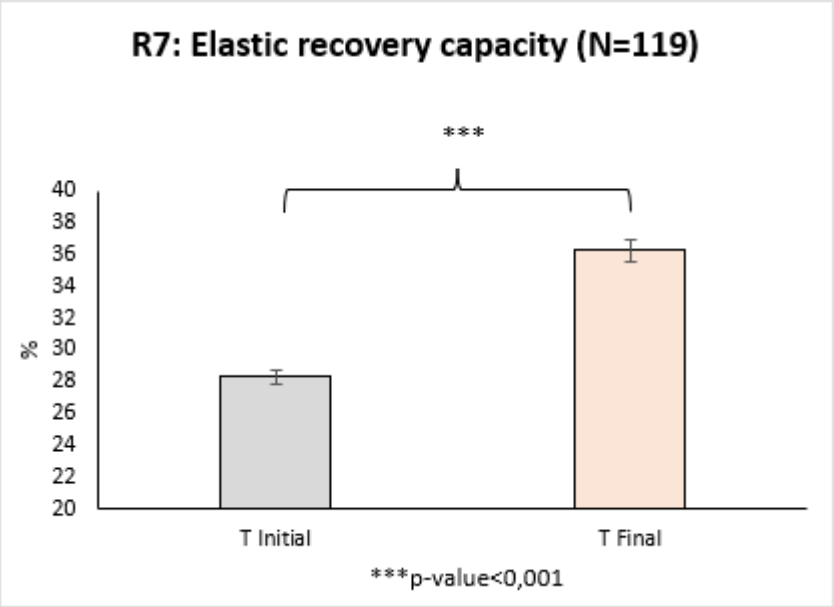

Figure 6. Parameter of study R7 (Elastic recovery capacity). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

7. 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 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). Total recovery was found to improve by 35.82% with respect to baseline. The average increase between the two study time points was 0.045 ± 0.01 mm and was statistically significant ($p = 1.65 .10^{-20}$), 78.99% of participants showed an improvement in capacity for total recovery.

Figure 7

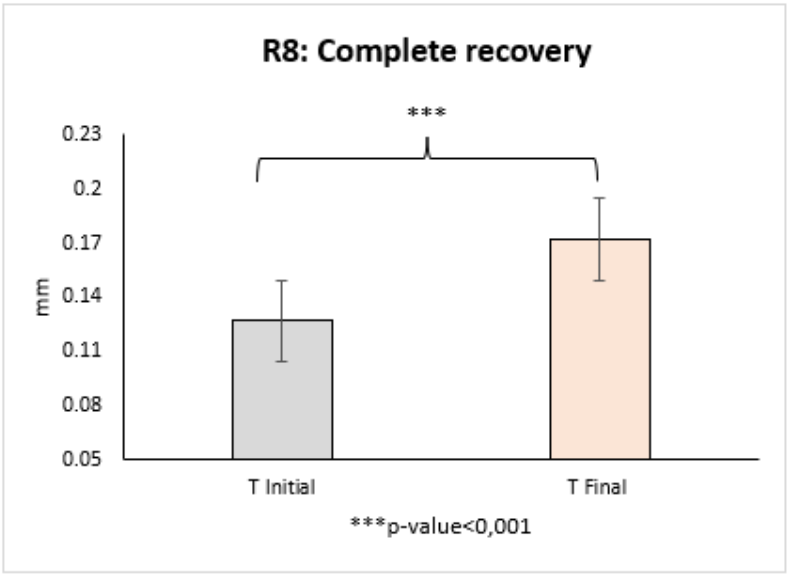

Figure 7. Parameter of study R8 (Complete recovery). The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Cutometer® probe in the preauricular area.

Annals of Dermatological Science (ISSN 2831-8129)

8. 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). Erythema was found to improve by 6.70% with respect to baseline. The average increase between the two study time points was 23.14 ± 2.86 AU and was statistically significant ($p = 1.03 .10^{-13}$), 79.83% of participants showed an improvement in erythema.

Figure 8

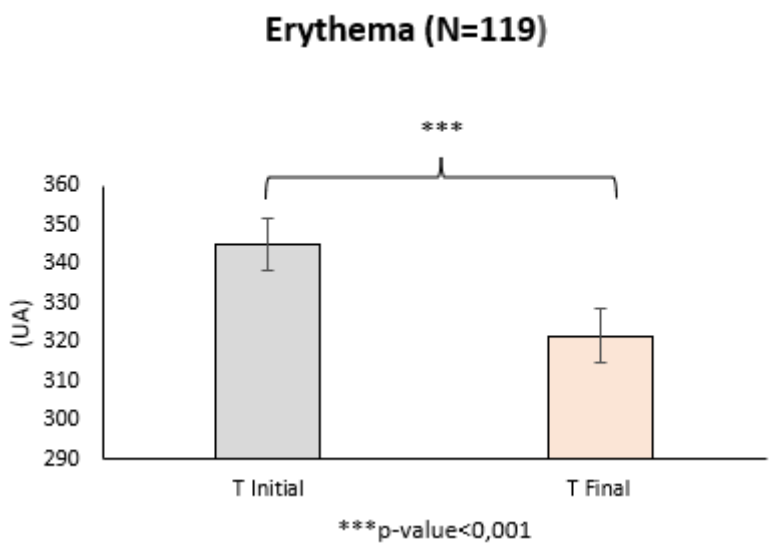

Figure 8. Parameter erythema. The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Mexameter® probe in the preauricular area.

9. Skin hydration

Figure 9 shows the results obtained (mean ± SEM). Skin hydration levels found to improve by 0.07% with respect to baseline. The average increase between the two study time points was 0.03 ± 0.93 AU and was not statistically significant (p = 0.95), 48.74% of participants showed an increase in skin hydration levels.

Figure 9

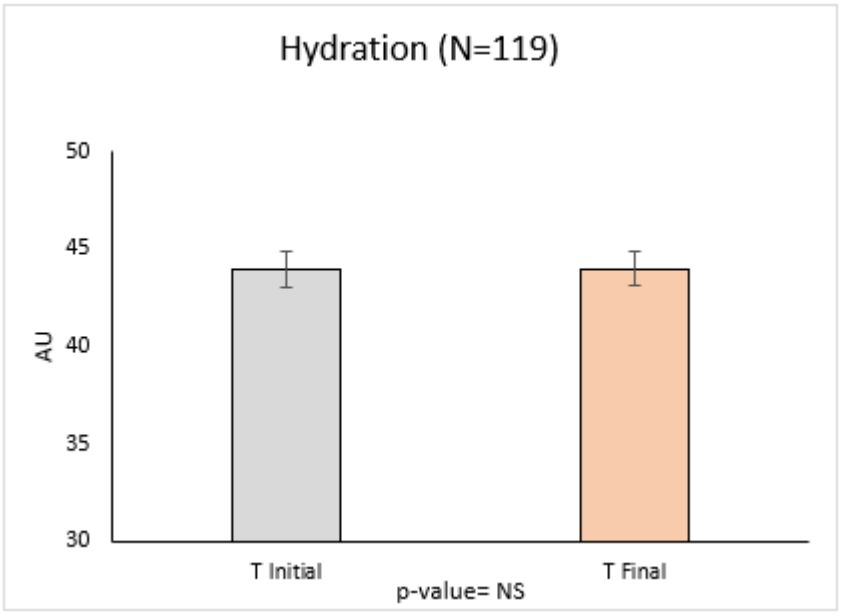

Figure 9. Parameter hydration. The results are shown on average and SEM corresponding to two temporal states of the patients before the study and 30 days after the third session, measured by the Corneometer® probe in the preauricular area.

Annals of Dermatological Science (ISSN 2831-8129)

10. Subjective parameters of the study (GAIS scale)

Data reported by patients based on the Subject Global Aesthetic Improvement Scale (SGAIS):

- After treatment radiance
  - 50.0% of participants reported a significant improvement
  - 38.9% of participants reported a considerable improvement
  - 10.1% of participants reported no change
  - 1.0% of participants reported worsening
- After treatment firmness
  - 42.0% of participants reported a significant improvement
  - 48.8% of participants reported a considerable improvement
  - 9.2% of participants reported no change
- After treatment hydration
  - 62.0% of participants reported a significant improvement
  - 28.6% of participants reported a considerable improvement
  - 9.4% of participants reported no change
- After treatment wrinkle reduction
  - 41.1% of participants reported a significant improvement
  - 41.1% of participants reported a considerable improvement
  - 17.8% of participants reported no change
- Overall effect of treatment
  - 47.9% of participants reported a significant improvement
  - 47.9% of participants reported a considerable improvement
  - 4.2% of participants reported no change
- After treatment social impairment
  - 83% of subjects were able to continue with their life as normal
  - 17% reported minor bruising that was difficult to cover with make-up

The subjects reported that their skin firmness, brightness, wrinkles and hydration were improved or much improved following 3 sessions of treatment with TKN HA3® (91.5%, 89.0%, 58.4% and 90.7%, respectively). Regarding the overall improvement of the skin, the subjects concluded that this was 96%.

Data reported by the investigators based on the Investigator Global Aesthetic Improvement Scale (IGAIS):

- After treatment radiance as assessed by the practitioner
  - 19.6% of participants showed a significant improvement
  - 70.6% of participants showed a considerable improvement
  - 5.4% of participants showed no improvement
  - 4.4% of participants showed worsening
- After treatment firmness as assessed by the practitioner
  - 9.3% of participants showed a significant improvement
  - 57.7% of participants showed a considerable improvement
  - 22.1% of participants showed no improvement
  - 10.9% of participants showed worsening
- After treatment hydration as assessed by the practitioner
  - 17.4% of participants showed a significant improvement
  - 72.2% of participants showed a considerable improvement
  - 5.1% of participants showed no change
  - 5.3% of participants showed worsening
- After treatment wrinkle reduction as assessed by the practitioner
  - 7.6% of participants showed a significant improvement in wrinkles
  - 44.9% of participants showed an average improvement in wrinkles
  - 35.8% of participants showed no change
  - 11.7% of participants showed worsening
- Overall post-treatment effect as assessed by the practitioner
  - 16.2% of participants showed a significant overall improvement
  - 67.5% of participants showed a considerable overall improvement
  - 8.5% of participants showed no change
  - 7.8% of participants showed worsening

Three independent investigators reported on average that their skin firmness, brightness, wrinkles and hydration were improved or much improved following 3 sessions of treatment with TKN HA3® (67.0%, 90.2%, 52.5% and 89.6%, respectively). Regarding the overall improvement of the skin, the subjects concluded that this was 83.7%.

Below are some cases of the photographic results obtained using the QuantifiCare 3D LifeViz® equipment. Figures 8, 9, 10 and 11.

Annals of Dermatological Science (ISSN 2831-8129)

Figure 8

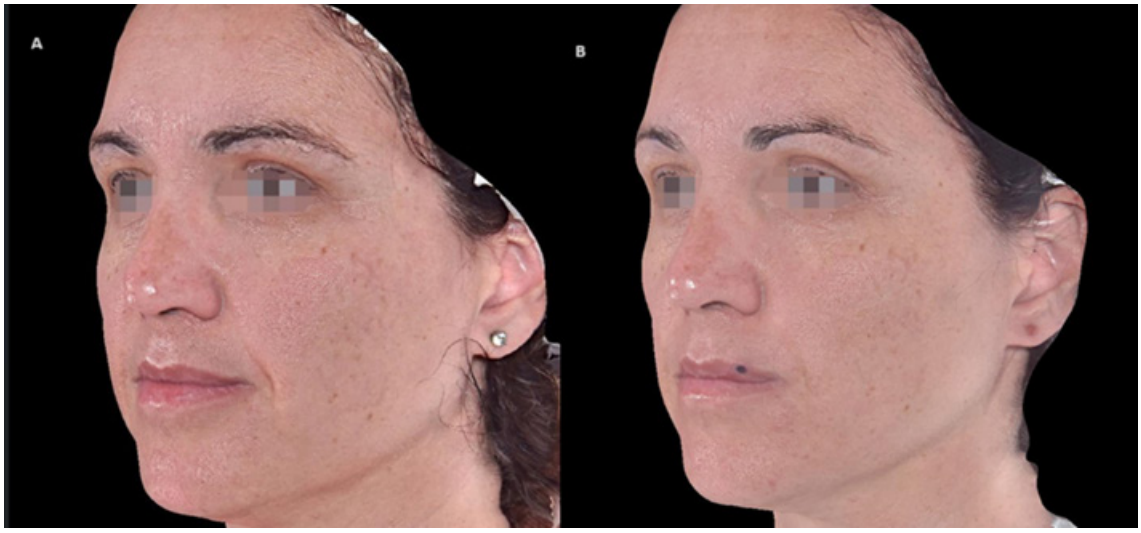

Figure 8. A: before the start of treatment. B: 30 days after the last treatment session, for a 45-year-old patient

Figure 9

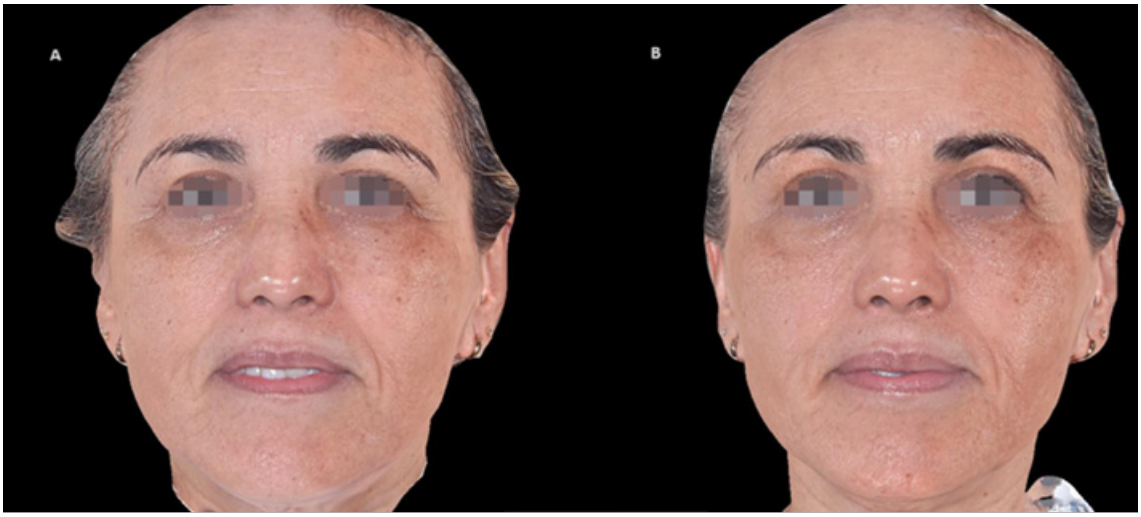

Figure 9. A: before the start of treatment. B: 30 days after the last treatment session, for a 55-year-old patient.

Figure 10

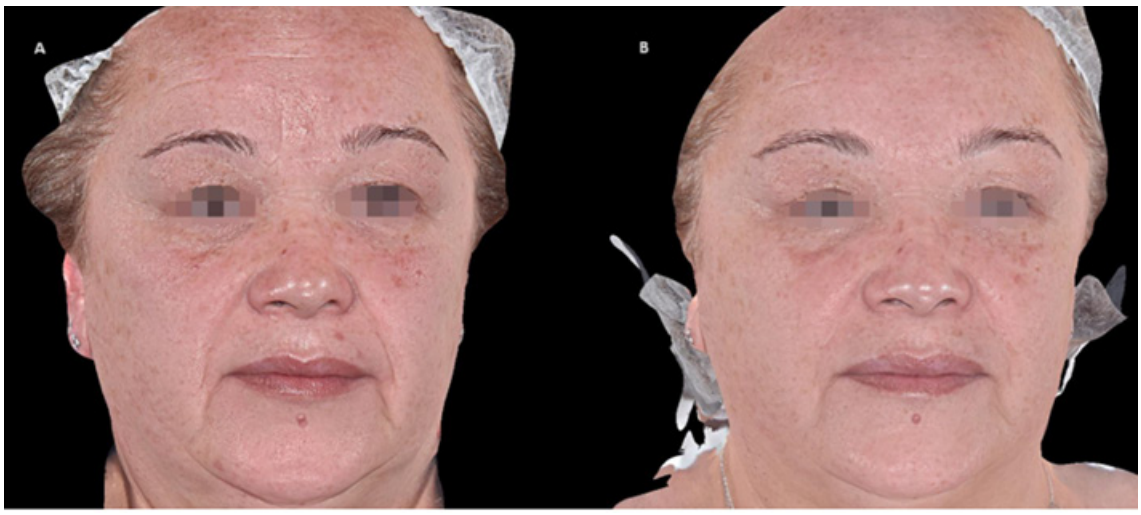

Figure 10. A: before the start of treatment. B: 30 days after the last treatment session, for a 52-year-old patient.

Annals of Dermatological Science (ISSN 2831-8129)

Figure 11

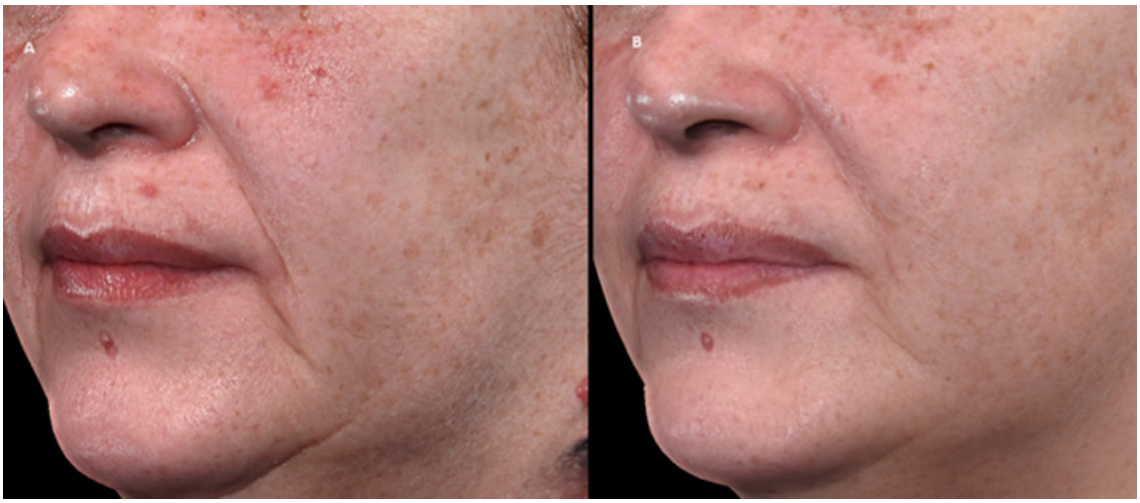

Figure 11. A: before the start of treatment. B: 30 days after the last treatment session for a close-up lateral view of a 52-year-old patient

DISCUSSION

This study was carried out to evaluate the improvement in skin elasticity in treatments performed with non-cross-linked very high molecular weight HA dermabooster (VHMWHA), specifically for HA of 3000kDa. Several efficacy and safety studies as well as literature reviews have been conducted on HA in its different indications in order to highlight the benefits of using it in its different forms (gels, creams, injectable intradermal fillers, dermal fillers, etc.). Studies concluded that the use of HA alone or in combination with other compounds showed effectiveness in improving skin firmness, elasticity and rejuvenation [24,30-31]. The results obtained showed that the use of VHMWHA improved skin elasticity in 84.03% of participants. Epidermal HA generation is influenced by the underlying dermis and is controlled by mechanisms distinct from those governing dermal HA generation [37-38]. Injecting VHMWHA into the dermis may give the impression that the epidermis is not benefiting from the treatment. The administration technique and subsequent massage of the injection site facilitate the spread of VHMWHA not only within the dermis but also to the epidermis [30]. This is confirmed by the improvements observed in terms of skin firmness and hydration, and bearing in mind that there were only 4 injection sites per hemiface, these findings are consistent with the technique and the amount of product delivered.

Age-related changes that occur in the dermis due to decreased HA synthesis. This is accompanied by gradual intertwining and stiffening of collagen fibres and loss of collagen production capacity [37]. When these changes manifest in the dermis, they contribute to dehydration, atrophy and loss of elasticity, which characterize aging skin. Several authors have reported that the higher the molecular weight of HA beyond 1000 kDa, the greater its water retention capacity [39] and therefore its ability to improve the viscoelastic characteristics of the skin. Based on the results obtained in this study for different parameters related to skin elasticity and viscoelasticity, treatment with VHMWHA generally improved the condition of the skin.

Several authors have reported that VHMWHA inhibits the inflammatory immune response and macrophage phagocytosis [29-33]. 79.83% of participants showed a decrease in erythema after injection of VHMWHA.

Mesotherapy causes tissue damage; this induces local proliferation of fibroblasts which orchestrate the repair process [42-43]. Fibrocytes produce cytokines, collagens, angiogenic and fibrogenic growth factors and matrix metalloproteinases that contribute to tissue repair after injury, so the action of the needle itself causes mechanical biostimulation [44-45]. This stimulation is further promoted and enhanced by the biological mechanisms triggered by VHMWHA; in this sense, it is known that the HA-CD44 interaction promotes the adhesion and motility of fibroblasts, facilitating the repair and remodelling of damaged tissues [46].

The present study did not measure the duration of the results. Therefore, further studies with longer follow-up periods are needed to determine its durability and the need and frequency to repeat treatment.

The sample size of the study was 119 participants and we consider the findings to be scientifically relevant and confirm the efficacy and safety of VHMWHA injection.

Although current evidence does not allow us to confirm the superiority of non-cross-linked VHMWHA over other intradermal

injectables based on non-cross-linked HA or other facial rejuvenation techniques, this study confirms that the proposed treatment and the 4-point technique used are effective, in treatments for improving skin elasticity.
These findings have positioned VHMWHA injection as an exceptionally effective material and therefore justify its use as a major component of biostimulator products.

CONCLUSIONS

Dermal injection of non-cross-linked VHMWHA (3000kDa) is a promising technique that has been shown to help counteract the aging process and improve skin elasticity.
This is an effective, safe and minimally invasive treatment option to improve skin elasticity and prevent and treat skin aging in a natural way by nourishing the skin with an HA of similar molecular weight to that mainly produced by the skin.
More studies are needed to establish whether the results obtained are attributable to the action of VHMWHA and/or the technique used, and to compare the results with other existing and marketed HA injection products, with longer follow-up periods.

Acknowledgements
The authors would like to thank ToskaniMED® (Barcelona, Spain) for providing the materials used in this study.

REFERENCES

1. Berneburg M, Trelles M, Friguet B, et al. (2008). How best to halt and/or revert UV-induced skin ageing: strategies, facts and fiction. Exp Dermatol. 17:228–40.

2. Klein J, Permana PA, Owecki M, Chaldakov GN, Böhm M, Hausman G, et al. What are subcutaneous adipocytes really good for? Exp Dermatol. 2007 Jan; 16(1): 45-70.

3. Klar AS, Zimoch J, Biedermann T. Skin Tissue engineering: Application of Adipose-Derived Stem Cells. Biomed Res Int. 2017; 2017:9747010.

4. Zhang LJ, Chen SX, Guerrero-Juarez CF, Li F, Tong Y, Liang Y, Liggins M, Chen X, Chen H, Li M, Haya T, Zheng Y, Plikus MV, Gallo RL. Age-related loss of innate immune antimicrobial function of dermal fat is mediated by transforming growth factor beta. Immunity. 2019 Jan 15; 50(1):121-136.e5.

5. Anderegg U, Simon JC, Averbeck M. More than just a filler – the role of hyaluronan for skin homeostasis. Exp Dermatol. 2014 May;23(5):295-303.

6. Quan T, Wang F, Shao Y, Rittié L, Xia W, Orringer IS, Voorhees JJ, Fisher GJ. Enhancing structural support of the dermal microenvironment activates fibroblasts, endothelial cells, and keratinocytes in aged human skin in vivo. J Invest Dermatol. 2013 Mar; 133(3):658-667.

7. Marion Salzer, Atefeh Lafzi, Antoni Berenguer-Llergo, Catrin Youssif, Andrés Castellanos, Guiomar Solanas, Francisca Oliveira Peixoto, Camille Stephan-Otto Attolini, Neus Prats, Mònica Aguilera, Juan Martín-Caballero, Holger Heyn and Salvador Aznar Benitah. Identity Noise and Adipogenic Traits Characterize Dermal Fibroblast Aging. Cell (2018): doi: 10.1016/j.cell.2018.10.012

8. Salles AG, Remigio do Nascimento AF, Liguori Zacchi VB, Saito OC, Castro Ferreira M. Avaliação clínica e da espessura cutâneaum ano após preenchimento de ácido hialurônico. 2011. Rev Bras Cir Plást (Impr). 26(1):66–9

9. R.D. Price, M.G. Berry, H.A. Navsaria. (2007) Hyaluronic acid: the scientific and clinical evidence. J. Plast. Reconstr. Aesthet. Surg. 60; 1110–1119

10. M.A. Keen, Hyaluronic acid in dermatology, Skinmed 15 (6) (2017) 441–448

11. M. Witting, A. Boreham, R. Brodwolf, K. Vávrová, U. Alexiev, W. Friess, S. Hedtrich, Interactions of hyaluronic acid with the skin and implications for the dermal delivery of biomacromolecules, Mol. Pharm. 12 (5) (2015) 1391–1401

12. L. Hong, M. Shen, J. Fang, Y. Wang, Z. Bao, S. Bu, Y. Zhu, Hyaluronic acid (HA)- based hydrogels for full-thickness wound repairing and skin regeneration, J. Mater. Sci. Mater. Med. 29 (9) (2018) 150.

13. Laurent TC, Fraser JRE. (1992) Hyaluronan. FASEB J. 6:2397-404.

14. Itano N, Kimata K. (2002) Mammalian hyaluronan synthases IUBMB Life.54:195-9

15. Kuo J-W (2005) Practical Aspects of Hyaluronan Based Medical Products. Boston: Taylor and Francis. pp 1–209.

16. Stern R, Jedrzejas MJ. (2006) Hyaluronidases: their genomics, structures, and mechanisms of action. Chem Rev.106:818-39.

17. Vigetti D, Karousou E, Viola M, Deleonibus S, De Luca G,Passi A. (2014) Hyaluronan: biosynthesis and signaling. Biochim Biophys Acta. 1840:2452-9.

18. Stern R, Asari AA, Sugahara KN. (2006) Hyaluronan fragments: an information-rich system. Eur J Cell Biol. 2006; 85:699-715.

19. Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic acid in the third millennium. Polymers. 2018;10(7):701. DOI: 10.3390/polym10070701.

20. Snetkov P, Zakharova K, Morozkina S, Olekhnovich R, Uspenskaya M. Hyaluronic acid: The influence of molecular weight on structural, physical, physico-chemical, and degradable properties of biopolymer. Polymers. 2020;12(8):1800.

21. Michaud T. Rheology of hyaluronic acid and dynamic facial rejuvenation: Topographical specificities. J Cosmet Dermatol. 2018;17(5):736-43. DOI: 10.1111/jocd.12774.

22. Erazo PJ, Carvalho AC de, Alexander T, Ramos M, Vianna P. Relleno facial con ácido hialurónico: Técnica de pilares y malla de sustentación. Principios básicos para obtener una remodelación facial. Cir Plást Ibero-Latinoam. 2009;35(3):181–94.

23. Bonté F, Girard D, Archambault JC, Desmoulière A. Skin changes during ageing. En: Harris J KV, editor. Biochemistry and cell biology of ageing: Part II Clinical science. Singapur: Springer; 2019. p. 249-80.

24. Bukhari SNA, Roswandi NL, Waqas M, Habib H, Hussain F, Khan S, Sohail M, Ramli NA, Thu HE, Hussain Z. Hyaluronic acid, a promising skin rejuvenating biomedicine: A review of recent updates and pre-clinical and clinical investigations on cosmetic and nutricosmetic effects. Int J Biol Macromol. 2018 Dec;120(Pt B):1682-1695.

25. Palmieri A, Avantaggiato A, Cura F, Casale M, Lopez MA, Bressi F, Scapoli L. Biorevitalization: an in vitro study on gingival fibroblast. J Biol Regul Homeost Agents. 2017 Dec 27; 31(4 Suppl 2):147-153.

26. Palmieri A, Avantaggiato A, Cura F, Papalia R, Casale M, Bressi F, Scapoli L. Effect of biostimulation on oral fibroblast: a pilot study. J Biol Regul Homeost Agents. 2017 Dec 27; 31(4 Suppl 2):139-145.

27. Fitzgerald R, Vleggaar D. Facial volume restoration of the aging face with poly-l-lactic acid. Dermatol Ther. 2011 Jan-Feb; 24(1):2-27.

28. Hu L, Nomura S, Sato Y, Takagi K, Ishii T, Honma Y, Watanabe K, Mizukami Y, Muto J. Anti-inflammatory effects of differential molecular weight Hyaluronic acids on UVB-induced calprotectin-mediated keratinocyte inflammation. J Dermatol Sci. 2022;107(1):24-31.

29. Muto J, Sayama K, Gallo RL, Kimata K. Emerging evidence for the essential role of hyaluronan in cutaneous biology. J Dermatol Sci. 2019;94(1):190-195.

30. Lorente, Enrique; Pérez, María Llanos. 2022. High molecular weight hyaluronic acid (HMWHA) for the treatment and prevention of skin aging. TKN HA3® for the skin aging. Aesthetic medicine. Volume 8, Nº 4, October - December 2022, pages 12-20

31. Liang J, Jiang D, Noble PW. Hyaluronan as a therapeutic target in human diseases. Adv Drug Deliv Rev 2017;97:186–203.

32. Vasvani S, Kulkarni P, Rawtani D. Hyaluronic acid: A review on its biology, aspects of drug 37 delivery, route of administrations and a special emphasis on its approved marketed products and recent clinical studies. Int J Biol Macromol. 2020;151:1012-29.

33. Kavasi R-M, Berdiaki A, Spyridaki I, Corsini E, Tsatsakis A, Tzanakakis G, et al. HA metabolism in skin homeostasis and inflammatory disease. Food Chem Toxicol. 2017;101:128-38.

34. Jang, M., Baek, S., Kang, G., Yang, H., Kim, S. and Jung, H. Dissolving microneedle with high molecular weight hyaluronic acid to improve skin wrinkles, dermal density and elasticity. Int J Cosmet Sci. 2020;42:302-309

35. Lepperdinger G, Strobl B, Kreil G. (1998) HYAL2, a human gene expressed in many cells, encodes a lysosomal hyaluronidase with a novel type of specificity. J Biol Chem. 273:22466–70.

36. Hemming R, Martin DC, Slominski E, Nagy JI, Halayko AJ, Pind S, et al. (2008) Mouse Hyal3 encodes a 45- to 56-kDa glycoprotein whose overexpression increases hyaluronidase 1 activity in cultured cells. Glycobiology. 18:280–9. doi: 10.1093/glycob/cwn006.

37. De Frutos Pachón E. Técnica de los 4 puntos, el concepto

Annals of Dermatological Science (ISSN 2831-8129)

de coste-efectividad en Medicina Estética. Medicina Estética. 2016;47(2):38 43.

38. Dobrev H. Cutometer®. In: Berardesca E, Maibach H, Wilhelm KP. Non-invasive diagnostic techniques in clinical dermatology. Berlin: Springer; 2014. pp. 315-338.

39. Holt B, Tripathi A, Morgan J. Viscoelastic response of human skin to low magnitude physiologically relevant shear. J Biomech. 2008; 41: 2689-2695.

40. Stern R, Maibach HI. (2008) Hyaluronan in skin: aspects of aging and its pharmacologic modulation. Clin Dermatol. 26:106–22.

41. Stuhlmeier KM, Pollaschek C. (2004) Differential effect of transforming growth factor beta lL(TGF-beta) on the genes encoding hyaluronan synthases and utilization of the p38 MAPK pathway in TGF-beta-induced hyaluronan synthase 1 activation. J Biol Chem. 279:8753–60.

42. Martin P. (1997) Wound healing–aiming for perfect skin regeneration. Science 276: 75–81.

43. Singer AJ, Clark RA. (1999) Cutaneous wound healing. N Engl J Med 341: 738–746.

44. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A (1994) Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1: 71–81.

45. Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, et al. (2001) Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. FASEB J 15: 2215–2224.

46. Svee K, White J, Vaillant P, Jessurun J, Roongta U, et al. (1996) Acute lung injury fibroblast migration and invasion of a fibrin matrix is mediated by CD44. J Clin Invest 98: 1713–1727.