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Facial Tissue Biomodulation Using Non-Cross-Linked Hyaluronic Acid: Clinical and Instrumental Outcomes of the Triple R Technique

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ABSTRACT

Skin aging is a multifactorial process characterized by the degradation of the extracellular matrix, reduced elasticity, and impaired skin barrier function. Biomodulation using non-crosslinked hyaluronic acid has established itself as a regenerative strategy aimed at restoring tissue homeostasis and improving overall skin quality. To evaluate the clinical and instrumental effects of the Triple R technique—a facial biomodulation protocol based on the sequential administration of non-crosslinked hyaluronic acid with different functional profiles— A prospective, observational, single-group clinical study was conducted in adult patients with signs of facial aging. Participants underwent a biomodulation procedure using INNO-CE® BI-DENS 2.5 and INNO-CE® MOIST 1.5, applied according to a standardized, anatomically guided protocol. a facial biomodulation treatment based on the sequential administration of non-crosslinked hyaluronic acid with different functional profiles. A prospective, observational, single-group clinical study was conducted in adult patients with signs of facial aging. Participants underwent a biomodulation procedure using INNO-CE® BI-DENS 2.5 and INNO-CE® MOIST 1.5, administered according to a standardized, anatomically guided protocol. The results included measurement of the hypoechoic subepidermal band (SLEB) using high-frequency ultrasound. Statistical analysis was performed using paired t-tests, with a significance level set at $p < 0.05$. The treatment resulted in a statistically significant reduction in SLEB thickness ($p < 0.0001$), indicating an improvement in dermal structure. No serious adverse events were observed. The Triple R technique demonstrated significant improvements in key skin quality parameters, suggesting a biomodulatory effect that goes beyond simple hydration. These findings support the role of combined non-crosslinked hyaluronic acid protocols as a safe and effective strategy for facial tissue regeneration. Further controlled studies are needed to confirm these results.

Keywords

Skin aging, Biomodulation, Hyaluronic acid, SLEB, Facial rejuvenation, Extracellular matrix.

Introduction

Cutaneous aging is a multifactorial biological process characterized by progressive degradation of the extracellular matrix (ECM), decreased levels of functional collagen and elastin, alterations in the dermal microenvironment, and reduced endogenous hyaluronic acid (HA) content, which clinically manifest as loss of elasticity, skin laxity, and impairment of barrier function [1,2]. This process results from the interplay between intrinsic factors, such as cellular senescence, and extrinsic factors, including ultraviolet radiation and oxidative stress, ultimately leading to cellular dysfunction and disruption of tissue homeostasis [1].

Over the last decade, the concept of skin quality has redefined the goals of facial rejuvenation, shifting the focus from volumetric correction to the comprehensive restoration of cutaneous architecture and function [3]. In this context, biomodulation has emerged as a regenerative therapeutic strategy aimed at activating endogenous biological mechanisms, including fibroblast stimulation, ECM reorganization, and the modulation of cell signaling pathways [2,7].

Hyaluronic acid (HA), a key glycosaminoglycan of the extracellular

matrix (ECM), plays a central role in these processes due to its hygroscopic capacity, biocompatibility, and interactions with cell surface receptors [7,8]. Beyond its **structural** function, HA acts as a biological modulator of cell proliferation, angiogenesis, and collagen synthesis, thereby contributing to the restoration of the cutaneous microenvironment [7,9]. Its effects are highly dependent on molecular weight, with high–molecular weight HA being associated with anti-inflammatory properties and structural support, whereas low–molecular weight HA fractions actively participate in cell signaling and tissue regulation [7].

Advances in the understanding of facial anatomy have enabled optimization of the safety and efficacy of aesthetic procedures, underscoring the relevance of fat compartments, retaining ligaments, and the stratigraphic organization of the face as key elements for clinical practice [4,6]. In this context, appropriate selection of treatment planes is crucial to maximize outcomes and minimize complications.pubmed.ncbi.nlm.nih+4

Combined protocols that integrate different hyaluronic acid (HA) profiles have demonstrated synergistic effects on skin quality, outperforming the results achieved with single-formulation approaches [10,11]. These strategies allow simultaneous modulation of functional hydration, dermal density, and the architecture of the connective tissue.

The Triple R Technique emerges as a facial tissue biomodulation approach based on the sequential, anatomically targeted administration of non-cross-linked hyaluronic acid (HA) with distinct functional profiles, aiming to act comprehensively on cutaneous architecture. Recent clinical evidence suggests that such strategies improve parameters including elasticity, barrier function, and overall skin quality, extending beyond a purely hydrating effect [11,12] pmc.ncbi.nlm.nih+6.

Nevertheless, important limitations persist in the current literature, including protocol heterogeneity and a lack of standardization in application techniques. In this context, the present study aims to evaluate the clinical and instrumental effects of the Triple R Technique on facial tissue biomodulation through the combined application of non-cross-linked HA, with the goal of providing objective evidence to support the optimization of regenerative strategies in aesthetic medicine

Materials and Methods

A preliminary, prospective, observational clinical study with a before–after design was conducted to analyze the effects of a facial tissue biomodulation technique based on the combined administration of non-cross-linked hyaluronic acid (HA). Clinical and instrumental follow-up was performed over a period of up to three months. The study population consisted of adult patients presenting clinical signs of facial aging, consecutively recruited from an aesthetic medicine outpatient clinic. The sample comprised a total of 10 patients who voluntarily agreed to participate and signed written informed consent.pmc.ncbi.nlm.nih+3

Inclusion criteria were age ≥30 years, presence of clinical signs of facial aging (skin laxity, loss of elasticity, cutaneous dehydration), availability to complete clinical follow-up, and provision of signed informed consent. Exclusion criteria were pregnancy or lactation, active autoimmune diseases, recent aesthetic treatments that could interfere with outcome assessment, presence of biopolymers or permanent fillers, and active cutaneous infections in the treatment area.

The study was conducted in accordance with the principles of the Declaration of Helsinki. All patients were informed of the procedures, risks, and benefits, and signed informed consent for the treatment, participation in the study, and the use of images for scientific and academic purposes.

Intervention: Triple R Technique

The procedure consisted of a facial biomodulation technique, the Triple R Technique, based on the sequential administration of two non-cross-linked hyaluronic acid (HA) formulations with distinct functional profiles: INNO-CE® BI-DENS 2.5, a high-molecular-weight HA preparation oriented toward structural biomodulation, and INNO-CE® MOIST 1.5, a lower-molecular-weight HA preparation focused on functional hydration. Both products were supplied in 3-ml prefilled syringes. innoaesthetics+4

Clinical Procedure

Before treatment, antisepsis of the skin was performed, and topical or infiltrative anesthesia was administered according to patient tolerance. A standardized facial marking was carried out, dividing each hemiface into anterior and posterior areas using anatomical reference lines. The intersection points were used as cannula entry sites, as illustrated in Figure 1.

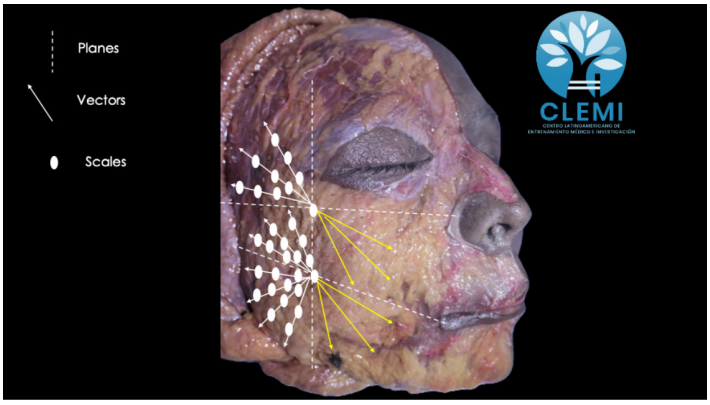

Figure 1: Superficial facial dissection illustrating the application of INNO-CE® BI-DENS 2.5, represented by vertical white scales, and INNO-CE® MOIST 1.5, depicted by yellow linear vectors in retrograde injection fashion. The dissection was performed at the Centro Latinoamericano de Entrenamiento Médico e Investigación (CLEMI), Bogotá, Colombia, in May 2026.

Application Technique

In the anterior area, INNO-CE® BI-DENS 2.5 was administered using a cannula, through micro-deposits in boluses of approximately 0.15 ml per point, directed to the dermal–hypodermal compartment. In the posterior area, INNO-CE® MOIST 1.5 was injected employing a fan-shaped retrograde technique, achieving homogeneous product distribution within the superficial subcutaneous plane. The procedure was performed with a slow injection rate, with prior aspiration when deemed necessary, and minimizing the number of entry points.

Post-Procedure Care

Standard post-procedure recommendations included local cold application for 10–15 minutes, avoidance of manipulation of the treated area for 24–48 hours, strict photoprotection, and individualized adjunctive dermocosmetic care.

Outcome Assessment – Instrumental

The primary instrumental parameter was the thickness of the subepidermal low-echogenic band (SLEB), measured by high-frequency ultrasound (≥18–22 MHz) in four predefined facial regions of interest (ROI). Imaging was interpreted as superficial soft-tissue sections of the face, corresponding to the aesthetic field of interest, using identical presets and depth settings in baseline and follow-up examinations.

Baseline ultrasounds revealed a stratified pattern: a thin superficial hypoechoic band, followed by a more heterogeneous layer compatible with subcutaneous cellular tissue, and a deeper, slightly more echogenic plane consistent with fibromuscular or fascial tissue (Figure 2).

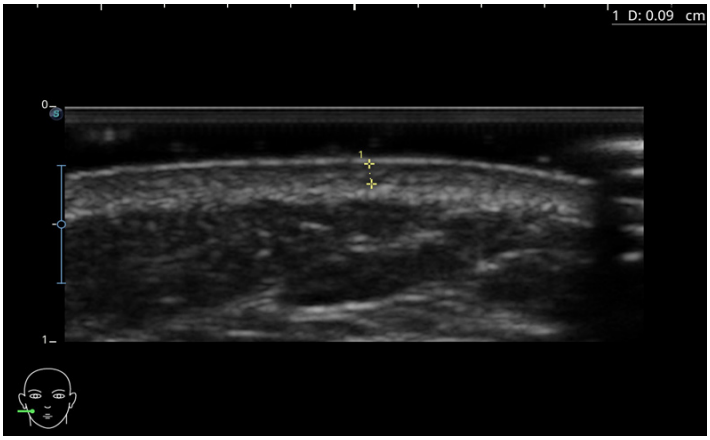

Figure 2: Baseline control ultrasound obtained before treatment in the patients included in the study, showing the subcutaneous cellular tissue and, more discretely, a fibromuscular plane; all selected patients presented similar patterns, and the subepidermal low-echogenic band (SLEB) was also evaluated.

On post-treatment ultrasound, the subcutaneous band exhibited a slightly brighter and more heterogeneous appearance, with an increased number of fine hyperechoic foci and a reduction in the previously observed homogeneous "granularity". These findings are consistent with a mild increase in matrix echogenicity and heterogeneity, potentially related to biostimulatory changes, subtle fibrous septa formation, or redistribution of microdroplets of the injected product.

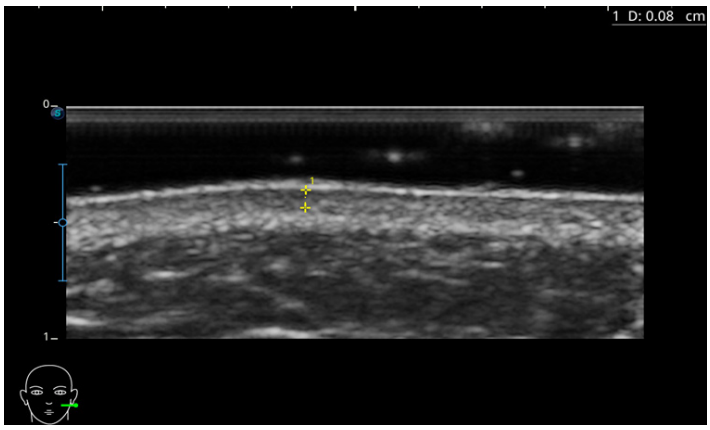

Figure 3: Post-injection ultrasound pattern following product placement. Note the changes compatible with biostimulation, characterized by a homogeneous granularity, as well as a clearly discernible moderate increase in the subepidermal low echogenic band (SLEB).

There are no clearly anechoic collections, well-defined cystic images, or frank acoustic shadows suggestive of dense calcifications in the planes evaluated, and the cutaneous interface remains linear and continuous in both scans, without marked irregularities. The main difference lies in the "amount of grey levels" within the subcutaneous tissue, which is greater in the second image.

The mean intensity increases from approximately 25 to 40 units, indicating that the tissue within the ROI is globally more echogenic/brighter in the second image. The standard deviation rises from 30 to 46, which reflects a greater dispersion of grey values and therefore a more heterogeneous texture (more visible "microstructure") in the second image. The maximum contrast of the ROI remains unchanged, suggesting that the overall range of grey levels does not vary, but their distribution does (intermediate levels are more densely populated).

Measurements were performed at three time points: baseline (T0), early post-treatment, and follow-up at 1 month (T1) and 3 months (T2). Values were expressed in millimetres as the mean of three measurements for each region.

Statistical Analysis

Data were entered into a spreadsheet and analysed using descriptive statistics (mean, standard deviation, and percentages). For comparison of pre- and post-treatment variables, a paired Student's t test was applied, with a threshold for statistical significance set at $p < 0.05$.

Results

A total of 10 patients aged 34 to 70 years were included. The

age distribution showed higher representation in the 30–39 and 60–69-year groups (30% each), followed by the 40–49 and 50–59-year groups (20% each). All patients completed the initial 1-month clinical follow-up, with partial availability of data at 3 months in some cases.

Instrumental Assessmen

Thickness of the subepidermal low echogenic band (SLEB) Analysis of the thickness of the subepidermal low echogenic band (SLEB) revealed a consistent post-treatment reduction in the evaluated regions, suggesting an improvement in overall skin quality.

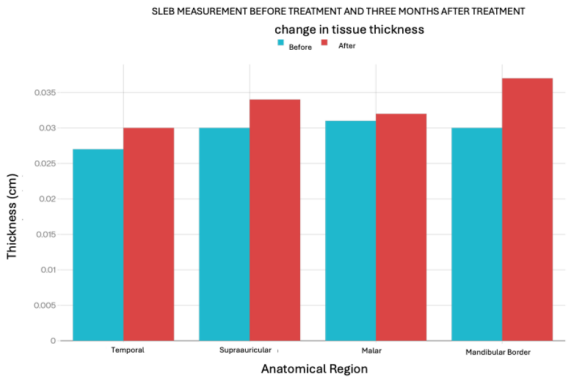

Figure 4: Graph illustrating the improvement in ultrasound-measured SLEB thickness.

Table 1: Evaluated regions with baseline and final SLEB measurements.

Region	Baseline	Final
Temporal	**0.027 cm**	**0.030 cm**
Supraauricular	**0.030 cm**	**0.034 cm**
Malar	**0.031 cm**	**0.032 cm**
Mandibular Border	**0.030 cm**	**0.037 cm**

Statistical analysis using a paired Student's t test (n = 7 patients with complete data; 28 measurements) demonstrated a statistically significant reduction in SLEB thickness after the intervention ($t = 8.94$; $p < 0.0001$).

Safety

The procedure exhibited a favorable safety profile. Adverse effects were minimal and transient, consisting of mild edema and localized erythema, both resolving spontaneously within less than 24 hours. No major complications or serious adverse events were reported during the study.

Discussion

This study shows that application of the Triple R Technique, based on the combined administration of non-cross-linked hyaluronic acid with different functional profiles, is associated with a significant improvement in key parameters of skin quality, including subepidermal low echogenic band (SLEB) thickness, cutaneous elasticity, and barrier function. These findings suggest that the observed treatment effect is not limited to simple hydration, but rather reflects a process of tissue biomodulation with structural and functional impact [7].

The significant reduction in SLEB observed in this study is a particularly relevant finding, as this ultrasound parameter has been proposed as an indirect marker of photoaging and chronic dermal inflammation [4-6]. The post-intervention decrease in its thickness suggests a reorganization of the dermal microenvironment and a possible attenuation of subclinical inflammatory processes, which is more consistent with mechanisms of tissue regeneration than with transient volumetric effects.

In line with this, the progressive increase in skin elasticity recorded throughout the protocol, with a peak after the third session followed by stabilization, supports the hypothesis of sustained fibroblast activation. This temporal pattern is coherent with previous studies showing that non-cross-linked hyaluronic acid can induce collagen synthesis and extracellular matrix remodeling via activation of signalling pathways such as TGF-β and MAPK, thereby enhancing the biomechanical properties of the tissue [7,8].

One of the most relevant aspects of this study is the use of a combined strategy integrating different molecular weights of hyaluronic acid. Current evidence indicates that high-molecular-weight HA contributes to structural stability and exerts anti-inflammatory and immunomodulatory effects, whereas lower-molecular-weight fractions are actively involved in cell signalling and interactions with fibroblasts and other cells within the dermal microenvironment. In this context, the Triple R Technique may harness these complementary actions, generating a synergistic response that translates into more consistent clinical and instrumental improvements [9,10].

Furthermore, the anatomically targeted application in subdermal and subcutaneous planes may partly account for the observed efficacy. The subcutaneous compartment and deep dermis play a key role in force transmission, extracellular matrix organization, and the functional coupling between dermis and hypodermis, contributing to contour, support, and mechanical integration of facial soft tissues. Intervention at these levels may facilitate an integrated tissue response, optimizing biomodulatory effects [11,12].

This study nonetheless has limitations that must be taken into account. The small sample size and observational design without a control group constrain the generalizability of the findings. In addition, heterogeneity in 3-month follow-up and the lack of histological biomarkers prevent definitive conclusions regarding the underlying cellular mechanisms. Future controlled studies with larger samples and long-term follow-up will be required to confirm these results and to standardize application protocols.

Conclusion

The use of the Triple R Technique, based on the combined administration of non-cross-linked hyaluronic acid, demonstrated a significant improvement in key parameters of skin quality,

including elasticity, barrier function, and dermal structure as assessed by SLEB. The findings suggest that this approach acts not merely as a hydrating agent, but as a biological modulator capable of inducing functional and structural changes in the cutaneous microenvironment. The combination of different hyaluronic acid profiles, together with anatomically targeted delivery, may generate synergistic effects that optimize tissue response in facial rejuvenation procedures. Controlled studies with larger sample sizes and extended follow-up are needed to validate these findings and to establish standardized protocols for clinical practice.

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