

An Innovative PN HPT™-based Medical Device for the Therapy of Deteriorated Periocular Skin Quality

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ABSTRACT

Objective: Polynucleotides Highly Purified Technology (PN HPT™) hydrate the extracellular matrix and fill the depressed dermal spaces in the periocular areas with skin quality problems; moreover, PN HPT™ replenish the nucleotide precursor pool. Within a long-term monitoring program, the questionnaire-based survey aimed to confirm the profile of known side effects and contraindications.

Methods: Survey base: A real-world cohort of 48 ambulatory outpatients with periocular skin quality problems after a three-session intradermal injection cycle with 15 mg of PN HPT™-based gel in disposable syringes PLINEST eye, Mastelli S.r.l., Sanremo, Italy). Study purpose: Confirming the PN HPT™ persisting efficacy and safety in improving facial and periocular skin quality and texture.

Assessment tools: Investigator-assessed Wrinkle Severity Rating Scale (WSRS) and investigator- and subject-assessed Global Aesthetic Improvement Scale (GAIS) at baseline and four weeks after the last PN HPT™ intradermal injection.

Results: The mean whole-cohort WSRS score improved from 3.3 ± 0.93 at baseline to 1.8 ± 0.90 at the final follow-up visit (-45.5% , $p < 0.0001$), including in the 39 cohort outpatients with hypotrophic skin (-46.9% , $p < 0.0001$). According to investigators, 87.5% of cohort subjects and outpatients were GAIS responders, with 50% “Much improved” or “Very much improved”. “Much improved” and “Very much improved” were the self-assessed GAIS ratings for 56.3% of surveyed subjects.

Limitations: Brief overall follow-up, lack of a control group.

Conclusion: PN HPT™ retain the usual excellent value and safety in improving skin quality in the periocular region with no variations over time and no differences in subjects with correctly structured or hypotrophic skin.

Keywords

Periocular area, PN HPT™, Polynucleotides, Real-world study, Skin quality.

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Introduction

Together with the eyes, the face region that includes the upper and lower eyelids and the brow-lid and lid-cheek complexes is crucial for social interactions and avoiding, even unconsciously, emotive misinterpretations and misunderstandings. The earliest signs of ageing also tend to appear in that area [1-3].

The ptosis of soft tissues is no longer considered the primary underlying trigger of facial ageing in the periocular areas: the loss and redistribution of volumes and bony resorption are probably more relevant determinants. Unsurprisingly, facial plastic surgeons and aesthetic medicine specialists have benefited from the wealth of volume-replacement medical devices available for cosmetic oculoplastic procedures to address age-related periorbital hollowing, skeletonization, and deep furrows in an office-based setting. Lack of downtime and few side effects are further benefits of those minimally invasive techniques [1,2].

The main caution is the need for a sound knowledge of the periocular anatomy to avoid adverse vascular effects. High-risk facial areas because of exposed large vessels include the glabella, temporalis fossa, tear trough, midface, nasolabial grooves, and nasal dorsum; caution should be maximum in the tear trough region. The thin skin in that area, closely attached to the underlying orbicularis oculi muscle with minimal subcutaneous fat, is especially prone to side effects—persistent irregular surface contours, festoons, and the bluish-hue Tyndall effect associated with a too superficial injection. Moreover, the infraorbital artery and nerve run just below the orbital rim after emerging through the infraorbital foramen about three cm lateral to the midline [1,2].

A periocular skin appearance that a dermatologist would deem as conveying a message of periocular good health needs fullness with sound brow conformation and minimal skin excess, pigmentation, and rhytidosis in agreement with the holistic concept of 360-degree skin quality [3]. Such a concept, founded on the four emergent perceptual categories of skin tone evenness, skin surface evenness, skin firmness, and skin glow, is independent of ethnicity [3].

According to the clinical presentation, one of four basic injection strategies applies. Retrograde injections in a continuous line to correct discrete rhytids are the most frequent (threading technique), followed by the crosshatching technique with overlapping horizontal and vertical lines superimposed on a threading line to

build volume [2]. Also used in periocular areas are the “fanning” technique (fan-shaped multiple injection lines) and the injection of discreet product aliquots [2]. The latter technique is helpful to correct profound deformities and avoid irregular surface contours in areas with minimal subcutaneous fat (serial puncture technique) [2]. Intradermal doses in the periocular area, preferably by the microdroplet or linear retrograde techniques, are one to two mL of the study formulation (15 mg/2 mL of Polynucleotides Highly Purified Technology or PN HPT™) like that used in the study every 14 to 21 days for a total of three to four sessions [5].

Polynucleotides Highly Purified Technology (PN HPT™) are a consolidated option in that delicate area [6-8]. The patented high-technology purification and high-temperature sterilizing technologies, performed on controlled-breeding, certified natural sources of polynucleotides, lead to a pure ingredient of highly fragmented residues between only fifty and two thousand polynucleotide base pairs without pharmacological activities, regulatorily unacceptable in medical devices, and free from allergically and biologically adversely active contaminants [6-8].

In the periocular environment, PN HPT™ rapidly promote the filling of depressed intradermal spaces while, over the longer term, contributing to a vital and optimal physiological environment, also thanks to its free radical scavenger properties [5,6,8]. The PN HPT™ action develops by the passive replenishment of the fibroblast pool of nitrogen bases and oligonucleotide precursors via the spontaneous degradation in periocular tissues of the PN HPT™ polynucleotide fragments [6,8]. The long-term outcome is supporting and promoting the dermal fibroblast viability and a sound extracellular matrix environment in the dermis of lids, tear troughs, and other hollow and atrophic periocular areas.

The PN HPT™ impact on the dermal environment is more potent than hyaluronic acid (HA) and, over the long term, complements the rapid volume-filling action that HA typically develops [6-8]. The specialists in aesthetic medicine and chronic wound and ulcer management have long exploited the PN HPT™ benefits on dermal viability [5,8-11]. The paper reports the outcomes of a survey study centred on a questionnaire distributed by the investigators, facial plastic surgeons, and dermatologists to those who spontaneously sought their help and office treatment to improve the facial and periocular skin texture and quality. To simulate a real-world situation as far as possible, the prospective enrollment involved minimal inclusion and exclusion criteria.

All investigators already used the investigated PN HPT™ periocular device according to regulatory prescriptions and expert indications, including the suggested long-term maintenance treatment—one PN HPT™ periocular syringe every one or two months or, as an alternative, two or three complete three-session intradermal treatment cycles per year [5]. The survey study was the first within a long-term monitoring program of the persisting efficacy performance, safety, and lack of emergent risks of a commercial Class III CE-mark PN HPT™-based medical device

for intradermal injections. Confirming the profile of known side effects and contraindications and identifying any unknown side effects or emergent risks was another purpose of the study and the long-term monitoring program. Resorting to real-world data independent from the tight inclusion and exclusion criteria of randomized clinical studies with their highly selected patient samples is the clue that supports the value of the investigation.

Methods

Design: a single-arm cohort of adults of both genders freely seeking specialist help to improve their facial and periocular skin texture and quality and prospectively enrolled in a real-world setting. Before the survey, all individuals had undergone a three-session treatment cycle with a Class-III CE-marked medical device containing 15 mg of polynucleotides PN HPT™ as a fluid gel in 2-mL prefilled disposable syringes with 30G½ needles for intradermal injection PLINEST eye, Mastelli S.r.l., Sanremo, Italy). All subjects underwent three facial PN HPT™ injection sessions in the periocular district at baseline (T0), 2 to 3 weeks after baseline (T1), and after 2 to 3 further weeks (T2) in the private-practice offices of investigators in agreement with regulatorily accepted procedures. The following survey was purely observational, with no active intervention, and all subjects freely agreed to answer the survey after being informed about its goals. Beyond monitoring the persisting efficacy and safety outcomes, the reasons for seeking ambulatory periocular rejuvenation procedures were also registered. Questionnaires allow information collection without time constraints for the investigator to answer questions thoroughly, faithfully, and more quickly than face-to-face interviews.

Observational Efficacy Assessments

Primary efficacy endpoint- Objective periocular skin-quality improvement based on the six-score Wrinkle Severity Rating Scale (WSRS) for periocular wrinkles (Table 1).

Table 1: Descriptors of the WSRS assessment tool for periocular wrinkles [13].

Score	Description
0	No wrinkles
1	Just perceptible wrinkles
2	Superficial wrinkles
3	Moderately deep wrinkle
4	Deep wrinkles, well-defined edges
5	Very deep wrinkles, redundant folds

The investigator attributed the semi-quantitative WSRS scores at baseline (T0), before the first PN HPT™ intradermal injection, and four weeks after T2 (end of the treatment cycle).

The WSRS is a validated scale initially developed as a reproducible wrinkle assessment tool for plastic surgeons, dermatologists, and aesthetic medicine specialists (see the Discussion section for details about WSRS as a primary efficacy endpoint assessment tool on skin texture and wrinkles). The WSRS purpose is to evaluate, objectively as far as possible within the limits of a semi-quantitative scoring scale, the skin benefits after using traditional

fillers. Applications have been in the skin quality grading of facial and periocular wrinkles, scars, and skin laxity [12].

Secondary efficacy endpoint

Improvement, assessed objectively and subjectively, of overall facial appearance based on the Global Aesthetic Improvement Scale (GAIS, Table 2), a five-score Likert scale rating global improvement in appearance, compared to pre-treatment.

Table 2: Rating categories and descriptors of the GAIS skin-quality and appearance assessment instrument.

Score	Description
1 (worse)	Appearance worse than the original condition
2 (no change)	Appearance is essentially the same as the original condition but not completely optimal for this patient.
3 (improved)	Obvious improvement in appearance from the initial condition, but a touch-up or retreatment is indicated
4 (much improved)	Marked improvement in appearance from the initial condition, but a touch-up or retreatment is indicated
5 (very much improved)	Optimal result for this patient

Compared with the face appearance assessed (investigator) or self-perceived (treated individuals) before the periocular treatment cycle, the investigators and outpatients attributed the respective GAIS scores four weeks after T2. Compared with other scales, GAIS is not limited by the lack of a mid-point, forcing raters using usual four-score scales to select a rating either above or below average, making them less sensitive.

Beyond being validated, the five-score and six-score WSRS and GAIS assessment instruments offer a further statistical benefit. Outcomes assessed on limited-score scales have unimodal and symmetric distributions; conversely, scales with a higher number of scores have highly skewed J and U-shaped distributions. Outcomes assessed on limited-score scales also have lower means and floor and ceiling effects. At the same time, regression analysis shows that assessment scales with few scores account for a significant fraction of total variance in floor and ceiling effects; moreover, scales with few scores minimize the contribution of unaccounted factors [13].

Observational Safety Assessments

Based on spontaneous reporting by cohort individuals, helped by open questions in the questionnaire, to identify known side effects, describe their presentation and severity with the help of an *impromptu* three-level scale (“mild”, “moderate”, “severe”), and identify any previously unknown adverse event or emergent risk. The investigator complemented the individual spontaneous reports by actively questioning for adverse events at the final assessment visit.

Statistics

The sample size was estimated with the G*Power statistical program version 3.14 based on the worst-case hypothesis and considering two effect sizes. The sample size calculation assumed a conservative 40% improvement in WSRS score after the PN HPT™ treatment cycle. Under this assumption, the statistical power to detect a significant (two-tailed) divergence in the evolution in

the WSRS score curves in 40 cohort individuals would have been greater than 0.91 [14].

For the primary efficacy endpoint, inferential statistics compared the mean WSRS scores before and two to four weeks after the end of the treatment cycle, using the Wilcoxon test, the non-parametric equivalent of the paired two-sample Student's t-test for within-subject variations, to assess whether the mean difference between the two sets of observations (baseline and end-of-treatment) is zero. Expression of secondary efficacy endpoint was as the per cent of treated individuals and outpatients showing an improvement of at least one aesthetic severity descriptor compared to pre-treatment both objectively (investigators) and subjectively (surveyed cohort subjects). Differences in the overall distribution of skin quality severity descriptors were assessed with the χ^2 test for proportions [15]. All statistical tests were two-sided with a 5% significance level; statistical program: StatPlus release v7 [15,16].

Results

Tables 3 and 4 illustrate the cohort demographics and individual characteristics of the 48 prospectively enrolled cohort subjects and the reasons that induced them to look for facial and periocular rejuvenation.

Table 3: Cohort demographics and individual characteristics of cohort individuals.

Mean age \pm SD (years old)	53.9 \pm 10.27
Median age (years old)	55,5
Age range (years old)	32 to 82
Women in the study cohort	38 (79.2%)
Men in the study cohort	10 (20.8%)
Smokers	16 (33.3%)
Fitzpatrick skin class 1	4 (8.3%)
Fitzpatrick skin class 2	20 (41.7%)
Fitzpatrick skin class 3	23 (47.9%)
Fitzpatrick skin class 4	1 (2.1%)

Table 4: Reasons for facial/periocular treatment.

Increasing the periocular soft tissue volumes	3 (5.3%)
Reconstructive purposes	5 (8.8%)
Hypotrophic skin	39 (68.4%)
Periocular area atrophy	10 (17.5%)

Multiple answers allowed; total answers reported: 57.

Efficacy outcomes

Figure 1 illustrates the change in the mean WSRS scores for periocular and facial wrinkles and skin quality for the whole population between baseline and the final follow-up visit two to four weeks after the last treatment session; Figure 2 shows the analogue changes in the subpopulation of 39 subjects and outpatients with hypotrophic skin at baseline. The mean WSRS score changes (-45.5% and -46.9% , respectively) were highly significant vs baseline and similar. Periocular and facial wrinkles of all severity and skin quality improved according to the investigator's assessment based on the WSRS severity descriptors. At the baseline visit, no barely noticeable periocular rhytide was apparent, while they appeared as superficial in 22%, moderately deep in 41%, deep in 19%, and very deep in 19% of the 48-strong cohort.

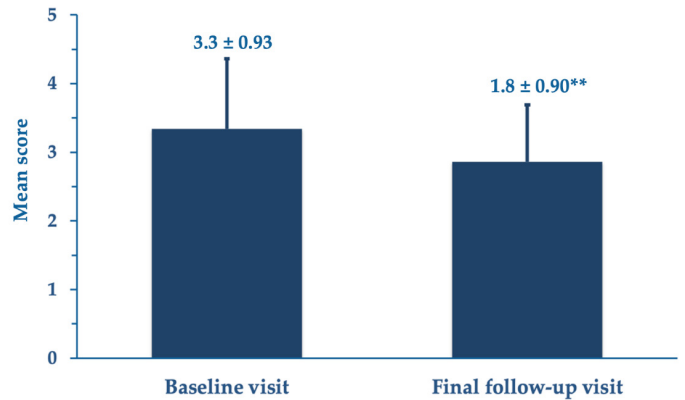


Figure 1: Comparison of the mean WSRS scores for periocular and facial wrinkles and skin quality at the baseline and the final follow-up visits for all cohort subjects and outpatients. ** $p < 0.001$ vs baseline.

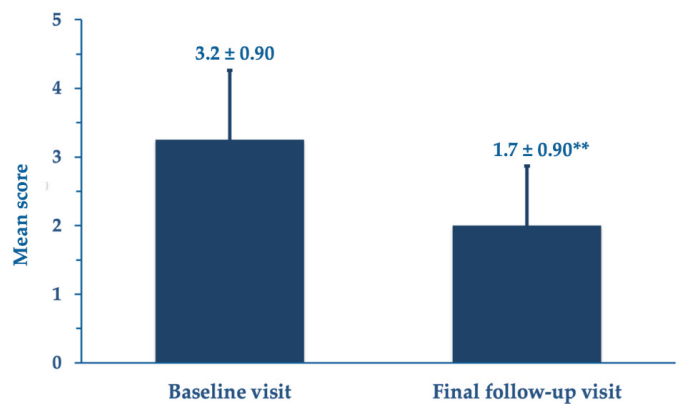


Figure 2: Comparison of the mean WSRS scores for periocular and facial wrinkles and skin quality between the baseline and the final follow-up visits for the 39 cohort outpatients with hypotrophic skin. ** $p < 0.001$ vs baseline.

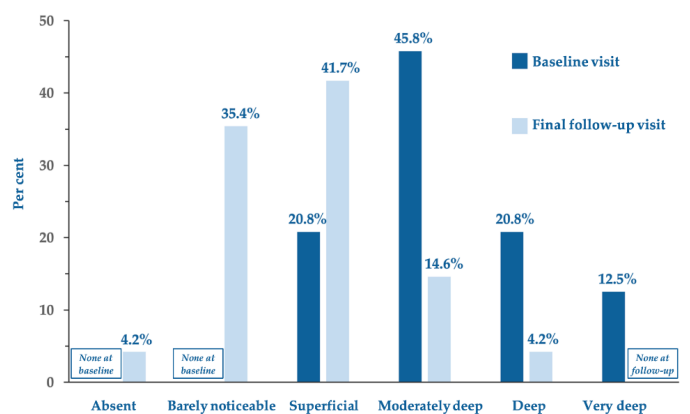


Figure 3: Comparison of the overall severity of periocular areas at the baseline and final follow-up visits (WSRS severity descriptors) in the 48 surveyed subjects and outpatients. Overall improvement in the distribution of severity: $p < 0.001$.



Figure 4: Photographic documentation of how the periocular regions (the upper two couples of photographs) and overall face (the lower couple of pictures) of three enrolled outpatients evolve between the baseline and final follow-up visits (photos on the left and the right, respectively).

Figure 3 illustrates the significant improvement in the overall distribution of aesthetic severity at the final follow-up visit in the 48 outpatients. All wrinkle categories improved with rhytides corresponding to the “Deep wrinkles” descriptor completely disappearing.

Figure 4 illustrates the evolution of three representative examples of periocular regions from enrolled subjects or outpatients between the baseline and the final follow-up visit.

The favourable evolution of the investigator-assessed GAIS scores confirmed the WSRS outcomes. At the end of the follow-up period, investigators labelled 87.5% of surveyed subjects and outpatients as GAIS responders with impressive efficacy outcomes for 50% of them—“Much improved” wrinkles and periocular skin quality in 18 of the surveyed individuals (37.5%), “Very much improved” in 6 (12.5%). The judgment by investigators was “Improved” for 18 cohort individuals (37.5%) and “No change” for 6 (12.5%). The GAIS score changes independently self-assessed mirrored the evaluations of investigators—self-perceived “Much improved”

wrinkle aesthetics in 19 of the surveyed individuals (39.6%), “Very much improved” in 8 (16.7%), “Improved” in 15 (31.3%). Regarding the subjective impressions, 87.5% of the cohort reported a clinically meaningful correction of the facial and periocular imperfections that led them to seek specialist help.

Safety outcomes

The treatment cycle with the PN HPT™ based periocular device for intradermal injections was well tolerated. A few mild local adverse effects at the injection sites were not unexpected in delicate skin areas like the periocular district and disappeared spontaneously in a few hours. Only bruising, a consequence of inadvertent and inevitable needle trauma, might have needed a few days to disappear (no more than ten). The most frequent minor, transient complications at the injection site were oedema in 25 outpatients (52.1%), erythema in 12 (25%), tumefaction/swelling in 5 (10.4%), and bruising in 17 (35.4%). No cohort individual reported pain after the injection, none required treatment, and there were no unexpected untoward events.

Discussion

Real-world studies, conceptually different from the somewhat artificial setting of randomized controlled trials, provide insights about efficacy and safety in conditions like routine clinical procedures [17]. Real-world studies are most valuable because they do not diverge from standard clinical practice and are instrumental in monitoring if the efficacy and safety of consolidated procedures and techniques persist over time [17].

In this typical real-world study, efficacy and safety procedures involved a cohort of Caucasian subjects and outpatients, predominantly women in their fifties, numerous enough to minimize the risk of a false negative outcome in the primary WSRS efficacy outcome, who sought specialist aesthetic treatment for their facial and periocular skin atrophy and wrinkles. The primary endpoint of persisting efficacy (mean WSRS score) improved by 45.5% in the overall cohort. The favorable WSRS outcome is independent of the underlying level of skin damage since periocular skin quality and trophism appeared similarly enhanced in the cohort subgroup of outpatients with hypertrophic skin.

The WSRS history led to choosing it as the ideal primary endpoint of persisting efficacy in the study. WSRS is a simple subjective assessment tool that requires no in-depth preliminary training [12]. Even more importantly, it was explicitly devised to assess skin quality and wrinkle changes after using fillers. A consolidated history of clinical use confirms that WSRS allows an accurate and reproducible grading of facial wrinkles, allowing the translation of qualitative treatment outcomes into quantitative terms trustfully and reproducibly. A further advantage is that each WSRS grade on the scale reflects a clinically meaningful change in wrinkle severity from adjacent grades. In that sense, WSRS is unique and a natural candidate as the primary endpoint assessment tool [12].

The evidence of the mean GAIS scores, the secondary endpoint of persisting efficacy, was similar at the end of the follow-up

period, with 87.5% of the cohort at least “Improved” according to investigators and the subjective self-assessment of surveyed individuals (50% “Much improved” or “Very much improved” according to investigators). The three-session cycle of intradermal injections in the delicate, thin-skin periocular region was well tolerated—no event beyond mild, rapidly transient, and expected episodes of local oedema, erythema, or bruising. In experienced hands, the PN HPT™ injections were always painless. Regarding the seemingly high incidence of local oedema and erythema, the problem appears mainly related to clinical anatomy. Due to the complex relationship of vessels, fatty tissue, and lymphatic drainage in the thin periocular skin dermis, low-level bruising and local inflammation are frequent even with a perfect intradermal injection technique and only unlikely related to the filler ingredient—for PN HPT™ even at high doses, as demonstrated by available ADME (Absorption, Distribution, Metabolism and Excretion) and toxicological documentation [18-20].

The study design three suffered from three main problems and liabilities: a too-short overall follow-up was the main one. Ten weeks at most, and often less, could not claim to investigate the long-term PN HPT™ benefits on skin texture satisfactorily. However, the idea behind the study was as a first step in a long-term monitoring program of the persisting efficacy and safety of the PN HPT™ device in helping to improve and maintain satisfactory skin quality and texture in periocular areas. An emphasis on short-term effectiveness and safety appeared adequate as a first step. In the future, the monitoring program will benefit from studies with more extended follow-up assessment periods.

The other two study limits are the lack of a control group and the risk of failing to detect a significant difference in skin quality improvement between baseline and end of study (β -risk of a falsely negative efficacy outcome). Using two validated and highly reliable assessment instruments like the WSRS and GAIS compensated for the first bias, at least partially. Moreover, the size of the surveyed cohort was more extensive than that estimated to reduce to almost zero the β -risk of a falsely negative efficacy outcome under the assumption of a mean 40% WSRS improvement. Conversely, the lack of a more extended follow-up period was not an actual bias. Replicating the study in the not-so-remote future within the medical device’s monitoring program will obviate this problem.

Conclusions

The real-world monitoring survey demonstrated that treatment with the PN HPT™-based medical device retains its usual efficacy in the periocular facial region with no variations over time and no differences in subjects and outpatients with still correctly structured and hypotrophic skin. Safety of use in the delicate periocular areas is also consistently excellent.

References

1. Ziai K, Lighthall JG. Periocular rejuvenation using hyaluronic acid fillers. *Plast Aesthet Res.* 2020; 7: 53.
2. Zein M, Tie-Shue R, Pirakitikulr N, et al. Complications after

cosmetic periocular filler: prevention and management. *Plast Aesthet Res.* 2020; 7: 44.

3. Goldie K, Kerscher M, Fabi SG, et al. Skin Quality – A holistic 360° view: consensus results. *Clin Cosmet Investig Dermatol.* 2021; 14: 643-654.
4. Mandal P, Gama F. The use of periocular fillers in aesthetic medicine. *J Plast Reconstr Aesthet Surg.* 2021; 74: 1602-1609.
5. Cavallini M, Bartoletti E, Maioli L. on behalf of The Polynucleotides HPT™ Priming Board, Collegio Italiano delle Società Scientifiche di Medicina Estetica (Italian College of the Aesthetic Medicine Scientific Societies) — SIME, AGORÀ, SIES. Consensus report on the use of PN HPT™ (polynucleotides highly purified technology) in aesthetic medicine. *J Cosmet Dermatol.* 2021; 20: 922-928.
6. Bartoletti E, Cavallini M, Maioli L, et al. Introduction to Polynucleotides Highly Purified Technology. *Aesthetic Medicine.* 2020; 6: 43-47.
7. Palmieri IP, Raichi M. Clinical commentary about foreign body complications over 20 years after polymethylmethacrylate face implants and control of late sequelae with Polynucleotides Highly Purified Technology (PN HPT™). *J Cosmet Dermatol.* 2022; 21: 5537-5542.
8. Colangelo MT, Govoni P, Belletti S, et al. Polynucleotide biogel enhances tissue repair, matrix deposition and organization. *J Biol Regul Homeost Agents.* 2021; 35: 355-362.
9. Araco A, Araco F. Preliminary prospective and randomized study of highly purified polynucleotide vs placebo in treatment of moderate to severe acne scars. *Aesthet Surg J.* 2021; 41: NP866-NP874.
10. De Caridi G, Massara M, Acri I, et al. Trophic effects of polynucleotides and hyaluronic acid in the healing of venous ulcers of the lower limbs: a clinical study. *Int Wound J.* 2014; 13: 754-758.
11. Segreto F, Carotti S, Marangi GF, et al. The use of acellular porcine dermis, hyaluronic acid and polynucleotides in the treatment of cutaneous ulcers: single-blind randomized clinical trial. *Int Wound J.* 2020; 17: 1702-1708.
12. Lemperle G, Holmes RE, Cohen SR, et al. A classification of facial wrinkles. *Plast Reconstr Surg.* 2001; 108: 1735-1750, discussion 1751–1752.
13. Garratt AM, Helgeland J, Gulbrandsen P. Five-point scales outperform 10-point scales in a randomized comparison of item scaling for the Patient Experiences Questionnaire. *J Clin Epidemiol.* 2011; 64: 200-207.
14. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods.* 2009; 41: 1149-1160.
15. <https://www.analystsoft.com/en/products/statplus/#stat-features> on May 29th, 2023.
16. Mishra P, Singh U, Pandey CM, et al. Application of Student’s t-test, analysis of variance, and covariance. *Ann Card Anaesth.* 2019; 22: 407-411.

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17. Miksad RA, Abernethy AP. Harnessing the power of real-world evidence (RWE): a checklist to ensure regulatory-grade data quality. *Clin Pharmacol Ther.* 2018; 103: 202-205.
 18. Gausas RE. Advances in applied anatomy of the eyelid and orbit. *Curr Opin Ophthalmol.* 2004; 15: 422-425.
 19. Shams PN, Ortiz-Pérez S, Joshi N. Clinical anatomy of the periocular region. *Facial Plast Surg.* 2013; 29: 255-263.
 20. AKROS BioScience Healthcare Consultants. Injectable gel medical devices toxicological assessment. Pomezia (Rome, Italy), February 9th, 2023.