Bionos

Testing Efficacy

CLINICAL ANTIWRINKLES ASSESSMENT OF "*FACE SERUM W / NE PURESTEROL RD7745*"

Bionos
Testing Efficacy

FINAL REPORT

INF.935.20.10

CLINICAL ANTIWRINKLES ASSESSMENT OF "*E@BD RDQTL V . MD OTQDRSDQNK QC6634*"

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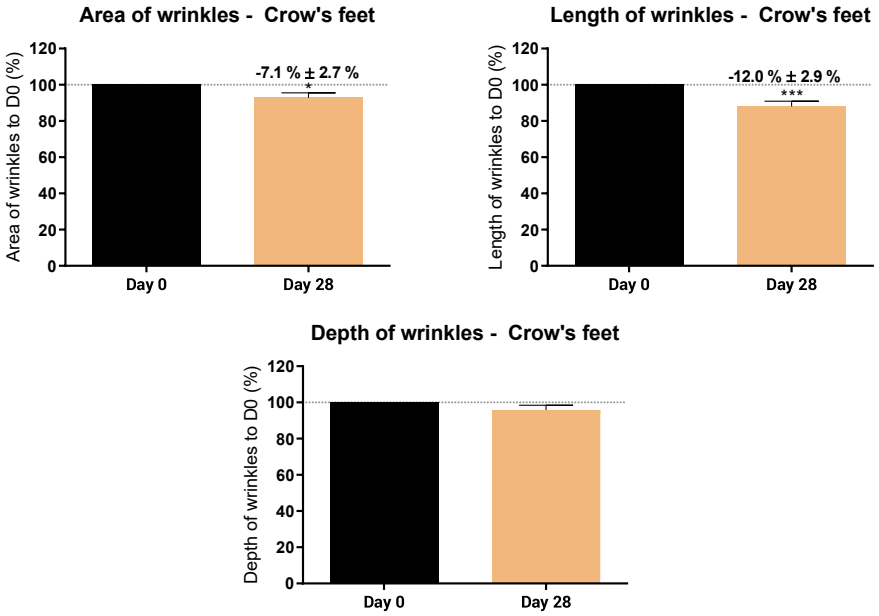
Executive Summary

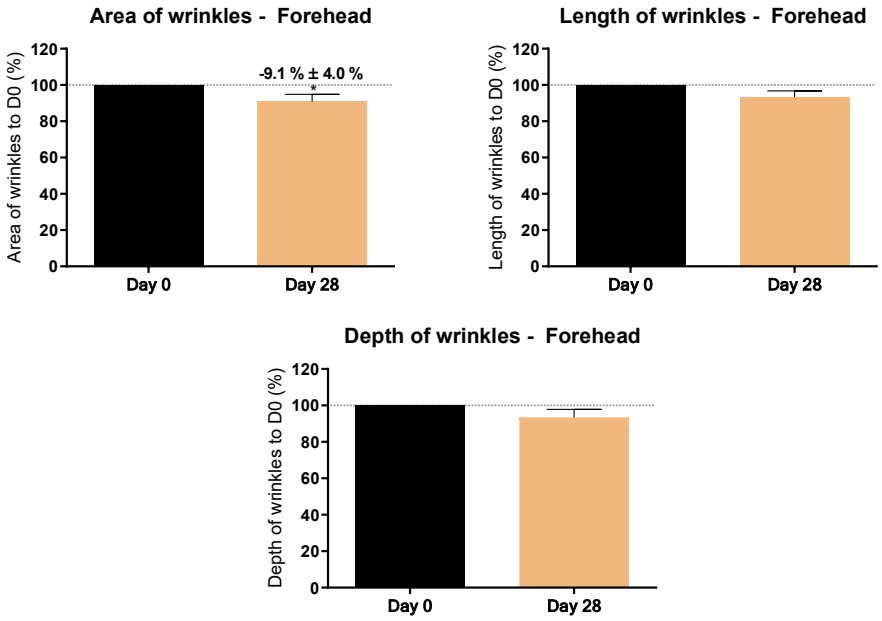
GOAL: Clinical antiaging assessment of "Face Serum w / NE Puresterol RD7745" (hereafter, "Face Serum") after a 28-day topical treatment in 21 volunteers, through analysis of forehead and crow's feet wrinkles using Bio3D Structured-light Scanner, use test through self-assessment questionnaire, and dermatological surveillance.

METHODOLOGY: 21 volunteers, aged from 30 to 65 were submitted to 28-day treatment with Face Serum on the facial area, twice per day. Images from the forehead and crow's feet areas were taken for each volunteer before (D0) and after 28 days (D28) with Bio3D Structured-light. Images were processed through specific software and 3 parameters (total area, length, and depth) were obtained for each of the areas. Furthermore, each volunteer answered a self-assessment questionnaire at the end of the treatment (D28). All data were statistically analyzed.

RESULTS: Results showed that treatment with Face Serum for 28 days significantly decreased the **area and length of crow's feet** by 7.1 ± 2.7 %, and 12.0 ± 2.9 %, respectively when compared to basal values at day 0 (D0). Similarly, the depth of crow's feet wrinkles was reduced by 4.1 ± 2.6 % when compared to basal values at day 0 (D0). However, these results were not statistically significant (Student's t-test, *p-value* > 0.05).



In addition, results showed that treatment with Face Serum for 28 days significantly decreased the **forehead wrinkles' area** by 9.1 ± 4.0 %, when compared to basal values at day 0 (D0). A similar decreasing trend was observed for both the length and depth of forehead wrinkles, with a reduction of 6.8 ± 3.5 %, and 6.6 ± 4.4 %, respectively. However, these results were not statistically significant (Student's t-test, *p-value* > 0.05).



Furthermore, these results were subjectively confirmed by the **self-assessment questionnaire**, which showed an overall acceptance average of 64% after 28 days of treatment (D28). Specifically, a positive evaluation was obtained for 4 out of 6 parameters on the product's cosmetic attributes, 1 out of 13 parameters on the product's cosmetic effectiveness, and 0 out of 4 parameters related to the consumer's opinion.

Finally, regarding skin compatibility and acceptability, none of the volunteers showed any skin acceptability problem, neither manifested any adverse symptom or cutaneous reaction during the period of treatment or the days after.

CONCLUSION: The **clinical treatment** with **"Face Serum w / NE Puresterol RD7745" displays antiwrinkle effects,** substantiated in a **significant decrease of forehead and crow's feet wrinkles (area and length), after 28 days of topical application**.

Concerning dermatological surveillance, the product **showed good skin compatibility** and may claim **"Dermatologically tested", "Clinically Tested", and "Tolerance Tested".**

1 Title

Clinical antiwrinkles assessment of "*Face Serum w / NE Puresterol RD7745*"

2 Product tested

The tested samples were received in Bionos on 23/04/2021 at room temperature, and labelled as indicated:

P.2023: Face Serum w/ NE Puresterol RD7745

The samples were stored at room temperature in our facilities and delivered to volunteers before the start of the treatment.

3 Registration dates

Study begins: 23/04/2021

Study ends: 08/06/2021

Experimental phase begins: 26/04/2021

Experimental phase ends: 25/05/2021

Dates of measurements: 1st 26/04/2021 2nd 25/05/2021

4 Platform

21 human volunteers enrolled in a 28 days treatment, self-applied twice a day (morning and evening).

5 Material and methods

5.1 Analytical equipment and software used

A structured-light 3D scanner is a 3D scanning device measuring the three-dimensional shape of an object using projected light patterns and a camera system [*Borko Furht, 2008*]. Projecting a narrow band of light onto a three-dimensional shaped surface produces a line of illumination that appears distorted from other perspectives than that of the project and can be used for geometric reconstruction of the surface shape (light section).

The evaluation of three-dimensional (3D) shapes by means of optical sensors has increasing importance in a number of applications because of the intrinsic noncontact nature of the measurement and the possibility of reducing the measurement time with respect to contact probes. Typical applications in the industrial field are production quality control, both in the micro range and in the macro range [*Docchio et al., 1999*], the digitalization of free-shape surfaces in the reverse engineering process [*El-Hakim and Pizzi, 1993*], and several 3D computer vision problems [*Poussart and Laurendeau, 1989*]. More recently, they have been successfully used in other fields, such as archeology, for measuring and preserving cultural heritage and in entertainment and 3D virtual reality frameworks [*Rioux et al., 1997*].

A number of publications exist now on the optical techniques developed for 3D measurement, both of the passive and the active nature. In passive methods, no controlled source of light is necessary. Surface reflectance, stereo disparity, and camera motion are examples of techniques based on this passive approach. However, the main drawback is represented by the high computation effort needed to get deep information [*Jarvis, 1983*]. The use of a pattern of radiation simplifies the problem of depth measurement in active methods, the use of a pattern of radiation simplifies the problem of depth measurement. Interferometric and more techniques achieve very accurate measurements over small depth ranges [*Kuwamura and Yamaguchi, 1997*], time-of-flight methods are suitable for medium and long distances, and triangulation-based methods match the short-distance interval. Within this frame, the systems based on the scanning of coherent light are widely used [*Rioux, 1984*], as well as whole-field profilometers, based on the projection of structured-light. A number of pattern projection schemes belong to this last category and differ from each other in the coding used to express the light directions. It has been developed a technique that combines

two methods for the projection and the demodulation of bidimensional patterns of light, known as the gray-code and the phase-shift methods [*Sansoni et al., 1999*]. The resulting technique, hereafter called **GCPS (Gray-code and Phase-shift)** has been integrated into a prototype for 3D vision developed to achieve a system that performs at optimal accuracy and speed over a wide typology of objects.

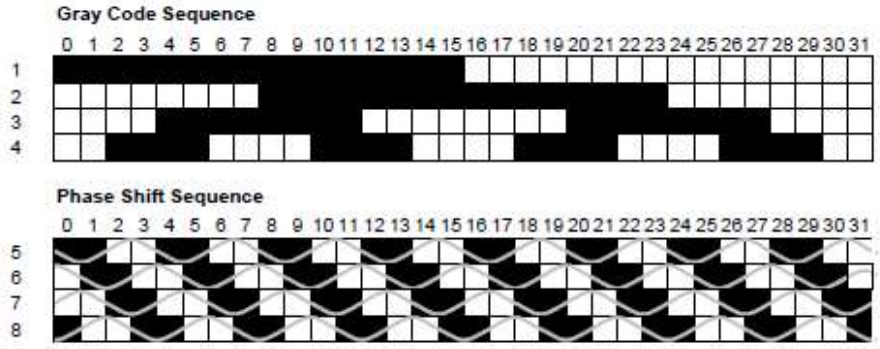


Figure 1. *Representation of Gray code and Phase shift patterns.*

The gray-code technique allows the unique description of 2 different directions of projection by means of the well-known one-distance gray code. The number of the directions of projection that can be unequivocally defined equals the number of the code words, thus, the larger this number, the wider the non-ambiguity height range. On the other hand, as each projection direction is associated with a code word, but not further decomposed, the measurement resolution is rather low [*Sansoni et al., 1997*]. With the phase-shift approach, the directions of projection are coded by phase values. Because the phase is continuously distributed within its range of non-ambiguity, a theoretically infinite height resolution can be obtained, actually limited only by the errors that are due to gray-level quantization and noise. On the other hand, the range of non-ambiguity is limited to the interval 0-2, and this fact strongly reduces the height range [*Sansoni et al., 1997*].

The combination of gray code and phase shift in GCPS has been proposed by several authors to exploit the positive features of each method and compensate for their drawbacks [*Krattenthaler et al., 1993*]. In principle, the latter is used to increase the information given by the former by adding a fractional contribution to the code words that identify each direction of projection. Consequently, the measurement performance is strongly improved as to the

resolution and the range of the measurement.

Two major methods of stripe pattern generation have been established: Laser interference and projection. The laser interference method works with two wide planar laser beam fronts. Their interference results in regular, equidistant line patterns. Different pattern sizes can be obtained by changing the angle between these beams. The method allows for the exact and easy generation of very fine patterns with unlimited depth of field. Disadvantages are high cost of implementation, difficulties providing the ideal beam geometry, and laser typical effects like speckle noise and the possible self-interference with beam parts reflected from objects. Typically, there is no means of modulating individual stripes, such as with Gray codes.

On the other hand, the projection method uses incoherent light and basically works like a video projector. Patterns are usually generated by passing light through a digital spatial light modulator, typically based on one of the three currently most widespread digital projection technologies, transmissive liquid crystal, reflective liquid crystal on silicon (LCOS) or digital light processing (DLP) modulators, which have various comparative advantages and disadvantages for this application.

A typical measuring assembly consists of one projector and at least one camera, as shown in Figure 2. For many application, two cameras on opposite sides of the projector have been established as useful.

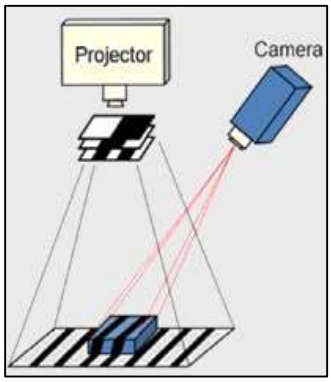


Figure 2. *Schematic representation of the Bio3D Structured-light Scanner set up.*

In Bionos Biotech, we have developed the **Bio3D Structured-light Scanner**, a 3D digitalization system based on structured-light fringe projection (gray code + phase shift

method), which works at 290 images per second, reducing the effects of movement and getting a higher resolution than current systems in the market. The 3D information obtained is analyzed with specific software, developed internally to perform different quantifications of the skin surface and profile [*Groves et al., 2014*].

The projector used is a high-performance and high-reliability device, with a very small size that uses DLP technology and a high-power white LED light source with a frame rate of 290 fps. The camera used is based on a 1.3 Mpixels CMOS sensor with GigE Interface for high-speed optical metrology applications. The purpose of using these devices is to synchronize the camera and projector to work at a frame rate of 290 fps, and in this way, be able to scan the scene in a time less than 0.15 sec. This scanning speed allows maintaining the precision of the method (> 0.1 mm) in the 3D reconstruction of *in vivo* objects.

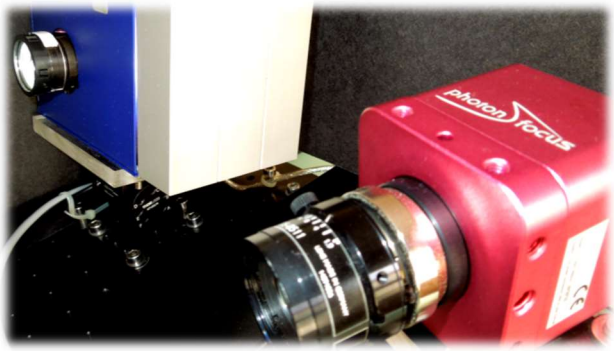


Figure 3*. Image from part of Bio3D Structured-light device used in this assay.*

2D images from the face (crow's feet areas and forehead) are processed in order to assess the effects of the treatment on the total area, length, and depth of wrinkles. State comparatives can be performed before and after a cosmetic or clinical treatment, through specific software, in order to estimate the level of improvement in each of the volunteers.

5.2 Procedure

The experimental area was defined as the forehead (glabellar lines) and crow's feet areas since these areas are the target of the specific treatment. Regarding the crow's feet area, the face side with more prevalence of wrinkles was selected and further processed. Different 2D images were taken through Bio3D Structured-light Scanner from each of the volunteers before the start of the treatment (Day 0) and after 56 days (Day 56) of Nova Fit device use (once a day).

Images were processed to generate 3D reconstructions and 3 parameters (total area, length, and depth) were obtained for each of the volunteers in each of the areas. Raw data is shown in **Attachment 3**. Unprocessed (2D) and processed (3D) images for each of the volunteers are included in a digital file.

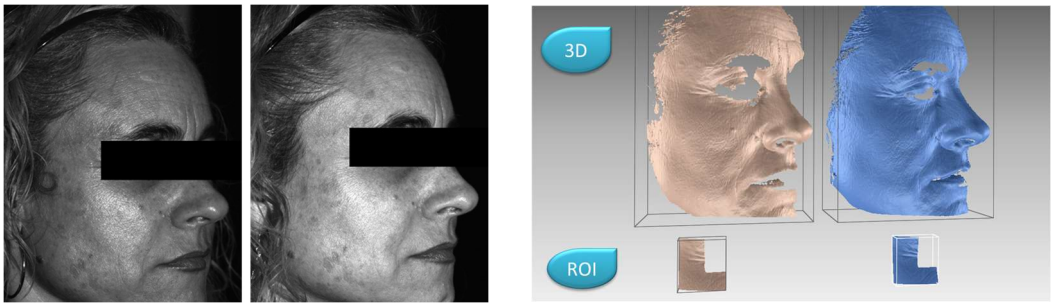


Figure 4. *2D pictures initially obtained from each of the volunteers (left) and 3D reconstructions (right) from crow's feet area obtained after image processing through specific software. ROI selected for analysis is shown below the 3D reconstructions.*

From the whole 3D reconstruction, a Region of Interest (ROI) is selected in which the wrinkles analysis is carried out. The analysis performed is a curvature analysis. The curvature of a point belonging to a surface is a measurement directly related to the geometry of the surrounding area of that determined point. The curvature is the inverse of the sphere's radius that best fits with the surrounding area of that assessed point. In Figure 5 it is shown the concept of curvature.

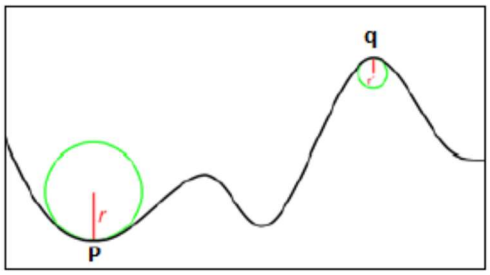


Figure 5. *Schematic representation of the curvature analysis, calculated as the inverse of the sphere's radius that best fits with the surrounding area of the assessed 3D point.*

Wrinkles correspond to the areas with minimum curvature. The total area, length, and depth are obtained from the 3D reconstructions (in mm^2 or mm). In Figure 6, it is shown the 3D area assessed (ROI), the curvature analysis (Topography), and the wrinkles detected from the analysis.

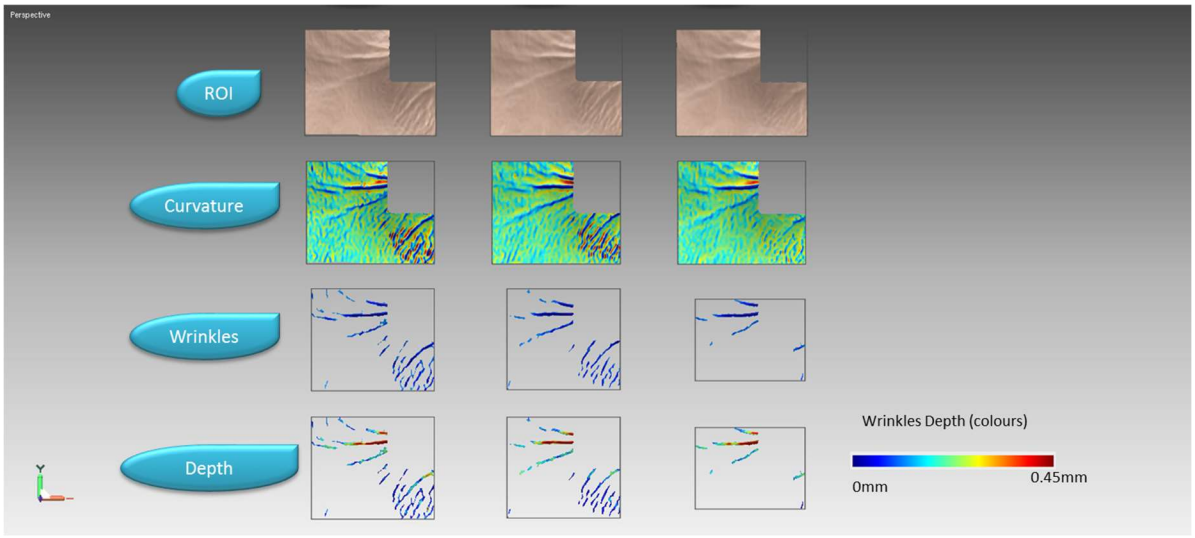


Figure 6. *Images showing 3D area selected (ROI), curvature analysis from that selected area (Topography) and the area of wrinkles detected from the analysis.*

It is important to remark that 2D images, obtained from 3D information, are shown in the document, so dimensions tend to appear smaller than reality, as shown in Figure 7.

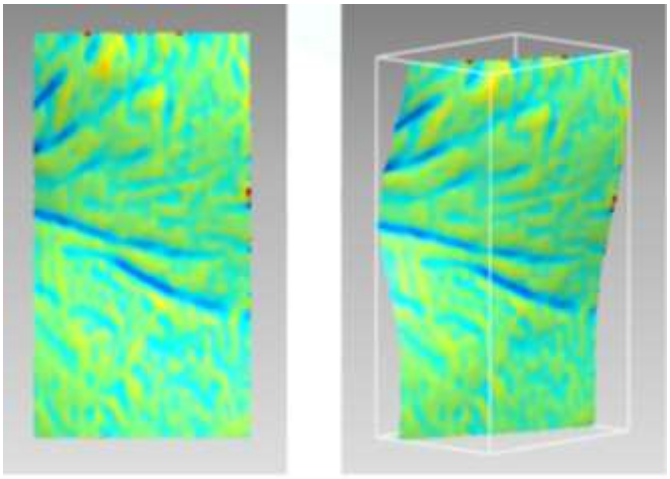


Figure 7. *Image comparison between 2D image processed and assessed (left) and real 3D information (right).*

USE CONDITIONS

The product was self-applied by the volunteers for 28 days twice a day (morning and evening). One pump of the product on a clean and dry face, avoiding contact with the eyes, following the client's guidelines.

PANEL

Panel represents the susceptible population to use the product. Inclusion criteria were:

- Presence of wrinkles on crow's feet and forehead.
- Relative frequency in the use of skin care cosmetic products.
- Gender: Female.
- Age: Between 35 and 65 years.
- Skin type: All skin types.
- No participation in any clinical assay in the previous month before the start of the study.
- Understanding and signature of Informed Consent (copy of original Informed Consent is shown in **Attachment 6**).

On the other hand, the criteria of exclusion were:

- Allergy or reactivity to some of the components of the product, or a product with a similar category than the tested one.
- Surgery at the tested area.
- Relevant cutaneous marks in the experimental areas, which could interfere with the measurements (scars, sunburns, etc.).
- In-use relevant pharmacological or hormonal treatment.
- Presence of skin diseases or melanomas.
- Forecast of change of routine or relevant way of life, during the period of study.

STUDY OBLIGATIONS

Obligations imposed to volunteers were the following:

- Do not apply other similar treatments (cosmetic or nutricosmetic) from the tested one in the experimental area (face) during the period of study.
- Do not apply aesthetic treatments (exfoliating, botox, clinical peelings, hyaluronic acid, etc.) in the experimental area from 3 months before the start of the experiment.
- To respect the use conditions of the tested treatment.
- Do not apply self-tanning products in the experimental area.
- Do not take drugs or dietetic supplements containing carotene.
- Conservation of hygiene and/or makeup habits.
- Do not receive any treatment based on Vit-A or its derivatives (in case of necessity, exclusion from the study) from 1 month before the start of the experiment.
- Do not use makeup in the experimental area during control days in the research center.
- Do not perform Turkish baths or sauna during the study.
- To avoid intense sun exposure (directly to the sun or in tanning studio), during the study period.

VOLUNTEERS

The number of volunteers according to the client's need was 20. 21 volunteers were initially recruited at the start of the treatment. The number of volunteers defined in the protocol was enough to assess the efficacy of the treatment. The volunteer's data are found in **Attachment 1**.

The self-assessment questionnaire was filled up by the 21 volunteers who completed the treatment. Raw data and additional comments from the volunteers are shown in **Attachment 4**.

INFORMED CONSENT

Informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment, and their limits of liability. The panelists signed and dated the informed consent document to indicate his authorization to proceed and acknowledge his understanding of the contents, before the start of the study. The informed consent model is shown in **Attachment 6**.

IMAGE AND PERSONALITY RIGHTS

The sponsor (Bio-Botanica) may use the pictures from all of the volunteers included in the study, for internal discussion of the results.

The sponsor (Bio-Botanica) can make commercial and marketing use of the pictures from the volunteers who gave the consent to transfer their image and personality rights (for this specific study), according to the information shown in the table of Attachment 1 (Volunteer's data).

The sponsor (Bio-Botanica) can make commercial and marketing use of the pictures from the volunteers who did NOT give the consent to transfer their image and personality rights (for this specific study), according to the information shown in the table of Attachment 1 (Volunteer's data), if they can assure the impossibility to recognize the person (e.g. using a black bar to completely cover the area of the eyes to avoid full personal recognition).

ETHICS

The study protocol is in accordance with the Scientific Committee on Consumer Safety (SCCS) guidance. It meets all international standards for research studies involving human subjects, the Good Clinical Practices (ICH-GCP), and World Medical Association. It has been conducted pursuant to the Declaration of Helsinki (1864), with the amendments of Tokyo (1975), Venice (1983), Hong Kong (1989), and Seoul (2008).

CHECKING OF THE ACCEPTABILITY

The subjects were requested to note every day any reaction observed, and sensation of discomfort felt. A skin examination of the experimental area under standard daylight source was performed by the responsible technician, the same days of the technical measurements.

Together with the clinical examinations performed during the treatment, each subject was questioned by the responsible technician about the possible sensations of discomfort he felt, at the end of the study.

CONSUMPTION CONTROL

Consumption control was carried out to verify that volunteers follow instructions and apply the treatment. Product containers were weighed before and after the treatment. Data is shown in **Attachment 2**.

5.3 Statistical analysis

Data were statistically analyzed applying **Paired Student's t-test**, since each volunteer shows values that can be assessed in pairs (before and during the treatment and before and after the treatment) [*David et al., 1997*]. By comparing the same subject's numbers before and after treatment, we are effectively using each volunteer as their control. That way, the correct rejection of the null hypothesis (no difference made by the treatment) can become much more likely, with statistical power increasing simply because the random between volunteer's variation has now been eliminated [*Walpole et al., 2002; Gross and Watkins, 1999*].

For the **self-assessment questionnaire**, opinions are given according to parameters from 0 to 3, (0 = completely disagree / 1 = disagree / 2 = agree / 3 = completely agree). For positive feedback, satisfaction is considered when volunteers scored parameters from 2 to 3. A significant percentage of volunteers is considered above 80 %.

6 Efficacy Results

6.1 Antiwrinkles results

Results showed that treatment with Face Serum for 28 days significantly decreased the **area and length of crow's feet** by 7.1 ± 2.7 %, and 12.0 ± 2.9 %, respectively when compared to basal values at day 0 (D0) (Figure 1, Table 8). Similarly, the depth of crow's feet wrinkles was reduced by 4.1 ± 2.6 % when compared to basal values at day 0 (D0). However, these results were not statistically significant (Student's t-test, *p-value* > 0.05).

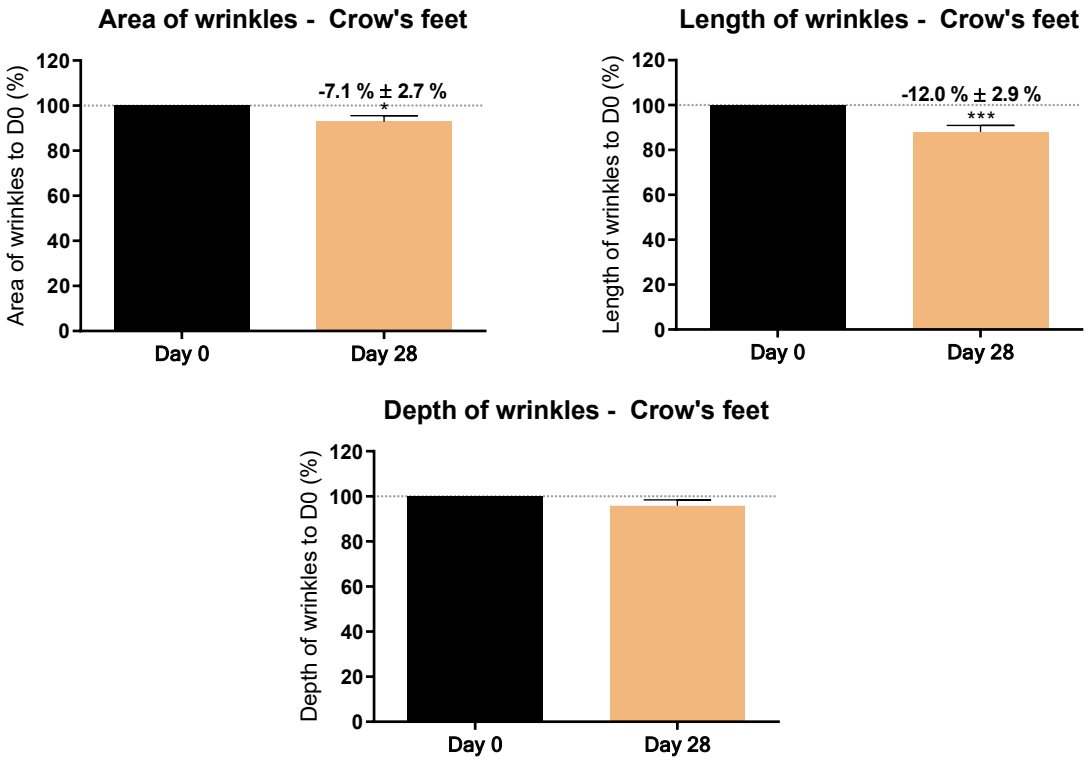


Figure 8. Crow's feet wrinkles results. *Graphical representation of the total area, length, and depth of crow's feet wrinkles, before (Day 0) and after 28 days of treatment (Day 28) with Face Serum in 21 volunteers. For all cases, values were normalized to the corresponding levels at D0 and subjected to paired Student's t-test for significance* Represents statistical significance with p-value < 0.05.*

Table Analyzed	Bio3D WRINKLES CROW's FEET AREA	Bio3D WRINKLES CROW's FEET LENGTH	Bio3D WRINKLES CROW's FEET DEPTH
Column B	Day 28	Day 28	Day 28
vs.	vs,	vs,	vs,
Column A	Day 0	Day 0	Day 0
Paired t test			
P value	0,0153	0,0005	0,1288
P value summary	*	***	ns
Significantly different (P < 0.05)?	**Yes**	**Yes**	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed
t, df	t=2,652, df=20	t=4,113, df=20	t=1,584, df=20
Number of pairs	21	21	21
How big is the difference?			
Mean of differences (B - A)	**-7,126**	**-11,97**	-4,104
SD of differences	12,31	13,33	11,87
SEM of differences	**2,687**	**2,909**	2,591
95% confidence interval	-12,73 to -1,521	-18,03 to -5,897	-9,508 to 1,300
R squared (partial eta squared)	0,2602	0,4582	0,1115

Table 1. *Statistical analysis of results shown in Figure 8.*

In addition, results showed that treatment with Face Serum for 28 days significantly decreased the **forehead wrinkles' area** by 9.1 ± 4.0 %, when compared to basal values at day 0 (D0). A similar decreasing trend was observed for both the length and depth of forehead wrinkles, with a reduction of 6.8 ± 3.5 %, and 6.6 ± 4.4 %, respectively. However, these results were not statistically significant (Student's t-test, *p-value* > 0.05).

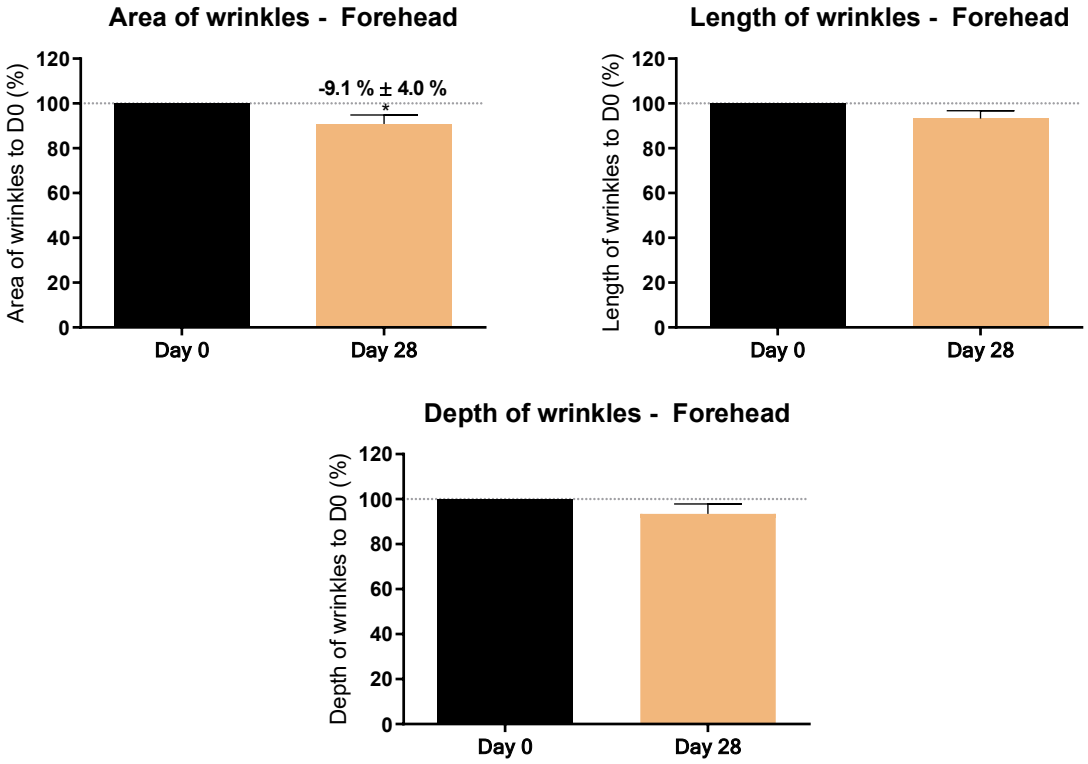


Figure 9. Forehead wrinkles results. *Graphical representation of the total area, length, and depth of forehead wrinkles, before (Day 0) and after 28 days of treatment (Day 28) with Face Serum in 21 volunteers. For all cases, values were normalized to the corresponding levels at D0 and subjected to paired Student's t-test for significance* Represents statistical significance with p-value < 0.05.*

Table Analyzed	Bio3D WRINKLES FOREHEAD AREA	Bio3D WRINKLES FOREHEAD LENGTH	Bio3D WRINKLES FOREHEAD DEPTH
Column B	Day 28	Day 28	Day 28
vs.	vs,	vs,	vs,
Column A	Day 0	Day 0	Day 0
P value	0,0325	0,0688	0,1514
P value summary	*	ns	ns
Significantly different (P < 0.05)?	**Yes**	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed
t, df	t=2,297, df=20	t=1,923, df=20	t=1,491, df=20
Number of pairs	21	21	21
Mean of differences (B - A)	**-9,134**	-6,784	-6,563
SD of differences	18,22	16,16	20,16
SEM of differences	**3,976**	3,527	4,4
95% confidence interval	-17,43 to -0,8398	-14,14 to 0,5736	-15,74 to 2,616
R squared (partial eta squared)	0,2088	0,1561	0,1001

Table 2. *Statistical analysis of results shown in Figure 9.*

6.2. Self-assessment questionnaire

The efficacy of the treatment was subjectively evaluated by a use test (self-assessment questionnaire), answered after 28 days of treatment.

For each attribute, the number and percentage of satisfied volunteers according to the punctuation are shown. For positive impressions, satisfaction was considered when volunteers scored 2 or 3. Complete results for each volunteer are shown in **Attachment 4.**

QUESTIONNAIRE AFTER 28 DAYS					
COSMETIC ATTRIBUTES	**0**	**1**	**2**	**3**	**% Satisfied**
1. The application of the product is easy	0	0	2	19	**100**
2. The texture of the product is pleasant	1	0	8	12	**95**
3. Product absorption is fast	0	0	4	17	**100**
4. The perfume of the product is pleasant	2	5	6	8	67
5. The colour of the product is nice	0	4	11	6	**81**
6. The product oils the skin	16	2	0	3	14
COSMETIC EFFECTIVENESS	**0**	**1**	**2**	**3**	**% Satisfied**
7. The contour of my face appears smoothed	1	3	11	6	**81**
8. My neck jowl has been reduced	2	8	9	2	52
9. My skin is pulped	2	10	5	4	43
10. My skin looks younger	2	6	11	2	62
11. My skin is firmer	2	5	11	3	67
12. My wrinkles appear reduced	2	6	11	2	62
13. My skin is smoother	1	5	11	4	71
14. My skin seems more radiant	2	6	10	3	62
15. My skin seems more flexible (elastic)	2	6	11	2	62
16. The treatment unifies the skin tone.	0	7	12	2	67
17. The treatment reduces the presence of dark spots	4	10	6	1	33
18. The treatment brightness my skin	5	9	7	0	33
19. The treatment improves visually the quality of my skin	0	8	10	3	62
CONSUMER OPINION	**0**	**1**	**2**	**3**	**% Satisfied**
20. I am satisfied with the treatment received	0	6	8	7	71
21. I would use the treatment again	1	6	7	7	67
22. I would recommend the treatment	1	7	6	7	62
23. I will buy the global treatment	3	7	7	4	52

Table 3*. Results were obtained for the different parameters of the cosmetic attributes, cosmetic effectiveness, and consumer opinion, after 28 days of treatment. The number of volunteers answering each of the options is shown. Bold writing represents values with ≥ 80 % acceptance (N ≥ 17*

volunteers).

A significant percentage of volunteers consider that:

- The application of the product is easy
- The texture of the product is pleasant
- Product absorption is fast
- The color of the product is nice
- The contour of their faces appears smoothed

No significant results (≥ 80 %) were obtained for the rest of the assessed parameters.

The results obtained from the self-assessment questionnaire are correlated with the wrinkles assessment (6.1), showing an overall acceptance average of 64 % after 28 days (D28) of treatment. Specifically, a positive evaluation was obtained for 4 out of 6 parameters on the product's cosmetic attributes, 1 out of 13 parameters on the product's cosmetic effectiveness, and 0 out of 4 parameters related to the consumer's opinion.

7 Discussion and conclusions

In this assay, we evaluated the antiwrinkles effect of the topical treatment with "**Face Serum w / NE Puresterol RD7745**" in 21 human volunteers during 28 days, through analysis of forehead and crow's feet wrinkles using Bio3D Structured-light Scanner, use test through self-assessment questionnaire, and dermatological surveillance.

To this end, 21 volunteers, aged from 30 to 65 were submitted to 28-day treatment with Face Serum on the facial area, twice per day. Images from the forehead and crow's feet areas were taken for each volunteer before (D0) and after 28 days (D28) with Bio3D Structured-light. Images were processed through specific software and 3 parameters (total area, length, and depth) were obtained for each of the areas. Furthermore, each volunteer answered a self-assessment questionnaire at the end of the treatment (D28). All data were statistically analyzed.

Results showed that treatment with Face Serum for 28 days significantly decreased the **area and length of crow's feet** by 7.1 ± 2.7 %, and 12.0 ± 2.9 %, respectively when compared to basal values at day 0 (D0). Similarly, the depth of crow's feet wrinkles was reduced by 4.1 ± 2.6 % when compared to basal values at day 0 (D0). However, these results were not statistically significant (Student's t-test, *p-value* > 0.05).

In addition, results showed that treatment with Face Serum for 28 days significantly decreased the **forehead wrinkles' area** by 9.1 ± 4.0 %, when compared to basal values at day 0 (D0). A similar decreasing trend was observed for both the length and depth of forehead wrinkles, with a reduction of 6.8 ± 3.5 %, and 6.6 ± 4.4 %, respectively. However, these results were not statistically significant (Student's t-test, *p-value* > 0.05).

Furthermore, these results were subjectively confirmed by the **self-assessment questionnaire**, which showed an overall acceptance average of 64% after 28 days of treatment (D28). Specifically, a positive evaluation was obtained for 4 out of 6 parameters on the product's cosmetic attributes, 1 out of 13 parameters on the product's cosmetic effectiveness, and 0 out of 4 parameters related to the consumer's opinion.

Finally, regarding skin compatibility and acceptability, none of the volunteers showed any skin acceptability problem, neither manifested any adverse symptom or cutaneous reaction during the period of treatment or the days after.

In conclusion, the **clinical treatment** with **"Face Serum w / NE Puresterol RD7745" displays antiwrinkle effects,** substantiated in a **significant decrease of forehead and crow's feet wrinkles (area and length), after 28 days of topical application**.

Concerning dermatological surveillance, the product **showed good skin compatibility** and may claim **"Dermatologically tested", "Clinically Tested", and "Tolerance Tested".**

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9 Registry and Regulation

The final report, the raw data, and the assay protocol have been saved in computer format and a copy on paper. All the information provided by the Client, volunteers, and generated by Bionos Biotech will be considered as *confidential.* The information about materials, reagents, and protocols adopted by Bionos Biotech SL during the assays is confidential and will not be shared with a third party.

This study was performed under the principle of Good Clinical Practices (International Recommendations ICH Topic E6, CPMP/ICH/135/95 of May 1st, 1996, European Parliament and Council Guideline 2001/20/CE – DOCE OF May 1st, 2001).

The whole process involving this assay was performed under **Quality Management System UNE-EN-ISO 9001/2015.**

Article 20 of the EC Cosmetic Products Regulation 1223/2009 (CPR) frames the requirements for cosmetic claims. Furthermore, cosmetic claims have to comply with **EU Regulation 655/2013** that provides the Common Criteria to ensure that the information conveyed to the end-users through claims is useful, understandable, and reliable so that consumers can make informed decisions.

The third Common Criterion, 'Evidential support', states that "claims for cosmetic products, whether explicit or implicit, shall be supported by adequate and verifiable evidence regardless of the types of evidential support used to substantiate them, including where appropriate expert assessments. Evidence for claim substantiation shall take into account the state of the art practices. Where studies are being used as evidence, they shall be relevant to the product and the benefit claimed, shall follow well-designed, well-conducted methodologies (valid, reliable, and reproducible) and shall respect ethical considerations."

Experimental studies include (but are not limited to) studies *in silico*, *in vitro*, *ex-vivo*, with instrumental or biochemical methods, studies conducted on volunteers, investigator evaluations, sensory evaluations, etc. Different types of experimental studies can be used to provide data on the performance of cosmetic products. Such studies should comprise methods that are reliable and reproducible. The studies should follow a well-designed and scientifically valid methodology according to best practices. The criteria used for evaluation of product performance should be defined with accuracy and chosen in accordance with the aim of the test. The experimental aspect of studies calls for reliance on knowledge and awareness of statistical principles in the design and analysis of

the study, e.g. in terms of the number of subjects, test samples, etc. This is necessary in order to ensure that the studies achieve scientifically and statistically valid conclusions.

Studies conducted on volunteers should follow ethical principles and products tested should have been assessed as safe. Human studies should be conducted on the target population where necessary, and be defined by strict inclusion/exclusion criteria.

Products may bear claims that relate to the nature of experimental studies. Consumer expectations regarding these claims may vary depending, in particular, upon the presentation of the claim and its specific context. However, in all circumstances, consumers will expect that such claims are made only when the effects tested are favorable:

- The claim "tolerance tested" means that the product underwent tests under the supervision of a scientifically qualified professional intended to study its tolerance on a target group and that the results of those tests show that the product was well tolerated by this group.
- The claim "tested under medical supervision" indicates that the product underwent tests conducted under the supervision of a medically qualified professional, such as a medical doctor or a dentist. Depending on the presentation of the claim, it may, for (example, refer to a specific efficacy of the product or skin tolerance.
- The claim "dermatologically tested" implies that the product was tested on humans under the supervision of a dermatologist. Depending on the presentation of the claim, it may refer to a specific efficacy or tolerance of the product. Consumer self-perceptions studies are not appropriate to support such claims. The same logic would apply to a claim referring to any other medical discipline.
- The claim "clinically tested" refers to the expertise, process, or conditions under which the tests were carried out. "Clinically tested" means that the product was tested on humans under the supervision of a medically qualified professional or another scientifically qualified professional according to a clinical protocol or in a clinical setting.

The treatments tested in this **study 935.20.10** may claim:

- **"Dermatologically Tested".**
- "**Tolerance tested**".
- "**Clinically tested**".

A critical point for the validity of consumer tests is the wording of the questionnaire. The questions and proposed answers should be clear enough to be unequivocally understood by participants. The answers scale should be well balanced (e.g. same number of positive and negative answers (a nominal, ordinal or visual analogical notation scale may be used)) and not capable of influencing the answer.

Special attention should be paid to the wording of questions for which responses will be used to substantiate the claim: the claim should be directly substantiated by the results related to the relevant question without any questionable interpretation.

Data processing and the interpretation of results should be fair and should not overstep the limits of the test's significance. Data recording, transformations, and representation in tabular or graphical form should be transparent or clearly explained if complex. It should not be designed to overstate the effect(s) measured. An appropriate statistical analysis of the data should be performed.

Attachments

Attachment 1: Volunteers data

Nº Volunteer	ID Volunteer	Age	Gender	Type of skin	Transfer of image and personality rights
1	**1749**	55	Female	Combination	Yes
2	**453**	65	Female	Combination	Yes
3	**465**	57	Female	Combination	Yes
4	**638**	57	Female	Combination	Yes
5	**1322**	57	Female	Dry	Yes
6	**1542**	62	Female	Dry	Yes
7	**558**	58	Female	Combination	Yes
8	**1634**	65	Female	Combination	Yes
9	**1540**	58	Female	Dry	Yes
10	**1671**	65	Female	Dry	Yes
11	**1668**	64	Female	Combination	Yes
12	**1445**	55	Female	Dry	Yes
13	**699**	65	Female	Combination	Yes
14	**640**	60	Female	Combination	Yes
15	**1108**	62	Female	Combination	Yes
16	**593**	57	Female	Combination	Yes
17	**164**	61	Female	Dry	Yes
18	**1435**	57	Female	Combination	Yes
19	**600**	58	Female	Dry	Yes
20	**1706**	57	Female	Combination	Yes
21	**1619**	55	Female	Combination	Yes

Attachment 2: Control consumption

Nº Volunteer	BASAL WEIGHT (g)	WEIGHT AFTER 28 DAYS (g)	PRODUCT USED AFTER 28 DAYS (g)
1	139,92	129,24	10,68
2	140,03	119,65	20,38
3	141,31	123,21	18,10
4	139,36	11,36	128,00
5	138,56	128,56	10,00
6	141,11	117,69	23,42
7	139,30	99,95	39,35
8	142,01	114,28	27,73
9	141,50	102,53	38,97
10	140,25	52,31	87,94
11	141,24	38,21	103,03
12	141,02	111,83	29,19
13	141,42	122,86	18,56
14	141,46	119,46	22,00
15	139,55	109,27	30,28
16	142,39	127,95	14,44
17	137,24	120,16	17,08
18	143,04	133,44	9,60
19	142,00	118,41	23,59
20	141,06	93,92	47,14
21	140,68	115,28	25,40

Attachment 3: Raw data from face wrinkles

	WRINKLES_CROW`S FEET											
	RAW DATA						RELATIVE TO D0					
	Area (mm2)		Length (mm)		Depth (mm)		Area (%)		Length (%)		Depth (%)	
Nº Vol	D0	D28	D0	D28	D0	D28	D0	D28	D0	D28	D0	D28
1	21,51	20,72	69,03	63,50	106,48	105,91	100,00	96,33	100,00	91,99	100,00	99,46
2	56,89	57,43	117,73	116,35	430,41	439,78	100,00	100,95	100,00	98,83	100,00	102,18
3	12,37	13,46	24,21	24,59	130,27	147,51	100,00	108,81	100,00	101,57	100,00	113,23
4	23,09	20,22	37,24	27,00	355,05	326,82	100,00	87,57	100,00	72,50	100,00	92,05
5	25,67	24,72	48,81	40,52	277,03	276,95	100,00	96,30	100,00	83,02	100,00	99,97
6	19,68	17,62	29,21	24,36	264,63	258,00	100,00	89,53	100,00	83,40	100,00	97,49
7	77,45	74,72	117,1	117,20	888,89	849,55	100,00	96,48	100,00	100,09	100,00	95,57
8	50,05	43,47	95,55	77,03	426,76	409,83	100,00	86,85	100,00	80,62	100,00	96,03
9	16,55	16,50	29,89	30,61	169,59	163,14	100,00	99,70	100,00	102,41	100,00	96,20
10	35,95	30,89	84,78	63,62	300,97	280,98	100,00	85,92	100,00	75,04	100,00	93,36
11	53,56	53,73	102,93	107,69	414,71	383,93	100,00	100,32	100,00	104,62	100,00	92,58
12	37,47	38,91	73,2	68,34	316,36	349,07	100,00	103,84	100,00	93,36	100,00	110,34
13	32,47	30,16	76,45	69,33	255,91	236,54	100,00	92,89	100,00	90,69	100,00	92,43
14	32,13	24,55	70,36	52,03	310,39	239,40	100,00	76,41	100,00	73,95	100,00	77,13
15	12,86	7,42	27,98	16,86	66,89	38,47	100,00	57,70	100,00	60,26	100,00	57,51
16	17,09	13,57	31,9	23,64	213,29	190,24	100,00	79,40	100,00	74,11	100,00	89,19
17	51,83	51,73	81,87	80,03	652,48	649,94	100,00	99,81	100,00	97,75	100,00	99,61
18	20,72	16,98	52,36	36,02	130,41	137,48	100,00	81,95	100,00	68,79	100,00	105,42
19	23,26	22,73	33,56	32,46	278,13	270,59	100,00	97,72	100,00	96,72	100,00	97,29
20	25,52	28,91	54,41	57,96	219,61	242,94	100,00	113,28	100,00	106,52	100,00	110,62
21	23,62	23,29	40,96	37,88	276,69	266,04	100,00	98,60	100,00	92,48	100,00	96,15

	WRINKLES_FOREHEAD											
	RAW DATA						RELATIVE TO D0					
	Area (mm2)		Length (mm)		Depth (mm)		Area (%)		Length (%)		Depth (%)	
Nº Vol	D0	D28	D0	D28	D0	D28	D0	D28	D0	D28	D0	D28
1	151,66	125,88	334,29	312,90	604,66	529,85	100,00	83,00	100,00	93,60	100,00	87,63
2	27,02	26,90	66,64	65,02	160,51	147,41	100,00	99,56	100,00	97,57	100,00	91,84
3	9,48	8,95	26,85	30,27	34	33,07	100,00	94,41	100,00	112,74	100,00	97,26
4	100,3	104,83	247,22	251,66	377,57	383,49	100,00	104,52	100,00	101,80	100,00	101,57
5	91,37	53,64	192,9	130,69	408,79	255,03	100,00	58,71	100,00	67,75	100,00	62,39
6	71,71	70,81	163,39	172,75	356,99	340,00	100,00	98,74	100,00	105,73	100,00	95,24
7	48,23	51,04	118,47	116,96	192,37	215,13	100,00	105,83	100,00	98,73	100,00	111,83
8	51,35	50,69	130,09	136,57	271,3	286,13	100,00	98,71	100,00	104,98	100,00	105,47
9	222,91	166,01	464,75	361,40	754,53	557,56	100,00	74,47	100,00	77,76	100,00	73,90
10	128,08	65,97	246,32	144,52	649,33	309,55	100,00	51,51	100,00	58,67	100,00	47,67
11	60,69	42,21	137,94	98,27	259,1	181,45	100,00	69,55	100,00	71,24	100,00	70,03
12	97,73	99,99	233,54	238,24	347,93	361,25	100,00	102,31	100,00	102,01	100,00	103,83
13	20,64	19,25	57,26	56,87	82,67	89,44	100,00	93,27	100,00	99,32	100,00	108,19
14	90,44	69,83	218,02	169,54	377	327,19	100,00	77,21	100,00	77,76	100,00	86,79
15	20,13	23,30	50,83	59,94	65,29	77,42	100,00	115,75	100,00	117,92	100,00	118,58
16	37,17	38,21	101,69	102,13	95,84	99,95	100,00	102,80	100,00	100,43	100,00	104,29
17	64,84	67,63	153,09	163,28	235,59	258,88	100,00	104,30	100,00	106,66	100,00	109,89
18	68,87	45,35	118,44	85,46	330,43	203,42	100,00	65,85	100,00	72,15	100,00	61,56
19	18,04	17,40	57,71	49,61	59,97	55,81	100,00	96,45	100,00	85,96	100,00	93,06
20	29,54	27,63	85,57	88,17	79,51	84,93	100,00	93,53	100,00	103,04	100,00	106,82
21	57,25	67,39	110,01	111,90	244,47	303,97	100,00	117,71	100,00	101,72	100,00	124,34

Attachment 4: Self-assessment questionnaire

VOLUNTEER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
COSMETIC ATTRIBUTES																					
1. The application of the product is easy	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	2	3
2. The texture of the product is pleasant	3	3	3	3	2	2	2	3	0	3	3	3	3	2	2	3	3	2	2	2	3
3. Product absorption is fast	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	2	2	2	3
4. The perfume of the product is pleasant	3	2	2	3	1	1	2	3	0	3	3	2	3	3	0	3	2	1	2	1	1
5. The colour of the product is nice	3	2	2	2	2	2	2	3	1	3	3	2	3	3	1	1	2	2	2	1	2
6. The product oils the skin	0	0	0	0	0	0	1	0	0	3	0	0	3	0	0	3	0	0	0	1	0
COSMETIC EFFECTIVENESS																					
7. The contour of my face appears smoothed	2	0	2	2	1	2	2	2	2	3	3	1	2	3	3	3	3	2	2	1	2
8. My neck jowl has been reduced	2	0	1	2	0	2	1	1	2	3	3	1	1	2	2	1	2	1	2	2	1
9. My skin is pulped	2	0	1	2	0	1	1	1	2	3	3	2	1	3	1	1	3	1	2	1	1
10. My skin looks younger	2	0	1	2	0	2	2	1	2	2	3	2	1	2	3	1	2	1	2	1	2
11. My skin is firmer	2	0	1	2	0	2	2	1	2	2	3	2	1	3	2	1	3	1	2	2	2
12. My wrinkles appear reduced	2	0	0	2	1	2	2	1	2	3	2	2	1	3	2	1	2	1	2	2	1
13. My skin is smoother	2	0	1	2	1	2	2	1	2	3	3	2	1	3	3	2	2	1	2	2	2
14. My skin seems more radiant	2	0	1	2	0	2	1	1	2	3	2	2	1	3	3	2	2	1	2	2	1
15. My skin seems more flexible (elastic)	2	0	1	2	0	1	2	1	2	3	2	2	1	3	2	2	2	1	2	2	1
16. The treatment unifies the skin tone.	1	1	1	2	2	2	2	1	2	2	3	2	1	3	2	2	2	1	2	1	2
17. The treatment reduces the presence of dark spots	0	0	1	1	2	1	1	0	1	2	2	0	1	3	2	1	2	1	2	1	1
18. The treatment brightness my skin	0	0	2	1	1	1	2	0	2	2	2	0	1	2	1	1	1	1	2	1	0
19. The treatment improves visually the quality of my skin	2	1	1	2	1	2	2	1	2	3	3	1	2	3	2	1	2	1	2	2	1
CONSUMER OPINION																					
20. I am satisfied with the treatment received	3	1	1	2	2	2	2	1	3	3	3	1	1	3	2	3	3	2	2	1	2
21. I would use the treatment again	3	0	1	3	1	1	2	1	2	3	3	1	1	3	2	3	3	2	2	2	2
22. I would recommend the treatment	3	0	1	2	1	1	2	1	2	3	3	1	1	3	3	3	3	2	2	2	1
23. I will buy the global treatment	3	0	1	2	1	0	1	1	2	3	2	1	1	3	3	2	2	2	2	1	0

Attachment 5: Images

All images are enclosed in a digital file, together with this report, with the following folder structure: 935_25_10_Photos

Macroscopic Images (Digital Camera)

- **V_01**
  - V1_ D0_ FRONT_935.tif
  - V1_ D0_ RIGHT_935.tif
  - V1_ D0_ LEFT_935.tif
  - V1_ D28_ FRONT_935.tif
  - V1_ D28_ RIGHT_935.tif
  - V1_ D28_ LEFT_935.tif

Bio3D Structured-light Scanner

- **V_01**
  - Frontal .jpg
  - Frontal_0 .tif
  - Frontal_28 .tif
  - Lateral .jpg
  - Lateral_0 .tif
  - Lateral_28 .tif

Explanation of Figures:

- Frontal.jpg: Region of Interest (ROI) selected from the forehead area, extraction of wrinkles in the ROI (the bluer, the deeper wrinkles, the greener, the smooth area), wrinkles without background, wrinkles without background including depth analysis at each of the time points (above, D0 / below, D28).
- Frontal_0.tif: Frontal 2D picture included in the set used for the 3D reconstruction before the treatment (D0).
- Frontal_28_.tif: Frontal 2D picture included in the set used for the 3D reconstruction after 28 days of treatment.
- Lateral.jpg: Region of Interest (ROI) selected from the crow's feet area, extraction of wrinkles in the ROI (the bluer, the deeper wrinkles, the greener, the smooth area), wrinkles without background, wrinkles without background including depth analysis at each of the time points (above, D0 / below, D28).
- Lateral_0.tif: Lateral 2D picture included in the set used for the 3D reconstruction before the treatment (D0).
- Lateral_28_.tif: Lateral 2D picture included in the set used for the 3D reconstruction after 28 days of treatment.

Attachment 6: Informed Consent

INFORMACIÓN Y AUTORIZACIÓN DE PARTICIPACIÓN EN EL ENSAYO	
Código de Protocolo: Bio-Botanica ESTUDIO *IN VIVO* 935	Promotor: Bio-Botanica
Fecha de la versión: 23/04/2021	Investigador Principal: Dr Jose Luis Mullor SanJosé Dr Adela Serrano Gimeno
CENTRO: BIONOS BIOTECH S.L.	

CONSENTIMIENTO INFORMADO:

Yo, _____

- He comprendido la información que se me ha facilitado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- Comprendo que mi participación es voluntaria.

AUTORIZACIÓN DE CESIÓN DERECHOS DE IMAGEN:

Al amparo de lo dispuesto en la Ley Orgánica 1/ 1982, de 5 de Mayo, de protección civil del derecho al honor, a la intimidad personal y familiar y a la propia imagen, como parte del estudio citado y en la hipótesis de una futura explotación publicitaria de los productos testados:

El cedente autoriza la reproducción y difusión de las mencionadas imágenes, o partes de las mismas con las siguientes condiciones:

-La cesión se efectúa a título gratuito; el cedente no recibe contraprestación alguna a cambio de la cesión de sus derechos de imagen.
-El promotor únicamente podrá identificarme por mi código de voluntario y no tendrá acceso a mis datos personales
-Para marketing/ publicidad, con la única salvedad y limitación de aquellas utilizaciones o aplicaciones que puedan atentar al derecho al honor en términos previstos en la Ley orgánica 1/1982

Autorizo al Promotor del estudio a usar mi imagen/registros audiovisuales

SI ☐ NO* ☐

* Rechazar el uso de mi imagen para fines comerciales no prohíbe mi participación en el estudio.

Apruebo la presente autorización
Firmado en Valencia a _____/_____/2021