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Efficacy Test Conducted With 'LONGEVITY SERUM "O.S."'

Summary

Study Sponsor .....: **Catalent Cosmetics**
Via Nettunense, Km.20, 100
04011 Aprilia (LT)
Italy

Date of Order.....: 25.03.2009

Performance of Test .....: Derma Consult Concept GmbH
and Evaluation by Von-Weichs-Str. 9A
53121 Bonn

Supervisors of Study .....: H. P. Nissen, PhD
Dr. H. Prieur, Dermatologist – Allergist
Drs. B. Nissen

Study Code .....: DCC09W027

Test Product .....: The test product, which was coded as follows, was provided by Catalent Cosmetics:

A. LONGEVITY SERUM "O.S." (ref. 9RDA00046)

Subjects .....: Number of individuals.: 20 (+1 reserve subject)
Sex.....: female
Age range .....: 36-64 years (average: 46,5)

Test Area .....: Inner sides of forearms
Crows' feet (face)

Application.....: Duration....: 28 days
Frequency.: twice daily

Test Period .....: April 2008

Test Parameters .....: 1. Determination of *skin firmness and elasticity (biomechanical properties of the skin)* by means of Cutometer MPA 580 (Courage & Khazaka GmbH, Cologne)

2. Determination of *skin roughness* by means of PRIMOS® 5.6 high-res (GFMeßtechnik GmbH, Teltow, Germany)

3. Determination of *wrinkle depth* by means of PRIMOS® 5.6 high-res (GFMeßtechnik GmbH, Teltow, Germany)

4. *Subjective evaluation* / questionnaire

Design of Study .....: **Day 0**

- Determination of the parameters in the test areas
- First test product application

Day 14

- Determination of the parameters 8-12 hours following the last daily test product application

Day 28

- Determination of the parameters 8-12 hours following the last daily test product application
- Completion of questionnaire

Evaluation .....: Descriptive statistics (average, median, minimum, maximum, variance, standard error, standard deviation); Wilcoxon Rank Test

Results .....: **Biomechanical Properties of the Skin**

The test product was found to statistically significantly enhance the biomechanical properties of the skin towards the firm-elastic optimum. After 28 days of treatment, a mean increase by 15% (firmness) / 10% (elasticity) was observed and a positive effect of the test product was detected in 95% (firmness) / 95% (elasticity) of the volunteers.

Skin Roughness

The test product was found to statistically significantly increase smoothness. After 28 days of treatment, a mean increase by 19% was observed and a positive effect of the test product was detected in 100% of the volunteers.

Wrinkle Depth

The test product was found to statistically significantly decrease wrinkle depth. After 28 days of treatment, a mean decrease by 18% was observed and a positive effect of the test product was detected in 100% of the volunteers.

Subjective Evaluation / Questionnaire

The test product was received very favourably by the subjects and clear effects were attributed to its use.

Methods

Measurement of Biomechanical Properties (Elasticity, Firmness)

The biomechanical properties of the skin are assessed using the Cutometer MPA580 (Courage + Khazaka Electronic GmbH, Cologne; S/N 31050887 tube: S/N 07154103).

The measurement is based on the vacuum-suction principle. By applying a constant negative pressure for a given time period, skin is drawn into a hollow tube with an orifice of 2 mm in diameter. Then, at normal air pressure, the skin is allowed to retract. The penetration depth of the skin into the tube is recorded optically without friction and without mechanical influence. A number of standardized parameters can be calculated from the resulting penetration depth curve. Most of the parameters are a function of skin thickness and thus cannot be simply compared between subjects and regions.
To increase accuracy and to capture information on the properties of skin under repeated external stress, the cycle is repeated several times and parameters selected for evaluation are based on areas rather than individual measurement points.

The delineation of the areas is based on the fitted logarithmic envelope curves of the minimum and maximum extensions according to the equation:

$$y = \frac{\ln x + b}{a}$$

(x = repetitions, y = max. amplitude or min. amplitude).

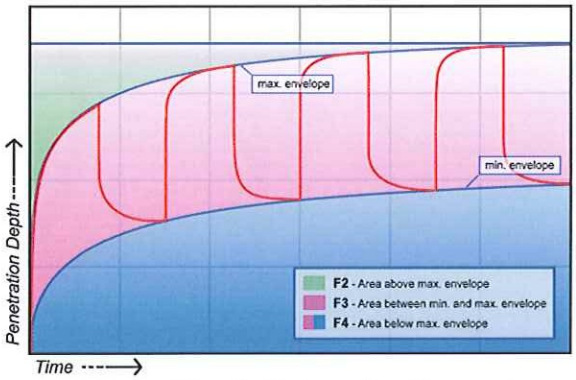

Fig. 1: Cutometer Parameters

The study was conducted with 20 successive measurement cycles, 1 second suction, 1 second retraction, with a 450 mbar vacuum. The following parameters were selected to assess changes in the biomechanical properties of the skin:

Skin firmness
Skin firmness is assessed by the parameter F4, the area below the approximated envelope function of the maximum extensions. → A decrease in F4 corresponds to an increase in skin firmness.

Skin elasticity
Skin elasticity is assessed by the ratio F3 / F4. The larger F3 in comparison to F4, the larger are the restoring forces and the smaller is the remaining residual deformation. → The closer the resulting value is to 1, the more elastic the skin.

The calculation of the parameters was conducted by WinCT (Courage & Khazaka GmbH, Cologne - Germany)

Measurement of Skin Roughness

PRIMOS (Phase-Shifting rapid in vivo measurement of skin) is a non-contact measurement device, which allows for real-time three-dimensional in vivo measurement of the micro topography of human skin based on the technology of active image triangulation. The measurement head consists of a digital micromirror device as projection unit and a CCD-camera as recording unit, mounted onto an adjustable rack. For active image triangulation an intensity encoded point M is projected onto the surface under investigation. Its image on the surface is recorded by the CCD-camera from a specific angle. The point M is a function of parameters like intensity, triangulation angle between projection system and camera and some other inner respectively outer coordinates of the camera and projection plane. The height information of the structured surface is coded in the distorted intensity pattern, which is recorded. The resolution and accuracy depends on the optical and topographical characteristics of the measured surface and on the noise characteristics of the measurement system. For accurate in vivo measurements of human skin, depending on the measured part of the human body (inner forearm, forehead, eye zone), different parameters of effective wavelength and amplification factor should be used.
To regard the differences of human skin and avoid undesired distortions by movements, the fast phase-shift technique was used for the measurement (phase width: 16 & 64 pixels). For each measurement, a minimum of 3 recordings were made and the clearest image without movement distortions or artefacts was selected for further processing
At the end of the study, distortions due to body hairs were digitally removed and the macro structure (calculated by polynomial approximation), i.e. the curvature of the entire test area, subtracted to allow a proper analysis of the microstructure, i.e. surface roughness.
Skin roughness was then assessed by means of the parameter R_Z (mean depth of roughness). To mitigate potential directional effects, the evaluation was conducted using the arithmetic average of R_Z from 32 radial cuts. The mean depth of roughness is defined as:

$$R_Z = \frac{1}{n}\sum_{i=1}^{n} R_{Zi} ,$$

where n is the number of equal segments into which the scan length l has been divided into and R_{Zi} is the maximum peak to valley depth within each of the segments. In accordance with the German Standard Din 4768/1, R_Z was calculated using 5 segments of equal length.

Measurement of Wrinkle Depth

The crows' feet area is recorded as a 3D topography using the PRIMOS system as outlined above. To accurately detect deeper structures different settings for the fast phase-shift were used (phase width: 16, 64 & 128 pixels). For each measurement, a minimum of 3 recordings were made and the clearest image without movement distortions or artefacts was selected for further processing. On follow-up visits, the original captured data was projected onto the skin of the volunteers to help in the relocation process of the test area.
At the end of the study, distortions due to body hairs were digitally removed and the macro structure (calculated by polynomial approximation), i.e. the curvature of the entire test area, subtracted to allow a proper analysis of the microstructure, i.e. wrinkles and surface roughness.
Wrinkle depth was then assessed by means of the parameter R_{Max} that is defined as the maximum vertical distance from the highest peak to the lowest valley of five segments of equal length. To mitigate locational effects, the evaluation was conducted using the arithmetic average of R_{max} from 50 parallel cuts.

Subjective Evaluation / Questionnaire

Questionnaire design and evaluation were conducted using Keypoint 5.5 (Cambridge Software Publishing, UK). In the design of the questionnaire and the wording of the items, specific attention was paid to comprehensibility and appropriate vocabulary, as well as the volunteers' ability to articulate the answers. For maximum compliance and ease in evaluation and to additionally minimize item nonresponse by making it easier for subjects to complete the questionnaire, closed dichotomous and multichotomous questions were used, the latter mostly in the form of a Likert scale (expression of agreement to disagreement to fixed statements).
The German original questionnaire was first designed according to the sponsor's requirements and then an English translation created. The German original and the English translation are included in paper form in the appendix; the subjects were presented the German version of the questionnaire on a touch sensitive kiosk computer terminal with on-screen keyboard for open questions.

Performance of Test

The subjects were selected from the Derma Consult Concept GmbH database. They were informed about importance and meaning of the study. Written informed consent was obtained from all the subjects prior to entry into the trial. Following criteria were used for selection of subjects:

for inclusion in study:
- female (≥ 35 years of age, visible crow's feet)
- clinically healthy

for exclusion from study:
- skin diseases
- pregnancy

The subjects of this study were between 36-64 years of age (average: 46,5). A reserve subject, to compensate for potential drop-outs started the study on the same day as the original 20 subjects. They could withdraw from the study at any time without giving any reason.
The subjects were instructed not to use any topical preparations on the test areas starting from seven days prior to testing and until the end of the test. For cleansing, water or a mild syndet (Eubos® flüssig – blau; manufacturer: Dr. Hobein, D-53340 Meckenheim-Merl, Germany) was allowed only (whole study inclusive the run-in phase).
Prior to the first application of the test product, measurements were taken at clearly defined sites of the inner sides of the forearms (biomechanical properties, skin roughness) and in the crows' feet region (wrinkle depth). One area on the inner side of the forearms remained untreated and served as control. Further measuring was performed after 14 and 28 days of application 8-12 hours following the last daily application (adaptation time: 30 min, room temperature: 21±1°C, relative humidity: 50± 5%). After a demonstration of the correct product application procedure by a Derma Consult staff member, the subjects used the test product (approximately 2 mg/cm²) twice daily (in the morning and evening) and in a manner corresponding as largely as possible to that to be practised by the future consumer.
On the final visit to the test institute, the subjects were asked to complete the questionnaire asking for their experience with the test product and their perception of its effects.

Biometry

Measurement data is automatically computerised and after validity check and quality assurance stored centrally in a database. Evaluation is conducted using the software NAG® Statistical Add-Ins for Excel – NAG Ltd., United Kingdom. The data were analyzed by Wilcoxon Rank Test. The 0.05 level was selected as the point of minimal acceptance of statistical significance.

During the second week of treatment one of the original 20 subjects reported incompatibilities in the form of dry, scaly spots in the facial area. After a consultation with the supervising dermatologist, she dropped out of the study. Her initial measurement data was discarded and she was replaced by the reserve subject. Data evaluation is hence conducted on 20 cases.

Results

Biomechanical Properties (Skin Firmness / Skin Elasticity)

In assessing skin firmness, evaluated are changes in the parameter F4 in the treated area in comparison to the changes in the untreated area. The absolute changes by area and time point are shown below in figure two. A decrease in F4 corresponds to an increase in skin firmness.

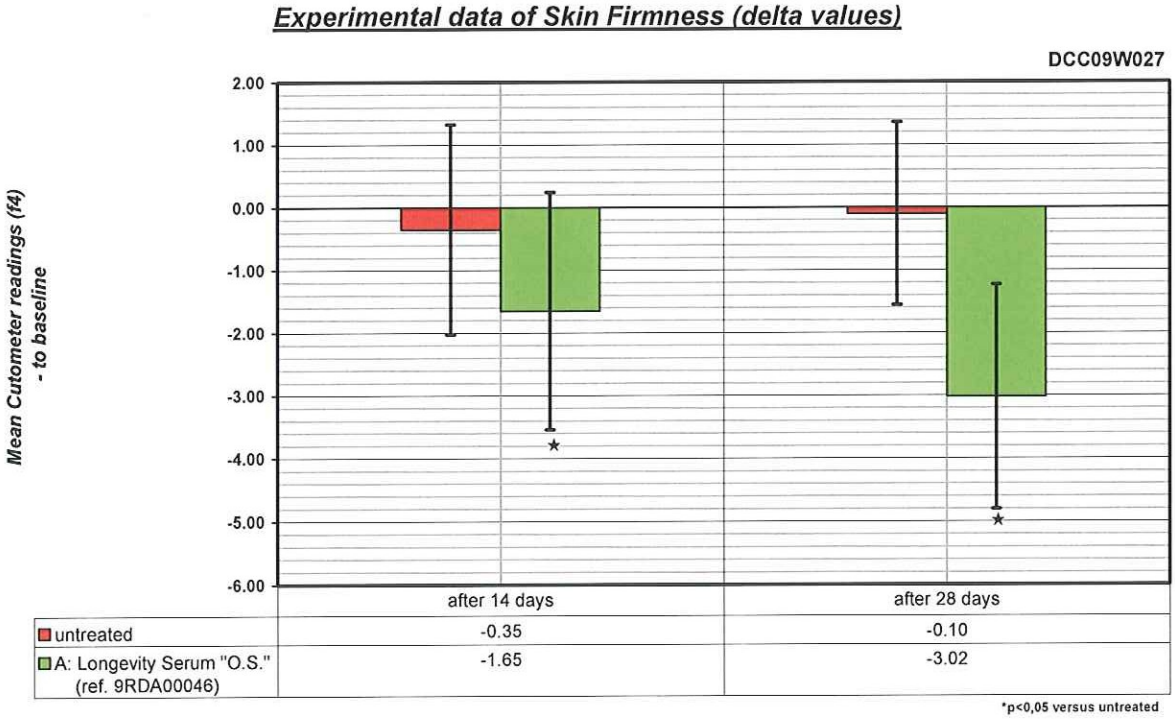

Fig. 2: Δ F4 Values

After both 14 and 28 days of treatment, a statistically significant ($p<0.05$) decrease in F4 was observed in the product treated test area as compared to the changes in the untreated area.
The test product was found to statistically significantly increase skin firmness; after 28 days of treatment a positive effect could be detected in 95% of the study participants. The respective percentage changes as compared to the initial condition and with regard of the changes in the untreated area are shown in figure three below.

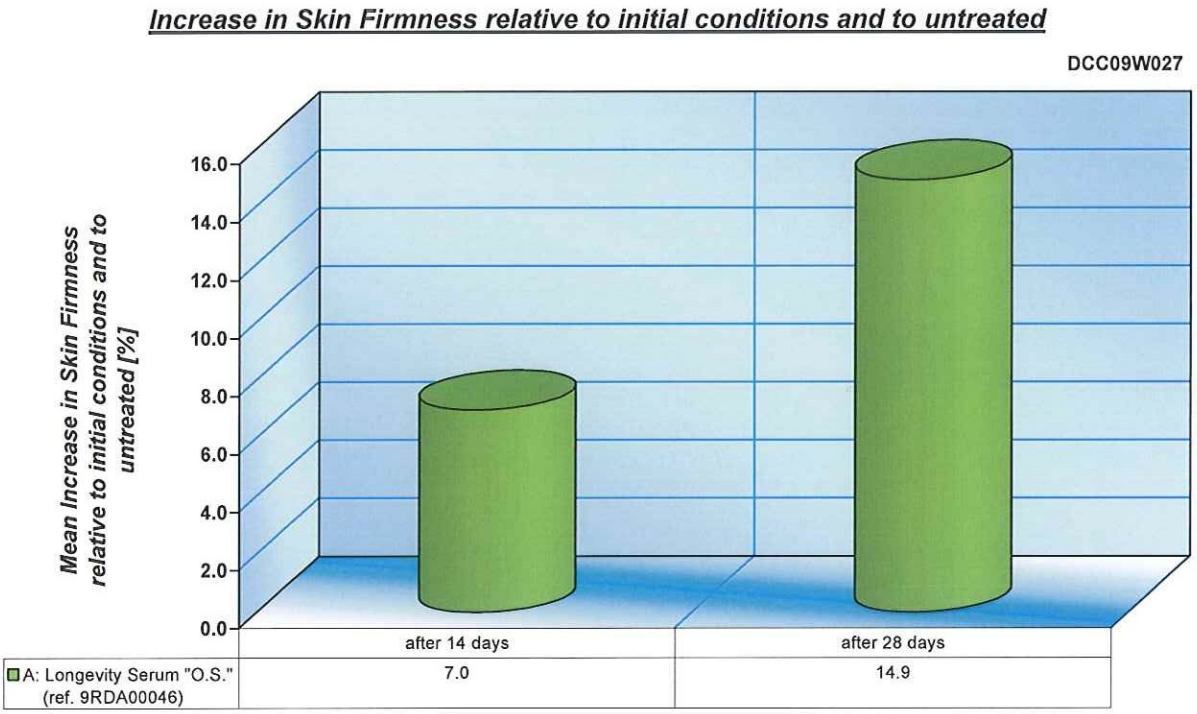

Fig. 3: Increase in Skin Firmness in %

In assessing skin elasticity, evaluated are the changes in the fraction F3 divided by F4 in the treated area in comparison to the changes in the untreated area. The absolute changes by area and time point are shown below in figure four. An increase in F3/F4 corresponds to an increase in skin elasticity.

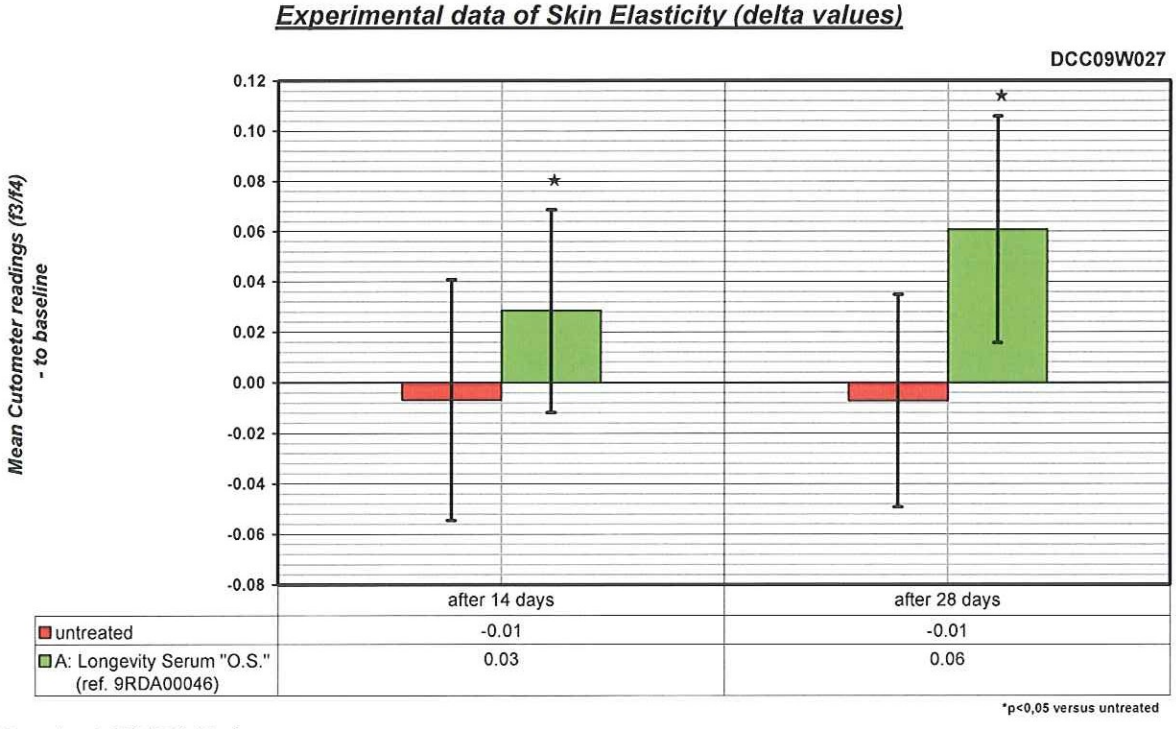

Fig. 4: Δ F3/F4 Values

After both 14 and 28 days of treatment, a statistically significant ($p<0.05$) increase in F3/F4 was observed in the product treated test area as compared to the changes in the untreated area.
The test product was found to statistically significantly increase skin elasticity; after 28 days of treatment a positive effect could be detected in 95% of the study participants. The respective percentage changes as compared to the initial condition and with regard of the changes in the untreated area are shown in figure five below.

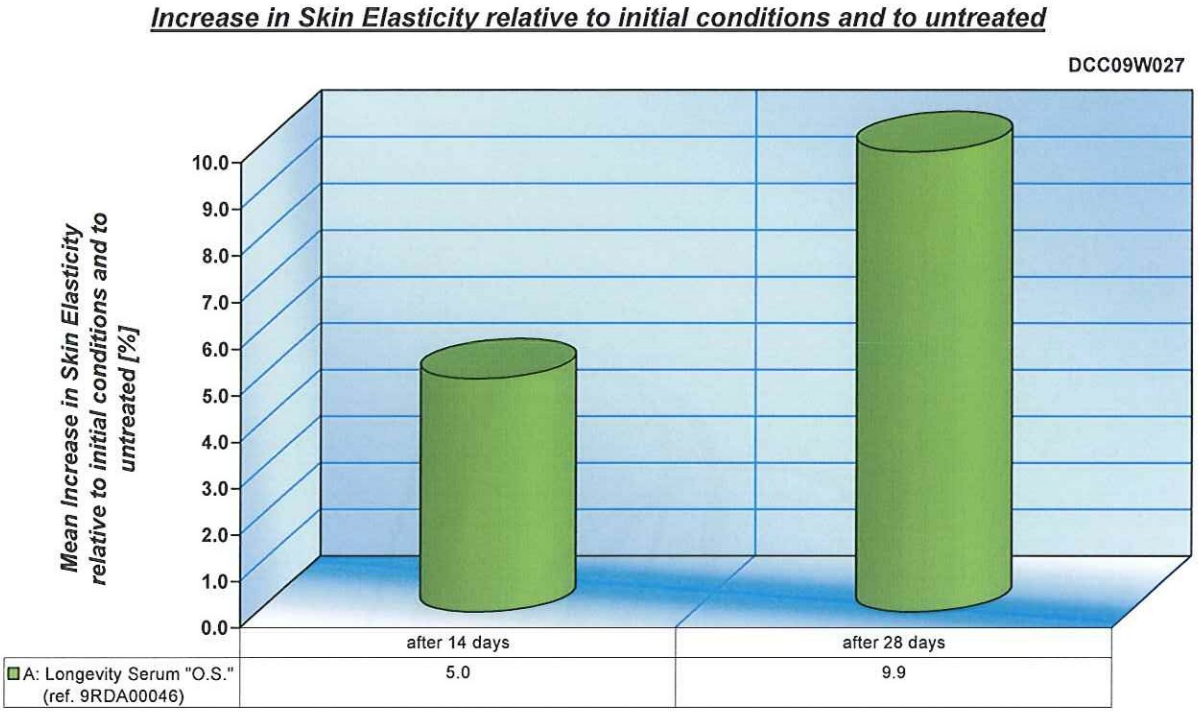

Fig. 5: Increase in Skin Elasticity in %

The test product was found not only to increase skin firmness, but at the same time to also increase skin elasticity. Overall, it induced a change in the biomechanical properties of the skin towards the firm-elastic optimum.

Skin Roughness (Rz)

Evaluated are the changes in the parameter R_Z in the treated area in comparison to the changes in the untreated area. The absolute changes by area and time point are shown below in figure six. A decrease in R_Z corresponds to an increase in skin smoothness.

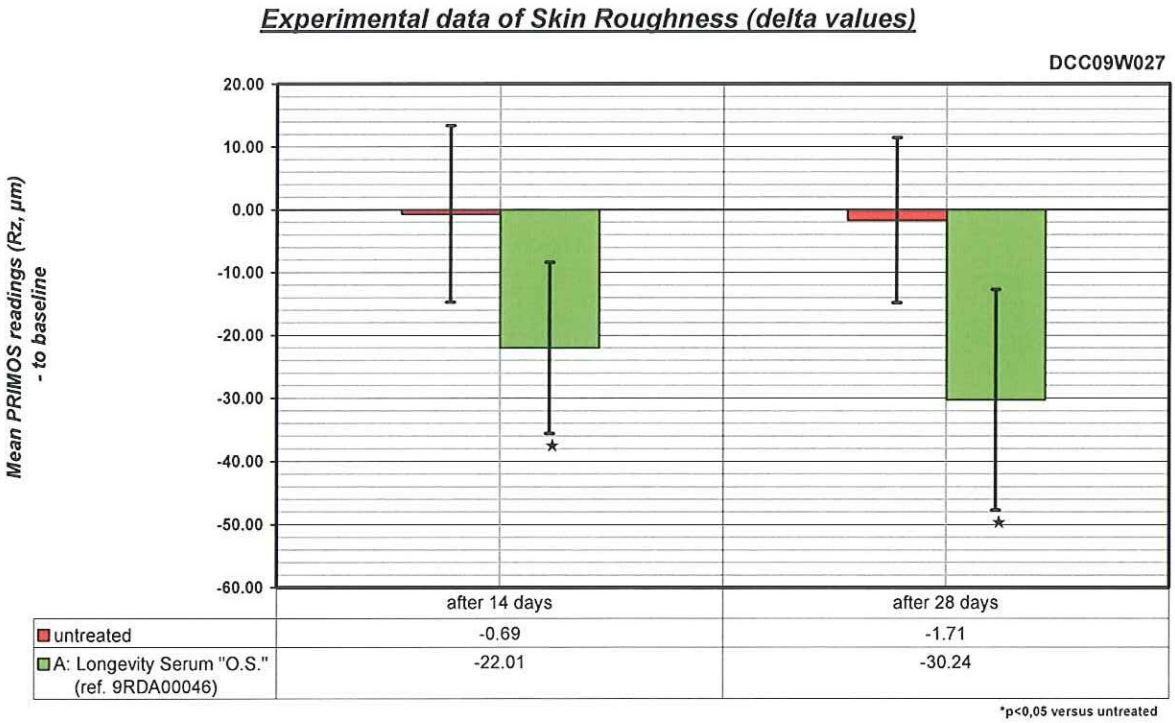

Fig. 6: ΔR_Z Values

After both 14 and 28 days of treatment, a statistically significant ($p<0.05$) decrease in R_z was observed in the product treated test area as compared to the changes in the untreated area.
The test product was found to statistically significantly increase skin smoothness; after 28 days of treatment a positive effect could be detected in 100% of the study participants. The respective percentage changes as compared to the initial condition and with regard of the changes in the untreated area are shown in figure seven below.

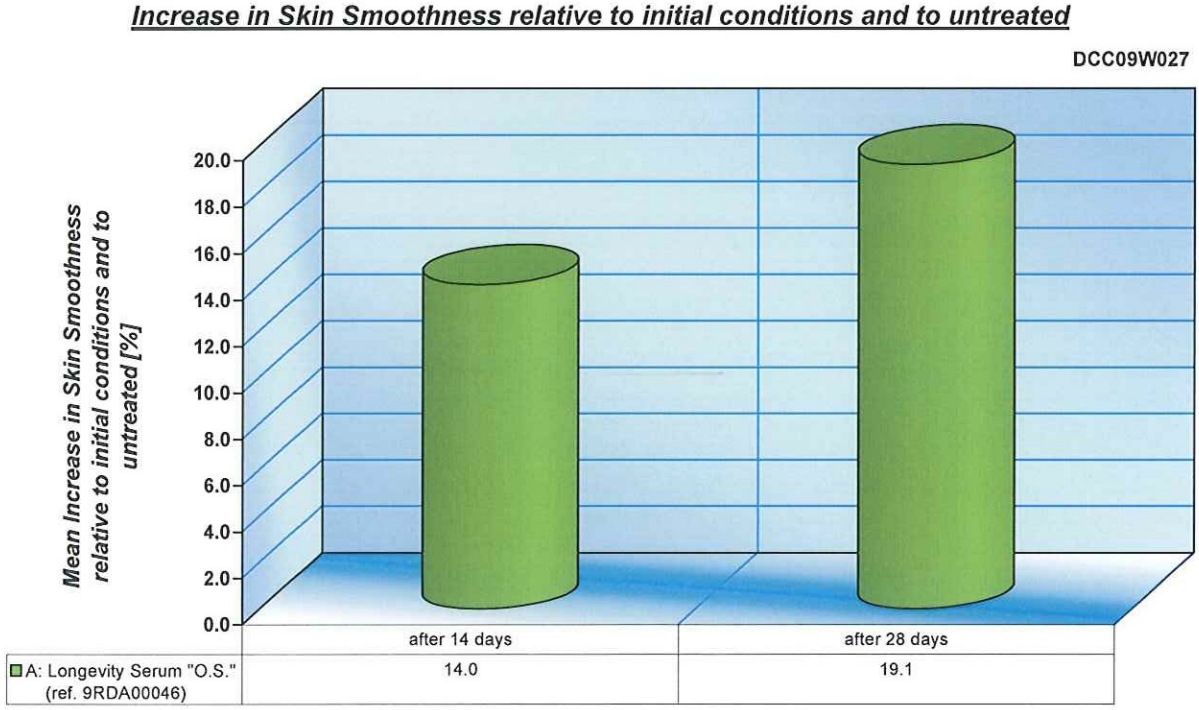

Fig. 7: Increase in Skin smoothness in %

Wrinkle Depth

Evaluated is the parameter R_{Max} in comparison to the initial condition in the same respective test area. The absolute changes by area and time point are shown below in figure eight. A decrease in R_{Max} corresponds to a decrease in wrinkle depth.

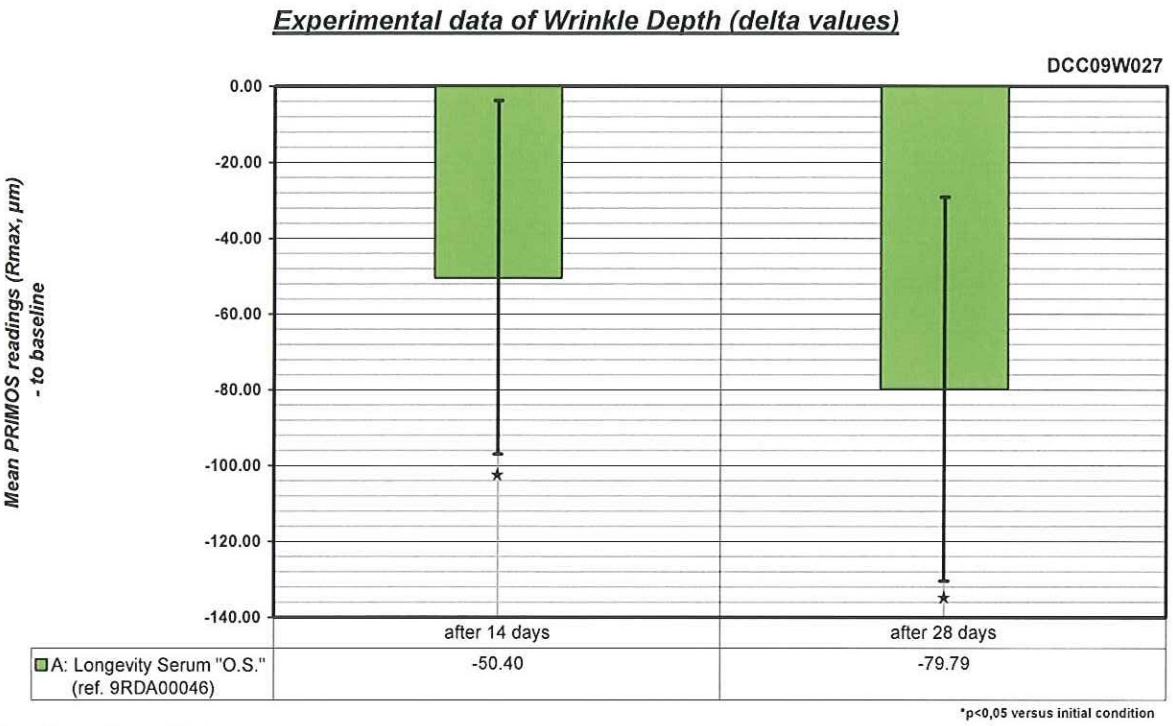

Fig. 8: ΔR_{Max} Values

After both 14 and 28 days of treatment, a statistically significant ($p<0.05$) decrease in R_{Max} was observed in the product treated test area as compared to the initial condition.
The test product was found to statistically significantly decrease wrinkle depth; after 28 days of treatment a positive effect could be detected in 100% of the study participants. The respective percentage changes as compared to the initial condition are shown in figure nine below.

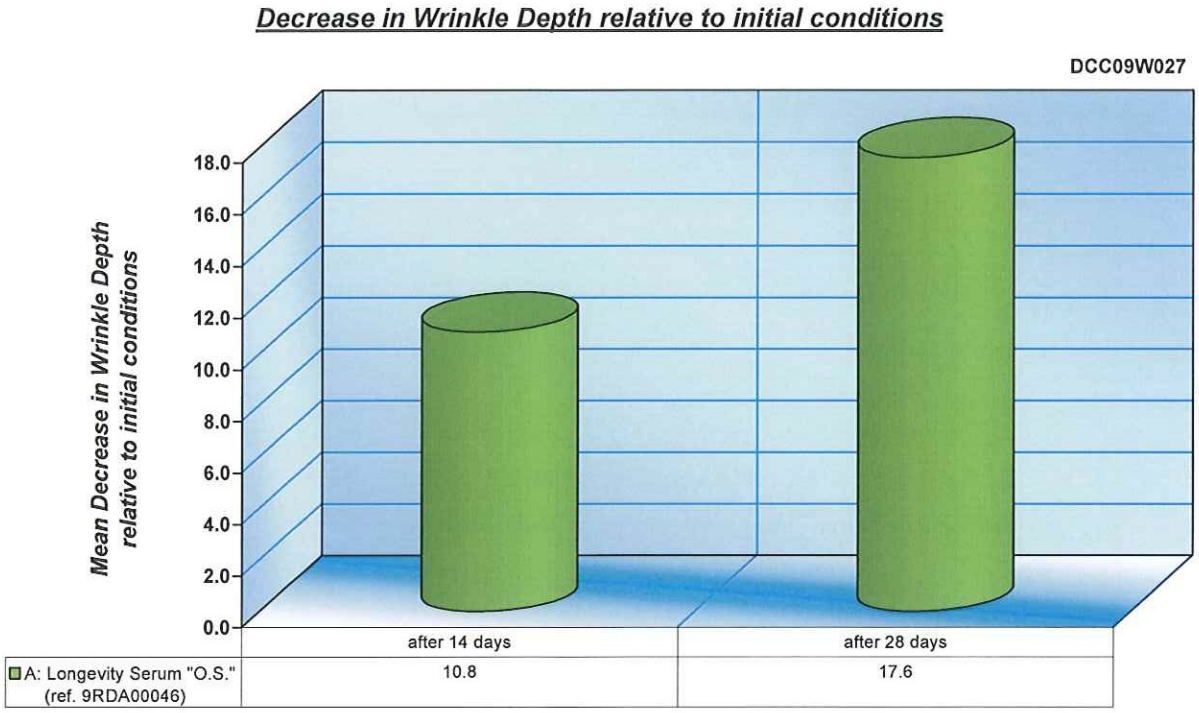

Fig. 9: Decrease in Wrinkle Depth in %

Subjective Evaluation / Questionnaire

After 28 days of regular use, the test product was rated overall very favorably by the subjects. A full graphical review and a paper version of the questionnaire can be found in the appendix.

The subjects rated their skin as 25% very dry, 25% dry, 40% normal, 10% combination skin and their skin sensitivity as 25% very sensitive, 15% sensitive, 45% normal, 15% insensitive.

Concerning the application experience, the product was found (almost) unanimously to be easy to open (95%), easy to apply (100%) and to create a pleasant skin sensation (100%).

Regarding the efficacy of the test product, the great majority of subjects found their skin to feel smoother (80%), softer (85%) and revitalized (75%), their skin to look more radiant (75%) and younger (60%). The great majority was satisfied with the overall skin feel of the product (90%) and found the product to be very comfortable (90%). A majority also found their skin to feel firmer (50%), their skin tone to look more even (55%) and their facial contour redefined (55%).
There was almost unanimous agreement on a good skin compatibility of the product (95% - one subject experienced a short period of mild irritations as detailed below) and in line the subjects expressed that they would like to continue using the product (80%), would buy the product (85%), would recommend it to their friends (90%) and gave the product an overall grade of "good" (60%) to excellent (25%).

Incompatibility

During the second week of treatment subject 8 developed dry, scaly spots in the treatment area in the face and hence was excused from the study by the supervising study dermatologist. A second subject, no. 11, reported similar, but less strongly developed symptoms at the same time. After a consultation, she decided to continue the treatment and the symptoms disappeared within the following four days. Both subjects had no history with regard to prior adverse reactions to topical cosmetic formulations. No further incompatibility was observed in or reported by any of the volunteers.

Overall, the test product showed good skin compatibility under the given test conditions.

Signature:

Drs. B. Nissen
Manager Derma Consult Concept

Signature:

Dr. H. P. Nissen
Chemist – Ph.D.

Signature:

Dr. med. H. Prieur
Dermatologist - Allergist

Enclosures: Measuring values, statistics, summary statistics, graphic representations