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CHARACTERISATION OF BIOMECHANICAL PROPERTIES WITH A NOVEL DEVICE AND THROUGH EVALUATION OF TENSION LINES INDEX IN VERY MATURE SENESCENT SKINS

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1. Introduction

The aging of the face after the age of 60 leads to changes in the skin. Fibroblasts secrete regenerative growth factors essential for cell renewal and matrix synthesis, but their secretion declines with age, disrupting communication [1]. Cellular senescence, during which cells stop dividing and release harmful SASPs, is a key factor in aging. After the age of 60, senescent fibroblasts escape immune defenses, leading to their accumulation. These immuno-evasive cells continuously release pro-aging SASPs, rendering healthy cells senescent and reducing extracellular matrix synthesis [2]. The result is reduced dermal density, impaired epidermal renewal, increased wrinkling and loss of skin tone uniformity. The skin surface also changes, losing its mechanical properties and becoming stiffer, less elastic and firm with a decrease of the natural tension [2]. In this study, we propose a quantitative characterization of skin tension *in vivo* using a new model. This model consists in calculating the tension indices based on the equilibrium equation, and uses the Fourier transform. Tension indices are calculated primarily from skin topology replicas performed on the face. We also combine an innovative indentation device (Adhelaskin) to study the biomechanical properties of the skin, as its device's ruby ball sensor and patented indentation technique detect the tiniest changes in skin properties, providing an in-depth understanding of the skin's response to age, treatments and environmental factors. We also performed more conventional methods to describe the effect of the investigational product on the skin firmness.

2. Materials and Methods

2.1 Panel and investigational product

The study, single blinded, enrolled 20 Caucasian female subjects between 60 to 74 years old with all face skin types, presenting with a fine, dull and yellowish skin tone, with wrinkles on the crow's feet (score under 5 on the Bazin scale [3]). A total of 19 subjects with a mean age of 66 years old completed the study and were kept for analysis.

They were asked to use the tested serum in place of all of their skincare routine (only cleanser allowed) for two months twice a day on the face. Each subject was placed in a temperature controlled rest room for 15 minutes before performing all measurements at each time point. All assessments were made before the first application and after 28 and 56 days of use, on a bare skin.

The study was conducted following the principles of the Declaration of Helsinki and compliance with local applicable laws, regulatory and ethics requirements. All the subjects provided written consent to allow their participation prior to the study.

2.2 Measurement of biomechanical properties with Adhelaskin

Adhelaskin is a new and innovative portable and connected device, quantifying the human skin's elasticity properties, with a force sensor of 1mN and a movement system allowing measurement with a precision of 1 μ m, provided to measure the mechanical characteristic of the skin in surface and in depth as firmness and elasticity. The device's ruby ball sensor and patented indentation technique enable it to assess the contact stiffness (K(N/m), reduced modulus of elasticity in KPa, lateral tensile stress in KPa and lateral pile-up height thanks to the low pressure vertical palpation principle by creating a load curve and a discharge curve with the sphere (figures 1 and 2). The elastic modulus reflects the density of collagen and elastin fibres, with age the skin is less resistant to the penetration of the ruby ball in the skin. Three repeated measures were taken on one cheekbone with Adhelaskin and the mean was used for the analysis.

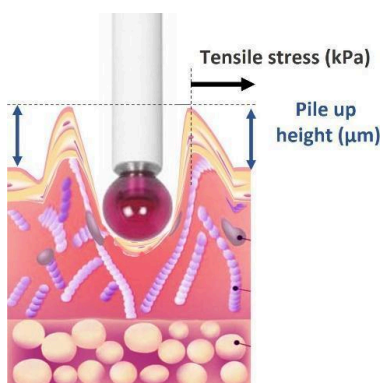
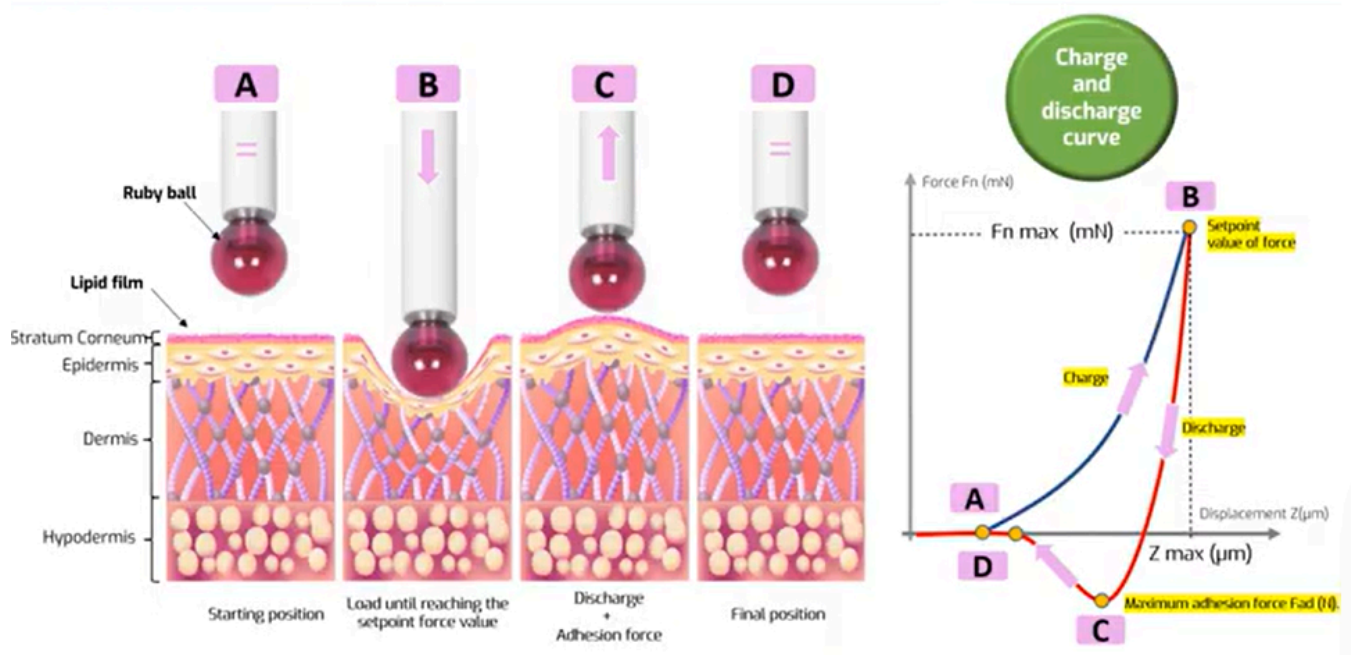


Figure 1: visualization of the pile up height and tensile stress with the Adhelaskin



2.3 Skin replicas and evaluation of tension lines

A skin replica using silicone SILFLO (MONADERM, Monaco) was made on the other cheekbone in order to assess the skin tension index under white light confocal microscope images.

The three-dimensional skin topology was then reconstructed using the chromatic confocal microscopy system (from AltiMet—AltiSurf 500®, France). The 3D images obtained were studied with a program developed by Zahouani [4,5] using TopoSurf®. This approach allows the identification of the anisotropy of the line network at different scales of depth and with different orientations [6]. For each plane at a certain depth of the skin surface, are determined three parameters of the point belonging to the line of tension: the density of depth z , the width of the line and the rise of directions between 0 and 180° (Figure 3 a).

For the calculation of tension indices (Figure 3 b), the global image A is decomposed into two sub-images oriented 0-90° (Image D) and 90-180° (Image C). Each image corresponds to a representation of the skin tension patterns (Global Image B), Image E (90-180°) and Image F (0-90°). For each image C and D, the wavelengths are statistically quantified along the X and Y axes. The difference between the wavelengths (C and D) along the two X and Y axes allows the quantification of the effect of tension forces and the quantification of tension indices [7].

The lower this index, the greater the tension, this is the case of the skin, the more the skin is loose, the stronger this index is, this is the case of the skin of elderly subjects.

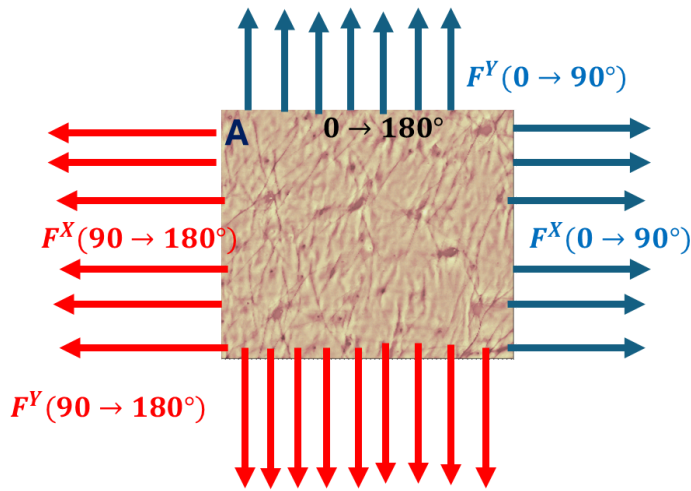


Figure 3: (a) The skin is subjected to tension forces along the X and Y axes of the image

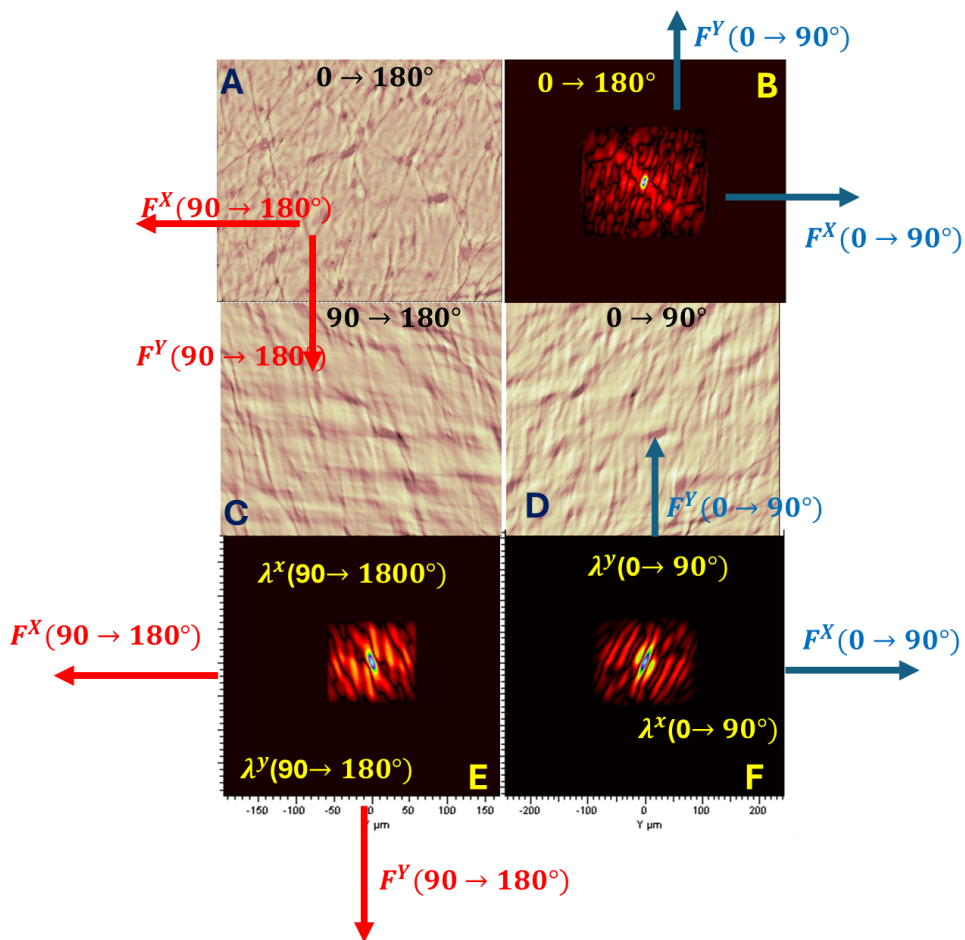


Figure 3 (b): the global image A is decomposed into two sub-images oriented 0-90° (Image D) and 90-180° (Image C). Each image corresponds to a representation of the skin tension patterns (Global Image B), Image E (90-180°) and Image F (0-90°). For each image C and D, the wavelengths are statistically quantified along the X and Y axes.

2.5 Fringe projection

Measures using the non-invasive 3D fringe projection method, allowing to characterize the skin surface by a full-face acquisition were performed (AEVA-HE V4, Eotech, with sensor 250). After the calibration, subjects were installed precisely on the bench in order to have positioning at each measurement time.

The positive volume parameter in mm³ was analyzed by AEVA-HE V4 software and the data were compared before using the product after one and two months of use. This parameter reflects the intensity of ptosis of the lower part of the face.

2.6 Expert clinical scoring and satisfaction

A clinical assessment was realised by an expert regarding ptosis on the oval of the lower part of the face. A scale from 0 (oval of the lower part of the face defined, firm and lifted skin) to 10 (oval of the lower part of the face not defined, loose skin) was used and the expert chose for each subject the stage of ptosis with the help of the scale at each time point.

Satisfaction was also asked to the subjects as regard several benefits, after one month of use of the investigational product, linked to the firmness and the ptosis. 4 modalities of answer were proposed (agree, somewhat agree, not agree, not agree at all).

2.7 Statistical analysis

For tension lines, a statistical analysis was performed using the XLStat software (Addinsoft, France). A descriptive analysis was performed upstream to identify data trends and anomalies, and to provide essential information such as: the minimum, maximum, mean, median, and standard deviation of a sample of data.

For the other data (adhelaskin, fringe projection and scoring):

Determination of the mean values of the different parameters, at each time point of the study, by the calculation of the means and the standard deviations (Sd) of individual data. . Comparison of the data obtained at considered time points of the study to the initial values.

To analyze the data, the Shapiro-Wilk test was used to determine if the data follows a normal distribution. If normality is confirmed, a paired Student's t-test (two-tailed) was used; otherwise, the non-parametric Wilcoxon test (two-tailed) was applied. Statistical significance was set at (p-value < 0.05), while results with (0.05 ≤ p-value < 0.10) were considered limit significant, and those with (p-value ≥ 0.10) were deemed non-significant.

In case of statistically significant evolution, calculation of the corresponding variation percentage, from the mean values.

For satisfaction percentage, results correspond to sum of the 2 positive answer modalities.

3. Results

3.1 Measurement of biomechanical properties with Adhelaskin

A significant improvement was obtained after 1 month of application of the serum versus the baseline on all parameters. The stiffness increased by 14.24% ($p<0,002$) (figure 4), the tensile stress increased by 17.87% ($p<0,007$) (figure 5), the elastic modulus was improved by 24.90% ($p<0,003$) (figure 6) and the pile up height was reduced by 12.11% ($p<0,009$) (figure 7).

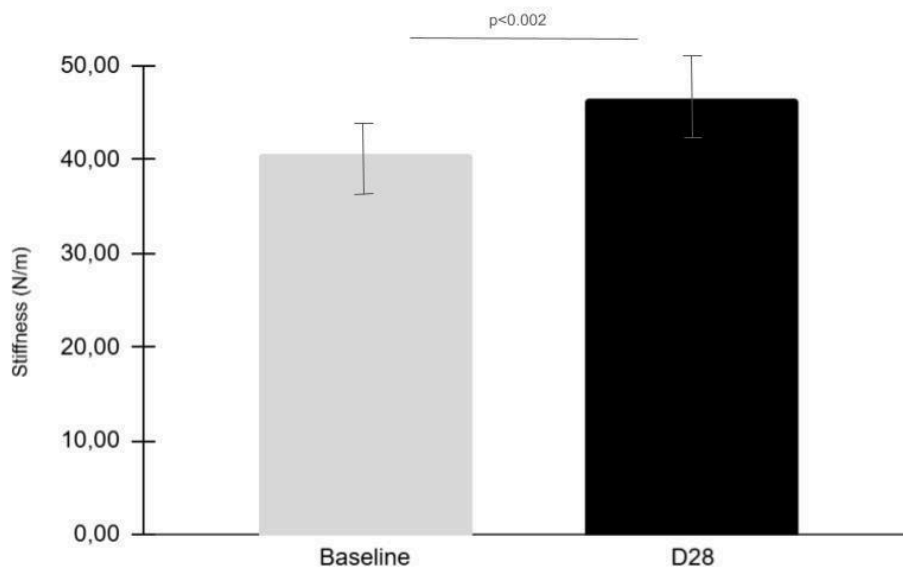


Figure 4: value of the stiffness (N/m) before (baseline) and after one month (D28) of use (mean and standard deviation), p value $p<0.002$

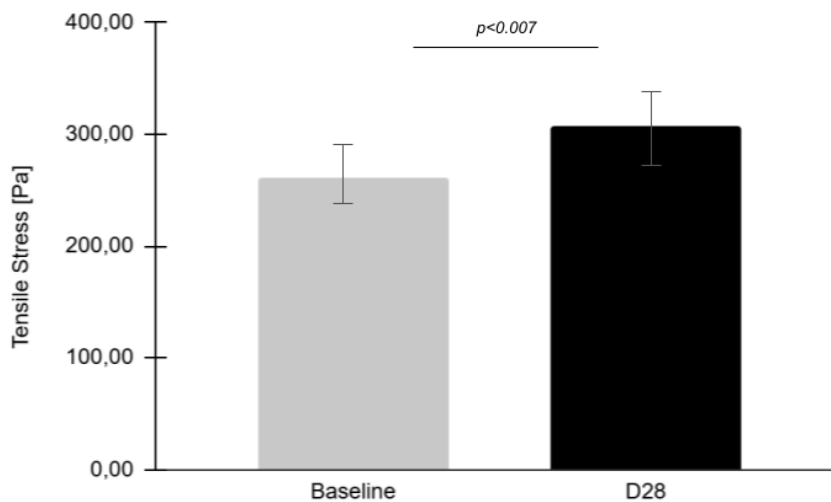


Figure 5: value of the tensile stress (Pa) before (baseline) and after one month (D28) of use (mean and standard deviation), p value $p<0.007$

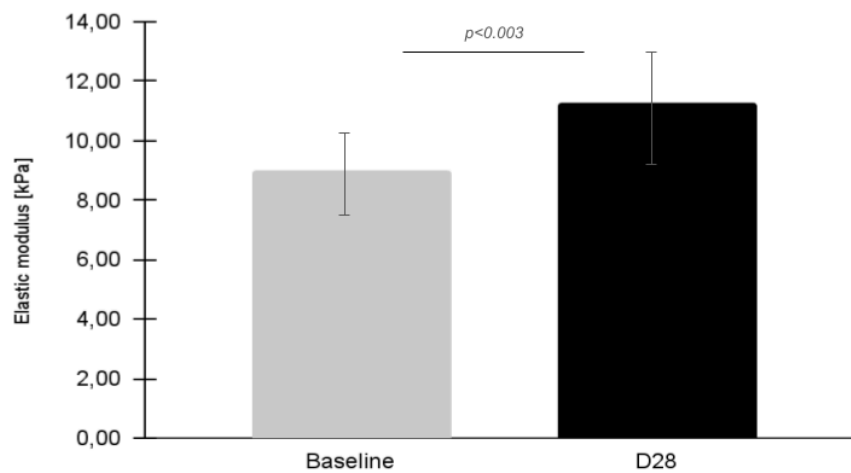


Figure 6: value of the elastic modulus (kPa) before (baseline) and after one month (D28) of use (mean and standard deviation), p value < 0.003

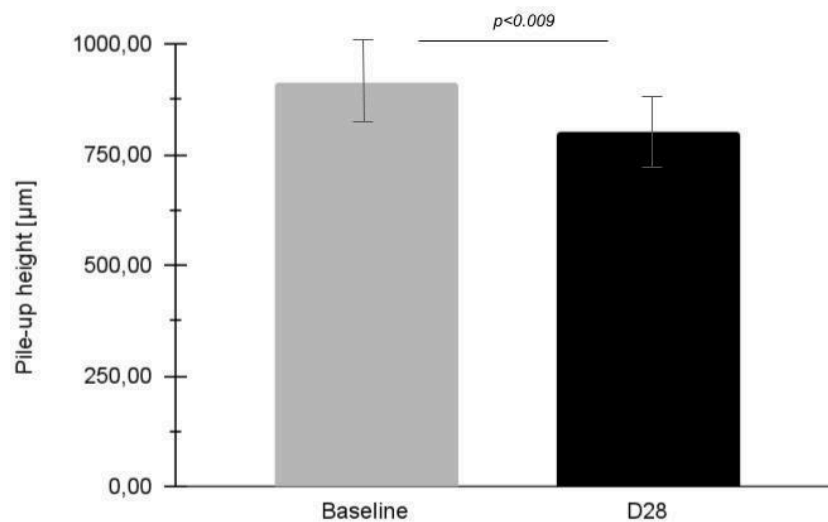


Figure 7: value of the pile up height before (baseline) and after one month (D28) of use (mean and standard deviation), p value < 0.009

3.3 Evaluation of tension lines

The skin tension index statistically decreased of 27% after one month and of 28% after two months of use, meaning an increase in skin tension (figure 8).

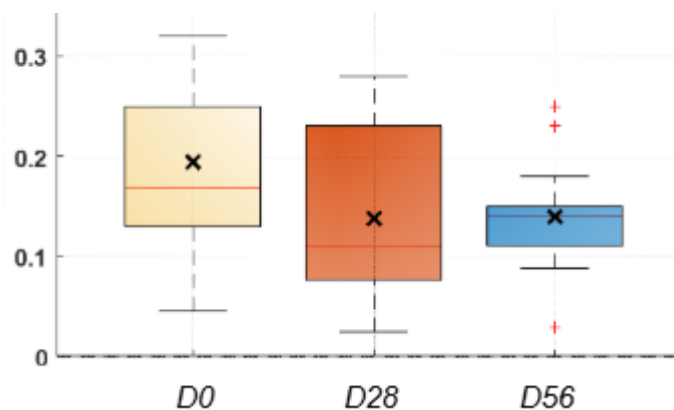


Figure 8: value of the tension index before (D0) and after one (D28) and 2 months (D56) of use (x: mean, red line: median and standard deviation)

3.4 Fringe projection

The results obtained showed a decrease at the limit of significance of the positive volume (in mm³) after one month of use which became significant after 2 months of application. These results reflect an improvement of the intensity of ptosis of the lower part of the face by 12% after 56 days (figure 9).

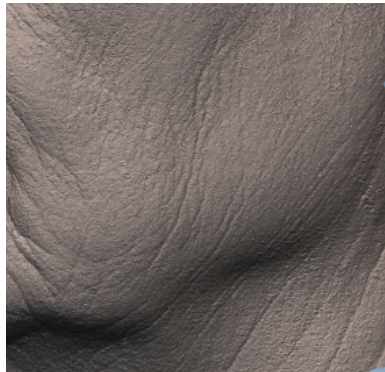
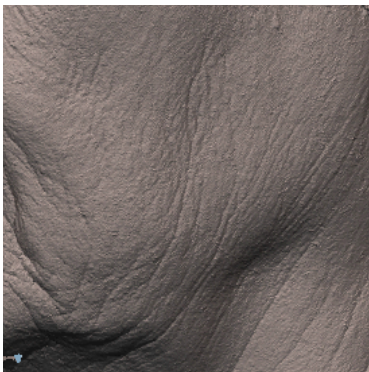


Figure 9: 3D reconstitution by AEVA-HE V4 software representing a tendency to improvement in ptosis between D0 (left picture) and D28 (right picture)

3.5 Expert scoring and satisfaction

The expert scoring showed an improvement of 8% on the face ptosis (figure 10).

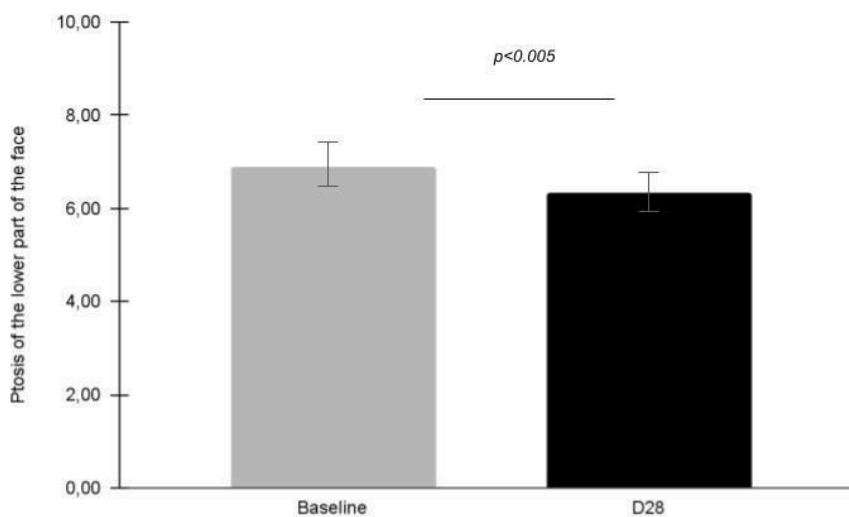


Figure 10: value of expert scoring of ptosis of the lower part of the face before (baseline) and after one month (D28) of use (mean and standard deviation), p value < 0.005

The subjects declared to have skin more tonic and firmer for 89% of them.

4. Discussion

The ageing signs of the mature skins are in particular due to problems of cell communication as fibroblastic secretion decreased. This cellular senescence leads to modifications of the biomechanical properties of the skin and thus of the skin tension. Our study with subjects over 60 years old and with the help of several *in vivo* methods evaluating the tension effect of the skincare product tested have built up a solid body of evidence. We used two innovative methods. The first was to use the brand-new patented indentation device, Adhelaskin. It significantly demonstrated that our skin care product improved skin firmness thanks to several well-known parameters, such as the skin's elasticity modulus, as well as a new parameter, stacking height. The innovation of using tension lines via replicas is that no skin deformation system is used to assess the biomechanical properties of the skin. The tension index observed after one and two months of use proved that skin tension had improved. The average skin tension at these evaluation times is quite similar, but the data are more compact after 2 months' use, showing a more effective response from the subjects to our care. More conventional methods such as fringe projection also showed good performance on the tension effect via the positive volume parameter. All these instrumental methods are also confirmed by the expert on the ptosis and by the subject's perception, with very good satisfaction on a firmer and tonic skin.

5. Conclusion

Through the results obtained in this work, the anti-age serum highlights its efficacy on biomechanical properties by innovative methods in mature senescent skins.

These new methods, whatever it is a new indentation device or a new application of a reference method (in vivo tension index), are pertinent to show that cosmetic products can act on a senescent skin as regards its biomechanical properties and are convergent to the results obtained with more conventional methods.

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