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Biomechanical Improvement and Dermal Regeneration: The Transformative Potential of a Novel Cosmetic Formulation

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1. Introduction

Skin aging is a multifactorial process involving intrinsic and extrinsic factors. Intrinsic aging stems from genetic and hormonal changes—such as reduced estrogen and progesterone—and oxidative stress from reactive oxygen species (ROS), leading to thinning, dryness, and barrier dysfunction [1–2]. Extrinsic aging, mainly from chronic UV exposure, increases ROS production, DNA damage, glycation, and inflammation, resulting in wrinkles, pigmentation changes, and loss of elasticity [3–4]. Aging also induces structural changes, including reduced epidermal and dermal thickness [5], and extracellular matrix (ECM) degradation—especially of collagen, elastin, and hyaluronic acid (HA)—which compromises skin structure and elasticity [6].

Anti-aging cosmetic formulations have emerged as a key strategy to prevent and mitigate the effects of skin aging. These products aim to protect the skin from environmental stressors, neutralize intracellular free radicals, promote cellular repair, and prevent the degradation of dermal collagen [7–8].

In this context, the present study aimed to clinically evaluate the efficacy of the skincare formulation F4565.33896 in reducing visible signs of skin aging. This evaluation was performed through a combination of biophysical measurements, high-resolution imaging analyses, and immunofluorescence assessment of skin biopsies to investigate biological markers associated with the aging process.

2. Materials and Methods

2.1. Ethical approval

This study was approved by Ethics Committee, in accordance with research project number 63574322.4.0000.5514, and was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

2.2. Test product

A skincare formulation - F4565.33896, was based on a moisturizer formula composed by tocopherol, hydrolyzed hyaluronic acid, Camellia sinensis leaf extract, Schinus terebinthifolius leaf extract, Theobroma cacao seed extract, and other plant-derived bioactive.

2.3. Study design

A total of 33 healthy female participants, aged 60 to 69 years, skin types II to V according Fitzpatrick [9] were enrolled. They refrained from using any cosmetics for 7 days prior to the study and during the study period. Participants applied the skincare formulation F4565.33896 to their face and one forearm daily for 56 days, while the other forearm served as a control. Assessments were conducted at baseline (D0), day 28 (D28), and day 56 (D56) using cutometric measurements, digital imaging (Visia® system), 3D imaging (Vectra® and Primos® devices), and ultrasonography. Skin biopsies for biomarker analysis were collected at D0 and D28 from 12 participants (aged 60-68 years). All measurements were performed in a controlled environment (18-22°C, 45-55% humidity). All participants were acclimatized to room conditions for 30 minutes prior to measurements.

Skin firmness and elasticity were assessed using the Cutometer® MPA 580 (Courage & Khazaka, Germany) with parameters R5 (Ur/Ue) and R7 (Ur/Uf) for firmness and elasticity, respectively. Measurements were conducted with a 2 mm probe under 400 mbar negative pressure, applying 3-second suction and 3-second relaxation for three cycles.

Skin texture and wrinkles were analyzed using the Visia® system (Canfield Scientific Inc., NJ, USA). Skin tone homogeneity was assessed through direct analysis of the color histogram in high-resolution digital images, using ImageJ software.

Facial contour reduction was assessed with Vectra® XT (Canfield Scientific Inc., NJ, USA). The facial angle was calculated from images of the face and neckline; a reduced angle indicates contour improvement. Periocular wrinkle (crow's feet) depth was evaluated using the Primos® CR device (Canfield Scientific GmbH, Germany). Wrinkle depth was measured as the maximum furrow length, while roughness (R) represented the average absolute profile height, with higher R values indicating deeper wrinkles. Dermal density was assessed using the Ultrascan UC 22 MHz ultrasound (Courage & Khazaka, Germany), with images captured from the forearms and analyzed through dedicated software.

Skin biopsies were collected (2mm dermatological punch) at the initial time and after 28 days and fixed in 4% paraformaldehyde (pH 7.4) for 24 hours and cryoprotected in 30% sucrose solution for 72 hours. Serial 12 µm sections were obtained using a cryostat (Leica CRYOCUT 1800) and mounted on silanized slides. Sections were washed with phosphate buffer and incubated overnight with primary antibodies targeting type I pro-collagen and elastin. After washing, sections were incubated with Alexa Fluor 488-conjugated secondary antibody (1:1000), followed by DAPI staining for nuclear visualization. Slides were mounted with a specific medium and analyzed using a fluorescence microscope (Olympus BX53) with CellSens software. Fluorescence intensity was quantified using ImageJ software (version 1.48) and expressed in arbitrary units (A.U.). Data were analyzed using Unpaired T-Test. A significance level of 5% was adopted (GraphPad Prism v8).

3. Results

3.1. Clinical effects on skin firmness and elasticity

Figure 1 illustrates the effects of the skincare formulation on forearm skin firmness and elasticity over a 56-day period. Significant increases in the R5 and R7 parameters were observed at days 28, and 56 compared to baseline (T0), indicating that the F4565.33896 effectively enhanced skin firmness and elasticity.

Treatment with F4565.33896 significantly increased skin firmness by 19.7% at 28 days, and 20.9% at 56 days. Similarly, skin elasticity improved by 12.7% at 28 days, and 13.4% at 56 days.

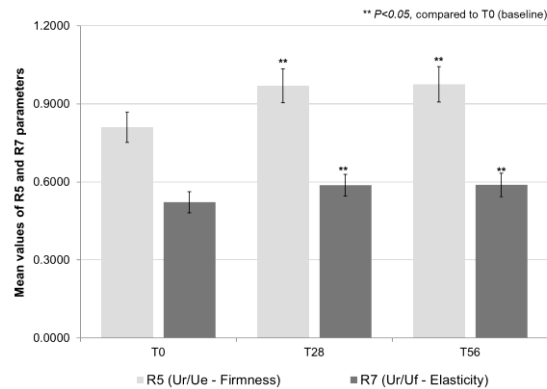


Figure 1. Mean values of R5 and R7 parameters at baseline (T0), T28 and T56. Data are expressed as mean \pm SD ($n = 33$ participants; paired Student's t-test).

3.2. Clinical effects on skin texture and reduction of wrinkles

Texture analysis was performed considering skin smoothness, based on color gradations relative to the surrounding skin tone, as well as the topographic profile of the surface, including elevations and depressions. Wrinkles and expression lines were identified by the software as furrows, folds, or creases and quantified accordingly. Both parameters—texture and wrinkles—were analyzed based on the 'Score' values generated by the Visia® imaging system, as presented in Figure 2.

Treatment with F4565.33896 led to a significant improvement in facial skin texture ($P < 0.05$), with an average enhancement of 4.7%—reaching up to 8.1%—at T28, and 6.0%—reaching up to 9.1%—at T56, compared to baseline. Notably, 67% of participants exhibited improved skin texture at T28, increasing to 70% by T56.

Regarding to wrinkle reduction, F4565.33896 significantly decreased the appearance of wrinkles and expression lines ($P < 0.05$), with mean reductions of 9.2% (up to 13.7%) at T28, and 11.0% (up to 16.2%) at T56. Correspondingly, 68% of participants showed improvement at T28, rising to 79% at T56.

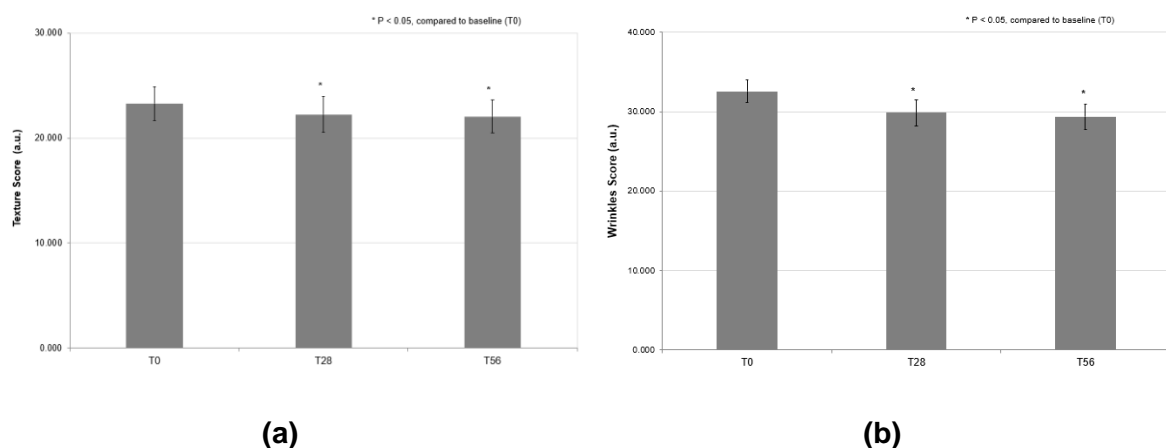


Figure 2. Mean score values for skin texture (a) and wrinkles (b) at T0, T28 and T56. Data are expressed as mean \pm standard error (SE) ($n = 33$ participants; paired Student's t-test).

3.3. Clinical effects on skin tone homogeneity

Skin color variation in the region of interest was assessed by direct analysis of the color histogram. A narrower histogram reflects greater uniformity of skin tone, indicating improved color homogeneity. Figure 3 shows the mean values of skin color variation obtained through histogram analysis at different time points.

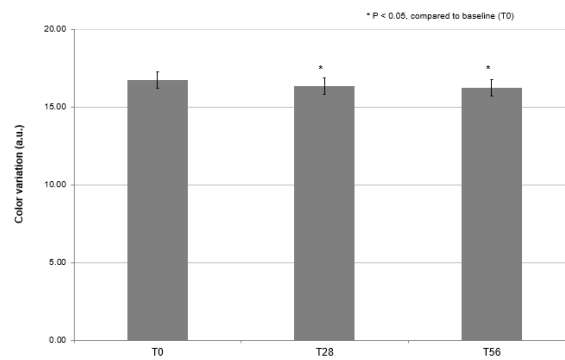


Figure 3. Mean values of color variation at T0, T28 and T56. Data are expressed as mean \pm SE (n = 33 participants; paired Student's t-test).

Treatment with the product led to a significant reduction ($P < 0.05$) in skin color variation: 2.2% (up to 2.6%) at T28, and 3.0% (up to 3.4%) at T56. These results demonstrate a progressive improvement in skin tone evenness over time. Remarkably, 100% of participants exhibited enhanced color homogeneity from T28 through T56, reinforcing the product's efficacy in promoting a more uniform skin appearance.

3.4. Clinical effects on reduction of deep wrinkles

Deep wrinkles were analyzed through 3D images. The image obtained was analyzed by using the VAM software, version 5.9.7, Canfield Scientific Inc, by inserting parallel lines over the determined region and the intensity of the wrinkles were measured. Figure 4 shows the mean values of wrinkles intensity.

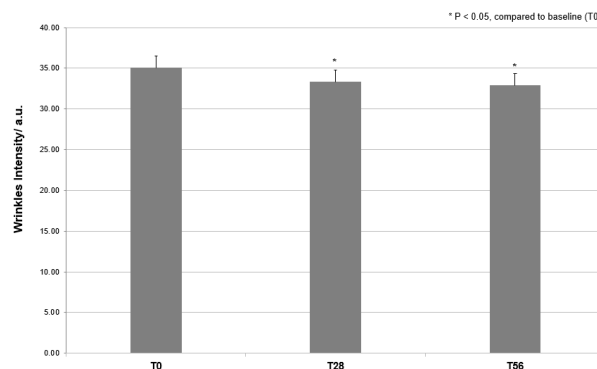


Figure 4. Mean values of wrinkles intensity at T0, T28 and T56. Data are expressed as mean \pm SE (n = 33 participants; paired Student's t-test).

The product F4565.33896 improved significantly the appearance of wrinkles ($P < 0.05$), with mean reductions of 4.9% (up to 6.9%) at T28, and 6.1% (up to 8.5%) at T56. Correspondingly, 76% of participants showed improvement at T28 and 73% at T56.

3.5. Clinical effects on reduction of contour definition

To evaluate the sagging improvement, the angle of the facial contour was calculated. The smaller the angle, the smaller the width of the face and lower the sagging. Therefore, this parameter can be related to the improvement of facial contour. Figure 5 shows the mean values of angle.

According to the results, F4565.33896 promoted a significant reduction of sagging ($P < 0.05$), with mean of increase of facial contour definition of 0.8% (up to 1.1%) at T28, and 1.1% (up to 1.4%) at T56. 91% of participants showed improvement at T28 and T56.

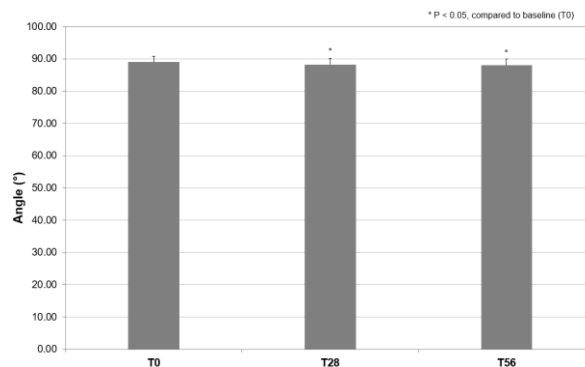


Figure 5. Mean values of angle at T0, T28 and T56. Data are expressed as mean \pm SE ($n = 33$ participants; paired Student's t-test).

3.6. Clinical effects on dermal density

Dermal density was measured and Figure 6 shows the mean values obtained.

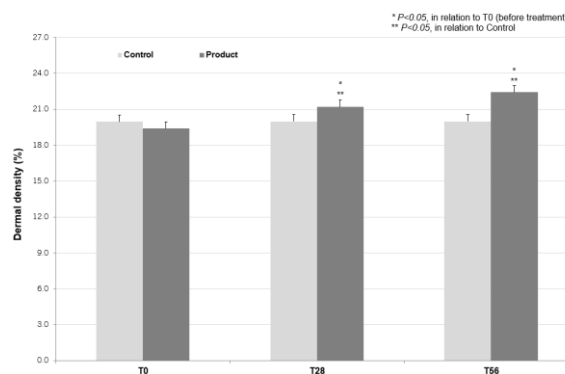


Figure 6. Mean values of dermal density at T0, T28 and T56. Data are expressed as mean \pm SE ($n = 33$ participants; paired Student's t-test).

The product F4565.33896 promoted a significant increase of skin density ($P < 0.05$), with mean reductions of 9.4% (up to 11.8%) at T28, and 16.1% (up to 19.5%) at T56. Correspondingly, 100% of participants showed improvement at T28 and T56.

3.7. Immunofluorescence marks evaluation on biopses

After 28 days of use, treatment with F4565.33896 significantly increased the expression of key dermal markers in human skin biopsies compared to baseline control. Specifically, a 191.85% increase in pro-collagen I production was observed, along with a 169.37% increase in elastin and a 283.96% increase in hyaluronic acid. All results were statistically significant (Figura 7; $P < 0.001$). The graphs representing mean values and standard deviations for each marker are presented in Figure 8.

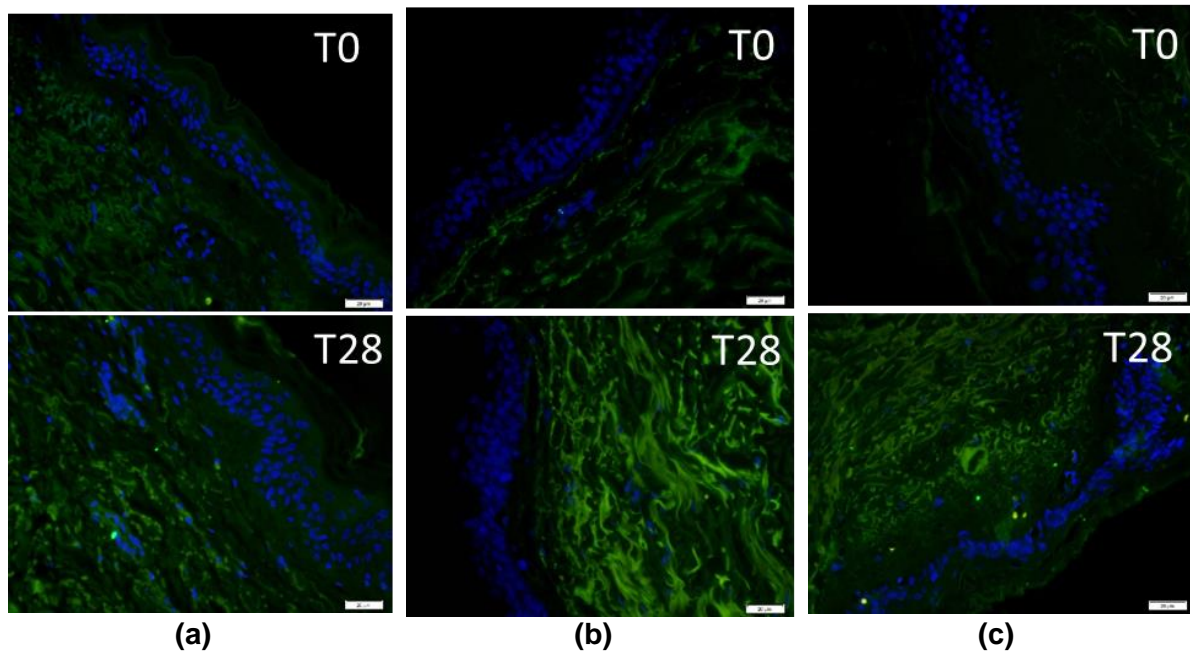


Figure 7. Evaluation of immunofluorescence of (a) type I pro-collagen, (b) elastin and (c) hyaluronic acid synthesis in biopsies. (T0) Untreated skin (T28) skin treated with the F4565.33896. The reference bar corresponds to 20 µm.

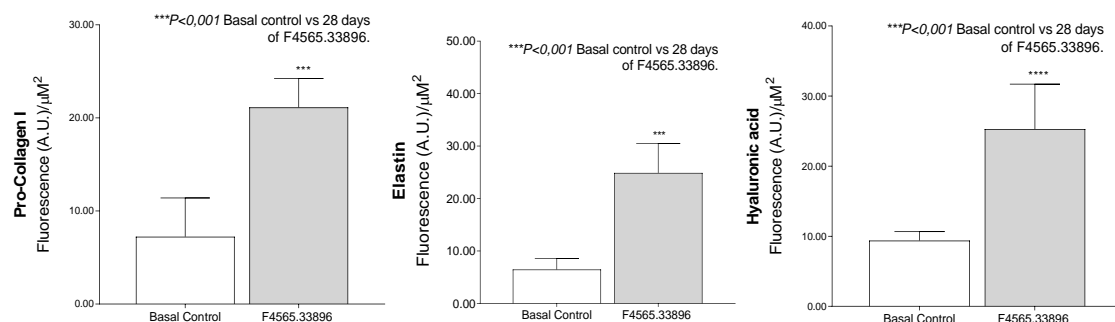


Figure 8. Semi-quantification of the fluorescence intensity of type I pro-collagen, elastin and hyaluronic acid synthesis in biopsies collected before and after 28 days of treatment with F4565.33896. Data represent the mean \pm standard error (T-test).

4. Discussion

Cutaneous aging is one of the most evident signs of the systemic aging of the organism. Unlike other organs, the skin is constantly exposed to environmental stress and damage, being particularly vulnerable to UV radiation, which exacerbates the natural degenerative processes associated with cutaneous aging [10]. The appearance of the skin has a significant impact on quality of life, with recognized effects on emotional and psychological aspects [4]. This increasing awareness has intensified scientific and clinical interest in skin aging, as the global population progressively ages. This phenomenon has driven the demand for effective therapeutic strategies for the prevention and management of clinical signs of skin aging [11]. In this context, the use of cosmetic products represents a strategic approach to improving appearance and mitigating the effects of both intrinsic and extrinsic cutaneous aging. Interventions for the management of skin aging include protection against environmental stressors, neutralization of free radicals, and the provision of bioactive nutrients essential for skin cell function [7].

This study evaluated the effects of the skincare formulation F4565.33896 on the reduction of visible signs of cutaneous aging over a 56-day period. The results demonstrated progressive improvements in skin elasticity and firmness, which are clinically relevant for maintaining skin integrity, as well as enhancing the appearance of wrinkles and skin laxity. Skin firmness increased by up to 20.9%, while elasticity showed an improvement of up to 13.4%. Improvements in both firmness and elasticity were observed as early as the first week of product use, and the increase in both parameters followed a progressive pattern throughout the 56-day treatment period, suggesting that continuous use of the F4565.33896 formulation induces a cumulative effect on the biomechanical properties of the skin.

The results of this study demonstrate that continuous use of Product F4565.33896 exerted clinically relevant and statistically significant effects on multiple skin parameters. Progressive improvement in skin texture and reduction of superficial wrinkles were observed, already noticeable in 67–70% of volunteers after 28 and 56 days. Similarly, there was a significant reduction in the intensity of deep wrinkles, highlighting the increased proportion of participants who benefited over time, thus validating the product's efficacy for both fine lines and deeper wrinkles.

Regarding skin tone evenness, all participants showed significant color homogenization, indicating the product's potential in reducing facial dyschromia. Improvement in facial contour was also observed, evidenced by both the reduction of sagging and the increase in facial definition in more than 90% of individuals. Furthermore, there was a marked increase in dermal density, an important indicator of structural skin integrity.

These results suggest that Product F4565.33896 promoted multidimensional effects on the skin, ranging from functional improvements to measurable morphological changes, reinforcing its potential as an effective intervention in anti-aging dermatological care.

The topical application of F4565.33896 for 28 days promoted significant increases in the production of pro-collagen I, elastin, and hyaluronic acid, key components involved in maintaining skin structure and function. Collagen is essential for providing mechanical strength and stability to the dermal matrix, and its decline is a hallmark of skin aging, leading to wrinkling and loss of firmness [12]. The increase in pro-collagen I observed in this study suggests that the product effectively stimulates collagen synthesis, contributing to improved skin firmness and resilience.

Similarly, the increase in elastin production supports the product's ability to restore elasticity, another critical aspect of youthful skin. Elastin degradation is strongly associated with reduced skin recoil and sagging with age [13], and interventions that stimulate elastogenesis are highly desirable in anti-aging strategies. Hyaluronic acid plays a central role in water retention within the dermal extracellular matrix, directly influencing skin turgor, smoothness, and barrier function [14]. Its natural depletion with age contributes to dryness and fine lines, making its replenishment a fundamental target in cosmetic formulations.

Overall, the statistically significant improvements across all evaluated markers reinforce the effectiveness of F4565.33896 in promoting dermal remodeling processes associated with youthful skin appearance[15].

5. Conclusion

This study, employing advanced evaluation methods, demonstrated that F4565.33896 significantly improved skin firmness and elasticity, reduced wrinkles and sagging, while also increasing dermal density and enhancing the synthesis of type I collagen, elastin, and hyaluronic acid, suggesting extracellular matrix regeneration. These findings highlight the potential of the active ingredients in the formulation to improve the biomechanical properties of the skin and mitigate signs of aging, consistent with the observed clinical results.

6. References

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