Clinical assessment of a skincare formulation in modulating biological mechanisms involved in skin barrier integrity

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Abstract

Skin barrier is greatly influenced by interconnected components that contribute to structure, organization and communication of epidermal layers. This study aimed the clinical evaluation of a skincare formulation (F4565.33347.116.3) on the production of biological markers involved in skin barrier integrity in skin biopsies samples. F4565.33347.116.3 was applied to the right forearm of research participants (n=9, 48±15y). Left forearm was considered a control site. Skin biopsies (2mm punch) were collected on T0, T7, T14 and T28 days of home use and immunostained with specific antibodies against the biomarkers: filaggrin (FLG), ceramide synthase (CerS), aquaporin-3 (Aqp3), integrin β1 (Int-β1) and hyaluronic acid (HA). F4565.33347.116.3 promoted a significant increase in FLG, CerS, Aqp3, Int-β1 and HA labeling after 7, 14 and/or 28 days of home use, compared both to compared to the same forearm before treatment (T0) and to corresponding untreated contralateral forearm. Skin biopsy is a valuable tool in dermatological research, enabling an in-depth exploration of the underlying mechanisms of topically applied cosmetics and medicines. Using this technique, our findings revealed the effects of a skincare formulation in modulating key biological pathways associated with strengthening the skin barrier.

Keywords: skin barrier; hydration; skin biopsy; skincare

Introduction

At the core of skin protective barrier lies the stratum corneum (SC), a sophisticated structure comprising corneocytes embedded in a lipid-rich matrix. This intricate arrangement, known as the skin barrier, plays a pivotal role in maintaining skin health and homeostasis. The integrity of the skin barrier is essential for regulating transepidermal water loss (TEWL), preventing the penetration of harmful substances, and orchestrating immune responses [1-3].

Several factors, such as aging, sun exposure, microbiota dysbiosis, certain skin ailments, and nutrient-poor diets, can compromise the integrity of skin barrier, precipitating a range of dermatological issues [4-5]. Due to this compromise, the skin loses its hydration, resulting in itching, eczema, opacity, loss of elasticity, exacerbation of immunological and inflammatory responses [1].

This natural barrier critically depends on its composition, represented mainly by proteins and lipids. Of the total proteins present in the SC, approximately 5% corresponds to a structural portion called the cornified epidermal envelope, which externally involve keratinocytes and play a fundamental role in structuring and organization of intracellular lipids [6]. The most highlighted constituent of cornified epidermal envelope is filaggrin [7]. The endogenously present ceramides (essentials sphingolipids) are derived from the cleavage of filaggrin and make up the natural hydration factor, act by retaining water in SC and, therefore, help maintain skin hydration [8]. Another important factor that plays a crucial role in skin hydration and integrity of the cornified epidermal envelope is the presence of aquaporins. Aquaporins are proteins that favor the transport of water, urea and glycerol across the plasma membrane. Among the aquaporins, aquaporin-3 is expressed in the basal layer of the epidermis and aquaporin-3 deficiency is related to xerosis, reduced hydration in the SC and decreased of skin elasticity [9].

Epidermal homeostasis depends on the balance between stem cell renewal and differentiation and is regulated by extrinsic signals from the extracellular matrix (ECM). Integrins are cell adhesion molecules found on the surface of cells, acting as bridges between the extracellular matrix (ECM) and the cytoskeleton, facilitating cell-cell and cell-ECM

interactions [10]. Integrins interact with HA through specific receptors, such as CD44, which plays a vital role in regulating cell behavior, including cell adhesion, migration, proliferation, and differentiation [11]. In the context of the skin, the interplay between integrins and HA influences several aspects of skin physiology.

Proper skin barrier function depends on a complex interaction of several biological markers, including filaggrin, ceramide, aquaporin, integrin, and hyaluronic acid. In this work, we investigated the effects of a skincare formulation on these key markers involved in skin barrier integrity using skin biopsy samples.

Materials and Methods

Test product: a skincare formulation - F4565.33347.116.3, was based on a sérum formula composed by *Fevillea trilobata*, tocopherol, *Gardenia florida*, hyaluronic acid, silanetriol, sodium hyaluronate and *Casearia sylvestris*.

Clinical study: this study was approved by the Ethics Committee of the University São Francisco – SP - CAAE 45889021.2.0000.5514, opinion nº 4.789.325. Nine research participants, female, aged 45 to 57 years, skin types II to V according Fitzpatrick [12], and regular users of facial cosmetic were selected for this study. Participants were instructed to stop using any cosmetics products 72 hours before the first day of the study and got instruction not to use any other products during the study period. During 28 days, F4565.33347.116.3 was applied to randomized forearm of research participants. The contralateral forearm was considered a control site. Skin biopsies from the forearms were collected before the beginning of study (T0), after 7 days (T7), 14 days (T14) and after 28 days (T28) of home use of serum, followed by immunofluorescence analysis.

Immunofluorescence evaluation: skin biopsies were fixed in paraformaldehyde and cryoprotected in sucrose solution. Serial sections were collected on silanized slides with cryostat (Leica - CRYOCUT 1800). Sections were washed with phosphate buffer and

incubated overnight with anti-hyaluronic acid, anti-filaggrin, anti-ceramide synthase, anti-integrin β1 and anti-aquaporin-3. Subsequently, the sections were washed and incubated with the secondary antibody Alexa Fluor 488. Further incubation was performed with DAPI (4'-6-diamidino-2-phenylindole; DNA marker). The slides were assembled using specific mounting medium and analyzed under the microscope (OLYMPUS BX53) using standard CellSens software (© 2010 OLYMPUS CORPORATION). The fluorescence intensity parameter emitted by the labeling of specific antibodies was evaluated. After obtaining the images, the fluorescence intensity was quantified using ImageJ software (Arbitrary Units - A.U.).

Statistical analysis: ANOVA test was used allowing the measurement of variation of the results, comparing the data between all the groups. The Bonferroni post-test was then applied, which reinforced and made even more accurate the result presented in the ANOVA test. For both analyses, the significance level of 5% (GraphPad Prism v6) was used.

Results

Clinical effects of F4565.33347.116.3 on filaggrin production: Figure 1 demonstrate the effects of 28 days-treatment with the skincare formulation on filaggrin staining in forearm biopsies. As we can see in Figure 1A, the sign for filaggrin is revealed in green in histological sections and it shows marking/distribution located in epidermis. The F4565.33347.116.3 treatment promoted a significant increase in filaggrin labeling after 14 and 28 days of home use, compared to the same forearm before treatment (T0 - dark green columns; 248.54 and 243.67%, respectively; Figure 1B). Respective increases of 80.12 and 56.31% in filaggrin production were also observed at T14 and T28, compared to the control (contralateral – light green) forearm at the same evaluation times.

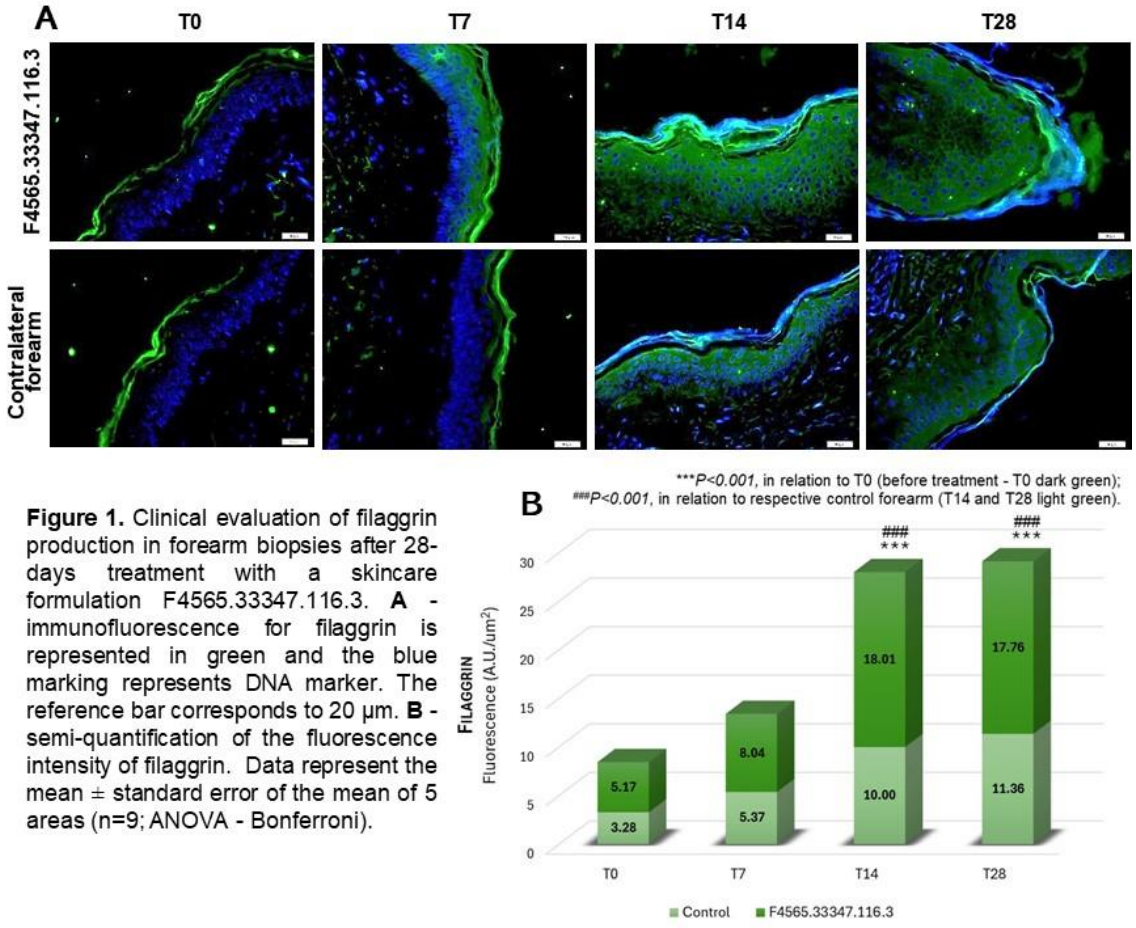


Figure 1. Clinical evaluation of filaggrin production in forearm biopsies after 28-days treatment with a skincare formulation F4565.33347.116.3. **A** - immunofluorescence for filaggrin is represented in green and the blue marking represents DNA marker. The reference bar corresponds to 20 µm. **B** - semi-quantification of the fluorescence intensity of filaggrin. Data represent the mean ± standard error of the mean of 5 areas (n=9; ANOVA - Bonferroni).

Clinical effects of F4565.33347.116.3 on ceramide synthase production: Figure 2A shows the results of ceramide synthase labeling (green) located in epidermis. As described in Figure 2B, F4565.33347.116.3 treatment promoted a significant increase in ceramide synthase production after 7 and 28 days of home use, compared to the respective contralateral control (T7 and T28 - light green) at the same evaluation times (87.60 and 57.62%, respectively).

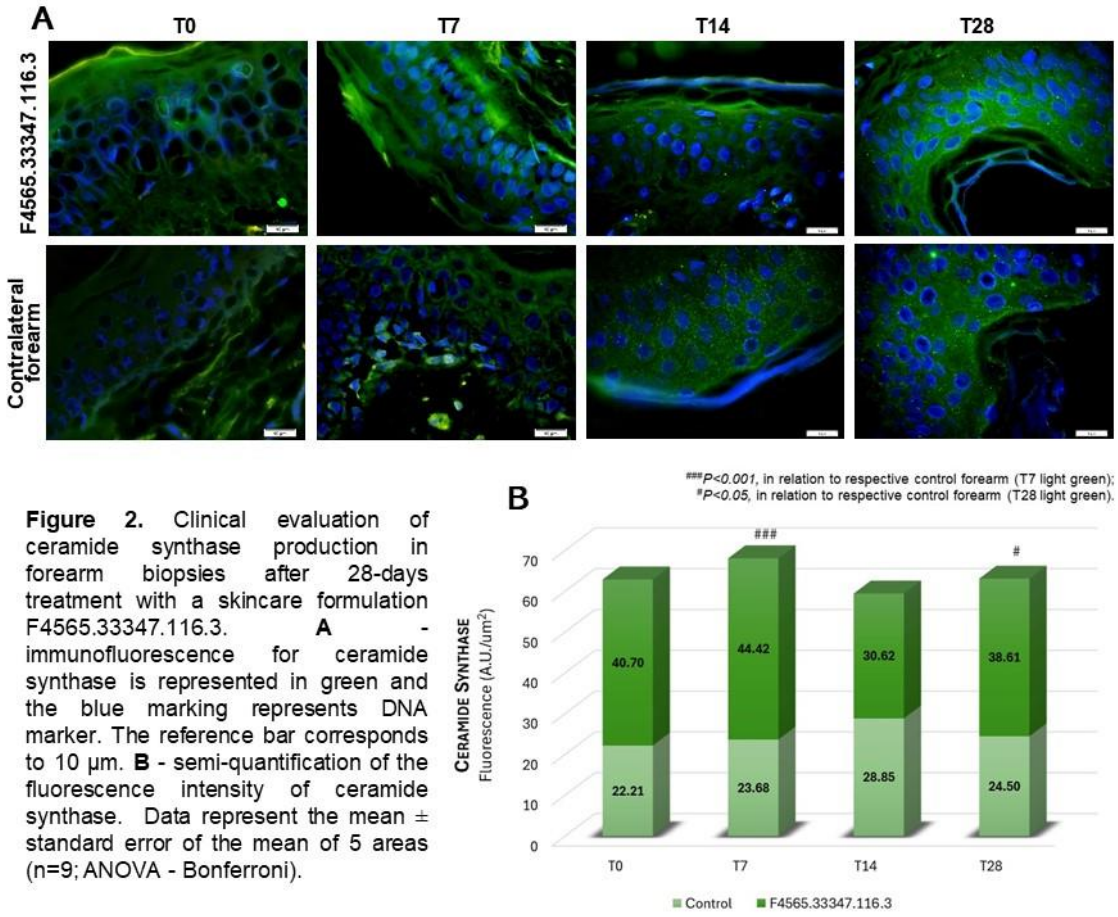
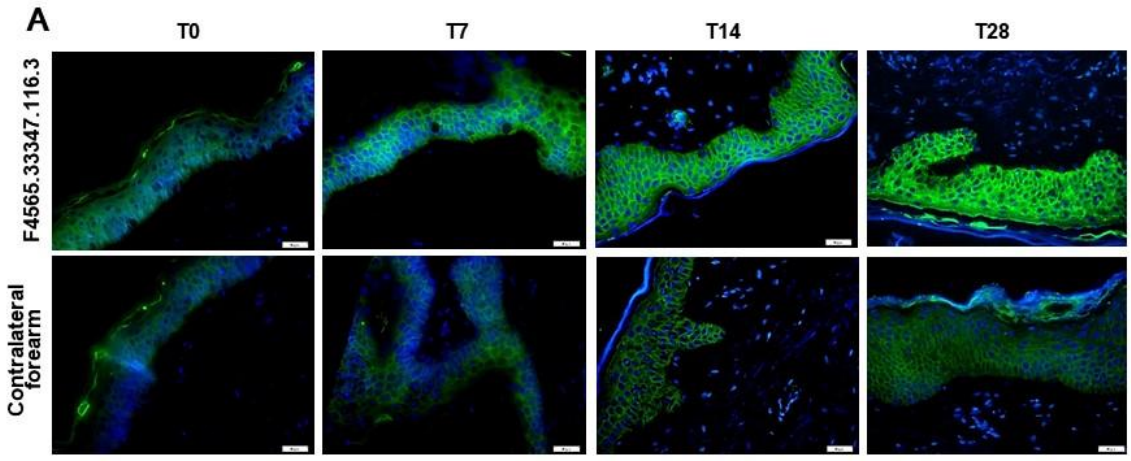


Figure 2. Clinical evaluation of ceramide synthase production in forearm biopsies after 28-days treatment with a skincare formulation F4565.33347.116.3. **A** - immunofluorescence for ceramide synthase is represented in green and the blue marking represents DNA marker. The reference bar corresponds to 10 µm. **B** - semi-quantification of the fluorescence intensity of ceramide synthase. Data represent the mean ± standard error of the mean of 5 areas (n=9; ANOVA - Bonferroni).

Clinical effects of F4565.33347.116.3 on aquaporin-3 production: Figure 3A represent the result of labeling aquaporin-3. At T14 and T28, F4565.33347.116.3 promoted an increase of 153.14% and 130.85% (*P<0.001*), respectively, in relation to the same forearm before treatment (T0 - dark green columns). In relation to the respective contralateral forearm (T14 and T28 – light green columns), the product promoted increases of 88.22 and 86.51%, respectively, over 14 and 28 days of treatment.



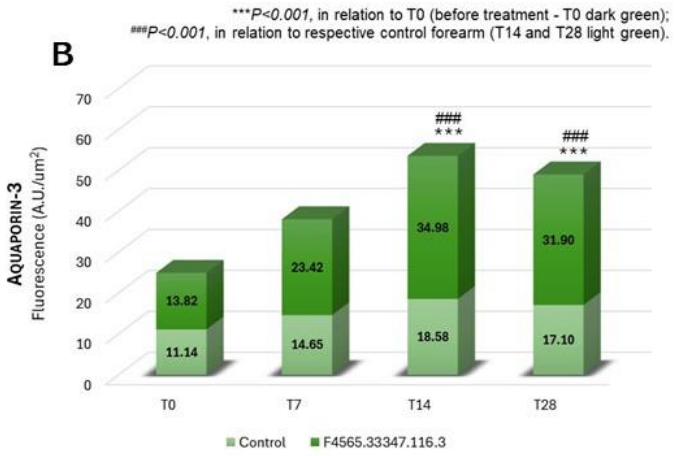


Figure 3. Clinical evaluation of aquaporin-3 production in forearm biopsies after 28-days treatment with a skincare formulation F4565.33347.116.3. **A** - immunofluorescence for aquaporin-3 is represented in green and the blue marking represents DNA marker. The reference bar corresponds to 10 µm. **B** - semi-quantification of the fluorescence intensity of aquaporin-3. Data represent the mean ± standard error of the mean of 5 areas (n=9; ANOVA - Bonferroni).

Clinical effects of F4565.33347.116.3 on integrin β1 production: the effects of treatment with F4565.33347.116.3 after 28 days are described in Figure 4. Figure 4A shows the labeling of the epidermis with integrin antibody. Statistical analysis revealed a significant stimulating action on integrin synthesis after 7, 14 and 28 days of clinical treatment with F4565.33347.116.3. On days 7, 14 and 28, the forearms treated with F4565.33347.116.3 showed greater staining for integrin, reaching 51.70%, 40.72% and 75.10%, respectively, in relation to the same forearm before of treatment (T0 - dark green columns). Additionally, increases of 56.43%, 102.02% and 98.54% (*P<0.001*) were observed compared to the respective contralateral forearm at T7, T14 and T28 (light green columns).

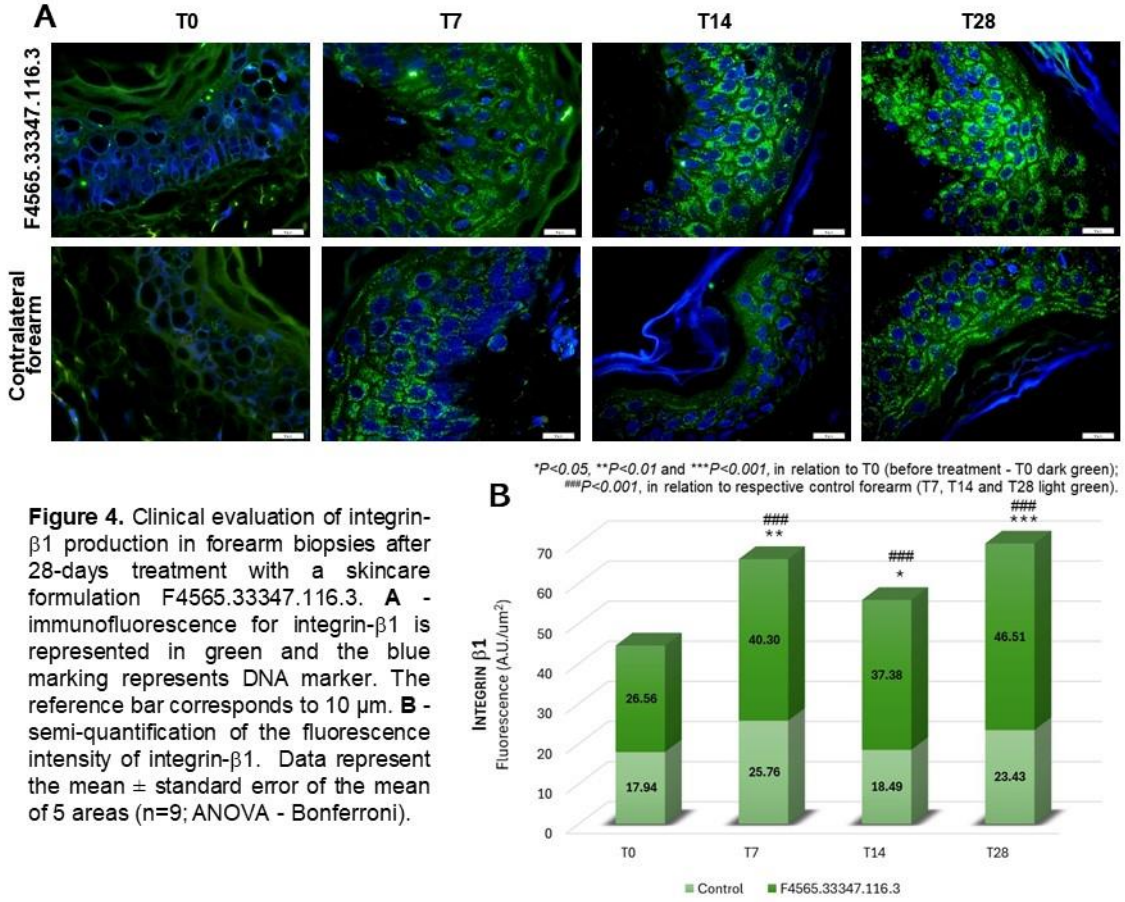


Figure 4. Clinical evaluation of integrin-β1 production in forearm biopsies after 28-days treatment with a skincare formulation F4565.33347.116.3. **A** - immunofluorescence for integrin-β1 is represented in green and the blue marking represents DNA marker. The reference bar corresponds to 10 µm. **B** - semi-quantification of the fluorescence intensity of integrin-β1. Data represent the mean ± standard error of the mean of 5 areas (n=9; ANOVA - Bonferroni).

Clinical effects of F4565.33347.116.3 on hyaluronic acid production: as we can see in Figure 5, the sign for hyaluronic acid is revealed in green in histological sections of biopsies, treated with F4565.33347.116.3 and is distributed throughout the dermis and epidermis (Figure 5A). Specifically in Figure 5B, the labeling intensity for hyaluronic acid is significantly increased throughout treatment, showing 4.10- and 6.14-fold increase at T14 and T28, respectively, in relation to the same forearm before of treatment (T0 - dark green columns). When compared to the contralateral forearm (T14 and T28 – light green columns), the treatment reveals increases of 77.67 and 115.45% (*P<0.001*), respectively.

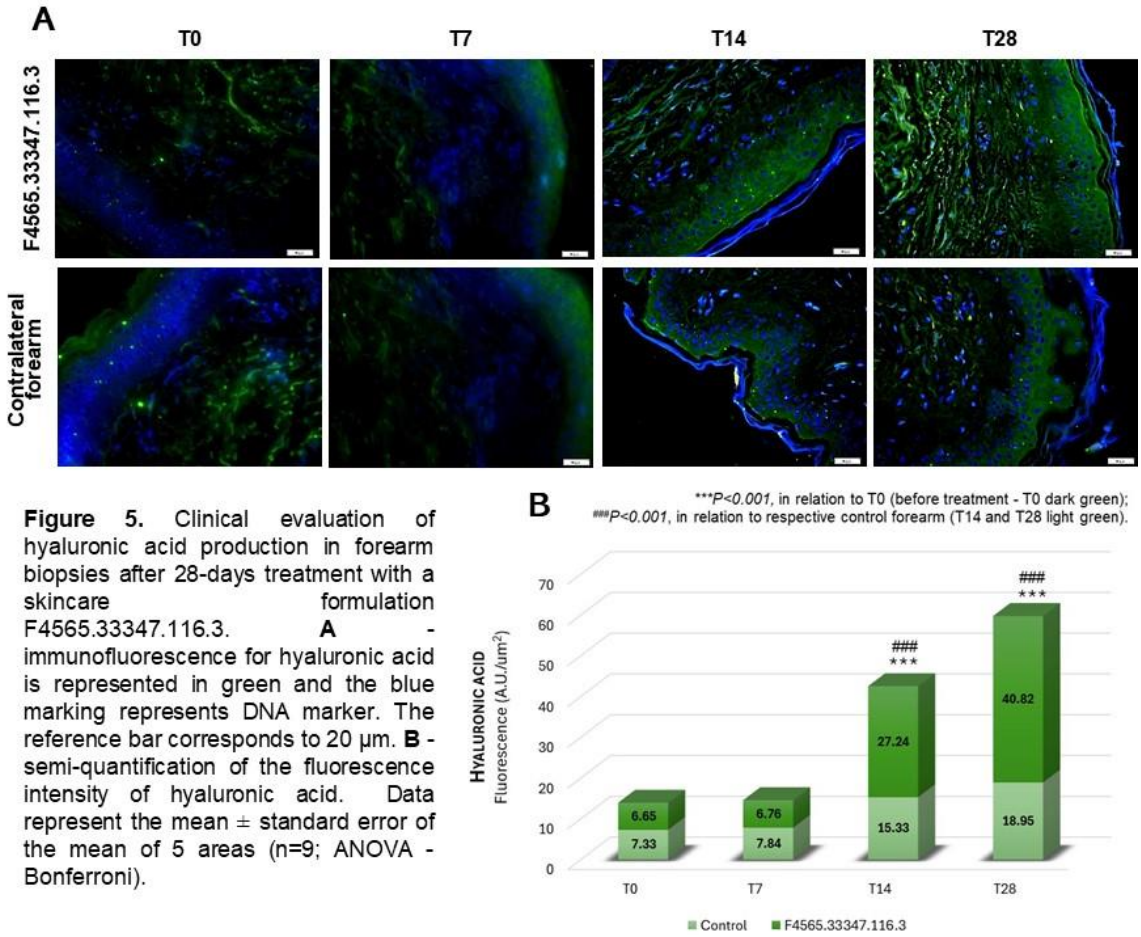


Figure 5. Clinical evaluation of hyaluronic acid production in forearm biopsies after 28-days treatment with a skincare formulation F4565.33347.116.3. **A** - immunofluorescence for hyaluronic acid is represented in green and the blue marking represents DNA marker. The reference bar corresponds to 20 µm. **B** - semi-quantification of the fluorescence intensity of hyaluronic acid. Data represent the mean ± standard error of the mean of 5 areas (n=9; ANOVA - Bonferroni).

Discussion

Chronological and extrinsic injuries promote disturbances in the epidermis, reducing the agility of prompt restoration mechanisms [13]. In unbalanced skin, the following structures and functions of the skin barrier are disrupted: skin barrier structure, permeability barrier function, epidermal calcium gradient, epidermal lipid synthesis, cytokine production and response after insults, antimicrobial barrier, and SC hydration [14]. Maintenance of optimal SC hydration is an important function of the epidermis and is dependent on some factors such as the amount of natural moisturizing factors (NMFs), which are a complex mixture of low molecular-weight substances produced within corneocytes by filaggrin degradation [3, 7, 14].

The epidermal protein profilaggrin is lately synthesized during epidermal differentiation and plays a key role in generating and maintaining SC flexibility and moisturizing [14]. Its main function is to bind to keratin fibers, helping to form a robust barrier that prevents moisture loss and protects against external aggressors.

Other important components of the skin barrier are ceramides and aquaporins. Ceramides are lipid molecules essential for the structure and function of the skin barrier and are synthetized by ceramide synthase. Ceramides play a key role in maintaining skin hydration, preventing TEWL, helping to strengthen the skin barrier, keeping it resistant and healthy [8]. Aquaporins are a family of membrane proteins that facilitate the transport of water across cell membranes. In the skin, aquaporins help regulate the movement of water within different layers of the epidermis and between the epidermis and the dermis. This regulation is vital for maintaining proper hydration levels in the skin and ensuring its overall health and function [9].

Connecting the epidermis to the dermis, integrins act as cell surface receptors and play an important role in cell adhesion and communication within the skin. They help anchor basal layer keratinocytes to the extracellular matrix, providing structural support and contributing to the overall integrity of the skin barrier [10]. Additionally, hyaluronic acid is a key component of the extracellular matrix, where it helps maintain hydration and structural integrity. It has the remarkable ability to retain up to a thousand times its weight in water,

which makes it essential for robust, hydrated skin. Additionally, hyaluronic acid also plays a role in wound healing and tissue repair [11, 15].

In this work we evaluated the ability of a skincare formulation - F4565.33347.116.3 to improve the integrity of skin barrier by mechanisms involving increases in FLG, CerS, Aqp3, Int-β1 and HA in human skin biopsies up to 28 days of clinical treatment.

Skin biopsies provide invaluable information in dermatological research on various aspects of skin biology, pathology and therapeutics [16-22]. Through histological and molecular analyzes of skin tissue obtained from punch biopsies, researchers can identify changes in several biomarkers involved in skin disorders, from aesthetic discomforts to more severe pathologies. By unveiling the mechanisms underlying skin disorders, it is possible to identify potential biomarkers for the diagnosis and prognosis of diseases and evaluate the effectiveness of new therapeutic approaches [17-20].

Cosmetic applications represent another area where skin biopsies can provide precious information. Clinical studies involving skin biopsies are often conducted by cosmetic companies to evaluate the safety and efficacy of skin care products, cosmetic procedures, and anti-aging products. Through meticulous analysis of changes in skin structure, hydration levels, elasticity, and collagen synthesis, researchers gain important insights into the efficacy of cosmetic interventions in enhancing skin health and aesthetic appearance. This analytical approach not only informs the refinement of existing products but also guides the trajectory of future innovations, facilitating the development of targeted skincare solutions and strategic marketing strategies [21-22].

Particularly in this work, skin biopsies allowed us to evaluate the main components of the skin barrier, such as the expression of barrier-related proteins and epidermal lipid composition. This understanding led us to the development of a formulation aimed at restoring barrier function and preventing skin diseases associated with epidermal involvement, such as dryness, itching and difficulty in wound healing. Therefore, maintaining the health and integrity of these essential components is crucial for overall skin health and well-being.

Conclusion

Skin biopsy is a valuable tool in dermatological research, enabling an in-depth exploration of the underlying mechanisms of topically applied cosmetics and medicines. Using this technique, our findings revealed the effects of a skincare formulation in modulating key biological pathways associated with strengthening the skin barrier. This was achieved by significantly enhancing the production of FLG, CerS, Int-β1, AQP3 and HA. This intricately orchestrated biological system plays a pivotal role in ensuring the structuring of the cornified epidermal envelope, synthesis of NMFs, ordered alignment of lipids, efficient water transport across the plasma membrane, cell-cell and cell-extracellular matrix interactions, and tissue repair. The elucidation of the underlying biological mechanisms of F4565.33347.116.3 is in line with clinical outcomes observed in terms of hydration and skin barrier improvement. These insights contribute significantly to the overall robustness of our findings, reinforcing the positive impact of the skincare formulation on skin health.

Acknowledgments

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Conflict of Interest Statement

Silas Arandas Monteiro e Silva and Leticia Gomes are employee at Natura&Co, study sponsor. Ana Lúcia Tabarini Alves Pinheiro, Gustavo Facchini, Barbara de Freitas Carli, Vania Renata Gonçalves and Samara Eberlin are employees at Kosmoscience Group, responsible for carrying out the study.

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