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Standardization of Skin Care Routine Design and Skin Phenotype Diagnosis Facilitates Machine Learning and AI

Leslie Baumann, MD; Skin Type Solutions

Introduction of research

The lack of standardization and hierarchical classification systems for skin types, skin care products and skin care regimens hinder the ability to collect the high-quality data sets needed for machine learning and deep learning processes that are required for accurate AI. Over the last 17 years, we have developed and tested a skin care routine recommendation engine based on a skin typing taxonomy with the help of over 100 dermatologists. This system that is independent from skincare brands, is discussed in the major dermatology and plastics surgery textbooks and used by over 280 doctors in the US. This system is also used commonly in Korea and is the subject of many dermatologic publications.

Body content here

Over 250 medical providers in the US, 100 of which are dermatologists, gave a scientifically validated self-administered skin type diagnostic questionnaire to patients seeking skincare advice. The results obtained include skin typing data on over 100,000 patients, finalization of over 40,000 skincare routine structures, and definition of the skincare product requirements for the "slots" in the skin care routine steps. My lecture will discuss the infrastructure used to collect this data.

Conclusion

A standardized taxonomy and structure allow for generalizability of collected skin type and skincare recommendation data regardless of skincare brand. This type of infrastructure is necessary for accurate machine learning and deep learning used in AI.

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About the speaker

Leslie Baumann, M.D. founded the University of Miami Cosmetic Medicine and Research Institute in 1997- the first academic institute in the US dedicated to cosmetic dermatology. She served as Professor of Dermatology at the University of Miami, Miller School of Medicine until 2006 when the Institute became independent from the University and the name changed to the Baumann Cosmetic and Research Institute. Dr. Baumann is the author of 3 bestselling books: *Cosmetic Dermatology: Principles and Practice* (McGraw-Hill 2009), *Cosmeceuticals and Cosmetic Ingredients* (McGraw Hill 2014), and *The Skin Type Solution* (Bantam 2005). Dr. Baumann has been involved in the Phase 3 FDA clinical research trials of the most notable products procedures in cosmetic medicine including Botox®, Dysport, ® Emervel®, Juvéderm®, Kybella® Restylane Silk®, Sculptra®, and Voluma®.

She earned her medical degree from Baylor College of Medicine in Houston, Texas, and completed her residency in Dermatology at the University of Miami, Miller School of Medicine. She is a board-certified dermatologist, member of the American Academy of Dermatology (AAD) and a Fellow of the prestigious American Dermatological Association (ADA).

Atopic dermatitis comprehension thanks to a new 3D microbial inflammatory skin model

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Introduction

Atopic dermatitis (AD) is a common multifactorial dermatosis due to complex interactions between genetic factors and the environment. It has been associated with disordered inflammatory type 2 immune reaction and impaired epidermal barrier integrity. A microbial dysbiosis characterized by a dominance of Staphylococcus aureus (Sa) is also documented [1].

Objective

In recent years, *in vitro* models reproducing some features of AD have been developed by challenging epidermis with either interleukin (IL) cocktails or Sa extracts, or by silencing the expression of pivotal genes encoding epidermal barrier proteins [2-3]. But none of them reproduced the whole pathophysiological AD features. Our objective was to develop a model reproducing the three main AD features: skin barrier disruption, inflammation and microbial dysbiosis to efficiently select bioactives able to help recovery from AD skin signs.

Methods

Reconstructed 3D Human Epidermis (RHE) were treated with an inflammatory cocktail (Inf C) made of IL-4, IL-13, IL-31 and TNF-alpha from 7 to 14 days at 5ng/ml. RHE were exposed at day 13 to an AD clinical isolate of Sa for 24h. Epidermal alterations were analyzed with a particular focus on keratinocyte differentiation markers, lamellar body trafficking and filaggrin processing, by immunohistology, transmission electron microscopy and western blotting. IL-8 was measured (AlphaLISA) as Sa adherence (1-hour post-seeding) and growth (24 hours post-seeding). A plant extract was also tested on the model for its capability to prevent AD skin features.

Results

Treated RHE exhibited alterations observed in AD: spongiosis, dysregulated expression of differentiation markers (loricrin, filaggrin), caspase 14 decrease and strong IL-8 release. Inflammatory cocktail effects get worse with the addition of Sa. (Fig.1A).

Moreover, we showed that Sa adhered and then grew better in the presence of the inflammatory cocktail (Fig. 1B).

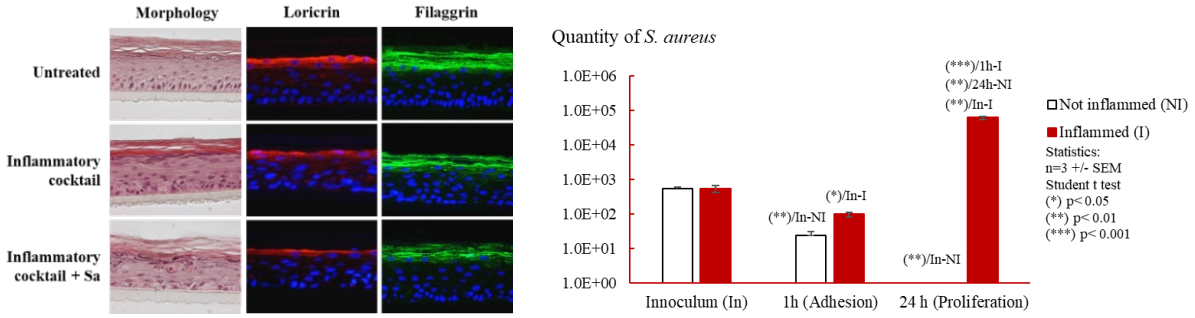

Figure 1 A: Modulation of epidermal morphology (hematoxylin eosin staining) and differentiation markers (loricrin immunostained in red and filaggrin in green) induced by a treatment with an inflammatory cocktail associated or not with Staphylococcus aureus**.** Figure 1B: *Staphylococcus aureus* adhesion and proliferation on atopic RHE.

Finally, the RHE model was used to study the effects of a plant extract pre-selected for its ability to limit virulence factors and biofilm formation (data not shown). In the 3D atopic epidermal model, the plant extract helped to prevent a better epidermal morphology and differentiation as evidenced by loricin and filaggrin staining (Fig. 2A). IL-8 level decrease was also observed (Fig. 2B)

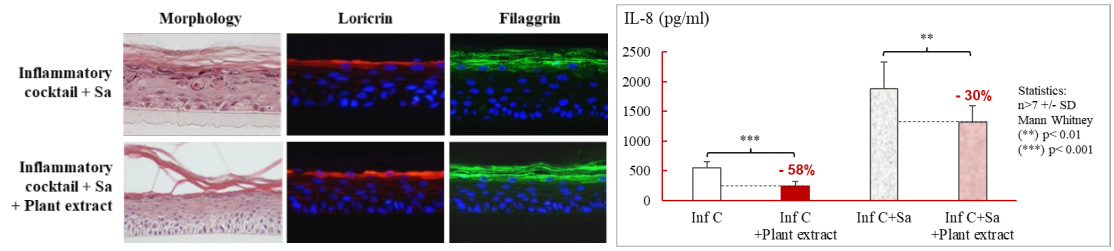

Figure 2A: Modulation by a plant extract of epidermal morphology (hematoxylin eosin staining), differentiation markers (loricrin and filaggrin immunostained in red and in green, respectively) and 2B: IL-8 level in RHEs induced with an inflammatory cocktail associated or not withSa

Conclusion

Our Sa-colonized inflammatory AD epidermal model efficiently mimics a mild to moderate AD skin phenotype. This model well mimics the impairments of skin barrier observed in AD, at the ultrastructural, microbial and immune levels. Moreover, our results strongly suggest that Sa acquires higher virulence potential as soon as the RHE were stressed with the inflammatory cocktail, and that IL-8 synthesis is increased when Sa and inflammation are combined, thus later contributing to enhance chronic inflammatory status.

Our innovative 3D epidermal model may help to improve scientific understanding of the role of skin microbiota in the inflammatory process characteristic of compromised skin. Consequently, it may be considered for *in vitro* screening of cosmetics or therapeutic compounds to open the way to new global preventive or therapeutic strategies for AD skin features.

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About the speaker

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Sébastien CADAU has a master's degree in biology and a PhD in development & oncology from Joseph Fournier University (Grenoble, France).
After a post-doc in Tissue Engineering to develop an innervated and endothelialized skin model at the LOEX in Laval University (Quebec, Canada), he joined BASF Beauty Care Solutions research team to lead the Tissue Engineering platform, implement the skin model portfolio and evaluate the efficacies of new cosmetic ingredients.

Ectopically expressed olfactory receptor OR2AG2 is involved in a skin stress response

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Introduction of research

Olfactory receptors (ORs), discovered in 1991 by Buck and Axel [1], are mainly located in the olfactory epithelium and mediate the first step in odor recognition [2]. Since their original discovery in the nasal cavity, ORs have been found in various other cell types throughout the body where they might regulate physiological cell functions beyond olfaction, including in the skin [3]. Nevertheless, the physiological roles of these new non-nasal ORs remains to be elucidated. In aromatherapy, essential oils and odorants are well-known for their relaxing and anti-stress effects in humans [4]. Therefore, we addressed the question of a potential involvement of skin ORs in the handling of chronic skin stress.

In cultured human primary keratinocytes (NHEK), the presence at the RNA level of randomly selected ORs using quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was investigated. Interestingly, we observed mRNA expression of three ORs (OR10A6, OR11H4, and OR2AG2) never previously described in skin. Their presences were confirmed by immunofluorescence microscopy analysis on NHEK and human skin explants. Surprisingly, these three ORs were localized at different epidermal layers of the skin.

Measurement of intracellular levels of cAMP on HEK293T-RTP1S/RTP2 cells transfected with a plasmid encoding the indicated odorant receptor (OR10A6, OR2AG2, or OR11H4), was used to detect olfactory receptor activity. A screening of 18 compounds allowed us to identify the Phenylethyl alcohol (PEA) as agonist of these three ORs. This molecule is highly present in rose flower. For this reason, a rose extract containing around 56% of PEA was tested. As expected, this product showed a transient increase in cAMP in cells transfected with OR11H4, OR2AG2 and OR10A6. Same results on NHEK with an increased cAMP level was obtained, suggesting that skin ORs could mediate the effect of volatile odorants.

Tabling on the documented positive effect of rose extract on human well-being and the activity found towards ORs, we decided to study its effect on epinephrine-induced stress in skin explants. After 9 days treatment with the latter (56 nM), three stress markers (loricrin, γH2AX, and G6PDH) were measured to estimate the stress response of skin. All showed an increase with fold change of 2.3, 1.7 and 1.26, respectively. Moreover, epinephrine-induced stress prompts a downregulation of mRNA levels of OR10A6, OR2AG2, and OR11H4 with fold change values of 0.45, 0.51, and 0.52, respectively. At the protein level, a significant decrease was only observed for OR10A6 and OR2AG2. Interestingly, co-treatment with epinephrine and rose extract (5×10^{-3}%) inhibited the upregulation of G6PDH, loricrin, and γH2AX expression that comes with epinephrine treatment alone. When looking at OR expression, while adding OR agonist with epinephrine inhibited the decrease for the 3 ORs at the mRNA expression, only OR2AG2

expression, at the protein level, was reestablished to the level seen in untreated skin. Therefore, we hypothesized that activation of ORs, probably OR2AG2, by rose extract may modify epinephrine receptor (β2 adrenergic receptor) conformation in a way that prevents its interaction with epinephrine and therefore avoids the stress response.

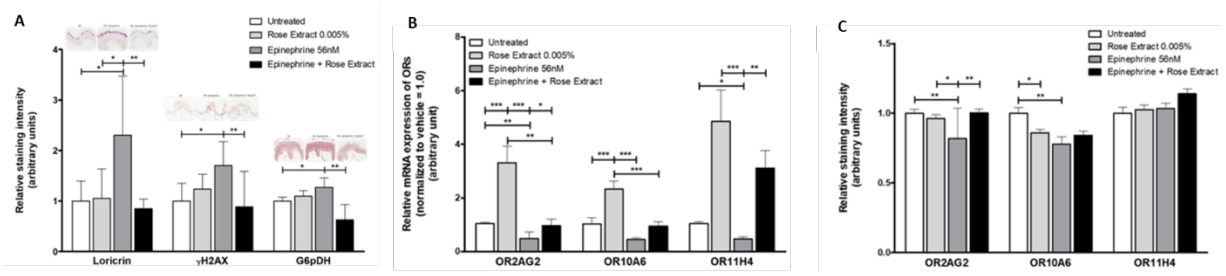

Figure 1. Effect of rose extract on epinephrine–induced stress markers (A) and OR expression in skin explants (B, C)

This positive effect of the rose extract was confirmed *in-vivo* in split-face study, on a panel of women volunteers with under-eye bags and dark circles and a stressful lifestyle. Results obtained with the Bio blue-light 3D scanning technology and images analysis showed that twice daily application of a cream containing rose extract for 28 days significantly improved the under-eye area and reduces the appearance of dark circles by 33%.

Conclusion

Our results identified three novel ORs expressed in human skin and found that a rose extract activate these receptors. We also provided evidence that OR2AG2 was involved into stress response mechanisms and that its activation protect skin against stress both *ex-vivo* and *in-vivo*. Therefore, these results reinforce the hypothesis that ORs could detect odor information, both in the skin and brain, to engage physiological functions in response to stress. In addition, we proved that the rose extract can be used for cosmetic applications to fight stress-induced skin fatigue and to boost natural skin defenses against external and internal daily stressors.

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Joan Attia, Global R&D Director

After my Master degree in Neurosciences and my PhD in Biomolecules & therapeutic pharmacology, I spent 9 years in the development of medical devices suitable for skin repair before to join LMC's R&D as a Project Manager in 2014. For 6 years, I led the development of the biological screening platform and the development of cosmetic ingredients. Since more than 1 year, I accepted this new challenge to be in charge of the global research and development of our new ingredients (active and functional ingredients and delivery systems) for the cosmetic market.

Approaching the Ideal of Beauty

Cristina Carreno; Lipotec S.A.U. (The Lubrizol Corporation)

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Introduction of research

Humans have an innate mechanism for subconsciously detecting beauty, based on symmetry and an ideal harmony of facial features. This perception of beauty is based on the ratio proportions of Phi known as the Golden ratio [1]. However, as we age, there is a deviation of facial proportions from the Phi number. Data on epidermal kinetics and cell compartmentalization strongly support a role for a golden ratio in epidermal organization [2]. Thus, by restoring skin proportions and bringing them closer to the golden ratio, we can help rejuvenate older skin.

Telocytes (TCs) are a relatively new cell type identified in the skin in 2012 [3] in charge of maintaining proper skin architecture. They can participate in the regulation of stem cells and contribute to reparative and regenerative mechanisms and maintenance of tissue homeostasis and organization [4].

Psychological stress is present in our daily lives and has a negative impact in the skin. Cortisol is the primary stress hormone, producing an array of effects in response to stress, leading to premature skin aging [5].

We have identified the impact of increased cortisol levels on TCs and proliferating epidermal cells, and how they may affect the right architecture of the skin and Phi beauty proportions. These negative effects could be minimized with an extract of Bacillus Ferment *in vitro* and *in vivo*.

Methodology

TCs isolation from human skin biopsies and identification through a triple immunocytochemistry using CD34, PDGFRa and vimentin antibodies. Measurement of TCs number after cortisol treatment in 2D culture. Measurement of p63 expression on human skin explants after cortisol treatment.

Facial features in women suffering psychological stress, assessed by salivary cortisol levels, were evaluated in two clinical studies after 14 and 28 days of product application. In each study, 2 groups of 40 Caucasian female volunteers (35-50 y.o) were recruited. One group applied a cream containing 2% of Bacillus Ferment (active cream) on the whole face and the second group applied a placebo cream in the same way. Macrophotographs of the face were taken with the software CameraScan. Vertical distance (in pixels) between eye corner point to mouth corner point and mouth corner point to chin point were measured with Image J software. The ratio between these distances was calculated and subtracted from Phi value to obtain the difference from Phi value for each volunteer. Macroscopic images were analyzed by using the software Luminosity and analyzed with Image J software. Symmetry in luminosity was calculated as the increase in luminosity uniformity between left and right cheeks. Acquisitions of the real 3D microtopography of the crow's feet area were taken with the PRIMOS lite system. 3D lineal confocal OCT (Line-field Confocal Optical Coherence Tomography, LC-OCT) was used at initial time and at 28 days in 5 volunteers of each group to obtain a vertical and horizontal reconstruction of the complete skin with cellular resolution. Stratum corneum and living epidermis thickness was calculated to study the Phi

proportional cellular architecture. The ratio between living epidermis and stratum corneum thickness was calculated to determine the proximity to the Phi[3] value [2].

Results

New protocol was developed to successfully isolate and culture TCs from human skin based on previous work [6]. Single adherent cells were observed in culture flasks a few days after the primary culture was established and showed the morphology characteristic of TCs with small cell body and long moniliform telopodes (Figure 1A). Connections between cells through telopodes were also observed in the established cultures after additional days in culture (Figure 1B). The isolated cells showed positivity for the three markers CD34, PDGFRa and vimentin corresponding to the phenotype previously described by others [6].

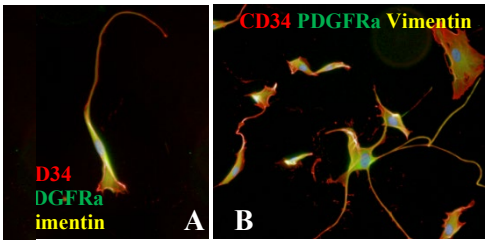

Figure 1. Triple immunofluorescent staining for CD34/PDFGRa/vimentin of skin TCs in culture.

Long-term chronic stress can accelerate the aging process and can lead to premature skin aging. To study if cortisol could have a negative impact on TCs, cell cultures of skin TCs were treated with 1 mM cortisol. As shown in Figure 2, results demonstrate that high levels of cortisol damage TCs and that this damage can be reduced in the presence of Bacillus Ferment.

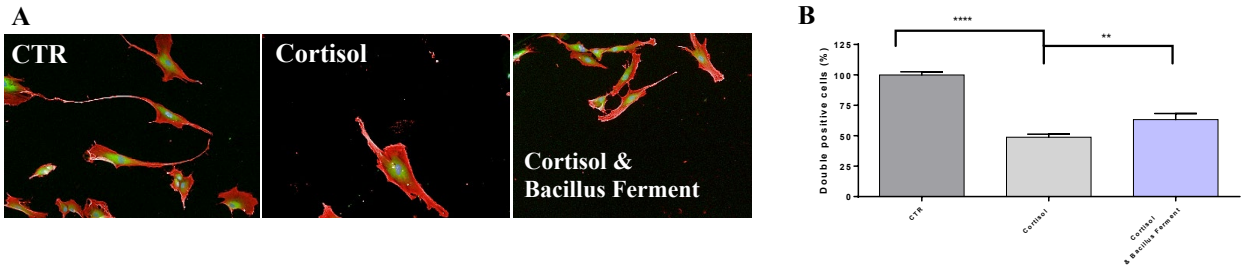

Figure 2. A) Double immunofluorescent staining for CD34/PDFGRa of skin TCs in culture in control condition (CTR), treated with cortisol and treated with cortisol and Bacillus Ferment. **B)** Mean of percentage of double positive cells in TCs culture after cortisol treatment and Bacillus Ferment treatment compared to control condition (CTR). Statistical significance: ****p<0.0001 and **p<0.01 calculated using unpaired t test.

The pattern of TC distribution in the skin augments the hypothesis that TCs are "nursing cells" where they may interact with resident cells in stem cell niche [4]. A marker of proliferative cells (p63) was quantified in skin explants, evaluating the effect of cortisol on p63 expression. Cortisol treatment significantly decreased the number of p63 positive cells, but this severe reduction was not observed in the presence of Bacillus Ferment (Figure 3).

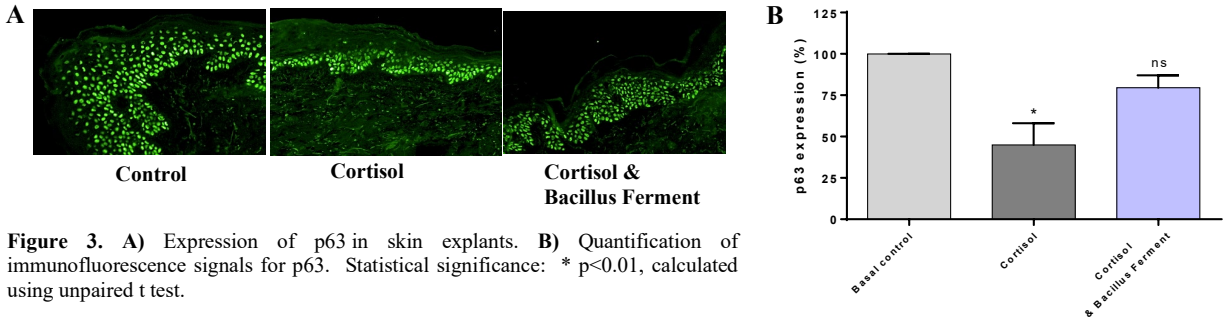

Figure 3. A) Expression of p63 in skin explants. **B)** Quantification of immunofluorescence signals for p63. Statistical significance: * p<0.01, calculated using unpaired t test.

Finally, clinical results (Figure 4) showed a statistically significant approximation to the ideal Phi beauty when volunteers applied Bacillus Ferment. The vertical ratio measured was closer to the Phi number at the end of the treatment and an increase in the symmetry of the luminosity determined as a reduction in

the difference of the skin luminosity of the left and right cheeks was also observed after 14 days of treatment. In addition, there was also an enhancement of skin luminosity of 13.9%. The results also showed a higher decrease of the wrinkle area parameter in crow's feet after 14 and 28 days of active cream use. Measurements done with LC-OCT showed an increase of both stratum corneum and living epidermis thickness after Bacillus Ferment application and a closer approximation to phi[3] value for the group that applied the active cream.

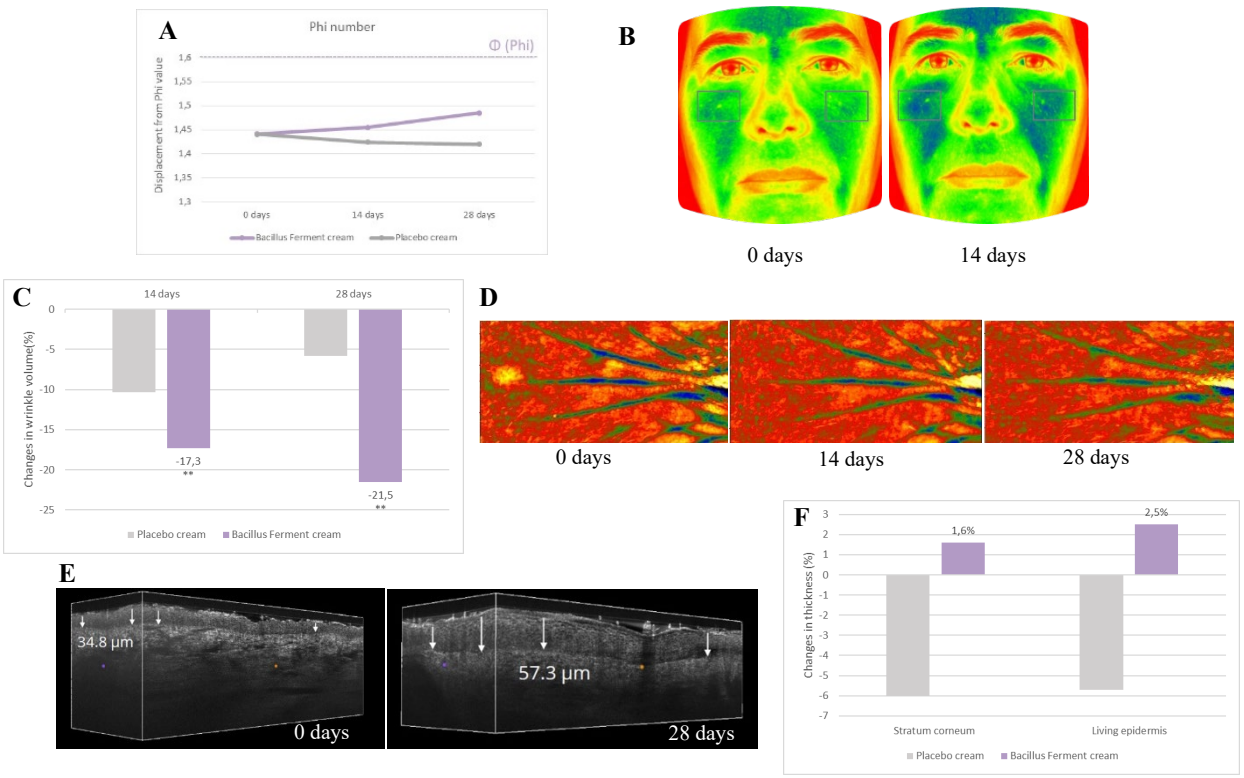

Figure 4. A) Displacement from Phi value for the vertical ratio. **B)** Volunteer nº 18. Images of luminosity obtained with CameraScan and processed with software Luminosity to generate colormap images (blue colored pixels). **C)** Changes in wrinkle volume. **D)** Volunteer nº 17. PRIMOS images of crow's feet area. **E)** Volunteer nº 37. Images obtained with LC-OCT. **F)** Changes in stratum corneum and living epidermis thickness.

Conclusion

Psychological stress affects the proportions of the skin, accelerating aging and affecting the perception of beauty. By protecting TCs from stress, we can preserve the functionality of stem cells, ensuring the right architecture of the skin approaching the ideal Phi beauty.

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About the speaker

Dr. Cristina Carreño is the Global Business Development Director of Lipotec™ Active Ingredients at Lubrizol.

She received her PhD in Organic Chemistry from the University of Barcelona in 1997, where she specialized in both peptide and combinatorial chemistry. For nine years she was in charge of the Peptide Synthesis Facility at the University of Barcelona, after which she joined the research company of Lipotec Group managing the discovery of new compounds for the pharmaceutical and cosmetic industry. Since 2008 she also took responsibility for the New Business Development area in the company on a global basis. Dr. Carreño is the author of several patents in the field of new cosmetic actives.