Natural regulation of skin hydration, a biological model to improve cosmetics efficiency and sustainability through biomimicry

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Introduction:

In the skin, gradient of water and homeostasis are crucial for maintaining physiological state and function [1]. If there are still many unknowns, hydration levels are recognized to affect not only visible microscopic parameters such as the suppleness and softness of skin, but also molecular parameters, enzyme activities and cellular signaling within the epidermis. As a result, facing various troubles, like sensitive skin, erythema, etc., being able to provide a product to reinforce hydration and barrier function with a good tolerance is at the center of attention for dermo cosmetic research[2,3].
As a first step towards biomimicry, we first focused on a bioinspired cream and balm. Thus, the design of these products is inspired by the regulatory processes that ensure an optimal hydration gradient in the skin, and more specifically on the structural and dynamic properties of the Stratum Corneum (SC) to increase the water residence and transit time in the SC along with a decrease in water exit.
Through in vitro and in vivo clinical studies, we then evaluated the benefits of these bioinspired products on the two above mentioned main requirements: how to improve the skin barrier function and adequate hydration to reach consumer's needs while limiting to a minimum the environmental impact.
In vitro methods including scanning electron microscopy (SEM) imaging and X-ray diffraction were used to analyze the formulations themselves, as well the impact of the formulations on the SC structure. The structure and special organization of the matrix can be visualized using X-ray diffraction. small-angle X-ray scattering (SAXS) provides information on the inter-bilayer distance in the multi-lamellar lipid structures, whereas wide-angle X-ray scattering (WAXS) provides information on the in-plane crystalline arrangement of the lipids (lattice type)[4].
Two complementary mass spectrometry imaging techniques, atmospheric-pressure matrix-assisted laser desorption/ionization high-resolution mass spectrometry (AP-MALDI-HRMS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS), were also used to compare the ingredients of the formulations with SC components, and to detect their distribution on and in the skin after topical application[5].
Clinical studies were performed to evaluate the effect of the formulations on skin hydration, as well as investigate potential adverse skin effects, such as irritation and sensitization.

Materials & Methods:

Mass Spectrometry imaging (MSI)
Fresh human skin was cut using a dermatome and punched out. Formulations were applied at 2 mg/cm² and skin samples were incubated at 37°C with 5% CO2 for 30 h. Punch-biopsies were frozen in liquid nitrogen. Samples were sliced into 10µm thick sections using a Cryo-Ultramicrotome.
Dried sections were coated with HCCA matrix using an HTX TM-sprayer. MALDI analysis was performed using an AP-MALDI UHR ion source coupled to an LTQ/Orbitrap Elite high-resolution mass spectrometer in positive ion mode. Data analysis and visualization was performed with Xcalibur 2.2 and MultimagingTM.
ToF-SIMS analysis was performed using an TOF.SIMS 5 with a 25kV Bi3+ primary analysis beam. Dried skin sections were analyzed in burst alignment, delayed extraction, positive ion mode Data analysis and visualization was performed using SurfaceLab 7.

X-ray diffraction
Human SC samples were placed on a polymeric support and 5 mg/cm2 of the formulation was applied. After 45 min, treated and non-treated SC were analysed. Global and Z-analysis diffraction analysis were carried out. SAXS and WAXS were visualized, and diffraction patterns were acquired in the scanning mode (vertical step was 10 µm and the horizontal step was 75 µm). Data were processed using ESRF FIT2D software.

Clinical studies: Hydration Index (HI)
The Hydration Index (HI) was measured in 26 female volunteers (HI ≤ 45). A single standardized topical application of the formulation (2 mg/cm²) was performed. The efficacy outcome measured was the change in cutaneous capacitance between the treated and the untreated control. The measurements were carried out before application of the product then 1, 2, 4, 6 and 24 h after application using a Corneometer.

Clinical studies: skin tolerance
Skin tolerance was measured with 32 volunteers for the balm and with 30 volunteers for the cream. A stinging test was performed before the first product application to confirm subject's inclusion. Each subject was given a dermatological and ophthalmic clinical evaluation before and after the first application. The cream and balm formulations were applied to the face, eye area, and neck at least twice a day for 3 weeks. Then, the dermatological evaluation was repeated, and the subjects asked to fill in a cosmetic acceptability questionnaire.

Results & Discussion:

MSI
Global overview of the composition of the formulations showed similarities with SC and epidermis composition. Penetration of the formulation components was analyzed. MSI allowed clear detection of the different layers of the skin using endogenous markers of each skin layers like ceramides and phospholipids. Penetration of the formulation ingredients did occur, especially monoglycerides, diglycerides and triglycerides (Fig. 1 A and B), which were detected mainly in the SC but also in the viable layers of the epidermis. The penetration of these components was higher after application of the balm compared to the cream, in particular, into the epidermis.

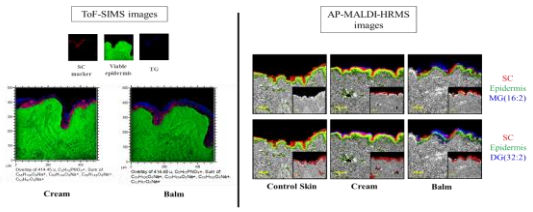

Fig.1.A. ToF-SIMS image analysis of penetration of triglyceride formulation components.

Fig.1.B. AP-MALDI-HRMS image analysis of penetration of monoglyceride, diglyceride formulation components. SC marker in red; Viable epidermis in green and summed-up triglyceride or mono- and diglyceride species in blue.

WAXS and SAXS analysis

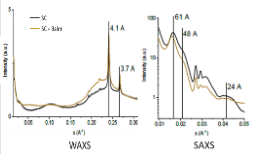

Fig.2. The global profiles of the SC with and without application of the formulations

The lipid organization of the formulations was similar to the SC.
WAXS and SAXS analysis indicate that the formulations did not modify the lamellar organization of the SC lipids but they did increase the relative proportion of the crystalized lipids in the orthorhombic lattice and some of the amorphous lipids.

Hydration Index
After application of both formulations, the HI was statistically increased by 65.1% (cream) and 60.2% (balm) compared the hydration level before application (Fig. 3). A high level of hydration was maintained over 4 h after application, such the HI of skin treated with cream or balm was at least 55% higher than at H0. The HI declined over the following 20 h but was still statistically significantly higher than the untreated control skin or H0.

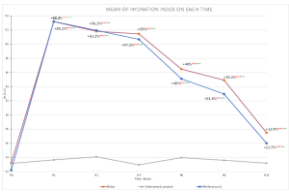

Fig.3. Hydration index of skin of subjects after application of the cream (blue symbols) and balm (orange symbols).

Skin tolerance
Both formulations exhibited excellent skin tolerance after twice daily applications for 3 weeks and could be labelled as "High Tolerance" (i.e. no reaction occurred during the study). The subjects of the in-use tolerance studies appreciated the soothing properties of both formulations (skin comfortable, soothed and relieved) with an agreement rating more over 90% immediately after application. The long-lasting soothing effect was sustained for the balm after a 21-day use period at the same level and decrease slightly to 87% and 83% for the cream.

Conclusions:

Formulations were well-tolerated and increased skin hydration *in vivo* as formulations were designed to imitate SC hydration regulation process. Results show a successful intake of various ingredients, leading to an increase in the SC hydration and a reinforced skin barrier. Bioinspiration process used to design these products allowed reduction of their environment impact, consistent with Biomimicry definition.

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