A marine bio active megasugar to activate “happiness” signaling pathways and improve skin appearance.

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ABSTRACT

The skin is an organ of perception and expression able to generate positive or negative feelings depending on our satisfaction with our overall image. Brain and skin interact constantly, both reacting to the perception of *stimuli* by producing mediators that induce a physiological and psychological response involved in the regulation of the emotions. Previous work on the high molecular weight exopolysaccharide Mo278 showed a unique lectin interaction signature associated with skin regeneration and potential improvement of well-being. It was also able to provide significant benefit *in vivo* by visibly improving the quality of the skin and the volunteer’s emotional state. To go further and decipher the biological mechanisms connected with these perceived benefits, an original experimental approach combining a proteomic evaluation on explants and an *in vitro* sensory neurons-keratinocytes study was set up. The proteomic analysis showed a positive impact on proteins linked to skin renewal and barrier homeostasis. The co-culture study demonstrated a protective effect of Mo278 under stress conditions on neurite length, Ki67, MOR expression levels and the dopamine level was increase by 16%. These results indicates that Mo278 activates well-being and happiness signaling pathways, leading to an overall improvement in psychological benefits such as self-esteem and allo-perception.

KEYWORDS

Exopolysaccharide; well-being; brain-skin relation; happiness; skin appearance.

INTRODUCTION

The skin is both an organ of perception and expression. The philosopher *Marcus Tullius Cicero*, also known as Cicero, said “If the face is the mirror of the soul, the eyes are its interpreters”. “The face is the mirror of the soul” means the state of our skin is also a reflection of our own mental state and physical health. The perception of our own image is the result of the combination from our brain and our skin perception, and the state of our skin is the reflect of our own mental state and physical health. There is a complex relationship between the brain and the skin, as highlighted in several scientific studies [1], [2], [3]: the mood can modify the structure and appearance of the skin by inducing messengers that have an impact on the skin. Emotion, from the Latin *movere* meaning “to set in motion”, refers to a dynamic notion. Indeed, man is guided by his emotions in response to the perception of stimuli generated by his sensory organs. They are transferred and interpreted by the brain that produces response signals in return. The skin is a primary sensory organ that perceives immediate stimuli *via* its numerous thermoreceptors, nociceptors and mechanoreceptors located on keratinocytes and cutaneous nerve endings. Skin and brain interact constantly, both reacting to the perception of *stimuli* by producing mediators such as hormones and neurotransmitters. These mediators induce a physiological response leading to automatic response mechanisms and are involved in the regulation of our emotions. For example, β-endorphins - nicknamed happiness hormones - provide pleasure sensations and help relieve pain. Dopamine provokes a sensation of immediate pleasure *via* the reward circuit, oxytocin is involved in social interactions and serotonin in serenity, while cortisol is linked to stress.

In addition to the barrier and immune functions, the skin, thanks to its constant crosstalk with the brain, relays and responds to signals from central nervous system by activating the endocrine and immune systems [4]. A clear link between psychological stress and skin physiology can therefore be established. Psychological stress, for its part, induces chronic cortisol production, which has an impact on the mood, but is also responsible for pro-inflammatory and oxidative effects on skin cells, impacting skin properties. Skin barrier

dysfunction has been reported as one of the negative consequences of chronic psychological stress [5], [6]. Chronic cortisol release also alters skin innervation and, consequently, its ability to interact with the brain.

In previous work [7], the potential of high molecular weight exopolysaccharides (EPS) as emotional-beauty-enhancer bioactive ingredients was explored. Among them, a specific EPS (coded “Mo278”) of more than 4.10^6 Da and rich in uronic acids was isolated and produced from a strain of *Alteromonas sp*. Its composition and molecular weight have been extensively studied by Magnetic nuclear resonance (NMR), gas chromatography (GC), High-performance Size-exclusion chromatography (HPSEC), Fourier-transform infrared spectroscopy (FTIR), as well as colorimetric analyses. An interaction profile study with 20 lectins was performed to decipher its unique signature reflecting the accessibility and specific recognition of glycan motifs by glycan-binding proteins, namely lectins, and the potentially associated biological activities. This study notably highlighted its ability to interact with the rhamnose-specific lectin CorM, which recognizes rhamnosylated glycan structures in the same manner as rhamnose-specific protein expressed at the surface of keratinocytes and fibroblasts. Recognition of rhamnose is known to be associated with increased release of β-endorphin by keratinocytes and improved feelings of well-being [8]. Besides, a double-blind study was carried out for 28 days with a cream containing 1% Mo278 *versus* placebo on 44 healthy Caucasian women divided into two groups. Biometric and emotional parameters were evaluated. The biometric measurements showed a 10.3% improvement in skin texture and an 8.2% improvement in moisturization with the use of Mo278. The evolution of the volunteer’s emotional state between D0 and D28 was assessed using an evaluation method called the “mirror test”, in which the volunteers were confronted with their own image in a mirror. It turned out that the voice intensity and frequency of the group of volunteers who had applied Mo278 was lower than that of the placebo group. The verbal lexicon used by the Mo278 group was also more positive and optimistic than at the start of the study and the placebo group. This study therefore showed that Mo278 provided significant benefits, visibly improving both the quality of the volunteers’

skin and their emotional state. These results were explained by the brain's ability to perceive certain changes in skin tissue very accurately and to amplify this signal through neurological rewards associated with improved self-esteem.

To go further and to decipher the biological mechanisms responsible for these perceived benefits, a global proteomic study on explants and a specific *in vitro* study, based on a co-culture of sensory neurons and keratinocytes under chronic psychological stress condition, were carried out. The aim of these studies, through these two approaches, is to assess Mo278's ability to modulate the pathways of well-being mediators and improve skin physiology, and to try to establish a link with the improvement in self-esteem observed in volunteers.

MATERIALS AND METHODS

Proteomic study

6 explants from the same surgery and the same Caucasian donor were divided in 2 experimental groups. The first half was treated twice a day for 3 days with 1% Mo278 and the second half was left untreated (control). All proteins in whole explants were extracted and their concentrations determined using the Bradford method. The same mass of protein extract from each explant was analyzed by LC-MS/MS. The spectra collected were computer-analyzed for protein identification and quantification. Relative expression levels were determined from the proteomic data set obtained and fold-change values were calculated to compare the Mo278 group with the control group. Proteins with a fold-change expression ratio greater than +1.3 or less than -1.3 (and a p-value inferior to 0.05) were considered up-regulated or down-regulated, respectively. The identification of biological pathways and functions specifically modulated by Mo278 was assessed by data mining analysis thanks to another statistic: the z-score. A positive z-score indicates a predicted activation and a negative z-score indicates a predicted inhibition of the enriched pathway or bio-function in relation to the binary comparison group.

***In vitro* co-culture of sensory neurons-keratinocytes**

An *in vitro* co-culture model based on human sensory neurons and keratinocytes was used. Human sensory neurons were derived from the differentiation and maturation of hiPS cells. The model was obtained following 2 main phases:

- After neuron differentiation, a maturation phase during which stress and Mo278 treatment were applied regularly, if applicable.
- A co-culture phase with the addition of keratinocytes, during which the application of stress and treatment with Mo278, if applicable, were continued.

The stress applied in this study corresponded to chronic exposure to 0.1 µM cortisol, with reiteration of its application at each renewal of the culture medium, to mimic chronic psychological stress.

A total of 6 experimental conditions were studied, with two main series: one stressed and the other unstressed. Each series comprised a trial without treatment, a trial treated with 0.25% Mo278 and a trial treated with 0.5% Mo278. Each experimental condition was replicated 6 times.

Both supernatants and cells were collected after the last medium renewal to analyze the targeted biomarkers.

To assess the effects of Mo278 on skin physiology, the expression rate of Ki-67, representative of the level of keratinocyte proliferation, and the density of the sensory neurons (*i.e.* neurite length normalized by the number of neurons) were quantified *in situ* by image analysis based on immunofluorescent staining.

The modulation of happiness pathways was studied by quantifying dopamine release using ELISA assay and by immunofluorescence quantification of the expression level of µ-opioid β-endorphin receptors, *i.e.* MOR receptors, normalized by the surface of sensory neurons.

For each parameter, values were compared with the control condition, *i.e.* untreated and unstressed.

RESULTS

Proteomic study

The proteomic study on explants showed the modulation of individual proteins involved in skin structure and barrier function (Table I). Thus, filamin-A, fibulin-1, decorin, lumican, the α2-chain of collagen VI, talin-1 and dermatopontin showed a statistically significant up-regulation. All these proteins are involved in cell adhesion and migration, assembly or organization of the cytoskeleton and components of the extracellular matrix (ECM).

Table I: List of individual up-regulated proteins relevant for skin benefits with application of 1% Mo278 compared to control.

Protein	Foldchange	p-value
Filamin-A	+1.55	< 0.05
Fibulin-1	+1.61	< 0.05
Decorin	+1.41	< 0.05
Lumican	+1.42	< 0.01
α2-chain of Col-VI	+1.32	< 0.01
Talin-1	+1.31	< 0.01
Dermatopontin	+1.49	< 0.05

Furthermore, the data mining analysis of the relevant individual proteins revealed that Mo278 can act on three predictive pathways of interest with:

- Tendency to activate the LRX/RXR (Liver X receptor-retinoid X receptor) pathway (positive z-score +2.45 *vs* control). In the skin, LXR activation induces keratinocyte differentiation, regulates lipid production and improves epidermal barrier homeostasis [9].
- Tendency to activate the “cell movement” biofunction (positive z-score +2.46 *vs* control). The movement of cells in the skin is a crucial process in maintaining skin integrity and facilitating wound healing. The main skin cell types involved in movement are keratinocytes, fibroblasts and immune cells. Keratinocytes migrate from the basal

layer of the epidermis to the surface, forming a protective barrier. Fibroblasts and immune cells move to the site of injury to repair damaged tissue.

- Predictive activation of TGF-β1 as the main upstream regulator (positive z-score of +3.64 *vs* control), this being a growth factor regulating various skin processes such as cell proliferation, differentiation, and migration.

The global proteomic study thus highlighted Mo278's ability to modulate, in the basal state, key pathways and proteins linked to overall skin structure and maintenance of homeostasis, promotion of cell renewal and proper skin barrier function.

Deciphering the effects of Mo278 on well-being pathways with an *in vitro* co-culture of sensory neurons and keratinocytes

The *in vitro* co-culture study of sensory neurons and keratinocytes allowed to study two main types of effect of Mo278: its impact in the basal state, *i.e.* without stress, and its impact under conditions of chronic psychological stress by regular application of cortisol.

Skin physiology biomarkers

The results showed that, overall, Mo278 had no deleterious impact on skin physiology, since neither Ki67 expression (Figure 1a) nor sensory neuron density (Figure 2a) were affected by its application at 0.25% and 0.5% in comparison with untreated and unstressed control.

However, in a context of chronic psychological stress, keratinocyte proliferation and nerve ending density were significantly impacted by chronic application of cortisol. Thus, the Ki-67 expression rate decreased by 31% with stress (Figure 1b), compared with the unstressed and untreated control. Similarly, the neurite length normalized by the number of neurons was reduced by 24% compared with the control (Figure 2b). Immunofluorescence images clearly show a decrease in neuronal density between the control (Figure 3a) and the untreated stressed assay (Figure 3b).

The application of 0.25% and 0.5% Mo278 counteracted these deleterious effects of stress and provided complete protection against the latter in terms of Ki-67 expression and neuronal length. This protective effect on neurons is visible in the number of neurons and the length of neurites observed by immunofluorescence (Figure 3c and Figure 3d).

In addition to providing complete protection against the deleterious effects of stress, Mo278 even tends to improve skin physiology, with Ki-67 expression rate increasing by 17% and 11% and neurite length by 16% and 36%, with 0.25% and 0.5% Mo278 respectively.

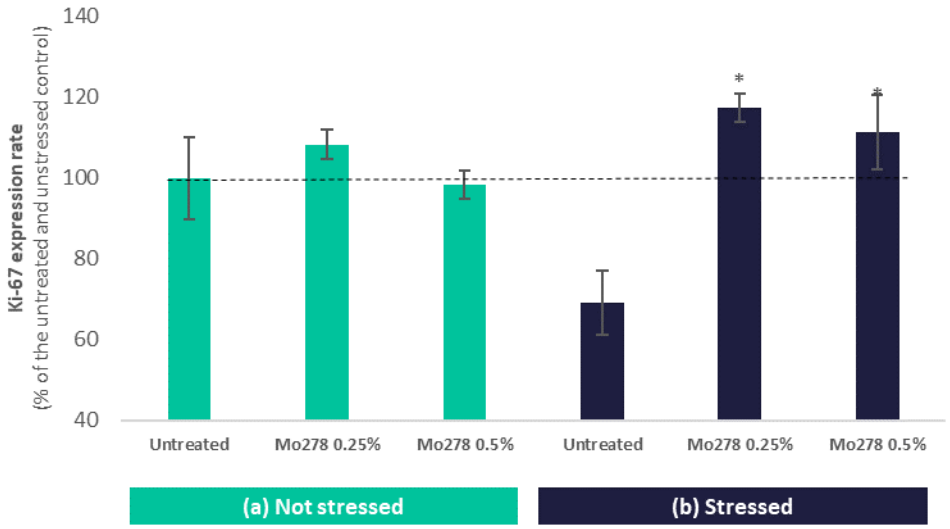

Figure 1: Comparison of the evolution of Ki67 levels expressed in sensory neurons-keratinocytes co-cultures (a) not stressed or (b) stressed with cortisol and untreated or treated with 0.25% or 0.5% Mo278. Data expressed as a percentage of untreated and unstressed control. *p-value < 0.05 *vs* stressed control. The y-axis starts at 40% for better data visualization.

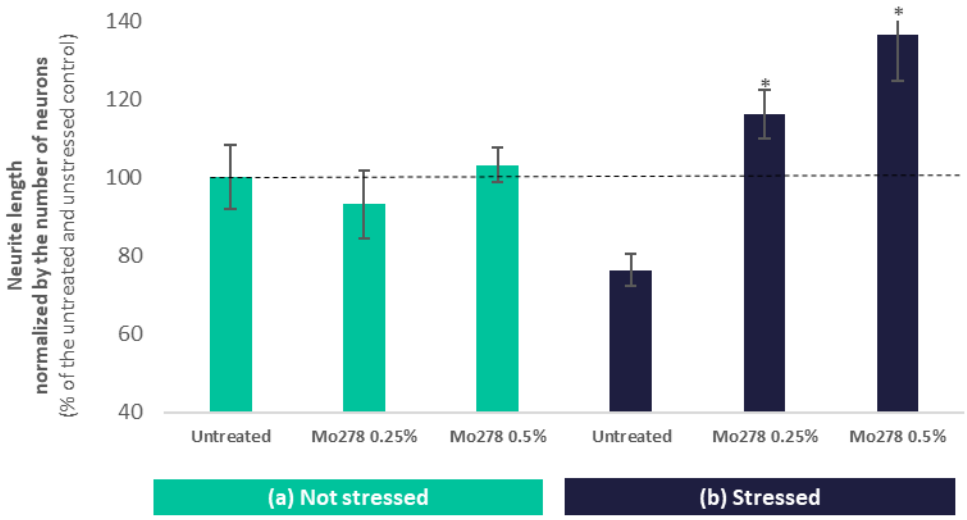

Figure 2: Comparison of the neurite length normalized by the number of neurons in sensory neurons-keratinocytes co-cultures (a) Not stressed or (b) stressed with cortisol and untreated or treated with 0.25% or 0.5% Mo278. Data expressed as a percentage of untreated and unstressed control. *p-value < 0.05 *vs* stressed control. The y-axis starts at 40% for better data visualization.

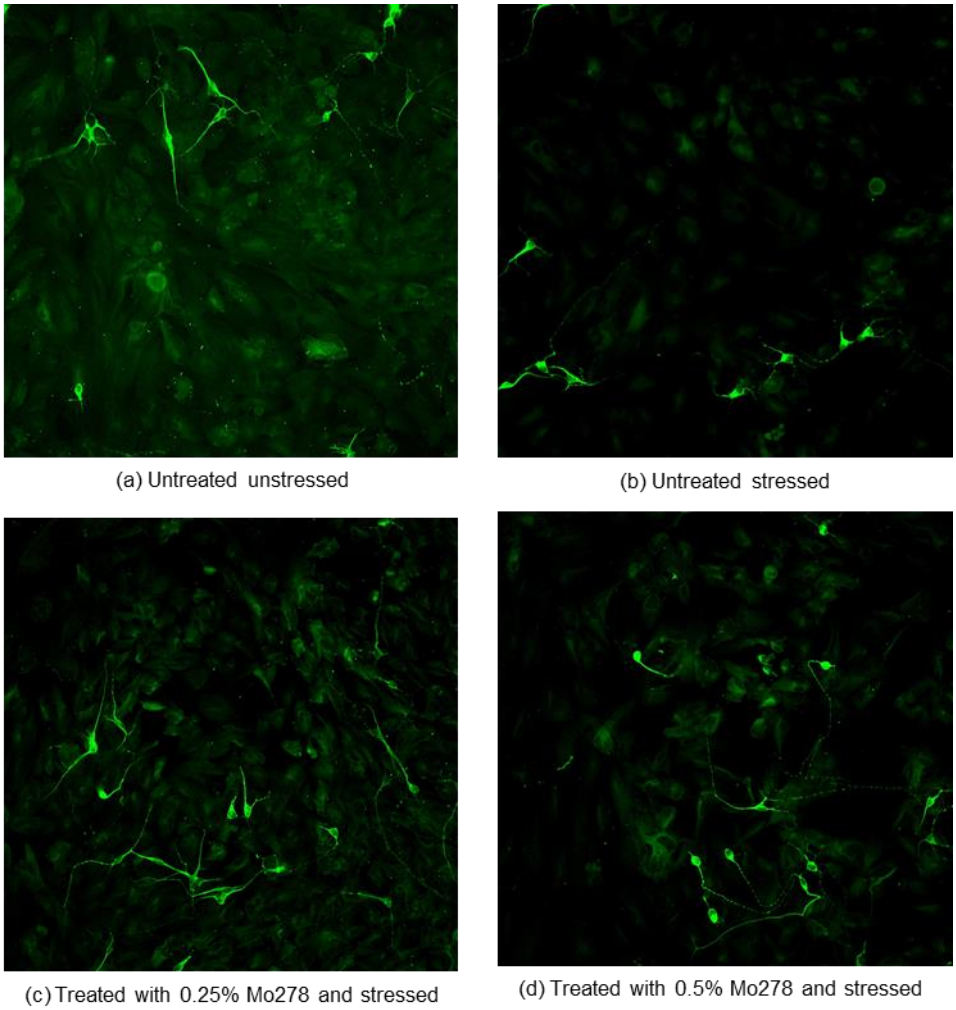

Figure 3: Sensitive neurons visualization by immunofluorescent staining

Well-being pathways

The β-endorphins pathway was studied through the expression of the μ-opioid receptors (MOR). The reduction in MOR expression in the stressed and untreated condition was not expected to be so important (-24%) compared to the untreated unstressed state (Figure 4b). Indeed, β-endorphins are in principle physiologically secreted under stress, because of their role in relieving unpleasant sensations. This phenomenon could be due to the methodology used to study this pathway, which was carried out indirectly by studying MOR receptors located on the neurons, themselves affected by the cortisol stress (Figure 3). Nevertheless, Mo278 seemed to activate the β-endorphins pathway, as MOR expression by sensory neurons increased both in the absence and in presence of stress, compared with the untreated

unstressed condition (Figure 4a) and the untreated stressed condition (Figure 4b), respectively. Thus, the MOR expression level under stress increased compared with the basal state by 20% and 22% with 0.25% and 0.5% Mo278, respectively.

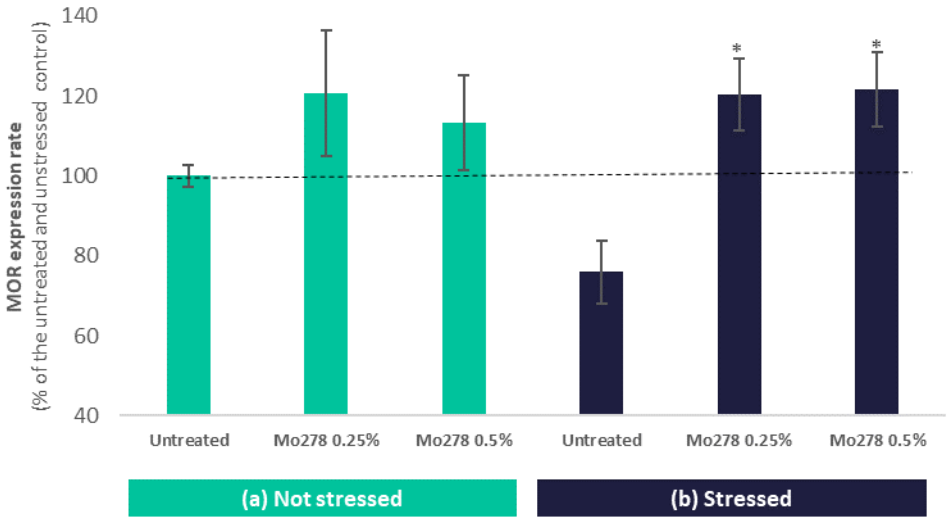

Figure 4: Comparison of the MOR expression rate in sensory neurons-keratinocytes co-cultures (a) not stressed or (b) stressed with cortisol and untreated or treated with 0.25% or 0.5% Mo278. Data expressed as a percentage of untreated and unstressed control. *p-value < 0.05 *vs* stressed control. The y-axis starts at 40% for better data visualization.

Finally, the effects of stress and Mo278 treatments were studied on dopamine release. In absence of stress, Mo278 did not modulate dopamine release in comparison with the untreated and unstressed control (Figure 5a). In contrast, the cortisol stress provoked a 9% decrease in dopamine release (Figure 5b), which was counteracted by the application of Mo278. Dopamine release was even higher with Mo278 than in the untreated and unstressed condition, namely 9% and 16% higher with 0.25% and 0.5% Mo278, respectively.

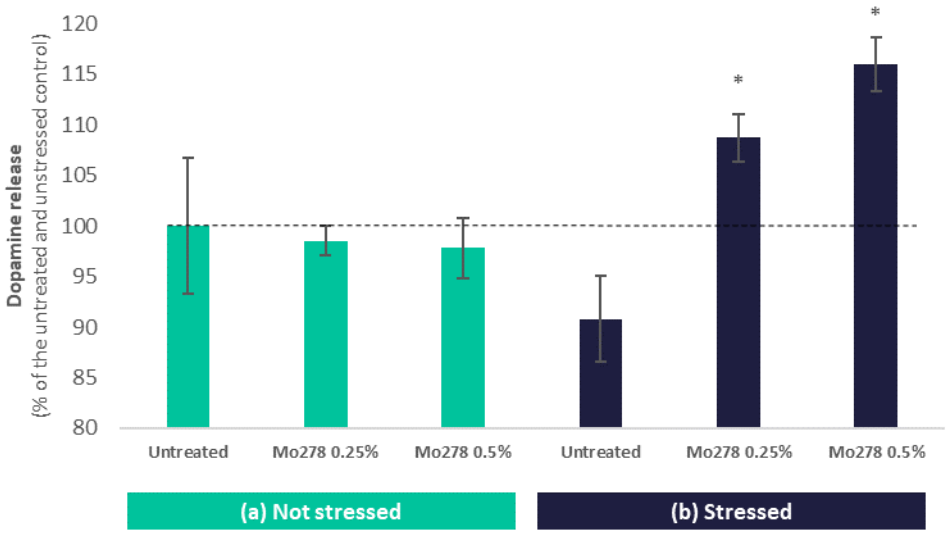

Figure 5: Comparison of dopamine release in sensory neurons-keratinocytes co-cultures (a) not stressed or (b) stressed with cortisol and untreated or treated with 0.25% or 0.5% Mo278. Data expressed as a percentage of untreated and unstressed control. *p-value < 0.05 *vs* stressed control. The y-axis starts at 80% for better data visualization.

DISCUSSION

A predictive evaluation using lectin interaction allowed to identify a potential active ingredient able to positively interact with the skin and to have an influence on mental well-being. This was confirmed during an evaluation on a panel of volunteers, with positive results both on physiological and psychological aspects. The results collected in these newly performed studies consolidate the activity of Mo278 and allow us to identify potential pathways to explain these findings.

On a physiological aspect, the proteomic study demonstrated that Mo278 is triggering cellular adhesion and more specifically keratinocytes proliferation and differentiation. This has a positive impact on the final cornification to reinforce the skin barrier. At the same time Mo278 stimulates lipid production, which is also crucial in the brick-and-mortar complex, still for the best barrier function. We even identify the stimulation of several proteins, involved in fibroblast stimulation and connected with the extracellular matrix and wound healing. These results,

obtained without stress, demonstrate that Mo278 has a positive impact on the skin barrier function and support skin global homeostasis.

The second part of the study is more connected to the perception aspect and designed to support the psychological benefits that were perceived during the mirror test. We evaluated the impact of Mo278 on a co-culture composed of keratinocytes and sensory neurons in the presence or absence of cortisol to reproduce a chronic stress. This allows us to have indications at cellular level of Mo278's mechanism of action. In absence of stress, Mo278 has no negative impact on Ki67 whatever the dosage and has a nice protecting effect in presence of cortisol. We can observe the same phenomenon on the neurite length, the MOR expression and the dopamine production. The protection of the neurons will avoid local chronic inflammation, the MOR expression ensuring that β-endorphins will be able to reduce external stress impact. Dopamine is well known to stimulate microcirculation and both differentiation and proliferation. This rare effect of local dopamine production is very interesting because generated *in situ* and only under stress. In these conditions, this will not perturbate the global natural homeostasis of the skin but will help protect and repair the daily life aggressions.

These results allow us to suppose that, concerning the neurons-keratinocytes interaction, Mo278's mode of action is more related to a protection pathway *versus* stress rather than to the stimulation of existing pathways. It will help restoring the global homeostasis of this complex system.

CONCLUSION

Our skin is our first barrier of defense against daily life aggression. It is now well established that it is a very complex organ composed with a lot of different cells communicating with each other, and that is also connected with our brain. The first connection is the visualization of our image in a mirror and can generate positive or negative feeling; the second is coming from the skin sensors. Mechanisms that will improve skin barrier or repair skin damages have been studied for decades, but the interaction between skin and nerves is a new field of research. It

is now possible to measure interaction at cellular level between keratinocytes and neurons to help understanding how actives ingredients can affect the skin.

Globally, these studies reinforce the results observed on volunteers and allow us to illustrate and explain why and how the application of Mo278 can visibly ameliorate skin texture and make people feel good. Many studies describe the vicious circle of chronic inflammation that must be broken to improve the skin. The approach developed during this study offers an additional solution to propose a virtuous circle that protects the skin from chronic psychological stress at the cellular level.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest related to the work presented in this article.

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