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In focus:
Anti-pollution Functionals Testing

특별호: 한국인 언어 부문

Holistic approach for environment-sensitive skin

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First suggested in 2005 by Dr. Christopher Wild of the International Agency for Research on Cancer, the exposome is a concept used to describe environmental exposures that an individual encounters throughout life, and how these exposures impact the skin and overall health.

The exposome impact is now well documented and incorporates several environmental factors such as UV, pollution, chemical climate, smoking, diet, use of cosmetic products, mechanical abrasions from shaving or frictions, microbiome with skin or atmospheric microbiota, as well as internal factors such as psychological and hormonal factors and gender.

According to the International Forum for the Study of Itch, sensitive skin is "a syndrome defined by the occurrence of unpleasant sensations (stinging, burning, pain, pruritus, and tingling sensations) in response to stimuli that normally should not provoke such sensations. These unpleasant sensations cannot be explained by lesions attributable to any skin disease. The skin can appear normal or be accompanied by erythema. Sensitive skin can affect all body locations, especially the face".[1]

Sensitive skin can be divided into two categories: naturally sensitive skin, which is related to genetics and associated with inflammatory skin conditions (eczema, rosacea, and psoriasis) and environmentally sensitive skin that is triggered by the exposome.

Biological approach of exposome impact on the skin

From a scientific perspective, the exposome is a source of inflammation that disrupts skin homeostasis. For example, it can affect the cellular production of cortisol by keratinocytes, which has a negative effect on keratinocyte proliferation.

This leads to a disruption of the skin and makes the skin more reactive to external aggression. Eventually, a vicious cycle of inflammation is established by repeated exposome aggression. A further consequence is the activation of inflammatory pathways that generate redness and inflammation.

In terms of discomfort, the β-endorphin levels will increase in response to external aggression and the specific sensory nerve receptors will also be affected, such as TRPV1 to trigger itch or pain sensations.[2]

At Seqens Personal Care, we decided to have a holistic approach towards environmentally sensitive skin. We have developed a methodology to evaluate several of our extracts on different exposome models and their impacts on the skin barrier, the inflammation and the neurosensory via many markers.[3]

Global and cellular approach of exposome impact

A proteomic study is a global evaluation of the different proteins impacted by the application of a stress or/and an active ingredient on skin. This technology allows us to evaluate, compared to a control, the impact of a *Crocus sativus* flower extract at 4% on skin protein expression levels.

Globally, our *Crocus sativus* flower extract can boost the chemical barrier function of the skin by improving fatty acid synthetase by 132% to maintain the epidermal lipids levels in the barrier. It also stimulates corneodesmosin by 142% reinforcing cell-cell adhesion when used at a 4% use level.

Our *Crocus sativus* flower extract reinforces the dermo-epidermal junction and protects the extracellular matrix from degradation.

To evaluate nerve sensitivity in environmentally sensitive skin, we utilized *in vitro* studies to assess the effect of our extract on the TRPV1 receptor in connection with itch or pain sensations. Some specific cell models have been designed to study this pathway.

To assess the efficacy of *Crocus sativus* flower extract on reducing the negative impact of the TRPV1 pathway, we modified human cell line 293T cells on the TRPV1 receptor. The active showed a significant reduction in the activity of the pathway.

We also evaluated the effect of the active on primary human keratinocytes (HK) under a global stress cocktail condition to mimic dysbiosis (microbiome perturbation) and irritated damaged skin.

Under stress conditions, both β-endorphin and cortisol productions increase. At 1% concentration, *Crocus sativus* flower extract can significantly reduce these markers by 15% to 18%, respectively demonstrating protection at the cellular level.

Holistic evaluation of several exposome impacts

Based on these results, we decided to further explore the potential of this extract on a more sophisticated model. A skin explant is a part of human skin recovered from surgeries and can be kept alive during a short period allowing an evaluation of several markers in a realistic model.

We built a holistic experimental study plan under different exposome stress types including dysbiosis,[4] UV-A irradiation,[5] and pollution with urban dust.[6] The study was divided into two different aspects of evaluation. The first one was a focus on several biological markers connected with the barrier function, the second aspect was focused on inflammation and the sensory pathway.

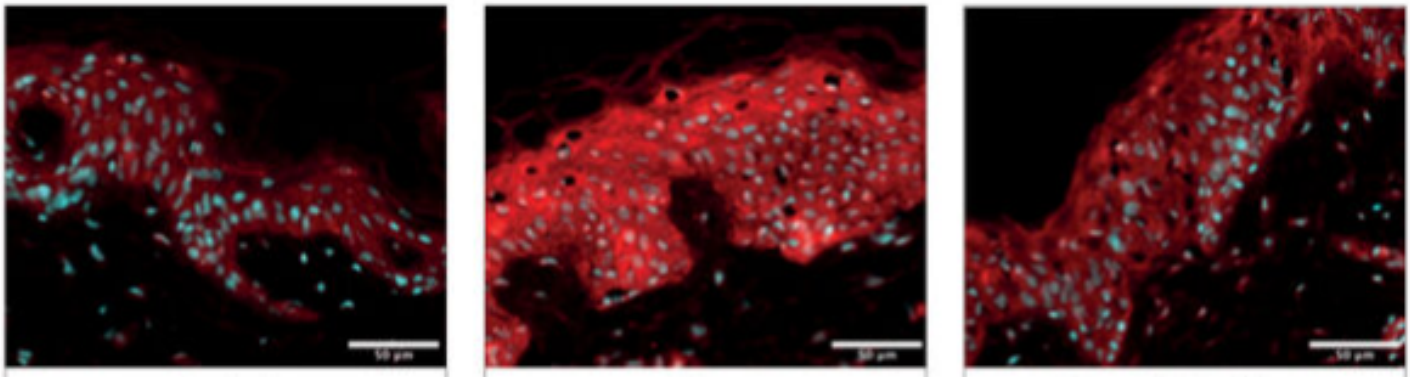

Figure 1: TSLP immunostaining: *in situ* visualization of TSLP levels by fluorescence microscopy (TSLP in red, nuclei in blue)

Exposome and barrier function

For the evaluation of the barrier function, a topical *S. aureus* protease (0.01 µg/mL sspA) and a systemic neuropeptide cocktail (10 µM substance P + 1µM αCGRP in culture medium) were both applied daily for 48 hours to explants from a 34 year-old Caucasian female.

Stressed explants were treated daily or not with *Crocus sativus* flower extract at 1% for 48 hours versus control. Twenty-four hours after the last treatment (on D3), explants were sampled and *in situ* visualization of filaggrin, loricrin, DSG1 (desmoglein-1) and DSC1 (desmocollin-1) was performed by specific immunostaining and image processing.

Loricrin is a marker connected with epidermal differentiation and its production is negatively impacted by the applied stress. In presence of the *Crocus Sativus* flower extract, we observed a 36% reduction in the intensity of the impact of stress on loricrin.

Filaggrin is also involved in the terminal differentiation process of the keratinocytes and participates in the global moisturization of the skin by generating the natural moisturization factor components. The stress was reducing filaggrin production by 24% versus control, while in presence of our extract and under the same stress condition, filaggrin production was improved by 16% versus control.

Similar benefits were observed for DSG1 with an improvement from -11% to +16% and for DSC1 with an improvement of -18% to -6%. Both of these markers are important in assessing desmosome composition connected with cell cohesion and barrier function. Combined with previous results, our data confirmed the *Crocus sativus* flower extract has a protective impact on barrier function when exposed to stress.

Exposome versus inflammation and sensory pathway

To assess inflammation and the sensory pathway, two different stress models were evaluated using skin explants from two different donors.

The first model was a dysbiosis cocktail composed with a *S. aureus* protease (0.01 µg/mL sspA) and a systemic neuropeptide cocktail (10 µM substance P + 1µM αCGRP in culture medium) that were both topically applied daily for 48 hours to explants from a 34-year-old Caucasian female.

Stressed explants were treated daily, with the *Crocus sativus* flower extract at 1% for 48 hours. Twenty-four hours after the last treatment (on D3), explants were sampled and *in situ* visualization of some S100A8/A9 and TSLP (Thymic stromal lymphopoietin) were performed by specific immunostaining and image processing.

The second model was a pollution and UV-A cocktail composed with a systemic neuropeptide cocktail (10 µM substance P + 1µM αCGRP in culture medium) applied daily for 48 hours and a topical urban dust treatment (30 minutes of contact with 0.375 µg/cm² PM10 and 20 µM benzo(a)pyrene + UV-A irradiation 6 J/cm² for 40 minutes) at D2 were applied to explants from a 31-year-old Caucasian donor.

Stressed explants were treated daily with *Crocus sativus* flower extract at 1% for 48 hours versus control. Twenty-four hours after the last treatment (on D3), explants were sampled and *in situ* visualization of IL-1β and IL-31 was performed by specific immunostaining and image processing. On both explant models, ELISA quantification on PGE-2 & β-endorphin levels.

PGE-2 is a prostaglandin produced by keratinocytes and is involved in the inflammatory response cascade. PGE-2 is responsible for increasing skin blood flow, which gives the skin its redness and was promoted in both stress model at different intensities. *Crocus sativus* flower extract was able to reduce PGE-2 production in both dysbiosis and pollution model by 51% and 27% respectively.

β-endorphin is an endogenous opioid peptide produced by the pituitary gland but also locally by keratinocytes. β-endorphin levels are higher in inflamed and stressed skin, providing a soothing effect on inflammatory pain while also inducing itching. Under our stress conditions, β-endorphin production was reduced by 62% and 24% under dysbiosis and pollution model respectively.

S100A8/A9 (also called calprotectin) is a member of the S100-alarmin family and is released actively and exerts a critical role in modulating the inflammatory response by stimulating leukocyte recruitment and inducing cytokine secretion. The *Crocus sativus* flower extract was able to reduce the production of S100A8/A9 by 39%.

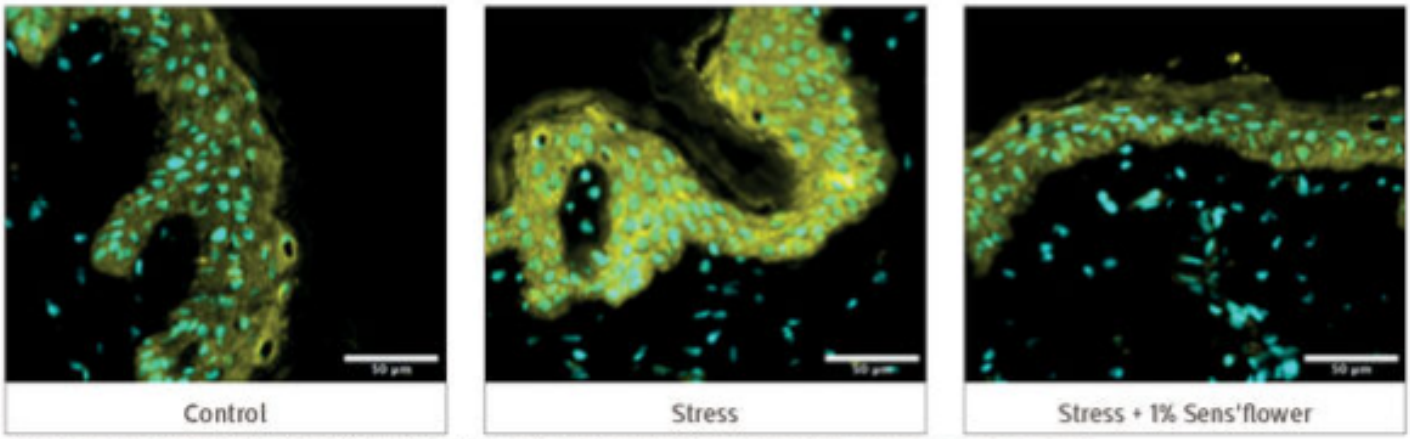

Figure 2: IL-31 immunostaining: *in situ* visualization of IL-31 levels by fluorescence microscopy (IL-31 in green, nuclei in blue)

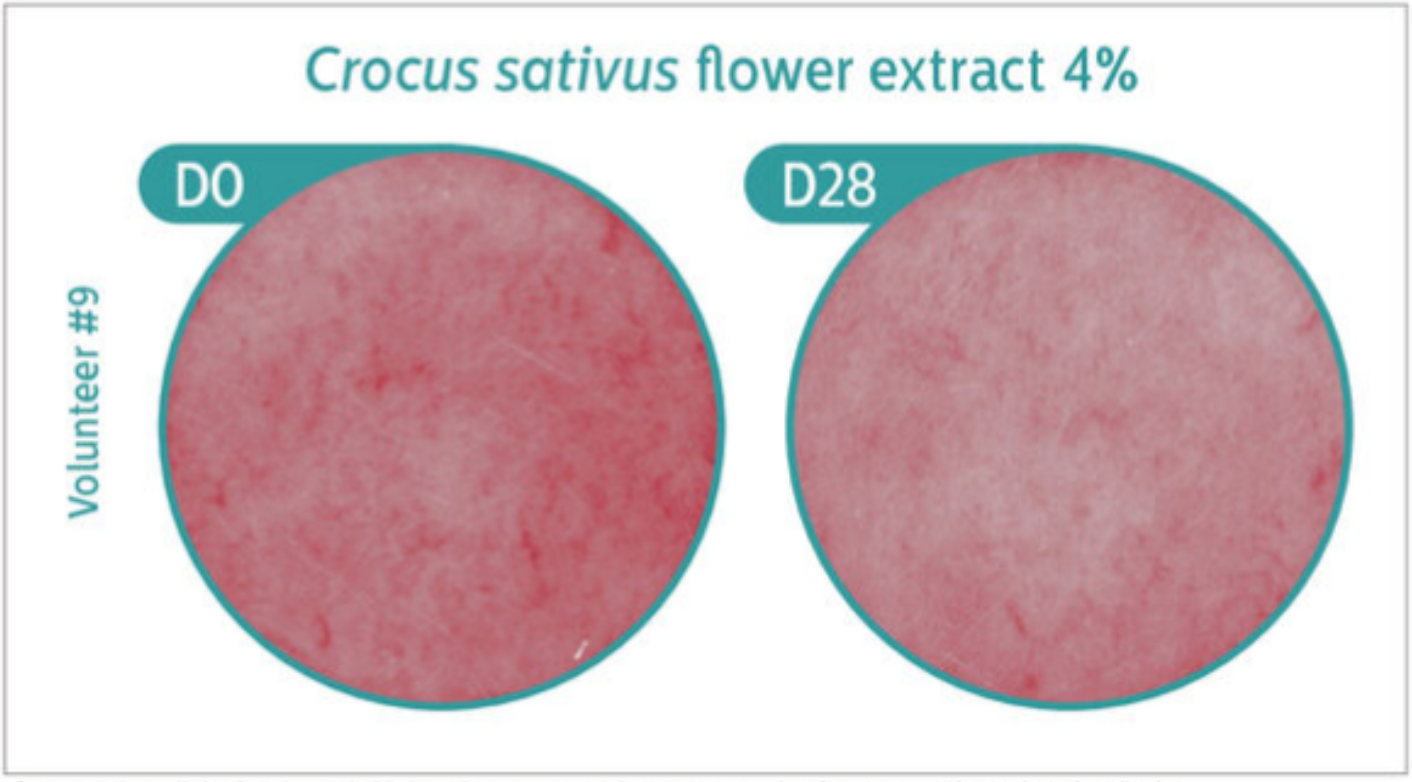

Figure 3: Redness diminution observed with the active cream containing 4% *Crocus sativus* flower extract after 28 days of application

Thymic stromal lymphopoietin (TSLP) is a cytokine primarily secreted by keratinocytes. It interacts with several immune cells and, when in contact with viruses, bacteria and parasites can potently increase TSLP expression. TSLP has been shown to be involved in skin hypersensitivity and itch initiation. In the dysbiosis model, TSLP was reduced by 67% in presence of the extract (Figure 1).

IL-1β is a major pro-inflammatory cytokine secreted by immune cells and keratinocytes that plays a critical role in the initiation and maintenance of skin inflammation. Its production under UV-A and pollution inflammation was reduced by 65% in presence of the extract.

Finally, we evaluated the level of IL-31, a cytokine constitutively produced by immune cells but also secreted by keratinocytes and involved in the pathogenesis of itch severity.[7] Under UV-A and pollution inflammation, IL-31 was reduced by 83% in presence of 1% *Crocus sativus* flower extract (Figure 2).

The final evaluation was performed with an *in vivo* test (redness reduction) on 20 Caucasian female volunteers with sensitive and/or reactive skin with atopic tendencies (45 ± 8 years old). A cream containing 4% *Crocus sativus* flower extract and a placebo were applied twice a day for 28 days with hemi-facial application.

Hemoglobin quantification was performed via SIAscope® to measure redness and a self-assessment on efficacy was realized. At the end of the evaluation, the *Crocus sativus* flower extract was able to reduce redness by 7% versus placebo on volunteers (Figure 3). During the self-assessment on efficacy, volunteers described their skin as more supple, softer, fresher and more comfortable.

Conclusion

Environmentally sensitive skin is an extension of sensitive skin that is a major concern for a wide range of the population. The exposome is very often the cause of skin perturbation that leads to discomfort due to a chronical stress exposure.

A vicious inflammation cycle is then created with the stimulation of biological pathways that impair the skin barrier, leading to dehydration of the skin and a vulnerability to pollution or any other irritants that will exacerbate the aggravation of the skin.

The skin responds to the aggressive factors of the exposome by upregulating inflammation, which affects nerves and sensory pathways to the point of discomfort. We wanted to take a holistic approach to the global exposome from a scientific perspective to identify potential solutions to protect and soothe the skin.

We carried out a complete study at the cellular level first to identify specific markers involved in keratinocyte differentiation impairment, such as cortisol. Then to understand more about perception pathways, we looked at TRPV1 and β-endorphin levels.

We then implemented an experiment using explants for a more complete model to corroborate the impact of our active on important markers such as PGE 2, loricrin, filaggrin, IL-1β, and more specialized markers of DSC1 and DSG1.

Then, our multiple stress models allowed us to illustrate the interaction with biomarkers involved in itch and discomfort using more distinct markers such as S100A8/A9, TSLP or IL-31; all these results confirmed the observations made during the *in vivo* evaluation performed on volunteers.

Environmentally sensitive skin and its effects can be reduced by a cosmetic solution containing active ingredients such as our botanical extract Sens'Flower (*Crocus sativus* flower extract). This active ingredient appears to be very effective in taking a holistic approach and will help break this vicious cycle of barrier dysfunction, chronic inflammation, and discomfort to restore overall skin homeostasis. **PC**

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