TOPICAL TREATMENTS IMPROVED PERIORBITAL AGING

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ABSTRACT

Facial aging is manifested by different symptoms. One area people pay more attention to and use additional cosmetic products to keep healthier is the eye area – the periorbital region. There are multiple reasons why the periorbital region manifests an unhealthy and aged look. The skin in the eye area is thinner than other parts of face, and repetitive muscle movements and UV exposure lead to skin aging. Periorbital aging includes eyelid drooping, dark circles, under eye bags, and fine lines/wrinkles. Topical and mechanical treatments to improve periorbital aging is challenging due to the proximity to the eyes. Hypersensitivity to treatment options is a common occurrence in the eyes. In order to improve the skin in the eye area, topical ingredients were evaluated for their potential to modulate different skin aging target genes, such as improving skin structures, hydration, anti-inflammation, antioxidant capacity and cell turnover, using normal human skin equivalent cultures. Some interesting finding includes modulation of Prostaglandin E2 (PGE2). Induction of cellular stress with 5mM or 10mM hydrogen peroxide increased PGE2 expression, which would increase inflammation. In addition, PGE2 increase has been found in the skin of the elderly and inhibition of PGE2 resulted in collagen production in skin organ culture. Experimental ingredients are able to suppress that H2O2-induced PGE2 expression, similar to ascorbic acid. After evaluating ingredients using in vitro models, a specific formula was developed to target the clinical signs of periorbital skin aging. Prior to running a clinical study, the formula was evaluated for safety, due to potential hypersensitivity. The eye formulation did not trigger any safety concerns during in vitro evaluations and non-ocular evaluations and the formula was further assessed for safety during the efficacy testing by both dermatological and ophthalmologic evaluations. By dermatological evaluations, the formula improved periorbital aging in 50% of subjects with sagging skin, eyelid firmness and puffiness after 12 weeks of application.

INTRODUCTION

Perceived age does not necessarily represent one's chronological age. Based on lifestyle choices, such as smoking or sun exposures, people may look older or younger than their chronological age. Being perceived as younger than their age is favored due to the impression that one is healthier. This perception of one's age is influenced by different attributes, including face, hair, posture and voice [1]. For example, uneven skin tone or gray hair gives the impression that one is older. An area of the face that plays a significant role in the perceived age is the periorbital region. It is an area where people are drawn for the first impression, as well as an area where one will see one of the first signs of skin aging. The periorbital skin is thinner and with repetitive muscle movements, the eye area develops lines and wrinkles sooner [2]. Signs of periorbital aging include eyelid drooping, dark circles, under eye bags, or fine lines/wrinkles [3]. There are multiple different causes for periorbital aging. Skin around the eyes becomes thinner and may swell or droop more easily, due to lack of the support from the underlying structures. The skin in the eye areas is more sensitive to irritants than other skin, partially due to thinner epidermis. Sleep deprivation or water retention is some of the known causes. Factors that would improve periorbital skin are antioxidants, anti-inflammation, firmness, etc. In order to assess improvement with cosmetic actives, in vitro gene expression analysis was done for skin-specific target genes in a skin equivalent model. In addition, a skin equivalent was also challenged with an oxidant, hydrogen peroxide, with or without the cosmetic actives, for the assessment of skin antioxidant and inflammation target gene modulations. After formulating the cosmetic actives in an eye cream, it was evaluated in clinical studies for tolerability by a dermatologist as well as an ophthalmologist, and for efficacy in improving the periorbital skin aging.

METHODS

In order to evaluate cosmetic ingredients on gene expression, full-thickness human skin equivalents were obtained from MatTek (Ashland, MA). These human skin equivalents were made from normal human fibroblasts and normal human keratinocytes. After equilibrating the skin equivalents according to the manufacturer's protocol, they were dosed with cosmetic actives in four replicates. For the active's antioxidant capacity evaluation, skin equivalents were dosed with hydrogen peroxide in the presence or the absence of the actives. After a 24-hour incubation period, the skin equivalents were harvested. Maxwell 16 LEV Simply RNA Tissue Kits (Promega, Madison, WI) were used to isolate RNA, followed by determination of RNA concentration and quality using a NanoDrop 2000 (Thermo Scientific, Waltham, MA). cDNA was synthesized using High Capacity DNA Synthesis Kits (Life Technologies, Grand Island, NY). 440 validated Taqman gene expression assays with endogenous control genes were put together in custom OpenArray (Life Technologies) formats and analyzed using the QuantStudio 12K Flex instrument (Life Technologies). Data was processed using RealTime StatMiner software v 4.2 for statistical analysis.

To assess the clinical efficacy and tolerability of the eye cream, Asian and Caucasian female subjects between 35 and 70 years old were recruited and evaluated by a dermatologist and an ophthalmologist. Subjects were instructed to apply the eye cream after cleansing the face, to the under eye and crow's feet areas twice daily for 12 weeks. 33 subjects were qualified by the dermatologist and the ophthalmologist to be in the study and 29 subjects completed the study. The study was approved by IRB.

RESULTS

A cosmetic active blend containing *Rhodiola rosea* root extract, *Saccharomyces cerevisiae* extract, betaine, pentylene glycol and water, was evaluated in the skin equivalent model. This active blend was shown to improve the periorbital skin by reducing the appearance of puffy eyes. 440 target genes were selected and custom arrays were prepared to evaluate different skin-specific target genes. Selected targets are shown in Figure 1. The actives stimulated skin barrier, antioxidant, proliferation and extracellular matrix genes, like filaggrin and superoxide dismutases, and suppressed pigmentation and inflammation related target genes.

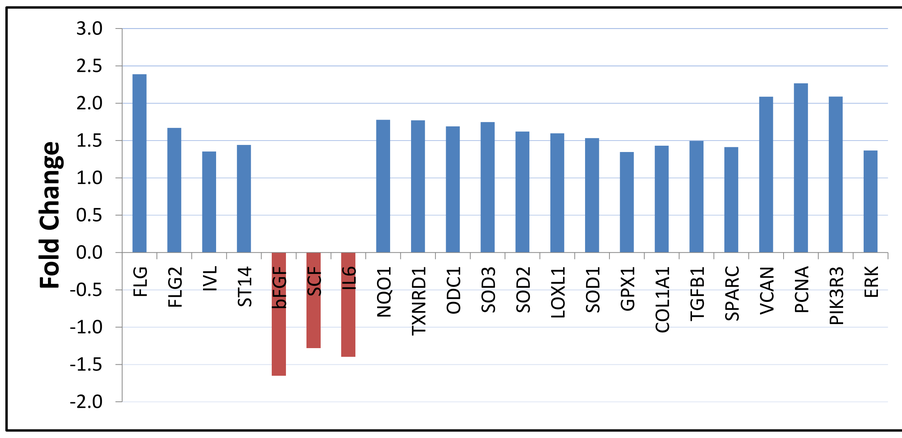
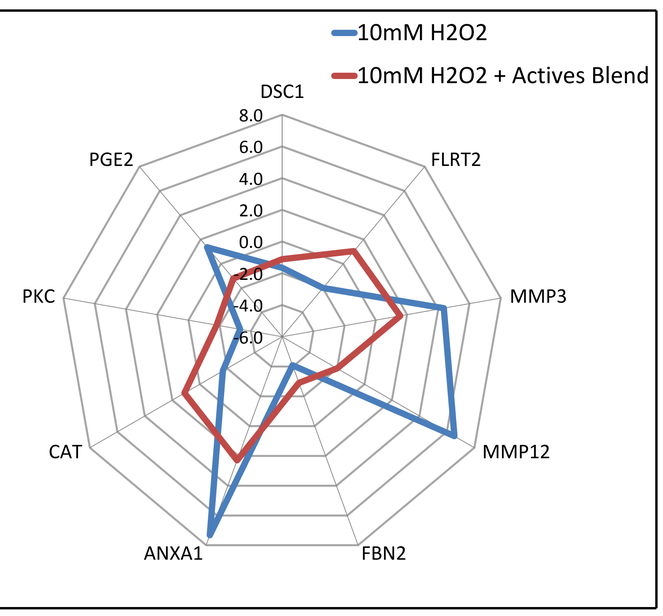


Figure 1. Selected target genes from OpenArray gene expression analysis of skin equivalents dosed with the cosmetic actives blend. Only statistically significant fold changes compared to the untreated samples were shown ($p<0.05$). Stimulated genes are shown in blue and suppressed genes are shown in red.

Figure 2. Selected target genes from OpenArray gene expression analysis of skin equivalents either challenged with 10mM hydrogen peroxide only, or 10mM hydrogen peroxide with the cosmetic actives blend. Only statistically significant fold changes compared to the untreated samples were shown ($p<0.05$). Hydrogen peroxide alone is shown in blue and hydrogen peroxide with the blend is shown in red.



In order to evaluate the antioxidant capacity of cosmetic actives blend, the skin equivalents were first challenged with a known oxidant, hydrogen peroxide at different concentrations, to modulate different target gene expression. In addition, skin equivalents were also dosed with a combination of hydrogen peroxide with the actives blend. Only 10mM hydrogen peroxide data is shown in Figure 2. Several target genes were modulated by hydrogen peroxide, which was countered by the actives blend, demonstrated by more neutralized gene expression.

Thirty three subjects were enrolled and 29 subjects have completed a clinical study, lasting for 12 weeks. Among the completed subjects, 20 were Asian and 9 were Caucasian. At week 12, multiple skin attributes for the efficacy of the eye cream were evaluated by a dermatologist investigator (Figure 3). Eyelid firmness was the skin attribute with the highest percent of improved subjects, with improvements on under eye puffiness, overall puffiness and sagging skin being close seconds. For safety and tolerability, subjects were evaluated by a dermatologist at baseline before and after product application, and at weeks 2, 4, 8 and 12, and by an ophthalmologist at baseline before and after product application and at week 4. There were no adverse events detected other than mild dryness and mild erythema, that diminished without intervention.

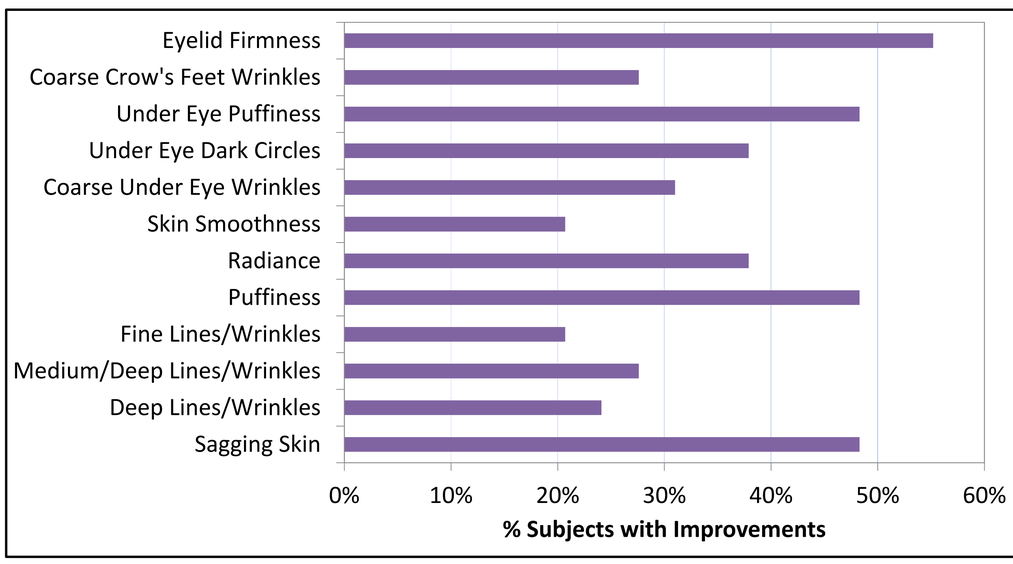


Figure 3. Percent of subjects with skin improvement by a clinical grade is shown according to different periorbital skin attributes at week 12, end-of-study.

CONCLUSIONS

- The cosmetic actives blend modulated skin specific target genes, improving skin barriers and matrix. In addition, challenging with hydrogen peroxide demonstrated the blend was able to oppose the effects of the oxidant in the skin equivalent model.
- The eye cream formulated with this actives blend improved the periorbital skin, demonstrated by the dermatologist grader.
- The eye cream was well tolerated by clinical subjects, both dermatologically and ophthalmologically.

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