Instant Wrinkles Reduction Products that Look and Feel Great

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Abstract

Background: Anti-aging cosmetics aim to reduce the appearance of wrinkles in the skin, benefits that typically accumulate over weeks and months. Our aim was to develop a formulation that 1) instantly reduced the appearance of wrinkles, 2) did not create undesired feelings of tightness, and 3) provided long-term benefits to the consumer. By combining a film-forming polymer that 'micro-textures' the skin and moisturizing components that diffuse into the skin, we optimize both appearance and sensation.

Methods: The stratum corneum (SC) was tested *in vitro* using a substrate curvature technique to measure SC stresses. Measurements were done before and after product application, and after product removal of the following three formulations: the full composition, the product without film-forming ingredients, and the product without moisturizing components.

In conjunction, a US clinical study was conducted with 41 participants aged 40-70. Volunteers were instructed to apply the full formulation daily on crow's feet and under eye areas for eight weeks, with clinical grading throughout the study.

Results: The product without film-forming ingredients decreased SC stresses, while the product without moisturizing components increased SC stresses. Together the two effects offset in the full formulation.

Results from the clinical study showed clear instantaneous and long-term benefits. In addition, over 70% stated that the product feels comfortable on skin directly after application.

Conclusion: We show that combining polymer films and moisturizing components in an optimal manner generates a product with the desired instant benefits, a pleasant sensorial feeling, and the ability to deliver long-term improvements to the appearance of fine wrinkles.

Keywords: anti-wrinkle, skin comfort, skin appearance, beauty

Introduction

Skin is a multifunctional composite designed to maintain water balance and protect against external aggressors. However, several processes degrade this natural tissue, damaging its structure, function, and appearance as a result. These damage mechanisms can be separated into two categories: intrinsic and extrinsic. Intrinsic, or chronological, ageing is characterized by atrophy of the skin affecting rate of desquamation, dermal thickness, thermoregulation, mechanical protection, sensory perception, and vitamin D synthesis. Extrinsic aging is superimposed on top as response to environmental damage, in particular UV radiation. Also known as photoaging, this process is associated with a thickened epidermis, increased melanogenesis, elastosis, collagen degeneration, and dilated microvasculature [1]. Notably, changes at both the surface and deeper layers contribute to and exacerbate the appearance of wrinkles.

Photoaging has a significant effect on dermal collagen, whose degeneration and loss is one of the main factors associated with wrinkle formation [2]–[4]. Specifically, UV radiation induces the expression of matrix metalloproteinases in fibroblasts of the connective tissue. These metalloproteinases target and degrade collagen fibers in the dermis; synthesis and repair of these fibers is always imperfect leaving subtle defects in the organization of the extracellular matrix, eventually leading to the formation of observable wrinkles on the surface [5]. However, the appearance of these wrinkles is accentuated by surface-level modifications.

The surface roughness at the microscale, also known as the micro-texture or microtopography, plays a pivotal role in how light interacts and reflects off the skin surface. Smoother surfaces produce more specular reflectance, while rougher surfaces result in more diffuse reflectance. It is well established that different body locations exhibit starkly different micro-textures [6]. However, of particular importance is the fact that skin slowly loses its micro-texture with age. Specifically, the number of peaks and troughs per unit surface area decreases with age; however, the height of these peaks and troughs may actually increase with age [7], [8]. If micro-texture helps mask or cover wrinkles, is it possible to artificially roughen the skin at the microscopic level using a cosmetic formulation?

Anti-aging cosmetics aim to reduce the appearance of fine-lines and wrinkles in the skin, benefits that typically accumulate over weeks and months. However, recent advances in skincare formulations have generated interest in products with instant anti-aging benefits (i.e., within a matter of minutes). There are several mechanisms through which such formulations can reduce wrinkles. One is the formation of a superficial polymer film that imposes a tensile stress on the skin surface, increases roughness at the microscale, and results in more diffuse light reflection that hides prominent wrinkles. Such products, while

demonstrating short-term benefits, often discard long-term benefits and have potentially damaging consequences, such as uncomfortable tightening and drying of the skin.

Recent work has demonstrated a clear link between sensations of tightness and mechanical stresses developed or imposed upon the uppermost layer of human skin: the stratum corneum (SC) [9]. Specifically, film stresses in the SC that arise due to drying, irritation, or damage, can propagate strain fields deep into underlying layers. Mechano-sensitive cells known as mechanoreceptors (MRs) respond to this deformation by sending neurological signals of discomfort to the central nervous system and brain. Superficial film-forming polymers are believed to impose a tensile stress on the SC to reduce wrinkles, however this is accompanied by activation of MRs and sensations of tightness. Reduction of these stresses corresponds to feelings of pleasantness and comfort.

Our aim was to develop a formulation that 1) instantly reduced the appearance of wrinkles, 2) avoided any undesired sensation of tightness, and 3) provided long-term benefits to the consumer. Two types of ingredients were used to achieve this goal: film-forming polymers and moisturizing components. The polymers leave an invisible coating on the skin surface with significant in-plane contractions. The resulting tensile stresses are believed to increase roughness at the microscale through local buckling; but these stresses also propagate strain waves into deeper layers where mechanoreceptors send signals of tightness to the brain [9]. In contrast, the moisturizing components diffuse into the topmost layers of skin, helping it reduce in-plane stresses [10]. By combining a film-forming polymer that ‘micro-textures’ the skin and hygroscopic moisturizing components that diffuse into the skin, we optimize both appearance and sensation.

Methods

Stratum Corneum Preparation

Full-thickness human cadaver skin from Caucasian females aged 30-90 years old were obtained through the National Disease Research Interchange (NDRI). Tissue from the abdomen was stored in a −80 °C freezer until processing. First, the subcutaneous tissue was cut away. Subsequently, the epidermis was separated from the dermis via heat treatment in 35°C water for 10 minutes followed by a one-minute soak in 60 °C water. The epidermis was then mechanically separated from the dermis using a flat-tipped spatula. The living epidermis was then removed from the SC using a trypsin enzymatic digest solution [0.1% (wt/wt) in 0.05 M, pH 8.3Tris buffer] at 35 °C for 180 minutes. The SC was then rinsed, dried, and subsequently removed to be stored in a low-humidity chamber (∼10–20% RH) at an ambient temperature of ∼18–23 °C. The work does not involve human subjects per standard guidelines and ethics approvals are therefore not required.

Drying Stress Profiles

Pieces of hydrated SC were adhered onto a 22 mm x 22 mm x 170 um cantilever beam made from borosilicate glass and coated with conductive Cr/Au (35 Å / 465 Å). As the SC dries in a chamber with controllable humidity (<5% RH), the substrate bends (Figure 1). The substrate curvature, K, is measured with a scanning laser substrate curvature instrument (FLX-2320, Tencor Instruments, Mountain View, CA, USA) and translated to an SC film stress, σ_{SC}, using Stoney's equation:

$$\sigma_{SC} = \left(\frac{E_{sub}}{1-\nu_{sub}}\right)\left(\frac{h_{sub}^2}{6h_{SC}}\right)K$$

where E_{sub}, ν_{sub}, h_{sub}, are the Young's modulus, Poisson's ratio, and thickness of the substrate. h_{SC} is the thickness of the stratum corneum and is measured with a digital micrometer.

After adhesion, samples were dried for ~20 hours without application of any formulation as a control. Specimens were then rehydrated in a 100% RH air chamber for 2 hours to return stresses to zero. Formulations were applied (approximately 2 mg/cm^2) at this point, and the specimen was once again left to dry for ~20 hours in the substrate curvature instrument. Measurements were done before and after product application, and immediately after product removal. Three formulations were investigated: the full composition, the product without film-forming ingredients, and the product without moisturizing components.

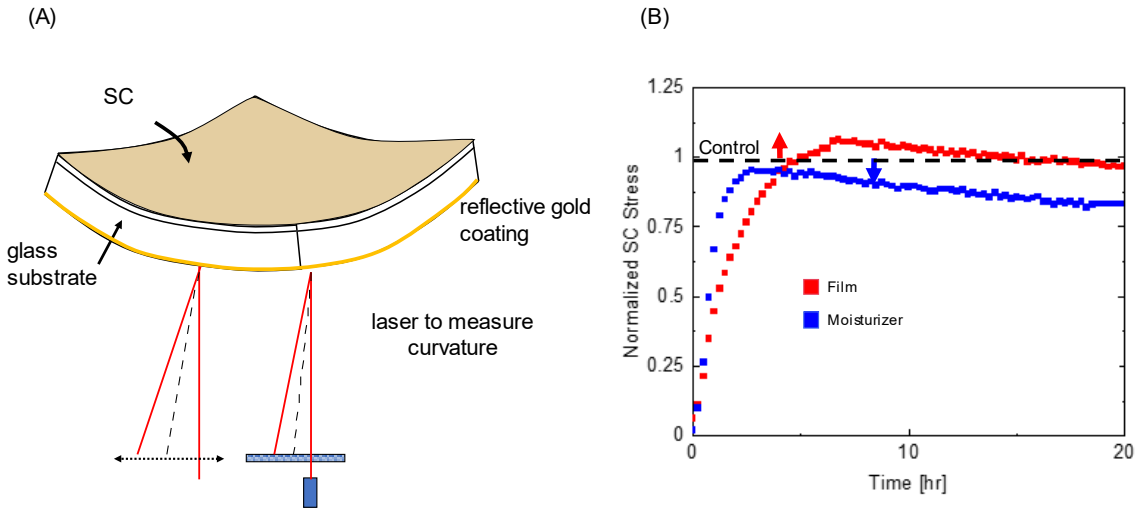

Figure 1: Drying stress setup and output. (A) A piece of fully-hydrated stratum corneum (SC) is adhered onto the backside of a glass substrate with a reflective gold coating. As the SC dries out, stresses develop in the tissue forcing the glass to bend. This curvature is measured with a laser and is directly proportional to the SC film stress. (B) Compared to control peak stresses, moisturizing components reduce SC stresses, while polymer films usually increase stresses.

Power Spectral Density

In conjunction to drying stress, the surface of the skin was analyzed to determine the spectral makeup of the roughness. Subcutaneous tissue was removed from cadaver skin, and the remaining skin layers (epidermis + dermis) were equilibrated to the room environment and constrained. Images were taken before and after formulation application using the Keyence VHX-7000 digital microscope. These images were analyzed using Power Spectral Density (PSD) theory to capture the contribution coming from wrinkles of different sizes (from about 1 μm to 1 mm) to the total skin roughness. This is a well-established technique to characterize and understand images of surface topography [11], [12]. PSD decomposes the signal (in this case surface topography) into a superposition of waves of varying frequencies (Figure 2). The power from a specific frequency is related to how much that particular wavelength contributes to the total signal. In particular, the discrete Fourier transform of the images is taken. The power is then defined as:

$$P(l,k) = \frac{|F(l,k)|^2}{MN}$$

where F(l,k) is the Fourier value of a pixel located at (l,k); M and N are the width and height of the image in pixels. The resulting array is usually averaged radially. Pixels closer to the center of the image correspond to large wavelengths (~1 mm) and often contribute the most power to the total signal. Smaller wavelength (~1 μm) correspond to pixels further away.

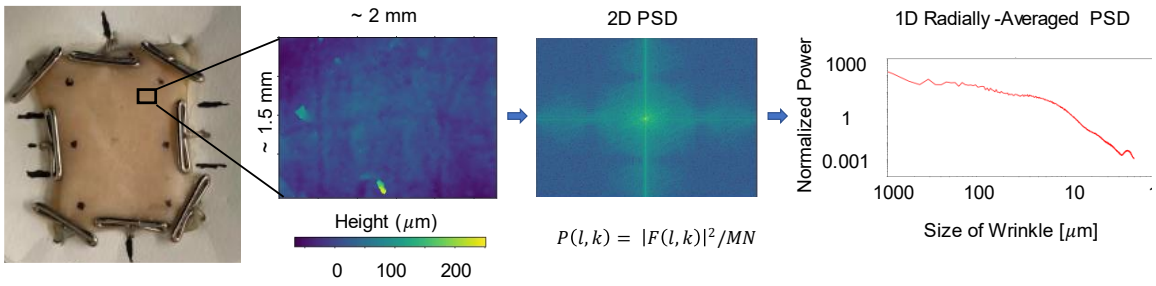

Figure 2: A roughly 3x3 cm^2 piece of skin (epidermis + dermis) was constrained and imaged with 100x magnification. The resulting surface map was Fourier-transformed and the power spectral distribution (PSD) was calculated. The 2D PSD was radially averaged to produce the 1D PSD, which is used to compare microtopography roughness of skin before and after application of various topical creams.

Clinical Study

In conjunction to these *in vitro* measurements, a US clinical study was conducted with male and female participants (n=41, <10% males) aged 40-70. Participants had moderate to severe periocular fine lines and wrinkles at the start of the study. Volunteers were instructed to apply the full formulation daily on crow's feet and under eye areas for eight weeks, with clinical grading occurring periodically throughout the study.

Results

In vitro testing of SC was able to distinguish between the different formulations, emphasizing the complementary roles of the film and moisturizing components. Upon application, the product without film-forming ingredients (in blue) decreased SC stresses by 5%, while the product without moisturizing components (in red) increased SC stresses by 6%. Together the two effects offset as seen in the full formulation (in grey) which increased SC stresses by 1% (Figure 3A).

Moisturizing components that diffuse into the skin cannot be easily removed and should therefore have a lasting effect on stresses. In contrast, the film which is a superficial layer can be readily removed and stresses should return to control levels. This is what we see upon removal, as the product without film-forming ingredients reduced SC stresses by an additional 7% (a total reduction of 12% to control), indicating that hygroscopic components diffused further into the skin. However, upon removal of the product without moisturizing components, stresses returned to control levels indicating that the film-forming ingredients remained solely on the surface of the skin. Upon removal of the full formulation, stresses decreased by 10% compared to control (Figure 3B). This corresponds with the fact that after removing the film, the full formulation should be the same as the product with no film former. The formation of these film surfaces, and the accompanying tensile stress acting upon the skin, affect microtopography.

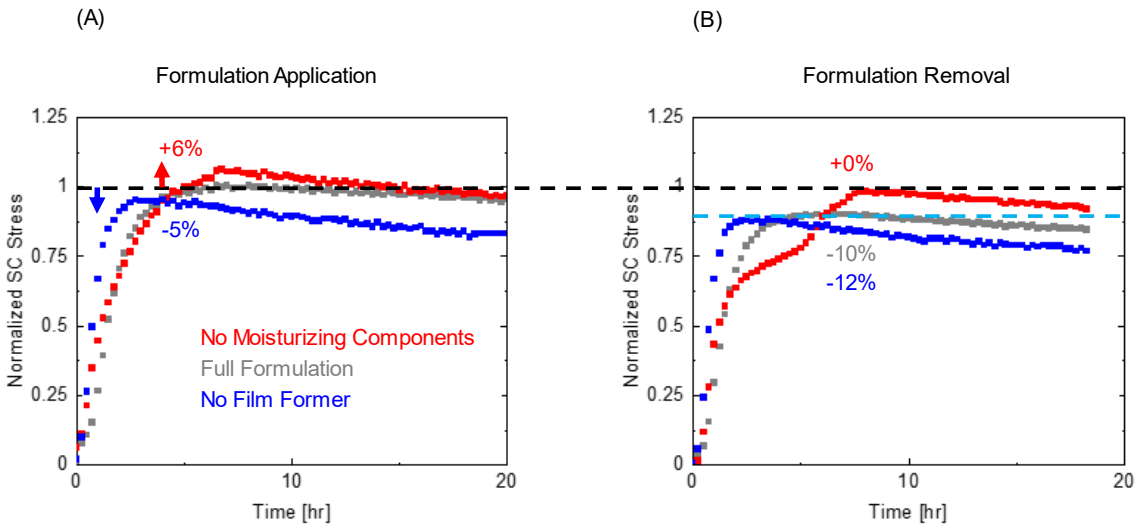

Figure 3: Drying stress curves for different formulations. (A) Upon application, we see that a product containing a film-former, but no moisturizing components (in red) increases stresses by 6%. A product with moisturizing components, but no film-former (in blue) decreases stresses by 5%. The full formulation that contains both film former and moisturizing components (in grey) lies between and increased stresses by 1%. (B) Upon removal, the product containing no moisturizing components returned to control levels as the superficial film was removed. In contrast, the product containing no film former actually sees a further decrease in stress (-12%) as the moisturizing components diffuse deeper into the skin. The full formulation, after removing the superficial film, becomes identical to the formulation that had no film-forming ingredients to begin with. This is seen as both the grey and blue curves reduce stresses by similar amounts.

Using PSD theory, the micro-roughness or microtopography of the skin was analyzed. Application of either the full formulation or the product without moisturizing components both demonstrate an increase in high-frequency contributions corresponding to micro-buckling and a decrease in low-frequency contributions corresponding to flattening of large wrinkles (Figure 4). Interestingly, the rate of these effects is different with the full formulation inducing micro-buckling and macro-flattening nearly instantly versus the much slower development of the product without the moisturizing components. This suggests that softening of the SC due to diffusion of hygroscopic components may play a pivotal role in effective and quick activation of the polymer film.

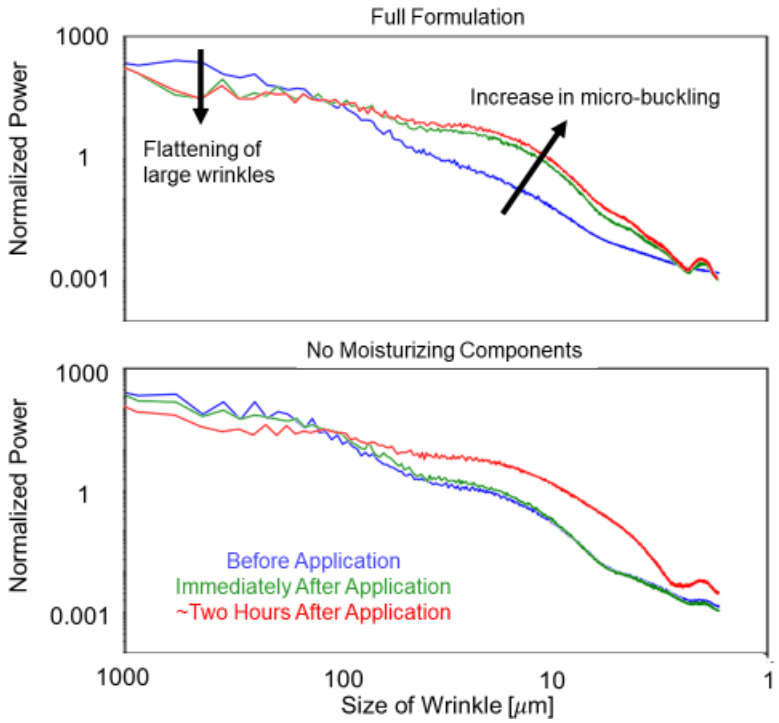

Figure 4: PSD results for full formulation and formulation that does not contain moisturizing components, but does contain the film-forming ingredients. Both products increase roughness at the microscale (10-100 μm) from before (blue) to two hours after application (red). Both products also appear to flatten large wrinkles (100-1000 μm). However, the full formulation containing the moisturizing components appears to immediately cause these effects, in contrast to the product without these components.

To better understand why increased SC stresses cause sensations of tightness, a finite element model was used to simulate strain field propagation using laboratory stress measurements as inputs. Since superficial polymer films increase SC stresses, we expect more activation of mechanoreceptors at the epidermal-dermal boundary. The finite element model corroborates this as we see larger levels of strain with the film than without the film (Figure 5).

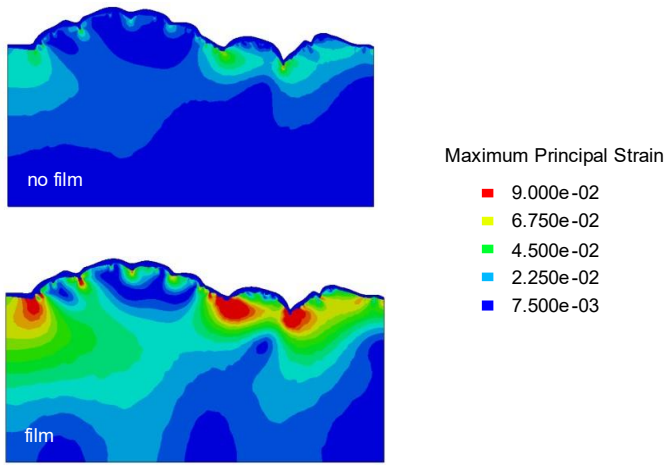

Figure 5: Finite element simulation showing the tightening effect of a film on top of the skin. Red corresponds to high strains; blue corresponds to low strains. Higher strains activate more mechanoreceptors located at the epidermal-dermal boundary, resulting in increased sensations of tightness.

A clinical study on the full formulation product was conducted simultaneously to compare to laboratory measurements and computational simulations. Results from the clinical study showed that over 70% of participants experienced tensing associated with a taut and youthful appearance within five minutes of application, 81% had fewer under eye fine lines (via clinical grading) and 78% had fewer crow's feet fine lines (via clinical grading) by the end of the study, illustrating clear long-term benefits. In addition, over 70% stated that the product feels comfortable on skin immediately after application, and 85% stated that the product is gentle enough for daily use after 4 weeks. No other tolerability parameters, visual acuity, or slit-lamp ophthalmological examination parameters (including subjective sensation) changed significantly between pre- and post-application, indicating comfort and safety of the formulation.

Discussion

In this work, we examine the mechanisms behind how anti-aging formulations function and investigate ways to optimize both comfort and appearance when designing these products. A clear link was established between increased SC stresses and the film-forming ingredients in these formulations. Removal of the film lowers stresses back to control levels, however, also diminishes any anti-aging benefits the film might have. Therefore, modifications to the composition, by adding moisturizing components, helped alleviate some of these increased stresses while still maintaining the film intact (Figure 6).

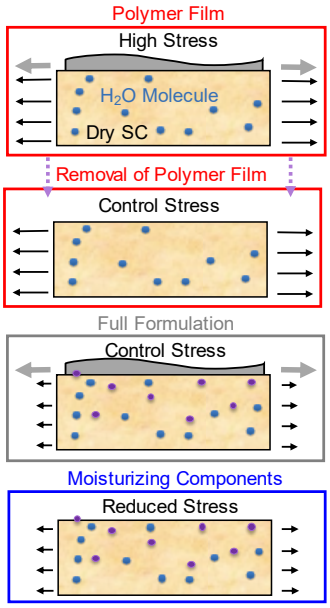

Figure 6: Skin stresses upon application and removal of different formulations. Polymer films (red rectangle) increase stresses when applied, but return to control levels when removed (dashed arrow). Moisturizing components (blue rectangle) decrease stresses. Full formulations (grey rectangle) combining film and molecules lies in between.

The film-forming ingredients impose a tensile stress on the skin that affects both appearance and comfort. Using PSD theory, we show that these films induce micro-buckling of the SC while also flattening larger millimeter-size wrinkles. Interestingly, by combining film-forming ingredients with moisturizing components, we achieve a much quicker and effective reduction of wrinkles. It is possible that the moisturizing components help to soften the SC, thereby making it easier for the superficial polymer film to change the microtopography, however more work must be done to definitively show this connection. Furthermore, while we show that increased micro-buckling measured in the lab corresponds to wrinkle reduction seen in the clinical study, there remains a lack of quantitative evidence to concretely explain this link. Would even more micro-buckling have a greater effect on skin appearance, or is there a limit to how much it can reduce wrinkles? If not, then comfort would be the limiting factor when designing these formulations.

Using a finite element model, we demonstrate that higher SC stresses (as a result of superficial film-formation), correspond to larger strains at mechanoreceptor locations. This would lead to more intense neurological signals being sent to the central nervous system, and therefore increased feelings of tightness. As discussed previously, one possible solution is to

add moisturizing components that act to reduce SC stresses by penetrating into the skin and occupying space between the corneocytes.

Lastly, this study shows the power in combining laboratory, computational, and clinical methods together to fully understand how cosmetic formulations interact with a consumer's skin. The clinical study not only was able to corroborate the *in vivo* and *in silico* results related to instant wrinkle reduction and skin comfort, but also found clear long-term benefits when using the product.

Conclusion

This study begins to explain how film-forming polymers combat skin wrinkles both instantaneously and long-term, while also providing remedies to the discomfort often accompanying these formulations. We demonstrate that these films by themselves increase the biomechanical stress experienced by the SC. These stresses (of a few MPa) micro-texture the skin to reduce the appearance of harsh wrinkles, but also activate mechanoreceptors located at the dermis-epidermis boundary. By including moisturizing components that relax skin stresses, it is possible to counterbalance the effect of film-forming polymers. We show that combining these two approaches in an optimal manner generates a product with the desired instant benefits, a pleasant sensorial feeling, and the ability to deliver long-term improvements to the appearance of fine lines around the eyes.

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Conflict of Interest Statement. TO, SC, DO, SB, are employees of Rodan+Fields, San Francisco, CA, USA.

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