INTRODUCTION

Skin is under constant challenge from a variety of external aggressors. 
Although aggressors like ozone\(^1\) and urban pollution\(^2\) have gained 
considerable attention in recent years, UV radiation remains the most 
well-studied and ubiquitous source of long-term skin damage.\(^3,4\) Skin 
photoaging leads to a host of deleterious effects including collagen and 
extracellular matrix breakdown, skin discoloration, and carcinogenesis.

Abstract

Background: Effective skin lightening remains an unmet need in over-the-counter 
formulations.

Aims: This research examined a topical facial formulation containing hexylresorcinol, 
silymarin, 20% vitamin C, and 5% vitamin E in a proprietary anhydrous vehicle in skin explants for UVB photoprotective effects and clinical benefits.

Patients/Method: In vitro investigation examined 12 skin explants to assess the test 
product and vehicle. Six skin explants received 10 \(\mu\)L of the study product, and six 
skin explants received the 10 \(\mu\)L of the vehicle. After 96 hours, half the skin samples 
were exposed to 250 mJ/cm\(^2\) of UVB radiation while the other half unexposed. 
Clinically, 42 female subjects with normal or dry skin 35-55 years with skin types I-VI 
were enrolled possessing discoloration, uneven skin tone, and fine lines. The dermatologist investigator evaluated brightening, evenness, fine lines, wrinkles, and global appearance.

Results: Explants treated with the study product experienced no significant change 
in gene marker expression of pro-collagen and pro-inflammatory gene markers upon 
UVB exposure. In contrast, skin explants treated with the vehicle experienced sig-
ificant decreases in pro-collagen expression and significant increases in pro-inflamm-
atory gene marker expression. Clinically, the greatest improvement as compared to 
baseline was seen at week 12 \((P < .001)\) with 45% improvement in brightening, 27% 
improvement in evenness, 25% improvement in lines, and 25% improvement in global facial appearance.

Conclusion: Hexylresorcinol, silymarin, 20% vitamin C, and 5% vitamin E in a propri-
etary anhydrous vehicle are effective in decreasing UVB-induced photodamage in 
skin explants while clinically producing skin brightness improvement.

KEYWORDS

hexylresorcinol, pigment lightening, silymarin, UV damage, vitamin C, vitamin E
Antioxidants such as ascorbic acid (vitamin C) and tocopherol (vitamin E) have documented effects in mitigating UV-induced photoaging. Although alone each of these antioxidants has beneficial properties, the combination of the two vitamins is known to induce a synergistic effect imparting enhanced protection against photoaging. Oral vitamin supplementation has limited effectiveness in protecting the skin as an optimal pharmacological antioxidant dose is not always present in skin. In contrast, topical application of vitamin C and/or vitamin E can protect the skin from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging. However, there are challenges in developing topical vitamin C and E formulations. Both actives are unstable from photoaging.

This research examined a formulation containing hexylresorcinol and silymarin in a proprietary anhydrous base incorporating 20% vitamin C and 5% vitamin E. This preparation was examined by measuring its ability to protect human skin explants from photograph-induced damages, via the expression of pro-collagen and pro-inflammatory gene markers. In addition, clinical testing was undertaken to demonstrate skin brightening appearance benefits.

2 | METHODS

2.1 | In vitro method

Twelve human skin models (NativeSkin®, Genoskin) produced from one donor were used to test both the product and the vehicle. Six skin explants received 10 μL of the test product, and 6 skin explants received the 10 μL of the vehicle rubbed on for 30 seconds. The process was repeated for four consecutive days. After 96 hours, half the skin samples were exposed to 250 mJ/cm² of UVB radiation while the other half unexposed. After treatment, all skin samples were cultured for an additional 17 hours prior to q-PCR analysis. N = 3 replicates were tested for each of the four groups (Table 1).

| TABLE 1 Cultured skin model assignments |
|-------------------------------|-----------------|
| Vehicle | Study product | UVB exposure |
| Group 1 (N = 3) | ✓ | ✓ |
| Group 2 (N = 3) | ✓ | ✓ |
| Group 3 (N = 3) | ✓ | ✓ |
| Group 4 (N = 3) | ✓ | |

At the completion of the culture period, skin samples were flash frozen in liquid nitrogen, immersed in RNAlater, and frozen at −80°C. Quantitative PCR was performed using TaqMan assays to measure expression of the following four gene markers: COL1A1, COL3A1, IL-6, and IL-8. The COL1A1 and COL3A1 gene markers encode for type I collagen and type III collagen, respectively. Type I collagen is the most prevalent form of collagen in the body, including the skin. Type III collagen is the newly synthesized form of collagen; therefore, up- or downregulation of this gene marker can indicate disruptions in the skin’s normal ability to synthesize collagen. The IL-6 and IL-8 gene markers encode for interleukin 6 and interleukin 8, respectively. These two proteins are cytokines whose synthesis is increased during inflammatory events, infection, or external damage. This research examined the ability of topical skincare products containing vitamin C and E to suppress the expression of interleukin gene markers in skin stressed with UV radiation.

The ratio of the expression for each individual gene marker was calculated between skin samples with and without UVB exposure (group 1 vs. 2 and group 3 vs. 4, Table 1). Unpaired t tests were conducted to determine the statistical significance. Linear fold-change values of 2 or higher were considered biologically relevant.

2.2 | In vivo method

Forty-two female subjects with normal or dry skin 35-55 years of age with Fitzpatrick skin types I-VI were enrolled. These subjects possessed signs of aging to include discoloration, uneven skin tone, and fine lines. Following completion of an IRB approved informed consent (AIRB) and after meeting all inclusion criteria and none of the exclusion criteria, subjects with lack of skin tone brightening, lack of skin tone evenness, fine lines, wrinkles, and global appearance issues were enrolled in the study. Subjects were not allowed to use topical retinoids or other cosmeceutical preparation within 2 weeks of study enrollment containing ingredients such as kojic acid, hydroquinone, vitamin C, vitamin E, licorice extracts, alpha hydroxy acids, etc.

Subjects were provided with the study product (PCA Skin C&E Advanced, Colgate-Palmolive Co. and PCA Skin) for once daily facial use and a facial sunscreen (Neutrogena Clear Face Broad Spectrum SPF 55) for use as needed during the study as required by IRB. Subjects continued to use their own self-selected moisturizer and cleanser without any anti-aging ingredients unchanged for the duration of the study. A compliance diary was provided to record the use of the study cream.

The dermatologist investigator evaluated lack of skin tone brightening, lack of skin tone evenness, fine lines, wrinkles, and global appearance issues at baseline, week 2, week 4, week 8, and week 12. Investigator tolerability was assessed in terms of erythema, edema, dryness, and peeling. Subject tolerability was assessed in terms of stinging, tingling, itching, and burning. All criteria were evaluated at baseline, week 2, week 4, week 8, and week 12 on a 5-point ordinal scale (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe).
VISIA CR4.3 (Canfield Scientific, Parsippany, NJ) photography was conducted of the front, right, and left face with visible light at baseline, week 4, week 8, and week 12, and the investigator assessed the photographs comparing facial pigmentation at baseline to week 12 using the following ordinal scale: 0 = excellent improvement, 1 = modest improvement, 2 = slight improvement, 3 = no change, 4 = worsening.

Statistical significance was defined as P-value less than or equal to .05. The ordinal nonparametric data obtained were evaluated as a change from baseline using a Wilcoxon signed rank test.

### 3 | RESULTS

#### 3.1 | In vitro results

The ratio of expression for relevant gene markers was determined between the UVB radiation exposed and unexposed groups (Table 2). The vehicle offered poor protection from UVB radiation, as the skin samples treated with the vehicle/UVB experienced a greater than twofold decrease in pro-collagen gene markers and a greater than twofold increase in pro-inflammatory gene markers compared to the samples treated with vehicle and no UVB radiation. In contrast, the study product containing hexylresorcinol, silymarin, and vitamins C and E provided excellent protection against UVB radiation. Samples that were treated with the study product over a four-day period and subsequently exposed to UVB demonstrated no significant changes in pro-collagen or pro-inflammatory related gene markers compared to samples that were treated with the study product, but not exposed to UVB radiation. These results indicate that the antioxidant ingredients in the study product provided protection against UVB-induced cellular damage. Since collagen is produced by fibroblasts in dermis,\textsuperscript{16} it may indicate the active ingredient combination can produce dermal protective effects.

#### 3.2 | In vivo results

42/42 subjects successfully completed the 12-week study with no adverse events. There was a statistically significant increase in subject reported facial stinging (P = .008), tingling (P = .004), and burning (P = .016) at 2 weeks. These noxious sensory stimuli improved with continued use at week 12 with only stinging being a statistically significant concern (P = .008). These sensory issues could be due to the hexylresorcinol, an ingredient used in face peels as a penetration enhancer. In order to decrease sensory issues, the dermatologist investigator advised subjects not to over apply the product, not to apply the product to a wet face, and not to apply the product too close to the eyes, nose, or mouth.

After 2 weeks of use, the dermatologist investigator noted a statistically significant 18% improvement in facial brightening (P < .001). More cumulative improvement was seen at week 4, with a statistically significant 26% improvement in brightening (P < .001) and 6% improvement in evenness (P = .016). At week 8, there was a significant improvement (P < .001) in brightening (38%), evenness (19%), and global facial appearance (18%) with improvement also seen in fine lines (P = .008, 7%). The greatest cumulative improvement as compared to baseline was seen at week 12 (P < .001) with an average 45% improvement in skin brightening, 27% improvement in skin evenness, 25% improvement in fine lines, and 25% improvement in global facial appearance (Figure 1).

The investigator compared the week 12 photographs to baseline for facial pigmentation and rated 36% of the subjects with excellent improvement, 21% with modest improvement, 29% with slight improvement, and 14% demonstrated no change. No subject demonstrated pigment worsening. Figures 2-4 present relevant images from the study demonstrating skin brightening.

### 4 | DISCUSSION

Facial skin brightening encompasses abundant light reflection from an evenly pigmented skin surface conferring the visual appearance of healthy skin. Since skin brightening is multifactorial, this formulation was designed to brighten the skin by containing an innovative combination of active ingredients. Hexylresorcinol is a time-tested penetration enhancing ingredient in dermatology that also induces stratum corneum exfoliation. The hexylresorcinol in this product produced peeling of the pigment containing corneocytes while enhancing penetration of vitamin C. This formulation contained 20% Vitamin C which was incorporated as an antioxidant and a tyrosinase inhibitor, the rate-limiting step in melanin synthesis, improving skin pigmentation. Other potent antioxidants included in the formulation were 5% vitamin E and silymarin. These ingredients were placed in an anhydrous silicone polymer base (cyclopentasiloxane, polysilicone-11, polymethylsiloxane) to create a smooth surface on the skin while delivering the active ingredients. Finally, the formulation contained a variety of antioxidants (Hordeum Distichon (barley) extract, Citrus Aurantium Dulcis (orange) oil, Phellodendron Amurense bark extract, and Santalum Album (Sandalwood) wood extract) to stabilize the vitamin C and E, but also provide oxidative protection to the skin.

The value of the antioxidant combination was demonstrated in human skin explants. Skin explants treated with the study formulation experienced no significant change in gene marker expression of biologically relevant pro-collagen and pro-inflammatory gene markers upon UVB exposure. In contrast, skin explants treated with the...
vehicle and subsequently exposed to UVB radiation experienced significant decreases in pro-collagen gene marker expression and significant increases in pro-inflammatory gene marker expression. This supports the value of the study formulation to both stabilize the active ingredients and deliver relevant antioxidant/anti-inflammatory doses to viable regions of the skin.
Although there are many topical skincare products on the market with both vitamin C and E, the anhydrous backbone of the study formulation allows incorporation of very high levels of each active (20% vitamin C, 5% vitamin E) in a stable manner. Because of the stabilizing effect of the anhydrous backbone, the product can use the pure, more potent form of ascorbic acid and alpha tocopherol rather than resorting to more stable less bioactive derivatives. Additionally, the presence of 1% hexylresorcinol provides further skin brightening benefits, while the presence of 1% silymarin provides additional skin protection benefits and prevents skin irritation.
A limitation of the in vitro study is the assumption that changes in gene expression will lead to changes in overall protein production; however, it takes time to see significant changes in protein production and the skin explants only have limited viability. Given the technical constraints of the materials used, measuring gene expression is the best option. A limitation of the in vivo study was the use of sunscreen; however, this was mandated for subject protection by the IRB. The study was run in North Carolina during fall and winter months (September 17, 2019 through December 10, 2019) where UVB exposure and outdoor activities were minimized.

Currently, there is a need for other innovative formulations to induce skin brightening and improve skin pigmentation evenness. Many skin lightening formulations abound, but are not necessarily supported by both in vitro and in vivo data. This blend of hexylresorcinol, silymarin, vitamin C, and vitamin E was found to be a stable antioxidant formulation possessing anti-inflammatory and skin brightening properties clinically demonstrated in women of all Fitzpatrick skin types.

5 | CONCLUSIONS

Skin explants treated with the study product for 4 days demonstrated no UVB-induced changes in pro-collagen or pro-inflammatory gene marker expression; in contrast, the skin explants treated with the vehicle demonstrated downregulation of pro-collagen gene markers and upregulation of pro-inflammatory gene markers. Hexylresorcinol, silymarin, 20% vitamin C, and 5% vitamin E in a proprietary anhydrous silicone polymer vehicle were effective in producing skin brightening in women of all Fitzpatrick skin types.

CONFLICT OF INTEREST

Zoe Diana Draelos, MD, received an educational grant from Colgate Palmolive to conduct this research. Isabel Diaz, BA, is an employee of Colgate-Palmolive Company. Colgate-Palmolive Company (Piscataway, NJ, USA). Aaron Cohen, PhD, is an employee of Colgate-Palmolive Company. Colgate-Palmolive Company (Piscataway, NJ, USA). Junhong Mao, PhD, is an employee of Colgate-Palmolive Company. Colgate-Palmolive Company (Piscataway, NJ, USA). Thomas Boyd, PhD, is an employee of Colgate-Palmolive Company. Colgate-Palmolive Company (Piscataway, NJ, USA).

AUTHOR CONTRIBUTION

All authors participated in the conduct of the research and writing of the manuscript.

ETHICAL APPROVAL

All subjects who participated in this research signed an Institutional Review Board approved consent, and Good Clinical Practice guidelines were followed.

DATA AVAILABILITY STATEMENT

All data are available upon request.

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