A serum containing vitamins C & E and a matrix-repair tripeptide reduces facial signs of aging as evidenced by Primos® analysis and frequently repeated auto-perception

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Abstract
Background: Allegations on the benefits of incorporating vitamin C, vitamin E, and combinations thereof in topical skincare formulations are mostly based on in vitro and ex vivo experiments and/or limited protocols of specific stress conditions (pollution, UV exposure, laser irradiation, ...).

Objective: To evaluate the instrumentally measurable effects and quantitative consumer perceptions of a protective and reparative serum on a panel of volunteers under normal nonstressed conditions of use, employing FOITS technology and innovative self-assessment methods.

Method: In an open-label study women of ≥40 years with visible signs of photoaging applied a serum comprising l-ascorbic acid USP (15% w/v), tocopheryl acetate USP, and 5 ppm palmitoyl tripeptide-38 to the face once daily for 56 days. Skin roughness and isotropy changes were evaluated on days 0, 28, and 56, visual instrumental evaluation of skin-tone parameters was assessed on days 0 and 56. Subjects completed self-assessment questionnaires every third day of the trial period for radiance, homogeneity, and wrinkle appearance.

Results: Skin-roughness parameters decreased significantly by 8%-9% (P < .05) and subjects experienced a significant increase in skin isotropy (P < .05). Photographic analysis revealed significant improvements in skin tone, with a 9% decrease in redness and 8% increase in homogeneity (P < .0001 for both), in excellent agreement with subjects' perception of significant improvements of radiance, complexion, and wrinkles.

Conclusion: The study confirms statistically significant correlation between objectively measured and quantitative subjectively perceived benefits of the bespoke serum containing antioxidants and a matrix-restoring peptide.

KEYWORDS
matrix repair, peptide, photoaging, vitamin C, vitamin E
INTRODUCTION

Vitamins C and E are popular ingredients in nutritional supplements and skincare formulations. Topical l-ascorbic acid, the most biologically active and well-studied form of vitamin C, is a multifunctional ingredient best known for its preventative antioxidant and photoprotective benefits.\(^1,2\) Vitamin C is a lipophilic antioxidant known for its beneficial effects on the skin, including photoprotection, hydration, and accelerated epithelialization, among others.\(^3\) Evidence suggests that both vitamins C and E protect the skin against UV radiation and may have additive or synergistic protective effects, with a fourfold increase in antioxidant protection factor when used together.\(^4\) While various studies on the benefits of topical vitamin C alone or in combination with vitamin E\(^5\) can be found in the literature, only a limited number of them report actual perceivable benefits in human trials under normal conditions of use, as opposed to ex vivo experiments, animal skin studies or additional stress treatments,\(^6-9\) such as laser therapy or UV irradiation.\(^7\)

Synthetic bioactive peptides—short sequences of amino acids derived from endogenous proteins (matrixines\(^10\))—have been shown to afford remarkable skincare and repair properties both in vitro and in clinical studies via synthesis and remodeling of the extracellular matrix.\(^11,12\) Topical peptides improve skin tightness, elasticity, and firmness, reduce the appearance of rhytides, and lessen other manifestations of photodamage and hyperpigmentation.\(^13\) Palmitoyl-lysyl-dioxymethionyl-lysine (palmitoyl tripeptide-38) is one such matrixine-mimetic peptide. Extensive in vitro and ex vivo (human skin explants) experiments have shown the peptide to stimulate the synthesis of six matrix molecules including collagen I, III, and IV, hyaluronic acid, fibronectin, and laminin,\(^14\) whereas a randomized vehicle-controlled clinical trial of the peptide confirmed the smoothing of forehead and crow’s feet wrinkle features when used in a topical preparation containing 5 ppm palmitoyl tripeptide-38.\(^15\)

This study was designed to objectively evaluate the effect of once-daily application of a serum containing both antioxidants, vitamin C, and E, and the repair peptide palmitoyl tripeptide-38 on the face of volunteer subjects, under normal conditions of daily use, using both instrumental, and innovative self-assessment parameters.

METHODS AND MATERIALS

Volunteers 40 years and older with Fitzpatrick skin type I to III and signs of photoaging (3-6 on Griffith’s scale\(^16\)) were included in this 2-month, open-label, before-and-after study. Informed consent, photographic release, and a detailed profile were obtained for each subject prior to participation. Exclusion criteria included pregnancy or breastfeeding; cutaneous pathology in the study zone; use of topical or systemic treatment likely to interfere with assessment of product efficacy during the previous weeks; use of an anti-aging product within the previous 2 weeks; history of cosmetic injections or surgery; use of general anesthesia associated with any surgery within the previous month; and excessive exposure to sunlight or UV rays within the previous month.

Subjects received unbranded samples of a serum (EverActive C&E™ + Peptide; Alumier Labs, Inc) comprising l-ascorbic acid USP (15% w/v), tocopherol acetate USP, and palmitoyl tripeptide-38 (5 ppm) as well as propanediol, dimethyl isosorbide, polyglyceryl-6 caprylate & polyglyceryl-4 caprate, polyglyceryl-6 & caprylate & polyglyceryl-3 cocoate & polyglyceryl-4 caprate & polyglyceryl-6 ricinoleate, and glycerin as principal components other than water.

To ensure optimal stability and efficacy, the l-ascorbic acid crystals were contained separately in a unique fail-safe cap, from which they were mechanically dropped and mixed into the bottle at first use. Subjects were asked to apply the serum to the entire face once daily in the morning after cleansing, including the crow’s feet region and excluding the eyelids. Other than a cleanser and basic moisturizer without any anti-aging actives, subjects did not apply any additional topical products for the duration of the study.

OUTCOMES

Subjects were assessed for changes in periorbital rhytides and skin structure after once-daily application of the study serum for 56 days. Perceived effect on radiance, evenness, and wrinkles, along with cosmetic acceptability of the product was evaluated via regular self-assessment scoring.

Instrumental assessment

Study visits occurred at day 0, 28, and 56 to evaluate dermal changes. Changes in periorbital rhytides and dermal structure of the cheek were assessed by an optical, in vivo, three-dimensional skin-surface measurement device (PRIMOS® Lite; GFM, Germany) that uses fringe projection and isotropy to assess skin topography.\(^17\) Photographs were taken on day 0 and day 56 with a facial image analysis system (VISIA®; Canfield Scientific, Inc) that quantifies various skin-tone parameters, including individual typological angle, whitening angle, and skin homogeneity index, based on the CIELAB color space defined by the International Commission on Illumination (CIE).

Subject self-assessment

Perceived effect on skin radiance, evenness, and wrinkles, along with cosmetic acceptability were recorded by subjects via treatment logs and self-assessment questionnaires. Subjects were asked to record a score on three skin parameters (radiance, complexion evenness, and wrinkles) every three days at home first thing in the morning in front of a mirror using a nonstructured scale from 1 to 10. Scores on each item were averaged and plotted over time. Additionally, subjects maintained a daily log to document any adverse effects or...
undesirable sensations. Self-assessment questionnaires and daily logs were submitted to evaluators at the end of the 2-month study.

### 3.3 | Statistical analysis

Data obtained for each parameter at each measurement time and on each zone were presented in raw value tables and then analyzed with EXCEL and SAS 9.4 software. Shapiro-Wilk test ($\alpha = 0.01$) was used to determine the normality of the difference, with subsequent paired Student’s $t$ test or Wilcoxon signed-rank test performed according to the result of the normality test.

### 4 | RESULTS

A total of 35 female Caucasian subjects with a mean age of 64 years (range, 49-74 years) and Fitzpatrick phototypes II (46%) and III (54%) participated in the study.

#### 4.1 | Changes in periorbital rhytides

Three-dimensional skin-surface measurements of the periorbital region revealed significant improvements in rhytides and skin roughness over time in more than 70% of subjects (Figure 1). On day 28, average relief $R_z$, maximum relief amplitude $R_t$ and skin roughness ($Ra$) decreased by a mean of $9\% (P = .0001)$, $8\% (P < .05)$, and $8\% (P < .001)$, respectively.

#### 4.2 | Dermal structure

Dermal structure was assessed by analyzing the direction of lines in cutaneous relief to determine an increase in isotropy (lines oriented in several directions) vs anisotropy (a more homogenous orientation of lines) that would indicate an improvement in skin structure (Figure 2). Analysis of isotropy parameters in the periorbital region of study subjects revealed a significant mean $4\%$ increase in isotropy in $68\%$ of subjects on day 28 ($P < .05$; Figures 3 and 4, Table 1).

**FIGURE 1** Rhytide reduction over time illustrated by color-coded depth, where yellow indicates shallow, green is moderate, and blue represents deep wrinkles at day 0 (A), day 28 (B), and day 56 (C).
Photographs taken from each subject on days 0 and 56 were analyzed to observe the evolution of color and homogeneity parameters quantitatively over time. Analysis revealed a significant 3% increase in clarity ($P < .0001$), a mean 9% decrease in redness ($P < .0001$), an increase of 8% in the ITA angle indicating decreased pigmentation ($P < .0001$), all of which confirmed an overall skin brightening effect (Figures 5A–D and 6).

**Figure 2** Variation of cutaneous relief parameters compared with baseline at days 28 and 56.

**Figure 3** Comparison of isotropy seen in younger skin (A) vs the anisotropy of aged skin (B) based on a directional dependence in the propagation of visible and near-infrared light through human skin in vivo. Aged skin shows strong directional anisotropy of dermal fibers, whereas the extracellular matrix in young skin is well-structured, with less evidence of photodamage.

**Figure 4** Comparison of skin isotropy in one subject via orientation of lines in cutaneous relief. Treatment with the study serum produced beneficial changes in dermal structure, from a lower degree of isotropy at day 0 (A) to a higher degree of isotropy at day 28 (B).

### 4.3 Skin tone

Self-assessment scores over the trial period indicate significant and progressive perceived improvements in complexion. Novelty here resides in following consumers’ perception of changes continuously over 56 days, where the average scores show significant differences to baseline after 30 and 14 days, respectively (Figure 7A,B). In addition, a more classical questionnaire confirmed the perceptions, by comparing the overall efficacy between baseline and day 56: subjects noted complexion to be more even (92%), redensified (86%), more radiant (88%), supple (88%), firmer (83%), and smoother (80%).

### 4.4 Self-assessment
after 56 days of use. Subjects also appreciated the organoleptic characteristics of the serum in terms of its color, texture, consistency, and finish on the skin (Table 2). The product was well tolerated, and no adverse effects attributed to the serum were observed.

5 | DISCUSSION

Results of this study of 35 women over the age of 40 demonstrate that once-daily application of a serum containing vitamins C and E combined with palmitoyl tripeptide-38 under normal conditions of use (no specific stress applied) produced noticeable improvements in skin appearance and structure, as measured by instrumental analysis and daily self-assessment over 56 days of treatment. While the numerical data expressed in % of change may appear small compared with studies under various stress conditions, they are nevertheless analytically and subjectively significant, particularly given the fact that the serum was used only once per day.

Topical antioxidants represent a noninvasive approach to the treatment of aging skin. Vitamin C is one of the most potent antioxidants in the skin, neutralizing damaging oxidative stress caused by UV exposure, pollution, and smoking. By interacting with copper ions at the tyrosinase-active site, which inhibits the enzyme tyrosinase, production of collagen I and III, as well as enzymes important for the postproduction of collagen, vitamin C and its derivatives also decrease melanin production, leading to a more even and brighter skin tone via interference with the oxidative processes of melanogenesis. Vitamin E is a lipid-soluble antioxidant with photoprotective effects that has been demonstrated to reduce the signs of photoaging, enhance collagen synthesis, and inhibit collagen degradation. When used in combination, vitamins C and E appear to work synergistically to provide substantial UV protection and improvements in biomechanical parameters of the skin, although human trials are limited and use topical solutions containing other additives, such as alpha-hydroxy acid.

Increasingly, in vivo efficacy of peptides used in cosmetic preparations is documented. Palmitoyl tripeptide-38 belongs to a group of synthetic peptides modeled on repair signaling sequences to increase the proliferation of collagen, elastin, proteoglycan, glycosaminoglycan, and fibronectin for improvements in pigmentation, fine lines, and wrinkles in photodamaged skin. Studies of palmitoyl tripeptide-38 in topical solutions with additive ingredients have suggested beneficial effects, including significant increases in dermal density and elasticity, as well as improvements in rhytides.

The present study assessed changes in rhytides by collecting data points that characterize surface smoothness using a three-dimensional measurement device. The PRIMOS® Lite allows contactless in vivo capture and measures the macro- and microstructure evaluation of skin with high resolution, reproducibility, and objectivity. The system uses interference fringe projection to determine parameters of cutaneous relief on an identified zone of measurement. Parameters include Ra (average roughness), Rz (average relief), and Rt (maximum height of the relief or maximum relief amplitude). A decrease in Ra signifies smoother skin, while decreases in Rz and Rt represent a reduction in rhytides. Results of this study demonstrated significant improvements in rhytides and skin roughness in 71% of subjects after 28 days.

The PRIMOS® system also investigated changes in skin structure by analyzing isotropy parameters as a rather novel but finely precise individual indicator of skin age. The numerical isotropy endpoint is based on a directional dependence in the propagation of visible and

### TABLE 1
Change in isotropy at days 28 and 56 vs baseline

| Day | Mean change | % change | P value | % of responders |
|-----|-------------|----------|---------|----------------|
| 28  | 1.9 (0.9)   | 4        | <.05    | 68             |
| 56  | 0.3 (1.0)   | 1        | <.05    | 55             |

Abbreviation: SEM, standard error of mean.
near-infrared light through human skin in vivo, where the skin’s reduced scattering coefficient can vary between different directions of the light path at the same position. Isotropy defines the orientation of the lines in cutaneous relief. The aging process results in a change in skin structure and organization, from a homogenous isotropic state to an anisotropic state. This anisotropy is believed to be caused by the preferential orientation of collagen fibers in the dermis, as described by Langer’s skin tension lines. There are only a few reports regarding the relationship between dermal structure and skin isotropy, such as by Mizukoshi & Hirayama who studied age-related changes in dermal fiber-like structures in facial cheeks and concluded, as clinically confirmed here, on the age-related directionality of skin fibers. Sakai and coworkers reported the relationship between the progress of wrinkle and depth-dependent dermal birefringence in the crow’s feet area in vivo and evaluated the skin damage associated with photoaging. In these studies, depth-resolved en-face birefringence maps showed the heterogeneous distribution of birefringence in the skin in vivo. In the present study, vitamins C and E in combination with palmitoyl tripeptide-38 produced a significant increase in isotropy parameters after 28 days of use, suggesting a restructuring effect (P < .05). While the numerical change in percentage appears small, there is yet little basis for comparison and scaling of the isotropy parameter. Further exploration of the underlying mechanisms of redressing the ECM in relation to macroscopic measurable isotropy is ongoing.
TABLE 2 Proportion of subjects reporting positive, negative, or neutral appreciation for study serum characteristics via subjective evaluation questionnaire administered at day 56

| Product characteristics | Positive appreciation | Negative appreciation | Neutral |
|-------------------------|-----------------------|-----------------------|---------|
| Color                   | 53%                   | 0%                    | 47%     |
| Texture                 | 65%                   | 9%                    | 26%     |
| Consistency             | 59%                   | 9%                    | 32%     |
| Not greasy              | 67%                   | 33%                   | 0%      |
| Not tacky               | 67%                   | 33%                   | 0%      |

Skin tone was assessed by a facial analysis system that provides close-up pictures under controlled conditions, allowing for before and after comparisons in quantitative terms. Photographic analysis revealed a significant increase in clarity, reduction in redness, and a more even skin tone. Although only skin-tone improvements were quantified, photographs also demonstrated improved skin smoothness, reduced appearance of wrinkles, and a noticeable brightening effect vs baseline. These results are reflected in subject self-assessment scores. Contrary to simple in-use tests where consumers are asked to appreciate the alleged efficacy of the test products after the trial period, the present method of registering progressive consumer perception allows the investigator to analyze subjective impressions over the test period and to obtain useful quantitative data of increase in radiance and evenness, and decrease in the appearance of rhytides. The absence of vehicle control might be considered an example of evidence-based cosmetics. Overall, the combination of vitamins C and E with palmitoyl tripeptide-38 applied topically once daily for 56 days produced beneficial measurable and auto-perceivable results on the appearance and structure of the skin.

6 | CONCLUSION

Once-daily use of a serum containing both preventative antioxidants and a matrix-restoring peptide resulted in clinically visible and statistically significant improvements in the signs of aging with smoothing, skin-tone brightening, as well as anti-wrinkle and isotropy restructuring effects, as measured by objective instrumental protocols. Although mild, the changes in the isotropy/anisotropy balance merit further investigation.

Moreover, the particular method of registering regular and frequent self-assessment demonstrated progressive improvements of skin evenness, radiance, and the appearance of wrinkles. We, therefore, propose that joint consideration of the available data (in vitro and ex vivo experiments and in vivo protocols on vitamin C, on vitamin E and on the tripeptide, respectively) allows us to suggest that the clinical data observed for the present serum—while numerically small but coherent with in vitro mechanisms—constitute nevertheless an example of evidence-based cosmetics.

CONFLICT OF INTEREST
Karl Lintner, Francine Gerstein, and Nowell Solish are consultants for Alumier Labs.

AUTHOR CONTRIBUTIONS
Dr Lintner contributed to conceptualization, choice and optimization of methodology and writing the draft. Dr Gerstein contributed to visualization, validation, and writing. Dr Solish contributed to validation of methodology and results as well as critical review of the drafts.

ETHICAL APPROVAL
The study was conducted based on principles of Good Clinical Practice of noninvasive study methods and the Helsinki Declaration as revised in 2013. Written informed consent was obtained from the participants of the study, including the use of nonidentifiable photographs (part of the face) for publication. The regulations concerning privacy (GDPR) was observed. The study was conducted before the outbreak of COVID-19.

DATA AVAILABILITY STATEMENT
The data, basis of the article, will be made available upon reasonable request from professionals.

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