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ORIGINAL RESEARCH

In Vitro and In Vivo Assessment of an Innovative Peeling System with Azelaic and Tranexamic Acids for Targeted Hyperpigmentation Reduction

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ABSTRACT

***Introduction*:** Melanin, derived from tyrosine, plays a pivotal role in skin pigmentation through melanogenesis. Disruptions in this process lead to hyperpigmentation, a condition affecting skin tone and quality of life. Current treatments, including chemical peels, have limitations, highlighting the need for novel solutions. Here, we present an innovative peeling system, comprising a masque and moisturizer, formulated with a novel blend of acids, including azelaic acid (AZA) and tranexamic acid (TXA), alongside known brightening and penetration-enhancing agents for a comprehensive solution to target hyperpigmentation.

***Methods*:** In vitro studies assessed the ability of the novel moisturizer to inhibit ultraviolet-A (UVA)-induced melanin accumulation in human melanocytes. In a single-center, controlled study, we assessed the efficacy of the peeling system in 33 healthy female participants aged 30–55 years with moderate-to-severe hyperpigmentation over a 6-week treatment period. Skin condition was assessed using clinical photography, 3D skin topography, and clinical expert evaluation (CEE) at baseline and 6 weeks post-treatment. Participants completed a self-evaluation questionnaire at 6 weeks post-treatment.

***Results*:** In vitro findings demonstrated a concentration-dependent inhibition of melanin accumulation by the novel moisturizer. In vivo, significant reductions in dark spot number, area, and perimeter were observed at week 6, along with improvements in skin homogeneity, contrast, and brightness. Skin tone and roughness parameters also improved significantly from baseline. These findings were supported by self-evaluation findings and improvements in CEE parameters.

***Conclusion*:** These data provide evidence for the efficacy of the innovative peeling system in reducing the appearance of hyperpigmentation over a 6-week treatment regimen in females with healthy skin and moderate-to-severe

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hyperpigmentation. The inclusion of AZA and TXA within the peeling system, along with active brightening and penetration-enhancing ingredients, may have synergistically facilitated the observed improvements. This multifaceted approach may address hyperpigmentation at the source, contributing to overall improvements in the appearance of the skin.

Keywords: Azelaic acid; Hyperpigmentation; Melanogenesis; Tranexamic acid

Key Summary Points

Why carry out this study?

The extended treatment duration of commercially available topical applications for hyperpigmentation often causes adverse effects that limit their clinical utility, emphasizing the need for novel therapeutic options.

The aim of the study was to evaluate the efficacy of an innovative peeling system in reducing hyperpigmentation through both in vitro and in vivo research.

What was learned from the study?

The novel moisturizer demonstrated a concentration-dependent inhibition of ultraviolet-A-induced melanin accumulation in human melanocytes, as shown in the in vitro study.

The in vivo findings revealed that the innovative peeling system effectively reduced the appearance of hyperpigmentation and improved skin brightness, tone, and texture with a favorable safety and tolerability profile.

Azelaic acid and tranexamic acid, combined with other active ingredients in the innovative peeling system, such as retinol, niacinamide, 4-hexylresorcinol, and ethoxydiglycol, may enhance skin brightness, tone, and texture through their synergistic action.

INTRODUCTION

Melanin plays a pivotal role in skin pigmentation through melanogenesis [1], initiated by the activation of melanocytes in response to stimuli, particularly ultraviolet (UV) radiation [2]. Regulated by tyrosinase, melanogenesis involves the conversion of tyrosine into melanin precursors leading to the formation of mature melanin within melanosomes. These melanosomes are transferred from melanocytes to neighboring keratinocytes, influencing skin pigmentation, coloration, and tone [2]. Additionally, UV radiation triggers plasminogen activator synthesis, enhancing plasmin activity in keratinocytes, which promotes melanin synthesis and fibroblast growth factor release, leading to melanocyte proliferation [3]. Melanin in keratinocytes provides photoprotection and helps scavenge free radicals and reactive oxygen species, constituting a system central for maintaining skin health [1, 2, 4].

Disruptions in melanogenesis can lead to hyperpigmentation [1, 2, 4], such as sunspots, melasma, and post-inflammatory hyperpigmentation, which manifest as dark spots on the skin [5–7] and can substantially affect an individual's quality of life [8, 9]. Efforts to address hyperpigmentation have driven innovation in the cosmetic industry, yielding various treatments [1]—topical, oral, and procedural—tailored to target different aspects of its pathogenesis [10].

However, current treatments for hyperpigmentation often provide limited and temporary results. Topical products often cause adverse effects such as skin irritation, scarring, and erythema, limiting their clinical utility [11]. Moreover, their extended treatment duration, lasting several months to years, may result in poor patient compliance, emphasizing the need for novel therapeutic options [11]. Alternatively, chemical peels utilize hydroxy acids to remove damaged skin cells through chemoexfoliation, which stimulates epidermal growth, collagen remodeling, and a more uniform melanin distribution [12, 13]. However, they do not directly inhibit melanogenesis [13, 14] and are often used as an initial treatment or

in combination with other therapies to achieve comprehensive results.

Recognizing the multifaceted nature of hyperpigmentation and the limitations of current treatments, an innovative peeling system comprising a masque and moisturizer has been formulated. The system integrates the benefits of traditional chemoexfoliation with targeted ingredients, using lower concentrations of acids than typically used in chemical peels [13], over a 6-week treatment period. This approach aims to ensure a smooth transition from clinic to at-home care, aligning with the emerging consensus favoring combination therapies for enhanced efficacy in hyperpigmentation treatment [15].

The masque and moisturizer combine a novel blend of acids, including azelaic acid (AZA) and tranexamic acid (TXA), along with brightening ingredients like retinol and niacinamide, all known for their role in reducing melanogenesis [3, 15–18]. Additionally, the moisturizer contains 4-hexylresorcinol (4-HR), a tyrosinase inhibitor recognized for its skin-brightening properties and ability to enhance skin tone, reduce hyperpigmentation, and improve the appearance of photoaged skin [19–23]. It is also formulated with ethoxydiglycol, which facilitates the absorption of active ingredients [24]. We hypothesize that topical application of the innovative peeling system will provide a comprehensive solution for effectively reducing the appearance of dark spots while enhancing skin brightness, tone, and texture.

Here, we present findings from a research program evaluating the efficacy of the innovative peeling system for reducing hyperpigmentation. We investigated the in vitro inhibitory properties of the novel moisturizer on melanin biosynthesis and analyzed its effect on melanin levels. Furthermore, we examined the clinical efficacy of the masque and moisturizer forming the innovative peeling system over 6 weeks in healthy female participants with moderate-to-severe hyperpigmentation.

METHODS

In Vitro Methods

Study Period

The study was conducted from March 2023 to August 2023 at Servei Central de Suport a la Investigació Experimental Universitat Valencia, Valencia, Spain, according to the general conditions of the center established for research studies involving human cell lines.

Cell Viability Assay

Cell viability was assessed using the MTT reduction assay to evaluate the cytotoxicity of the moisturizer on normal human epidermal melanocytes M2. Cells were exposed to various concentrations of the moisturizer (0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, or 1% v/v) for 5 days, with daily medium changes. Controls included the pre-treatment status of cells to assess the cytotoxic, cytostatic, and proliferative effects of the tested sample (Control 0h), untreated cells (Control), and empty wells filled with product dilutions to control for non-specific MTT reduction. After incubation, absorbance was measured at 570 nm to assess cell viability. Full details of the cell viability assay are provided in the Supplementary Material.

Melanin Content Quantification

Melanin accumulation was quantified following daily sequential ultraviolet-A (UVA) exposure, which was delivered in three steps: 20 min on day 1, 30 min on day 2, and 40 min on day 3, delivering a total dose of 16 J/cm^2. During this period, cells were incubated with treatment at sub-cytotoxic concentrations of 0.001% and 0.0001%, with daily medium changes. After the 3 days of UVA irradiation, fresh medium was added, and the cells were incubated for an additional 2 days to allow for melanin accumulation in the UVA-exposed cells. Control groups included untreated cells with no additional components (Control) and untreated cells exposed

to daily UVA irradiation (Control + UVA). Melanin content was then extracted and quantified by measuring absorbance at 405 nm. The full procedure for melanin quantification is provided in the Supplementary Material.

Statistical Analyses

Statistical analyses were performed with the GraphPad Prism V9 software (Insightful Science and Dotmatics, Boston, MA, USA). Data were analyzed by one-way analysis of variance with Dunnett's multiple comparison test. All statistical analyses employed a significance level of $p < 0.05$. For the MTT assay, data were normalized versus the untreated control and are expressed as a percentage (%). For melanin quantification, both viability and melanin absorbance values were normalized against UVA-exposed cells (Control + UVA). Normalized data were represented in bar graphs as mean ± standard error of the mean (SEM).

In Vivo Methods

Ethical approval was granted by Dermaclaim Lab S.L., Spain. The study adhered to the principles of Good Clinical Practice and the Declaration of Helsinki and its subsequent amendments. Prior to enrollment in the study, written informed consent was obtained from all participants for the participation and for the publication and use of all participants' images.

Study Design

The single-center, controlled clinical study was conducted between March and June 2023 at Dermaclaim Lab S.L., Valencia, Spain. The study adhered to the principles of Good Clinical Practice and the Declaration of Helsinki and its subsequent amendments. The study was performed following UNE-EN-ISO 9001/2015 Quality Management System guidelines (reference code, EC-10984/22).

Study Population

Thirty-three females aged 30–55 years, with non-sensitive skin of various types (normal, combination, oily), and spanning Fitzpatrick phototypes I–VI, provided informed consent to participate in the study. All participants had moderate-to-severe facial hyperpigmentation spots, ranging from grades 2 to 4 according to the Eiben-Nielson photonumeric scale [25]. Participants undergoing medical treatments that could interfere with the study, as well as those who had received esthetic treatments on the face within 3 months before the study commencement, were excluded.

Procedure

The study was conducted for 6 weeks, with four visits in total (Fig. 1).

One week before the first study visit, as part of the 2-week washout period, participants were instructed to follow a specific morning skincare routine: cleansing, moisturizing, and applying sunscreen to the face. Make-up could be applied after 5 min, but no other creams or serums were permitted. If make-up was applied, it had to be removed before participants' evening skin care routine, which comprised cleansing and moisturizing the face. Participants arrived at the test site and spent 15 min acclimating to the clinic environment, where the temperature was maintained at 23 ± 1 °C and the relative humidity at 45 ± 10%, before baseline assessments. Following this, a licensed esthetician applied the novel masque evenly to the face, leaving it on for 3 h before rinsing it off with cold or lukewarm water, avoiding rubbing the skin. A toner was then applied and could be reapplied as needed to alleviate sensations of tightness, dryness, or itching. The following day, participants exclusively used the toner on their face and were told to avoid sun exposure. Participants visited the test site on day 3, where their skin condition was monitored for flaking and peeling by the Clinical Project Manager of the research center or by a qualified and experienced technician under their supervision. For the 6 weeks post-treatment, participants followed a skincare routine. In the mornings, they gently cleansed the

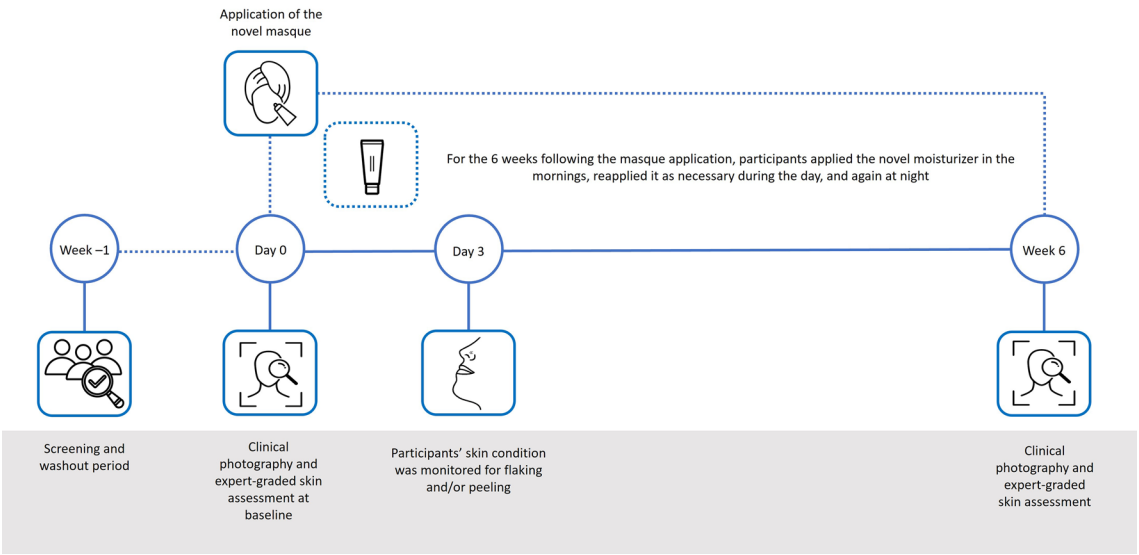


Fig. 1 Timeline and treatment schedule

skin and applied a toner (up until week 1). After applying the toner at week 1 and directly after cleansing from weeks 2 to 6, participants applied the novel moisturizer, which could be reapplied once more during the day if the skin felt very dry or irritated. After applying the moisturizer, participants were advised to wait a few minutes before applying sunscreen to the face and at least 5 min before applying make-up unless the skin was irritated. In such cases, it was recommended to avoid make-up entirely until the skin recovered. For the nightly routine, participants were instructed to remove make-up if applicable, gently cleanse the face, and apply the novel moisturizer.

Outcomes

Changes in skin condition and clinical photography were assessed at baseline and week 6 by the Clinical Project Manager or by the responsible technician under their supervision, both of whom were trained professionals experienced in clinical evaluations. High-resolution macroscopic pictures of the skin were obtained using a camera (Nikon D5600) installed in the HeadScan Bench Light Face.

Skin parameters, including dark spots (total number, area, perimeter, skin homogeneity, and skin contrast), tone (CieL* for lightness, CieA* for green–red, CieB* for blue–yellow in the CIELAB color space, and Individual Typology Angle), and brightness (specular brightness, diffuse brightness, skin lightness, and global brightness) were recorded from the region of interest (ROI) on each side of the participant's face. ROIs were first defined at the starting time for the chosen subject and then automatically replicated in the same position across all images. Notably, ROI sizes remained constant for reliable quantifications across different kinetics times for the same subject. FrameScan V4 software was used to quantitatively analyze digital 2D photographic images for colorimetric and morphologic characteristics.

Skin surface roughness of the nose–cheek area on both sides of the face was evaluated using the AEVA-HE V4 3D skin topography system, a high-resolution 3D scanning sensor used for measuring skin amplitude, roughness, and volume. Various parameters were analyzed to characterize skin surface roughness, including Sa (the average value of all height points), St (the maximum height deviation on the surface, peak to peak), Sr (the ratio of the real developed area to the apparent area), Sq (the standard deviation of the height points), and Stm (the average of the 25 local peak-to-peak values).

Dark spots, lack of radiance, and uneven skin tone were subjectively assessed by clinical expert evaluation (CEE). Dark spots were evaluated according to the Scientific Assessment Scale of Skin Quality, a 5-point ordinal scale ("0 = no pigmentation" to "4 = very severe pigmentation") for skin pigmentation [25]. Lack of radiance and uneven skin tone were evaluated according to the 10-point Modified Griffiths Scale ("0 = none" to "9 = severe")[26].

At the end of the treatment period, participants completed a questionnaire to evaluate the subjective efficacy of the innovative peeling system and their satisfaction with the treatment according to a 5-point ordinal scale ("1 = strongly disagree" to "5 = strongly agree"). The questionnaire was designed in collaboration with the sponsor.

Safety was monitored throughout the study. A visual examination of the experimental area was conducted by the responsible technician before and after treatment. Participants were instructed to report any adverse events observed or sensations of discomfort experienced following the use of the innovative peeling system to the technician overseeing the study.

Statistical Analyses

Statistical analyses were performed with the GraphPad Prism V9 software (Insightful Science and Dotmatics, Boston, MA, USA). Analyses included all participants who received treatment and completed the study (per protocol), and data were analyzed by applying the paired Student's *t*-test. Individual post-treatment values for dark spots, skin brightness, tone, and surface roughness were normalized against baseline measurements for the entire group to account for baseline variability among participants and ensure accurate interpretation of treatment effects. Results were expressed as a percentage relative to baseline values and the mean ± SEM was calculated. To maintain the integrity of subjective evaluations and avoid potential misinterpretation, CEE scores were not normalized against baseline measurements and are expressed as mean ± SEM. All statistical analyses employed a significance level of $p < 0.05$, with 95% confidence intervals (CIs) reported where available.

RESULTS

In Vitro Results

Cell Viability

Application of the novel moisturizer at 0.0003% demonstrated a moderate cytostatic effect, reducing cell viability by 15.2% compared

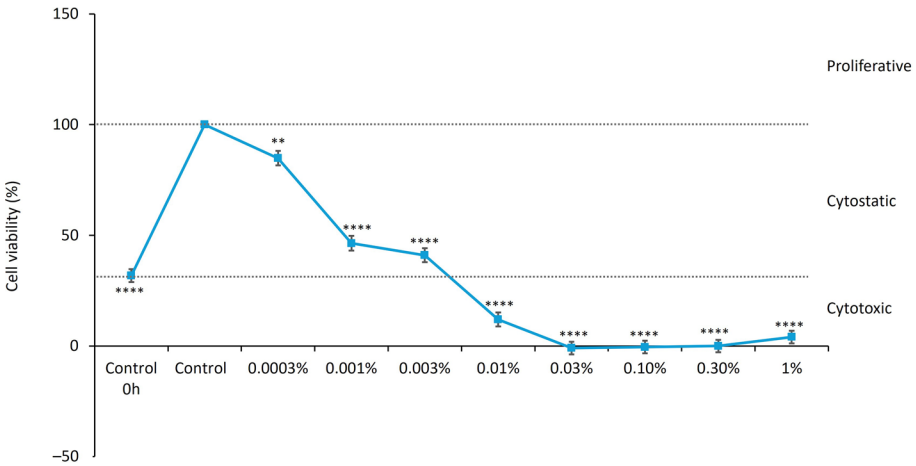


Fig. 2 Normalized cell viability levels in normal human epidermal melanocytes following application of a novel moisturizer at varying concentrations. $^{**}p < 0.01$, $^{****}p < 0.0001$

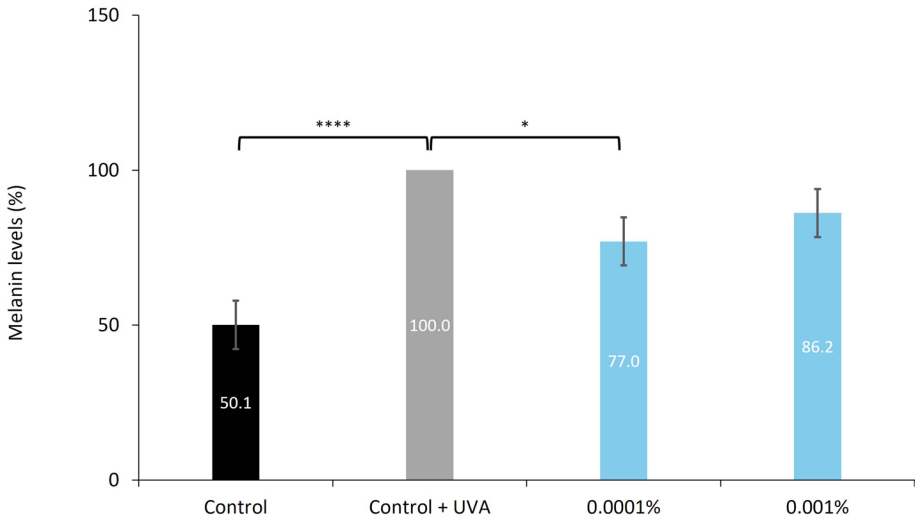


Fig. 3 Normalized melanin levels in normal human epidermal melanocytes following application of a novel moisturizer at 0.0001% and 0.001% and UVA exposure. $^{*}p < 0.05$, $^{****}p < 0.0001$. *UVA* ultraviolet-A

with control (– 26.0, – 4,4; $p=0.0049$) (Fig. 2). Higher concentrations showed greater cytostatic effects: 0.001% reduced viability by 53.6% (– 64.5, – 42.7; $p<0.0001$) and 0.003% by 59.0% (– 69.7, – 48.3; $p<0.0001$). Concentrations between 0.01% and 0.3% exhibited high cytotoxicity, reducing cell viability by 88.0% (– 98.6, – 77.4; $p<0.0001$) to – 100% (– 110.6, – 89.5; $p<0.0001$). Based on these findings, concentrations of 0.0001% and 0.001% were selected for the melanin quantification assay. The 0.0001% concentration was chosen for its potential to exhibit a greater cytostatic effect compared with 0.0003%, while likely preserving cell viability compared with the higher concentrations tested. The 0.001% concentration was selected for its demonstrated cytostatic effect and sufficient cell viability compared with higher concentrations.

Melanin Quantification

The sequential irradiation steps of UVA light, reaching a final dose of 16 J/cm^2, resulted in a significant 99.7% increase in the normalized pigment content in normal human epidermal melanocytes compared with control (68.4, 131.1; $p<0.0001$). This confirmed the effective pro-melanogenic stimulus of the UVA irradiation protocol.

Co-exposure of cells to UVA and 0.0001% moisturizer demonstrated a significant prevention of UVA-induced melanin accumulation, resulting in a 23.0% reduction compared with control cells exposed to UVA (3.2, 42.8; $p=0.0206$) (Fig. 3). A decrease in melanin levels by 13.8% was observed following treatment with 0.001% moisturizer compared with control cells exposed to UVA; however, this difference did not reach statistical significance ($p=0.2117$).

In Vivo Results

Of the 33 participants enrolled in the study, 30 were included in the per-protocol analyses. Two participants were excluded for not attending the baseline visit after pre-treatment. One participant discontinued the study on day 7 due to progressively worsening itching, stinging, desquamation, and purpled inflammation on the face. Symptoms were monitored by a dermatologist and resolved completely after 10 days of stopping treatment and initiating recovery with a dermatologically recommended moisturizer.

Demographic details of participants are given in Table 1.

Table 1 Characteristics of the participants at baseline

Characteristic	Participants ($N=30$)
Female, *n* (%)	30 (100)
Age	
Mean (SD)	44.3 (7.3)
Range	29–56
Race, *n* (%)	
White	16 (53.3)
Latin	14 (47.6)
Skin type (sensitivity), *n* (%)	
Normal	30 (100)
Sensitive	0
Skin type (sebum levels), *n* (%)	
Dry	13 (43.3)
Normal	17 (57.7)
Skin phototype (Fitzpatrick), *n* (%)	
II (white)	10 (33.3)
III (light brown)	13 (43.3)
IV (moderate brown)	7 (23.3)

SD standard deviation

Dark Spot Evaluation Using FrameScan Software

After 6 weeks of topical treatment, there was a significant reduction in the total number (– 9.7% [– 15.1, – 4.3]; $p=0.0010$), area (– 14.4% [– 22.8, – 5.9]; $p=0.0016$), and perimeter (– 3.5% [– 6.7, – 0.4]; $p=0.0292$) of dark spots compared with baseline (Fig. 4). A significant improvement in skin homogeneity (9.8% [1.3, 18.4]; $p=0.0253$) and reduction in skin contrast (– 9.2% [– 10.5, – 7.9]; $p=0.0001$) were observed at week 6 compared with baseline.

Specular and Global Brightness Evaluation Using FrameScan Software

A significant increase in specular brightness (28.6% [13.8, 43.4]; $p=0.0005$) was observed, along with a significant reduction in diffuse brightness (35.1% [– 53.3, – 16.9]; $p=0.0005$) and skin lightness (2.5% [– 3.8, – 1.2]; $p=0.0005$) at week 6 compared with baseline. Although global brightness increased from baseline by 5.3%, it did not reach statistical significance at week 6 (Fig. 5).

Skin Tone Evaluation Using FrameScan Software

Significant increases in CieL* (5.2% [3.3, 7.0]; $p<0.0001$) and ITA° (2.3% [1.5, 3.1]; $p<0.0001$) were observed at week 6 compared with baseline (Fig. 6). A significant decrease in CieA* (– 3.9% [– 5.4, – 2.3]; $p<0.0001$) was noted at week 6 compared with baseline, while the reduction in CieB* by 1.4% did not reach statistical significance.

Surface Roughness Evaluation Using AEVA-HE System

Significant reductions were observed in Stm, Sa, Sr, and Sq parameters by – 14.1% (– 23.2, – 5.0; $p=0.0037$), – 12.9% (– 20.5, – 5.3; $p=0.0016$), – 0.8% (– 1.3, – 0.3; $p=0.0029$), and – 13.0% ([– 20.9, – 5.1]; $p=0.0022$), respectively, at week 6 compared with baseline (Fig. 7). A reduction of 11.1% was observed for the St parameter; however, this did not reach statistical significance ($p=0.0579$).

CEE According to Scientific Assessment Scale of Skin Quality and Modified Griffiths Scale

CEE revealed significant reductions in the appearance of dark spots (2.8 vs 2.3; $p<0.0001$), lack of radiance (6.8 vs 4.9; $p<0.0001$), and uneven skin tone (5.9 vs 5.4; $p=0.0024$) at week 6 compared with baseline (Fig. 8).

Representative visual examples of differences in skin outcomes following treatment with the innovative peeling system after 6 weeks can be found in Fig. 9.

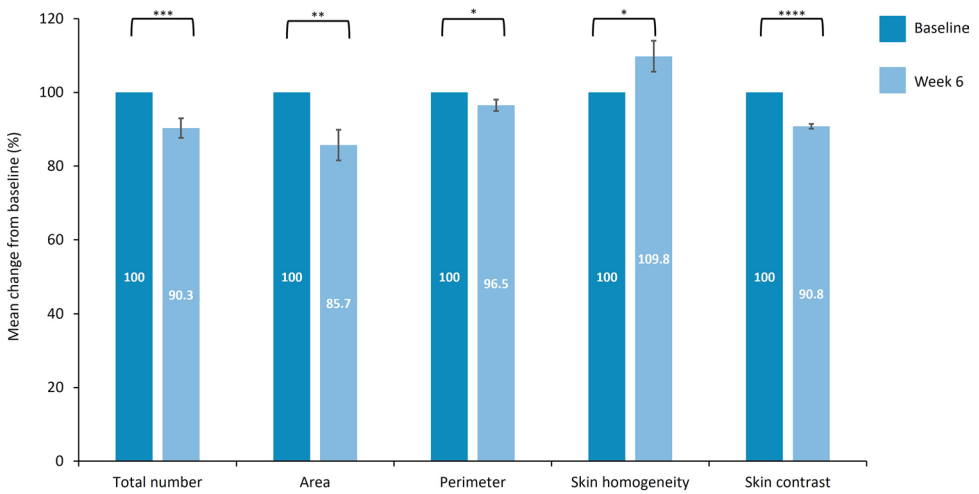


Fig. 4 Dark spot parameter mean change from baseline following 6 weeks of treatment with the innovative peeling system. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

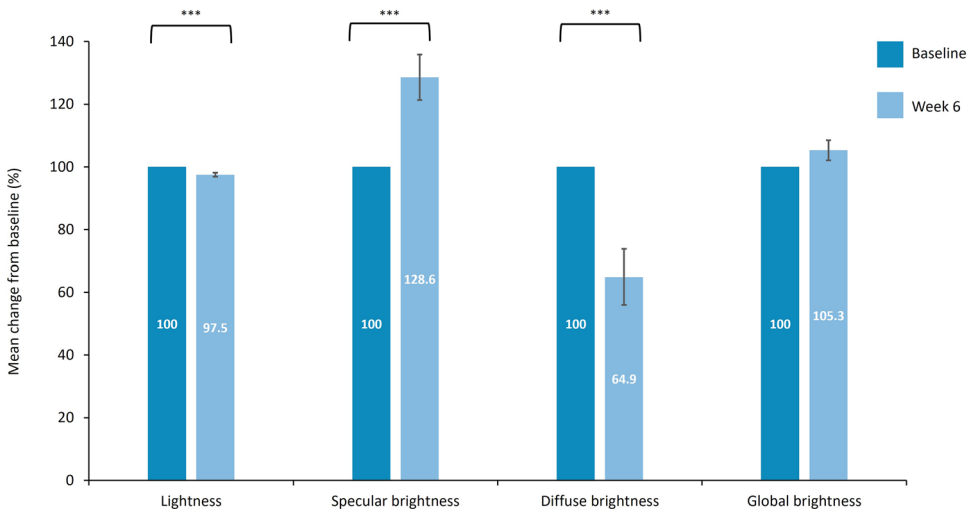


Fig. 5 Skin brightness mean change from baseline following 6 weeks of treatment with the innovative peeling system. $^{***}p < 0.001$

Self-Evaluation Questionnaire

Seventy-seven percent of participants (23/30) reported irritation, burning, or stinging while the masque was on and while using the moisturizer (Fig. 10). Similar experiences were reported for experiencing flaking and/or peeling, and tingling, itching, or flushing sensations. After more than a week of using the moisturizer, 40% (12/30) of participants experienced little to no irritation, burning, or stinging.

At week 6, participants reported smoother (70%, 21/30) and brighter skin (80%, 24/30), diminished appearance of uneven pigmentation (67%, 20/30), and an improvement in the overall appearance of their skin (77%, 23/30). Over half of the participants (57%, 17/30) reported that the system provided transformative results.

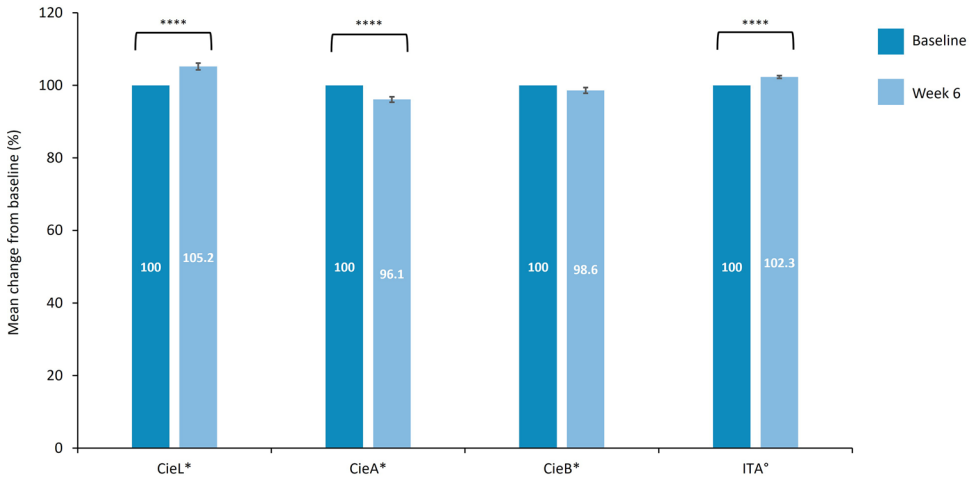


Fig. 6 Skin tone parameters mean change from baseline following 6 weeks of treatment with the innovative peeling system. ****$p < 0.0001$. *CieL** lightness component of the CIELAB color space, *CieA** green–red component of the CIELAB color space, CieB* blue–yellow component of the CIELAB color space, *ITA*, individual typology angle

DISCUSSION

Here, we describe in vitro evidence supporting the efficacy of the novel moisturizer in preventing UVA-associated increases in melanin. We also present in vivo evidence supporting the efficacy of the innovative peeling system for significantly reducing the appearance of dark spots and enhancing skin brightness, tone, and texture over 6 weeks. Given the negative impact of pigmentary disorders on health-related quality of life [27], the significant reduction in the appearance of dark spots and improvements in brightness, tone, and texture, along with a favorable safety and tolerability profile, are encouraging. Notably, over half of participants reported transformative results, with smoother,

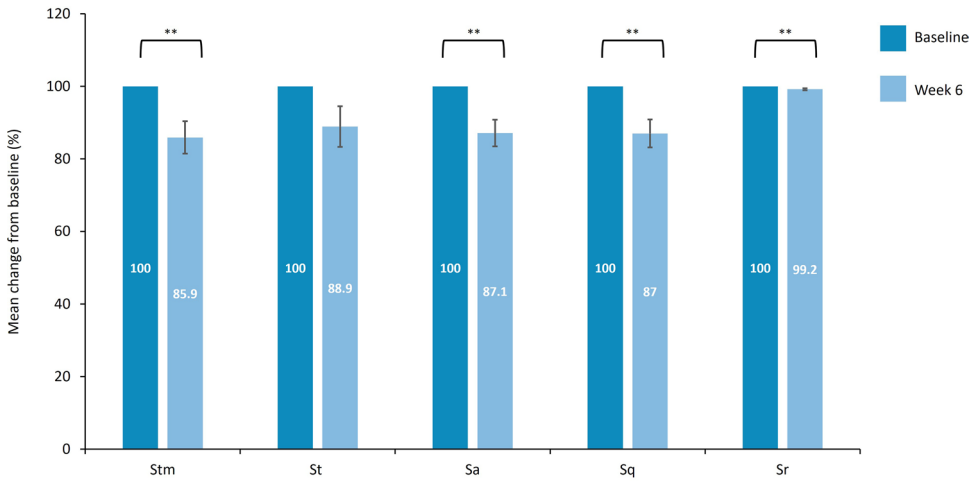


Fig. 7 Skin roughness parameters mean change from baseline following 6 weeks of treatment with the innovative peeling system. **$p < 0.01$, ****$p < 0.0001$. *Sa* average value of all height points, *St* maximum height deviation on the surface, peak to peak, *Stm* average of the 25 local peak-to-peak values, *Sq* standard deviation of the height points, *Sr* ratio of the real developed area to the apparent area

brighter skin and reduced pigmentation by week 6.

Hyperpigmentation disorders can result from abnormal melanocyte proliferation and hyperactivity, contributing to the increased production and accumulation of melanin [28, 29]. AZA suppresses melanocyte stimulation by inhibiting tyrosinase, the enzyme crucial for melanogenesis, and exhibiting selective toxicity toward hyperactive melanocytes [11, 16]. This targeted action reduces melanin production specifically in areas with abnormal pigmentation, while sparing normal melanocytes and therefore maintaining normal skin pigmentation. This selectivity may be associated with an increased permeability of hyperactive melanocyte cell membranes compared with normal melanocytes, making them more susceptible to AZA [30]. However, increased permeability may heighten the susceptibility of hyperactive melanocytes to cytotoxicity when exposed to high AZA concentrations, as observed in the in vitro study. The low solubility of AZA and poor skin penetrability often require higher topical doses (15–20%) to effectively reduce hyperpigmentation, which can lead to side effects like skin irritation and dryness [11]. This emphasizes the importance of using lower concentrations of AZA to minimize side effects. The lower AZA concentrations in the peeling system, combined with the ethoxydiglycol in the moisturizer, may synergistically enhance AZA penetration and efficacy, effectively reducing hyperpigmentation over 6 weeks while minimizing side effects. This presents a promising solution for addressing hyperpigmentation at its source.

Recent attention has also been directed toward TXA, recognized for its depigmentation properties and its inhibitory effect on plasmin synthesis [3]. TXA can be administered orally or topically as monotherapy or adjuvant treatment for hyperpigmentation disorders [18, 31]. Clinical studies have reported varying results, with some showing significant improvement in the appearance of melasma, while others find no effect, particularly in individuals with darker skin types [18, 31]. Notably, a comparative study indicated similar efficacy between the topical application of AZA cream and TXA solution in treating acne-related post-inflammatory hyperpigmentation, with TXA having a significantly better safety profile during the initial month of treatment [31]. To our knowledge, this is the first study assessing a formulation with both AZA and TXA for reducing the appearance of hyperpigmentation. The concentration-dependent reduction in melanin accumulation with the novel moisturizer in vitro aligns with the

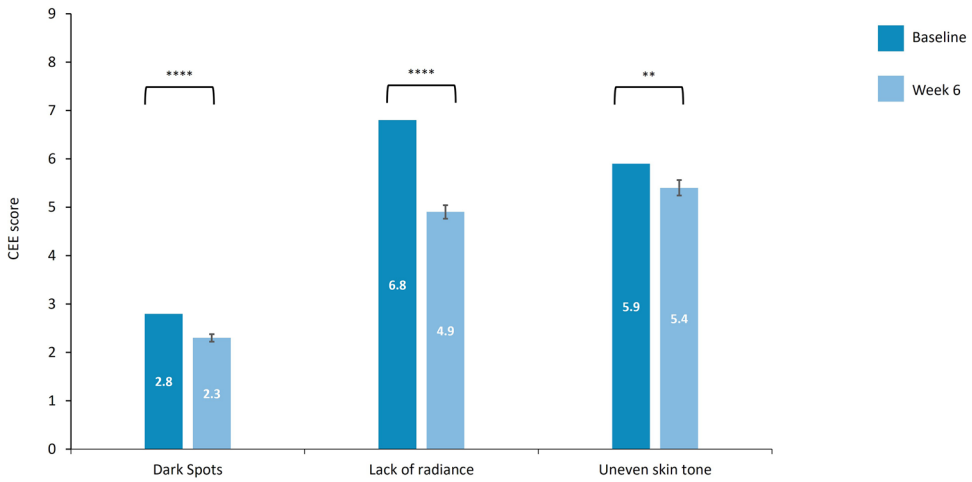
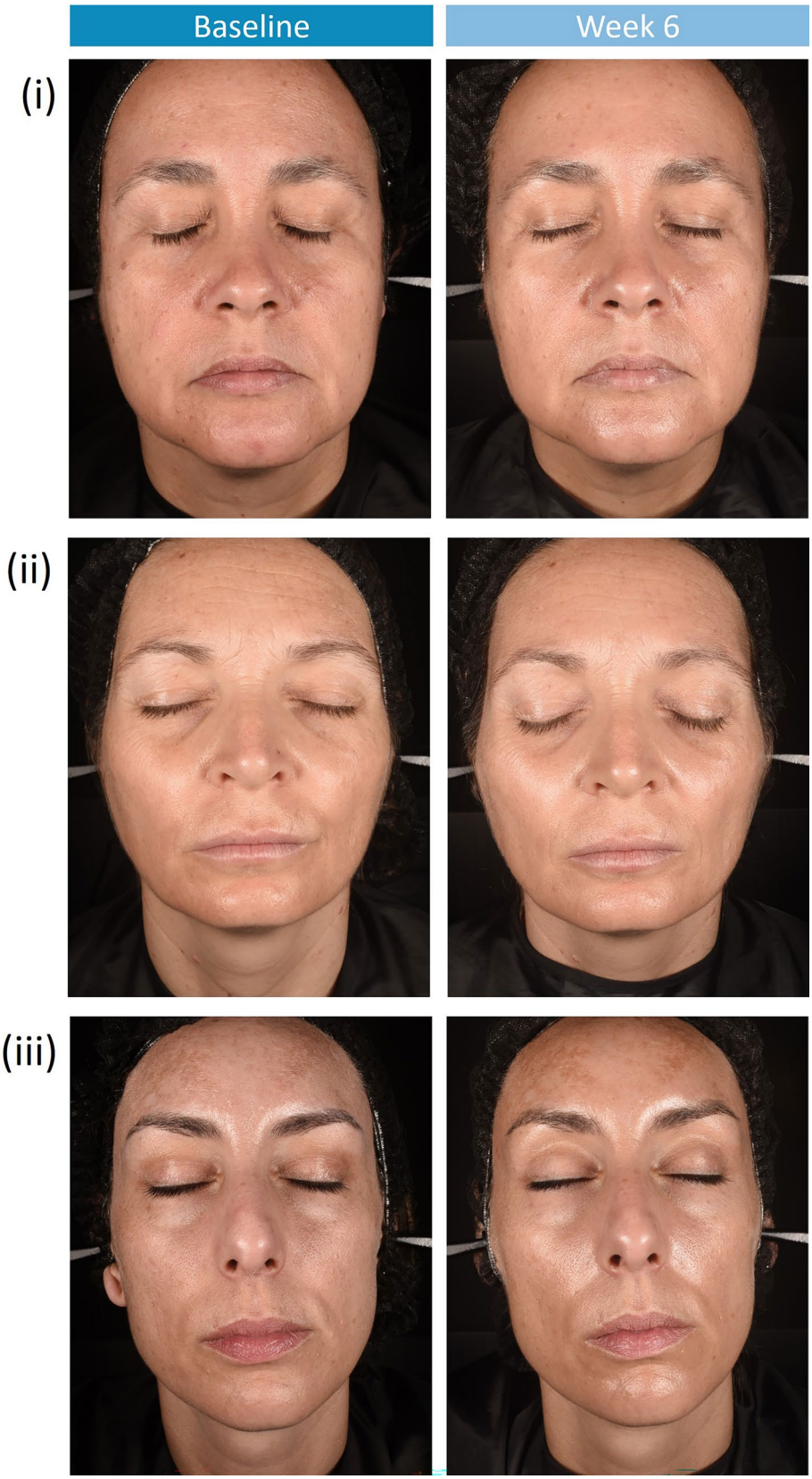


Fig. 8 CEE parameters at baseline and following 6 weeks of treatment with the innovative peeling system. CEE scores ranged from 0 to 4 for assessments of dark spots and from 0 to 9 for assessments of lack of radiance and skin tone. $^{**}p < 0.01$, $^{****}p < 0.0001$. *CEE* clinical expert evaluation


Baseline
Week 6
(i)
(ii)
(iii)

◂**Fig. 9** Representative parallel-polarized light clinical photographs of differences in skin outcomes at baseline (left) versus following the innovative peeling system at week 6 (right). (**i**) An increase in skin luminosity, texture, and tone is noted at week 6. (**ii**) An even skin tone and improved overall skin luminosity are achieved. (**iii**) Notable luminosity improvements and even skin tone are present on the cheeks and chin at week 6. Slight variations in lighting occurred, primarily due to minor differences in participants' expressions or positioning, which influenced how the camera flash reflected on their faces

documented inhibitory effects of both AZA and TXA on melanogenesis [15–18]. While not directly correlated, the in vitro study provides foundational insights into the mechanism of melanin accumulation inhibition, while the in vivo study demonstrates the clinical outcomes over 6 weeks.

The inhibitory effects of AZA and TXA on tyrosinase and plasmin synthesis, respectively, are supported by the actions of retinol and niacinamide, present in both the masque and moisturizer, along with the 4-HR in the moisturizer. Retinol downregulates tyrosinase activity, inhibiting melanin synthesis [32], and enhances skin brightness through increased cell turnover and texture through collagen synthesis [33]. Additionally, by slowing melanosome transfer and exerting anti-inflammatory and antioxidant effects, niacinamide reduces skin pigmentation while promoting skin brightness and an even skin tone [34]. Combinations of niacinamide and 4-HR have been found to improve skin tone and reduce the appearance of hyperpigmentation and the features of photoaging [19, 22], likely due to niacinamide's inhibition of melanosome transfer and the anti-tyrosinase enzyme activity of 4-HR [22]. Consequently, the diverse actions of the peeling system's active ingredients may improve pigmentation and contribute to a healthier, more radiant complexion, as seen in significant changes in specular brightness, skin tone, roughness, and CEEs, alongside a reduction in diffuse brightness. While the individual benefits of AZA, TXA, retinol, niacinamide, and 4-HR are well documented, further research into their synergistic effects could deepen understanding of how these ingredients interact to enhance melanin regulation. Comparative analyses of their individual and combined effects on melanogenesis and overall skin health may provide insights into their complementary mechanisms, further strengthening the rationale for their integration in the peeling system.

This study has limitations. While the innovative peeling system demonstrated improvements in skin parameters over 6 weeks, its long-term efficacy and safety remain unaddressed, which is particularly significant for hyperpigmentation treatments that often require extended use to maintain results [32]. The single-center design and limited sample size of the study reduce its statistical power. Larger, multi-center, randomized controlled trials or case–control studies, with more diverse samples, including participants from varied racial and ethnic backgrounds, are necessary to enhance the generalizability of findings and provide broader insights into the long-term efficacy and safety of the innovative peeling system across different skin types and tones. Furthermore, factors such as sebum, skin sensitivity, and Fitzpatrick skin type should be considered in future studies to provide more personalized insights and tailored treatment recommendations. Comparative studies using a split-face design could help to clarify the system's distinct effects compared with conventional treatments, while blinded study designs would reduce bias in subjective assessments. Additionally, the current in vitro study may not fully reflect in vivo outcomes because of factors such as skin barrier function, metabolism, microenvironment, and individual skin types, which can all affect how ingredients perform on the skin. External variables such as temperature and pH may further modulate in vivo efficacy [35]. Given the significant role of ultraviolet-B (UVB) in hyperpigmentation [36], investigating the combined effects of UVA and UVB using melanocyte–keratinocyte co-cultures to mimic in vivo conditions could provide further insights into the restorative effects of the moisturizer.

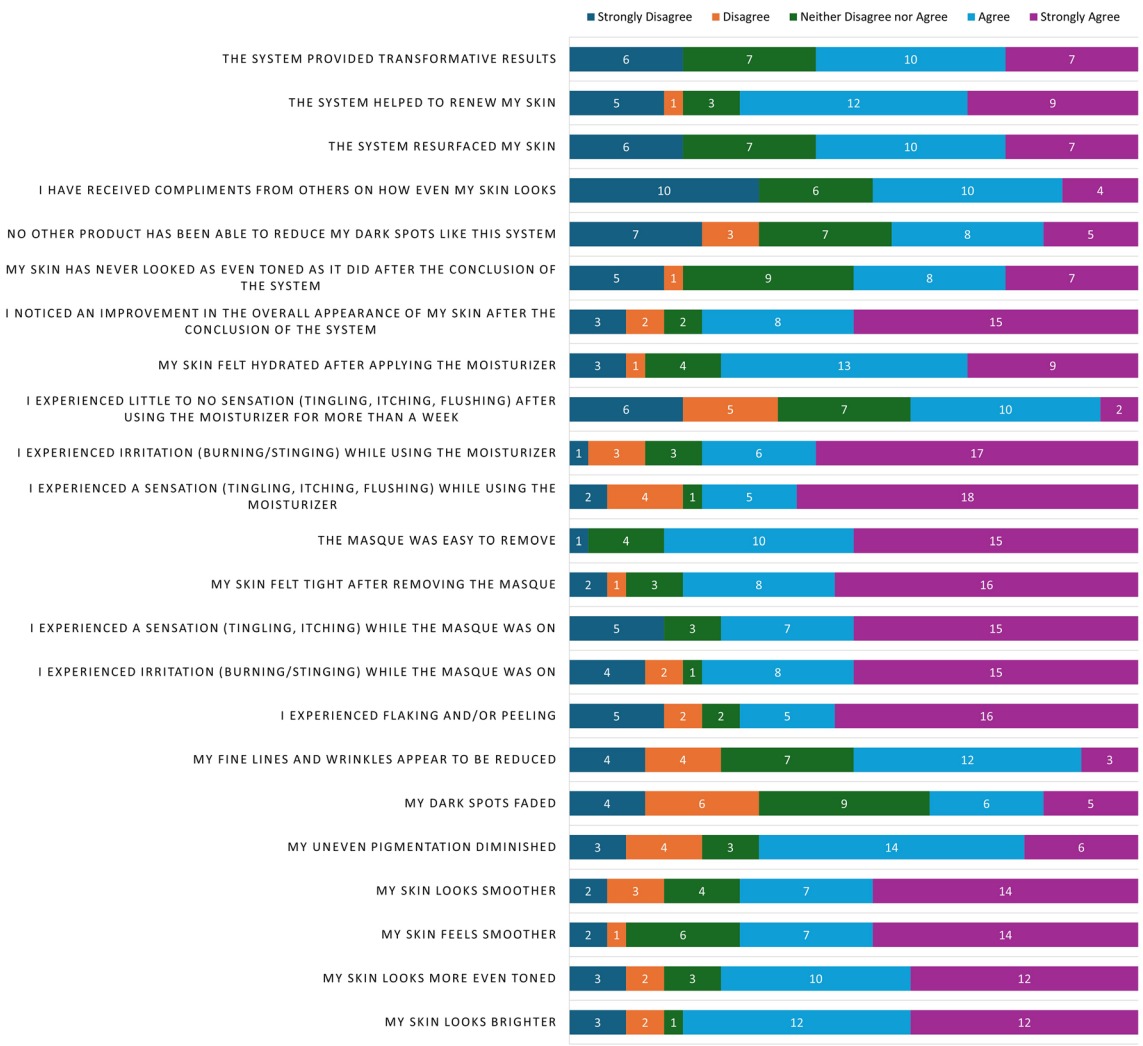


Fig. 10 Self-evaluation questionnaire outcomes following 6 weeks of treatment with the innovative peeling system

CONCLUSIONS

The study introduces an innovative peeling system designed to target hyperpigmentation at the cellular level by combining a synergistic blend of acids and brightening and penetration-enhancing ingredients for a comprehensive solution. The in vitro study demonstrated concentration-dependent cytostatic effects of the novel moisturizer, with promising inhibitory effects on UVA-induced melanin accumulation. The in vivo findings support the efficacy of the innovative peeling system for visibly improving skin pigmentation, brightness, tone, and texture with a favorable safety and tolerability profile over 6 weeks. While further research is warranted, this study provides valuable insights into the potential of the innovative peeling system for treating moderate-to-severe hyperpigmentation and improving overall skin health.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Russell Wong has received honoraria from Dermalogica, LLC. Mariangela G. de O. Sichmann and Kevin D. Hermanson are employees of Unilever Research & Development. James Sun, Alexis R. Kim, Robert J. Bianchini, and Louis Chabert are employees of Dermalogica, LLC.

Ethical Approval. Ethical approval was granted by Dermaclaim Lab S.L., Spain. The study adhered to the principles of Good Clinical Practice and the Declaration of Helsinki and its subsequent amendments. Prior to enrollment in the study, written informed consent was obtained from all participants' for the participation and for the publication and use of all participants' images.

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