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BEYOND BRIGHTENESS: A MULTI-TARGETED APPROACH TO SKIN TONE ENHANCEMENT WITH A NOVEL MASK FORMULA

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Abstract

Introduction: Hyperpigmentation requires multi-targeted approaches for comprehensive skin tone enhancement. This study investigated a novel face mask designed for this purpose.

Methods: The effects of a mask containing 3-O-ethyl ascorbic acid, tocopherol, and phenylethyl resorcinol were evaluated in vitro using reconstructed skin models and in vivo in a clinical trial with 65 Chinese women.

Results: In vitro, the mask mitigated UVA damage and protected against daily UV exposure. In vivo, it significantly improved skin brightness (22%), radiance (23%), and PIH (57%), respectively ($p<0.05$), with 100% of consumers reporting improvement.

Conclusion: This novel mask formulation offers a multi-targeted approach, delivering a more radiant complexion by addressing hyperpigmentation and improving overall skin quality.

Key words: Hyperpigmentation, skin tone, photoprotection, multi-target approach, 3D skin model, in vivo clinical trial.

1. Introduction

Hyperpigmentation, encompassing conditions like post-inflammatory hyperpigmentation (PIH), solar lentigines (age spots), and melasma, presents a significant concern for many individuals, particularly Asian women, desiring an even and radiant complexion [1]. Achieving comprehensive skin tone improvement requires a multi-targeted approach that extends beyond merely addressing discoloration. A holistic strategy must also enhance skin reflectivity (radiance) and improve skin texture (smoothness). This approach targets multiple factors contributing to uneven skin tone, ultimately creating a more luminous and healthy appearance.

An effective solution for overall skin tone management must therefore incorporate a combination of antioxidant, tyrosinase-inhibiting, anti-inflammatory, and photoprotective properties. This study investigates a novel mask formulation designed to address these multifaceted

aspects of skin tone enhancement. The mask utilizes a synergistic blend of three key ingredients: 3-O-ethyl ascorbic acid, tocopherol, and phenylethyl resorcinol (377Plus).

Phenylethyl resorcinol acts as a potent tyrosinase inhibitor, effectively reducing melanin production and mitigating hyperpigmentation [2]. 3-O-ethyl ascorbic acid, a stable and effective derivative of Vitamin C, works in concert with Vitamin E to provide robust antioxidant support [3,4,5]. This antioxidant action neutralizes reactive oxygen species (ROS) generated by UV exposure and inflammation, protecting against oxidative stress, a key contributor to both hyperpigmentation and premature aging [6]. By targeting these pathways, the mask aims to interrupt the cycle of damage that leads to uneven skin tone.

To comprehensively evaluate the efficacy of this novel mask formulation, a two-pronged approach was employed. In vitro studies using reconstructed skin models provided a controlled environment to investigate the mask's impact on key biomarkers associated with hyperpigmentation and photoaging, exploring the molecular mechanisms underlying the observed phenotypic changes. Complementing the in vitro work, an in vivo clinical trial will assess the mask's ability to deliver comprehensive skin tone improvement across crucial attributes, including a reduction in hyperpigmentation, increased radiance, and improved skin smoothness. This combined approach offers a comprehensive understanding of the mask's potential to address the complex issue of hyperpigmentation and promote a more even, healthy, and radiant complexion.

2. Materials and Methods

2.1. In vitro test with 3D Full-thickness (Soft-skin) skin model

Reconstructed full-thickness skin models were created following established protocols [7]. Briefly, a dermal equivalent of collagen and human dermal fibroblasts was contracted, seeded with normal human keratinocytes, and cultured for 7 days submerged, followed by 8 days at the air-liquid interface to allow for differentiation. The skin model was then cultured in a Falcon deep-6-well plate with culture medium for evaluation.

UVA exposure was performed using a 1550W Xenon arc solar simulator (Newport, California, USA) with a WG 335 filter [8]. Ingredients were added to the culture medium 48 hours before exposure. During exposure, skin models were transferred to fresh DPBS, and post-exposure, they were transferred to fresh medium with ingredients and incubated at 5% CO2. Skin samples were collected 48 hours later.

Skin model quality was confirmed via histological analysis and fibroblast quantification using Hematoxylin-Eosin staining. Culture medium samples were analyzed for inflammatory markers using the Olink Target 96 inflammation panel (Olink Proteomics AB) and for MMP-1 levels using a Human MMP-1 ELISA kit (Abcam), following the manufacturers' instructions.

2.2. In vitro test with Reconstructed Pigmented epidermis (ERP) skin Model

The assay was performed as described by Qiu et al. [9]. Briefly, normal human keratinocytes were isolated from adult foreskin of Chinese origin with informed consent, were cultured on irradiated 3T3 feeder cells. Normal human melanocytes were isolated and cultivated as described by Duval et al. [10]. Human pigmented epidermis was reconstructed using Duval's technique [10], involving an initial submerged culture for keratinocyte proliferation followed by air-liquid interface culture for differentiation. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO2.

Daily UV exposure was performed with a 1550W Xenon arc solar simulator (Newport, California, USA) and a WG320 filter [11], delivering a UVB/UVA spectrum simulating daily sunlight. Skin models were exposed to 7.5 J/cm2 Daily UVR, followed by culture in fresh medium with test RMs or solvent control . The exposure was repeated four times (Days 9-14), with sample collection on Day 15.

Histological analysis was performed to confirm ERP model quality and assess melanocyte morphology using Hematoxylin-Eosin & DOPA staining. Melanin content was quantified by scanning Fontana-Masson stained histological slides using Nanozoomer® (Hamamatsu, Japan). The area occupied by melanin (10-15 images per sample, white light, 20x magnification) was determined using Histolab® software (Microvision), while the area of living epidermis was quantified using ImageJ (W. Rasband, National Institutes of Health)

Gene expression analysis was initially performed using the QuantiGene RNA Multiplex assay (QGP, Affymetrix) on epidermis separated from BPER in each reconstructed skin, following manufacturer's specifications. Relative quantification was performed using the 2^-(ΔΔCt) method, with GAPDH as the HKG.

2.3. In vivo Chinese Clinical study

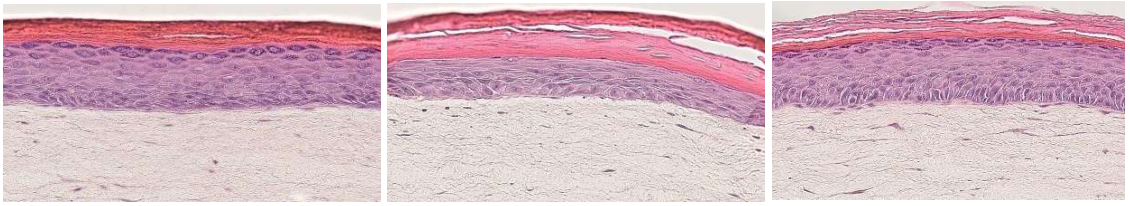
An in vivo clinical trial performed had the objective of assessing the skin tone and skin quality enhancing efficacy used in the real life under dermatological control through both clinical and consumer self assessment. The study involved 65 Chinese women (aged 25-45 years) with different hyperpigmentation (melanosis, PIH/PIE, spots) and skin quality concerns (skin eveness, smoothness, etc). The study protocol followed a 14-day washout period before the official treatment. Participants applied the test masks five times every two weeks for eight weeks. Clinical assessments and photo shooting were conducted at baseline, weeks 1, 2, 4, and 8. Consumer self-assessments were completed at week 8 and day 57 (next morning of week 8). The evaluations were performed by trained dermatologists using a 10-point sacle. All photo shooting was conducted by Visia-CR® under standard 1, standard 2 and UV modes from three directions.

3. Results

3.1. 377Plus exhibited an effective against photodamage effect on the 3D Full-thickness (Soft-skin) skin model

To evaluate the efficacy of 377Plus against photoaging, a full-thickness skin model was exposed to 30 J/cm² UVA and histologically examined 48 hours post-exposure. UVA irradiation induced changes in both dermal and epidermal compartments (Figure 1a) , including the disappearance of superficial dermal fibroblasts (Figure 1b), epidermal thinning (Figure 1d), and increased MMP-1 production, a marker of photoaging (Figure 1c). Treatment with 377Plus mitigated the UVA-induced damage, including restoring epidermal thickness and reducing MMP-1 levels. This demonstrates the efficacy of 377Plus in combating photoaging.

a



NT 30J/cm^2 UVA +Vehicle 30J/cm^2 UVA +377 Plus

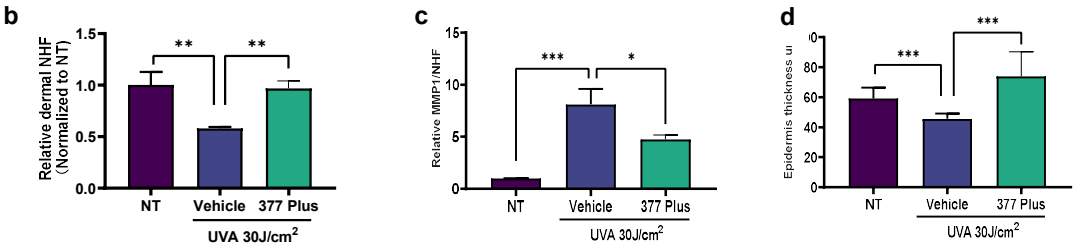


Figure 1: 3D restructured full-thickness skin tissue exposed to UVA

*a.Tissue morphology was observed by H&E staining after 48h of UVA exposure.b.Relative quantification of fibroblasts in dermis. b.Relative secretion of MMP1 in tissue culture medium exposed to UVA. c.The average thickness of epidermis. Note: * p<0.05, ** p<0.01 ,***p<0.001 One-Way ANOVA, n=3*

UVA irradiation, known to trigger inflammatory responses contributing to photoaging, was found to upregulate several key inflammatory mediators (IL-17C, EN-RAGE, CCL28, TNF, and TRAIL) in culture medium, as determined using the Olink Target 96 Inflammation panel (Figure 2). Subsequent treatment with 377Plus significantly downregulated these UVA-induced inflammatory factors, suggesting a mechanism by which 377Plus mitigates UVA-induced photoaging by potentially dampening the inflammatory cascade, protecting against collagen degradation, and improving skin barrier function and pigmentation.

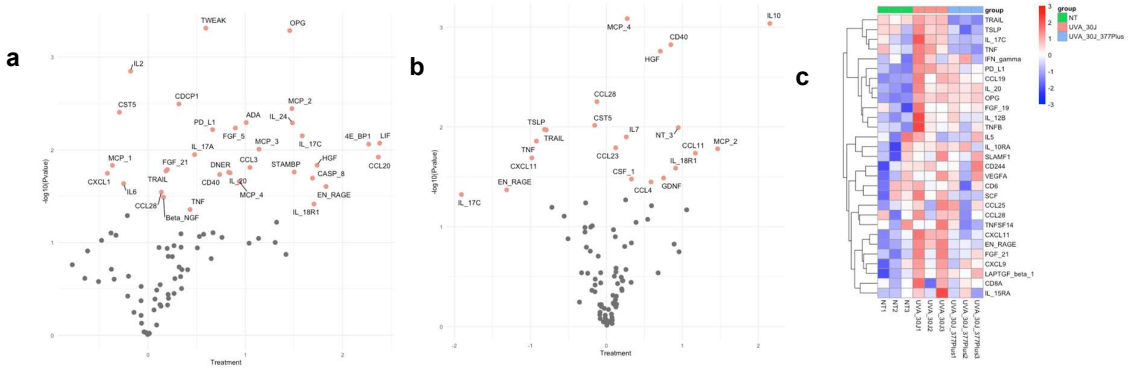


Figure 2: Differential Expression of Inflammatory proteins

a.Volcano plot of significant inflammatory proteins after 48h of UVA exposure. b.Volcano plot of significant inflammatory proteins with 377plus after 48h of UVA exposure. c. Heatmap of inflammatory proteins Mediates 377plus Protection Against UVA-Induced Skin Damage

3.2. 377Plus effectively reduced melanin accumulation in the 3D Reconstructed Pigmented Epidermal (ERP) skin model

The protective efficacy of 377Plus against photoaging was assessed using reconstructed pigmented epidermal skin models exposed to a simulated chronic, low-dose UV environment. The models were subjected to a "standard UV daylight" spectrum (UVA/UVB ratio of 27) mirroring cumulative daily UV exposure (5 J/cm² DUVR, four times between days 9 and 15). This UV exposure resulted in expected photoaging phenotypes, notably epidermal thinning and increased melanin content (Figure 3). Treatment with 377Plus effectively reversed these effects, demonstrably increasing epidermal thickness and reducing melanin accumulation.

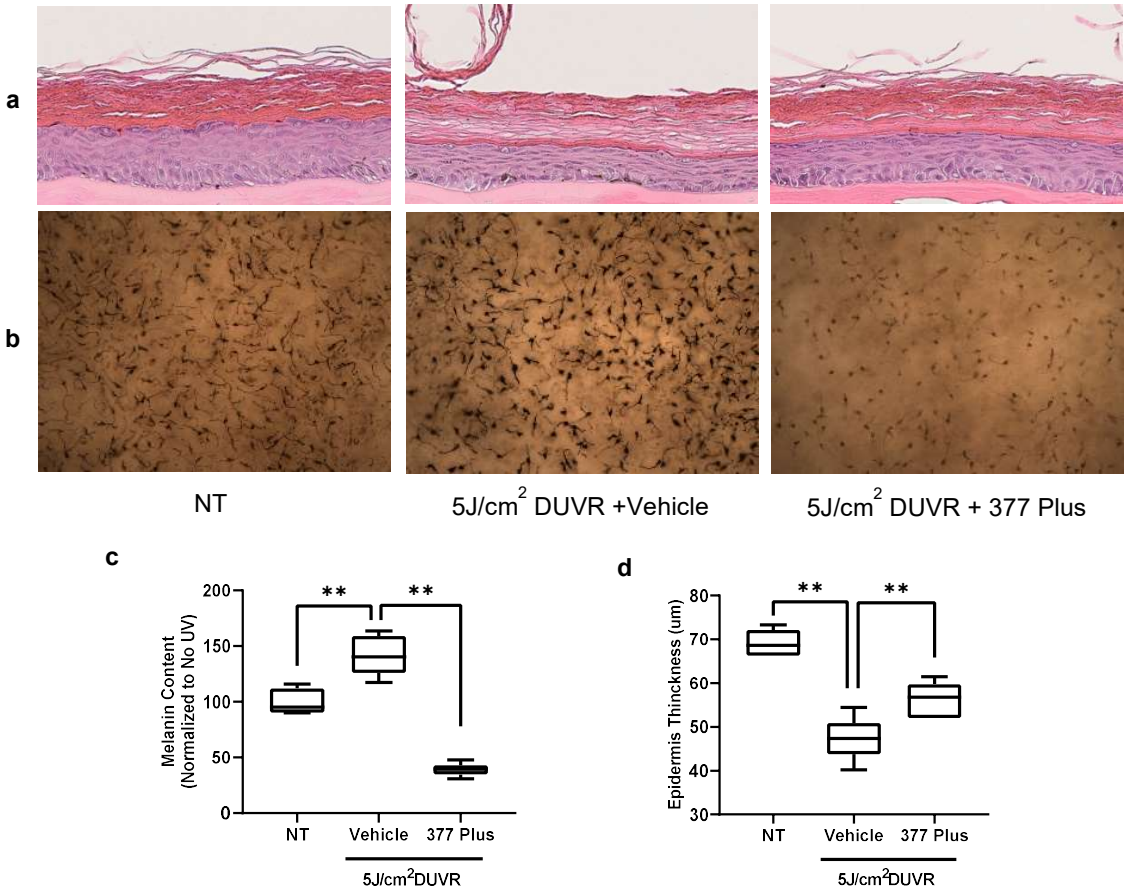


Figure 3: Chronic Daily UVR exposure to Pigmented Epidermis model

*a.Tissue morphology was observed by H&E staining. b.Tissue morphology was observed by Dopa staining. c.Quantification the melanin content with Fontana-Masson staining.d.Quantification the epidermis thickness. Note: ** p<0.01 Mann-Whitney U test, n=6*

To assess the impact of daily UV radiation (DUVR) exposure and the potential mitigating effects of 377Plus, the mRNA expression levels of four markers related to oxidative stress response and melanogenesis were quantified in reconstructed skin 18 hours post-exposure(Figure 4). The relative expression was calculated as fold change, normalized to the DUVR-only group. DUVR upregulated the melanogenesis-related genes MC1R and TYRP1. Treatment with 377Plus downregulated these genes. HMOX1, a gene associated with oxidative stress response, exhibited a similar trend, suggesting 377Plus may also mitigate DUVR-induced oxidative stress.

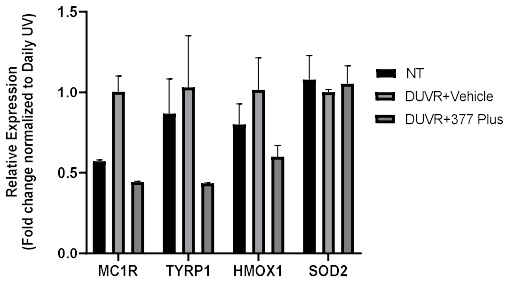


Figure 4: Gene expression at 18hr post- DUVR exposure

3.3.Enhanced Skin Tone and Texture with 377Plus Mask: A Chinese Clinical Study

Clinical assessments by trained dermatologists showed significant improvements in skin brightness, radiance, and post-inflammatory hyperpigmentation (PIH) intensity over the 8-week study (Figure 5), with continuous reductions in the point scale of all three attributes across all timepoints. Specifically, skin brightness improved by 22% ($p<0.05$), while skin radiance and PIH intensity improved by 23% and 57% ($p<0.05$), respectively. The image comparison is shown in figure 6.

	Skin Brightness	Radiance	PIH Intensity
T0	4.91	4.80	3.73
Timm	4.32	3.23	3.08
T1wk	4.48	4.09	2.97
T2wk	4.28	3.89	2.49
T4wk	4.07	3.83	2.03
T8wk	3.81	3.68	1.59

Figure 5. Clinical Improvements in Skin Brightness, Radiance, and PIH Intensity

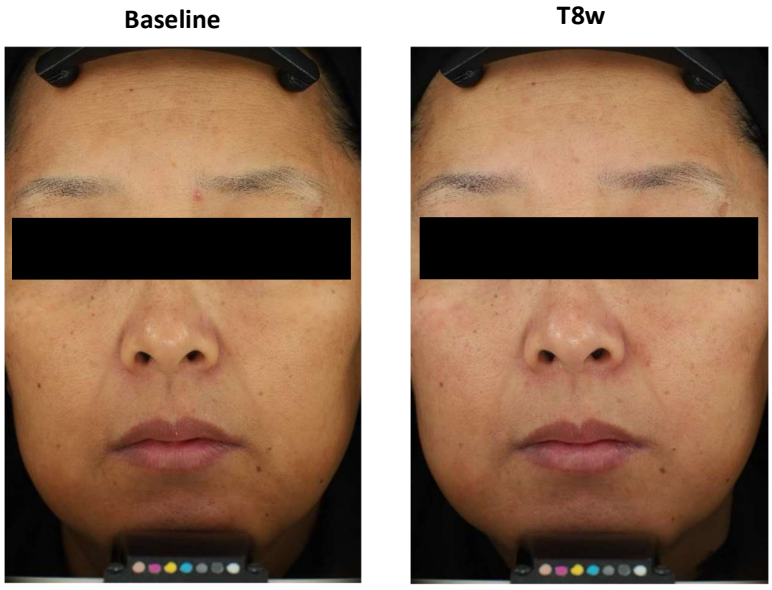


Figure 6. Average case comparison under VISIA-CR image after 8-week treatment

Skin texture relates to the overall skin reflection, which Figure 6 shows that the skin texture was overall improved. Skin elasticity, plumpness, smoothness, and softness improved significantly (35%, 36%, 31%, and 44%, respectively, $p<0.05$).

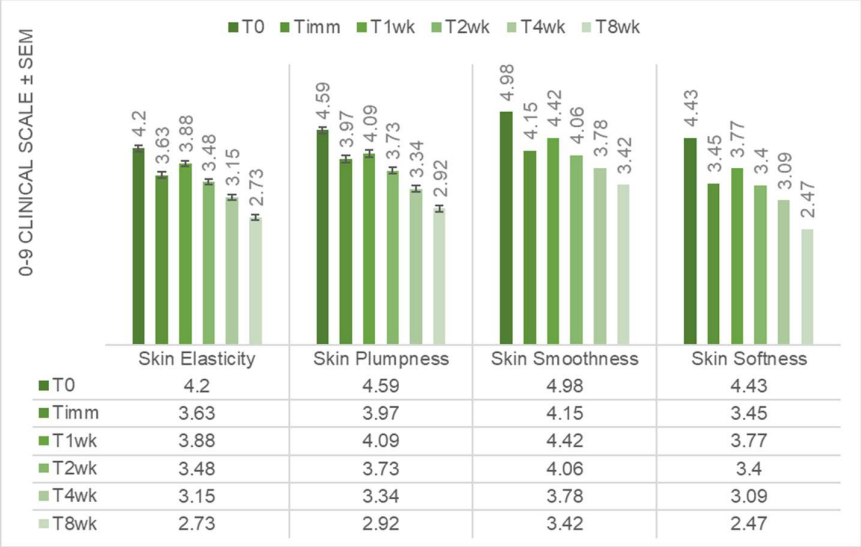


Figure 7. Clinical Improvements in skin elastictiy, plumpness,smoothness and softness

Moreover, consumer self-assessment corroborated these findings, with 100% of participants reporting improved skin dullness and quality at both week 8 and day 57. These results show that the improvement on both skin dullness and quality can be well perceived by consumers themselves and remain a long-lasting satisfication.

4. Discussion

This study demonstrates the efficacy of a novel mask formulation with EAC, tocopherol, and phenylethyl resorcinol for improving skin tone via a multi-targeted approach. Both in vitro (full-thickness and pigmented epidermal skin models) and in vivo (clinical trials) results highlight the mask's potential across multiple facets of skin tone.

In vitro, the mask effectively mitigated UVA-induced photoaging by restoring epidermal thickness and reducing MMP-1 levels, indicating protective and reparative capabilities. It also downregulated key inflammatory mediators, suggesting anti-inflammatory properties crucial for preventing UVA-induced damage and post-inflammatory hyperpigmentation. Furthermore, the mask demonstrated efficacy in protecting against chronic, low-dose UV exposure by increasing epidermal thickness and reducing melanin accumulation in pigmented epidermal skin models. Downregulation of melanogenesis-related genes and oxidative stress markers supports its multi-targeted action on underlying biological processes.

These effects are likely amplified by the combined antioxidant action of EAC and Vitamin E, neutralizing ROS and preventing oxidative stress.

These pre-clinical findings provided a strong foundation for clinical investigation and visible improvements in human skin. In vivo results further demonstrate that the mask can reduce hyperpigmentation, increase radiance, and improve skin smoothness in human subjects.

These findings suggest that the combination of EAC, tocopherol, and phenylethyl resorcinol in a mask formulation holds promise as a multi-targeted approach to improving skin tone and promoting healthier, more radiant complexion.

5. Conclusion

This study strongly supports the effectiveness of a novel mask formulation with 3-O-ethyl ascorbic acid, tocopherol, and phenylethyl resorcinol as a multi-targeted solution for comprehensive skin tone enhancement. By combining in vitro evidence of UV-induced photoaging and hyperpigmentation mitigation with in vivo clinical trial results demonstrating reduced hyperpigmentation, increased radiance, and improved skin smoothness, this research highlights the mask's potential to achieve a demonstrably healthier, more radiant complexion.

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Conflict of Interest Statement

NONE

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