Received: 21 June 2021 | Accepted: 8 October 2021

DOI: 10.1111/jocd.14544

ORIGINAL ARTICLE

JCD Journal of Cosmetic Dermatology WILEY

Amino acid complex (AAComplex) benefits in cosmetic products: *In vitro* and *in vivo* clinical studies

Isabel Diaz BA[1] | **Jin Namkoong PhD**[2] | **Joanna Qiang Wu PhD**[2] | **Giorgiana Giancola PhD**[3]

[1]Dermal Clinical, Colgate-Palmolive, Piscataway, New Jersey, USA

[2]Early Research, Colgate-Palmolive, Piscataway, New Jersey, USA

[3]Scientific Affairs, Colgate Palmolive, Piscataway, New Jersey, USA

Correspondence
Giorgiana Giancola, Scientific Affairs, Colgate Palmolive, 909 River Road, Piscataway, NJ 08855, USA.
Email: giorgiana_giancola@colpal.com

Funding information
Colgate-Palmolive Company; EltaMD

Abstract

Background: Amino acids are major components of skin's natural moisturizing factors and play a role in regulating skin hydration and skin pH.

Objective: This research examines a proprietary amino acid complex technology (AAComplex) designed to help reduce skin irritation and repair skin damage.

Methods

In- vitro Scratch Assay

HaCaT cells are scratched, and the wounds are imaged at different time points until the closure of the scratch wound is detected.

In-vitro 3D Reconstructed Human Tissue Evaluation

The concentration of heat shock protein 27 (HSP-27) extracted from 3D reconstructed human skin equivalent tissues and IL-1a released to the media was determined using enzyme-linked immunosorbent assays.

In Vivo Clinical Study

37 subjects were enrolled in a split-face study design. On test days 1, 2, 4, and 8, subjects visited the test facility to have their face assessed by facial swabbing and bio-instrumentation measurements.

Results

In- vitro Scratch Assay

The AAComplex demonstrated a strong cell renewal benefit in the HaCaT (human) cells scratch assay.

In Vitro 3D Reconstructed Human Tissue Evaluation

AAComplex demonstrated a significant skin repair benefit by quantifying Heat Shock Protein, HSP-27. Induced skin irritation was significantly reduced by quantifying interleukin-1 alpha biomarker, IL-1a.

In Vivo Clinical Study

The test products delivered skin benefits by reducing visual redness and skin irritation while increasing moisturization.

Conclusion: The *in vitro* and *in vivo* clinical studies demonstrated that the AAComplex technology formulated in the commercially available Skin Recovery System effectively reduced skin irritation and redness as well as accelerating the skin repair process.

KEYWORDS
AAComplex, amino acid, arginine, glycine, skin repair, taurine

1 | INTRODUCTION

The skin is the largest organ of the human body. As a physical barrier, the skin keeps vital chemicals and nutrients in the body, while preventing harmful substances such as UV, pollution, and microorganisms from getting inside.[1] Other important functions include regulating body temperature; maintaining water and electrolyte balance; sensing painful and pleasant stimuli and participating in vitamin D synthesis. The skin also operates as a highly active biofactory for the synthesis, processing, and/or metabolism of an astounding range of structural proteins, glycans, lipids, and signaling molecules and is an integral component of the immune, nervous, and endocrine systems.[2]

Skin is exposed to the external environment and under constant challenge from a variety of external aggressors. Sun radiation, especially UV, causes long-term skin damage is well studied,[3,4] and reactive oxygen species (ROS) from photodamage leads to collagen and extracellular matrix breakdown, skin discoloration, and even carcinogenesis. Pollution causes damage to the skin and begins to gain more and more attention nowadays.[5]

The COVID-19 pandemic breakout in 2020 brought the need for protection and repair of skin damage to a new level. According to the CDC's recommendation, masks play a vital role in reducing the spread of the coronavirus and therefore public face mask wearing is required in most places. However, masks can cause skin problems such as irritation, redness, rashes, and itchiness even acne and peeling.[6,7]

Amino acids are major components of skin's natural moisturizing factors and play an important role in regulating skin hydration and skin pH to keep skin healthy. Amino acids have been widely used in cosmetic skin care products, mostly for a skin hydration benefit. The current research examined a proprietary amino acid complex technology (AAComplex) featuring the key amino acids of taurine, arginine, and glycine that was designed to help reduce skin irritation and repair skin damage. Taurine is an amino sulfonic acid that is naturally produced by the human body and found in the brain, eyes, heart, and muscle. Taurine accelerates cell metabolism to stimulate regeneration and promote the healing of damaged skin.[10] It serves various important functions in human body including maintaining proper hydration and electrolyte balance in your cells; regulating minerals such as calcium within your cells; regulating immune system health and antioxidant function; moisturizing and nourishing the top layer of the skin (stratum corneum) from within. Arginine is a natural moisturizing factor component, a powerful antioxidant, and has the ability to support collagen production. Glycine works to improve the visible signs of aging; improves moisture retention; increases the production of collagen and strengthens the skin; and promotes skin repair and regeneration. The aim of the study was to evaluate the EltaMD facial skincare products—Skin Recovery System (toner, serum, and lotion) containing the AAComplex through the systematic *in vitro* to *in vivo* clinical studies to investigate the impact of it to skin irritation and damage repair.

2 | METHODS

2.1 | *In vitro* methods

2.1.1 | Scratch assay

Immortalized human keratinocyte cell line, HaCaT, was used in this scratch assay. HaCaT cells are maintained in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich), and penicillin/streptomycin (Sigma-Aldrich). First, cells are plated in a 24-well plate and grown to 100% confluency in 37°C and 5% CO_2. When cells are ready, cells are scratched off from the plates by drawing a vertical line using a P1000 pipette tip. Floating cells and debris are removed by rinsing with PBS. Cells are treated in triplicates with media or media containing testing ingredients, 0.001% AAComplex. The scratch wounds are imaged at different time points using EVOS FL AUTO (ThermoFisher) until the closure of the scratch wound is detected.

2.1.2 | 3D reconstructed human tissue evaluation

3D reconstructed human skin equivalent tissues from MatTek (EPI-200) were used to evaluate AAComplex technology. After equilibrating overnight after receiving EPI-200, irritation was induced by 0.15% sodium laureth sulfate (SLS), followed by incubation at 37°C with 5% CO_2 for 1 h. Tissue surface was rinsed 8 times with 1X phosphate-buffered saline (PBS) to wash away SLS after the incubation. An untreated control was not included in the experiment because the test products were compared with a respective placebo without the technology. This resulted in the true impact from the technology.

Test products:

1. Toner with AAComplex
2. Toner placebo (Same as test product 1 but no AAComplex)
3. Serum with AAComplex
4. Serum placebo (Same as test product 3 but no AAComplex)
5. Lotion with AAComplex
6. Lotion (Same as test product 5 but no AAComplex)

15 μL test products or placebo products were applied on top of the tissues and incubated overnight at 37°C with 5% CO_2. Treatments were done in triplicates. After 24 h of treatment, the media were collected and tissues were rinsed with PBS three times and collected. The epidermal lysates were prepared by protein extraction using TissueLyser II (Qiagen). The concentration of heat shock protein 27 (HSP-27) extracted from the tissues and IL-1a released to the media was determined using enzyme-linked immunosorbent assays (ELISAs) following the manufacturer's protocol (IL-1a R&D Systems, Minneapolis, MN and HSP-27 Enzo Life Sciences, Farmingdale, NY).

Two-way ANOVA was used to compare the difference between the test products to the respective placebo control for both HSP-27 and IL-1a.

2.2 | *In vivo* clinical study

37 female and male subjects 18–60 years of age were enrolled. Subjects were Caucasian and Asian with a variety of self-perceived facial skin types (normal, oily, dry, sensitive, or combination). Following completion of an IRB-approved informed consent (U.S. Investigational Review Board) and after meeting all inclusion criteria and none of the exclusion criteria, subjects with overall healthy-looking skin and no reported history of skin disease were enrolled in the study. Subjects were not allowed to use systemic or topical medications that may interfere with the study results such as anti-inflammatory medications. Prior to the inclusion in the study, subjects participated in a 3-day conditioning phase using only a commercial cleanser (EltaMD Foaming Facial Cleanser) on their face at home. They continued to use it as their daily facial cleanser throughout the study. Subjects were not allowed to use any other topical products on their face for the entire course of the study other than the test products provided.

A split-face study design was implemented to evaluate skin benefits for the EltaMD Skin Recovery System containing the AAComplex (toner, serum, and lotion). Subjects were provided all 3 products to use twice a day for 7 days at home according to the study instructions provided (only one application on certain days outlined in the study instructions).

During the 8-day Test Phase, subjects were asked to use the test products on their forehead and cheek area accordingly. On the forehead, subjects used the serum only on one side throughout the study, while the opposite side of the forehead remained untreated (washed with EltaMD Foaming Facial Cleanser only). On the cheek area, subjects used the regimen of products (toner, serum, and lotion) on one side throughout the study while the opposite cheek area remained untreated (washed only with the same cleanser). Only on Days 1 and 7, subjects were asked to use the 3 products on their cheek only once in the morning in order to evaluate for 24-h moisturization the next day.

On test days 1, 2, 4, and 8, subjects visited the test facility to have their face assessed by facial swabbings and bio-instrumentation measurements. Facial swabs were collected to evaluate irritation biomarker IL-1a. Skin moisturization was measured using a Corneometer (Corneometer CM 825 Courage + Khazaka), and photographs were captured using the VISIA (VISIA CR4.3 Photography Canfield Scientific). The VISIA captured high-resolution standardized photographs as well as images visualizing subsurface skin irritation/redness using Canfield's RBX Technology. On Day 1 (Baseline), tape stripping (10–12 strips) was conducted on both sides of the forehead utilizing D-squame tapes (Clinical & Derm) to induce damage to the skin barrier. The skin was then swabbed in the tape-stripped area (total of 2 sites) using a sterile flocked swab dipped into sterile PBS. All samples were kept frozen at −80°C until analyzed. Corneometer readings were captured in the cheek area, and photographs were taken of the frontal view of the face. The same assessments were conducted on Days 2 and 8 (except for tape stripping which was completed at Baseline only). On Day 4, only VISIA photographs were captured. Additionally, on Day 8, a questionnaire was completed by the subjects.

Prior to instrumental measurements, subjects rested for 20–30 min in a designated room at a temperature of 68–72°F and relative humidity <50%.

Statistical significance was defined as a p-value less than or equal to 0.05. Bio-instrument data obtained were evaluated using paired t tests to assess the within-treatment change from baseline at 8 and 24 h as appropriate. Between-treatment comparisons at post treatment time points were assessed by ANOVA with the baseline result included as a covariate. The questionnaire data included top two box (TTB) and bottom two box (BTB) counts which were tallied for the five self-assessment agree-disagree questions. TTB and BTB proportions were calculated by splitting the middle box counts between T2B and B2B tallies. A two-sample binomial test was used to compare the TTB and BTB proportions.

3 | RESULTS

3.1 | *In vitro* results

3.1.1 | Scratch assay

The AAComplex demonstrated a strong cell renewal benefit in the HaCaT (human) cells scratch assay compared with the control, helping to repair damaged skin faster and more effectively. Within 47 h, the AAComplex-treated skin cells significantly increased vs. the untreated control cells (Figure 1).

3.1.2 | 3D reconstructed human tissue evaluation

AAComplex demonstrated a significant skin repair benefit by all three skincare test products (toner, serum, and lotion) versus placebo controls by quantifying Heat Shock Protein, HSP-27 (Figure 2).

Induced skin irritation with 0.15% SLES (surfactant/irritant) was significantly reduced by toner, serum, and lotion with AAComplex compared with placebo controls by quantifying interleukin-1 alpha biomarker, IL-1a (Figure 3).

3.2 | *In vivo* results

37/37 subjects completed the study with no adverse events reported. The EltaMD Skin Recovery System delivered skin benefits by reducing visual redness and skin irritation while increasing moisturization.

After 1 day of serum use (2 applications) on the forehead, there was a visible reduction of skin redness and irritation as demonstrated by VISIA photographs using RBX Technology. These findings were consistent with the facial swabbing results demonstrating the serum significantly reduced skin irritation biomarker IL-1a vs. untreated skin by 21% ($p < 0.01$) after 24 h. The serum continued to significantly reduce signs of irritation induced at Baseline. (Figure 4A,B).

The product regimen which was used in the cheek area was consistent in delivering the same visible reduction of skin redness and irritation 24 h after a single use via VISIA photographs. In addition, the regimen significantly increased skin hydration (39% vs. untreated), providing 24-h moisturization after a single application ($p < 0.0001$). A cumulative improvement was seen on Day 8 ($p < 0.0001$). (Figure 4A,C).

Overall, questionnaire results showed subjects perceived very good product efficacy. After 7 days of serum use on the forehead, the majority of subjects (more than 50%) agreed their skin redness, irritation was reduced, and their skin felt soothed (83%) and calm (75%). After using the regimen of products on the cheek for 7 days, 91% of subjects agreed their skin felt moisturized.

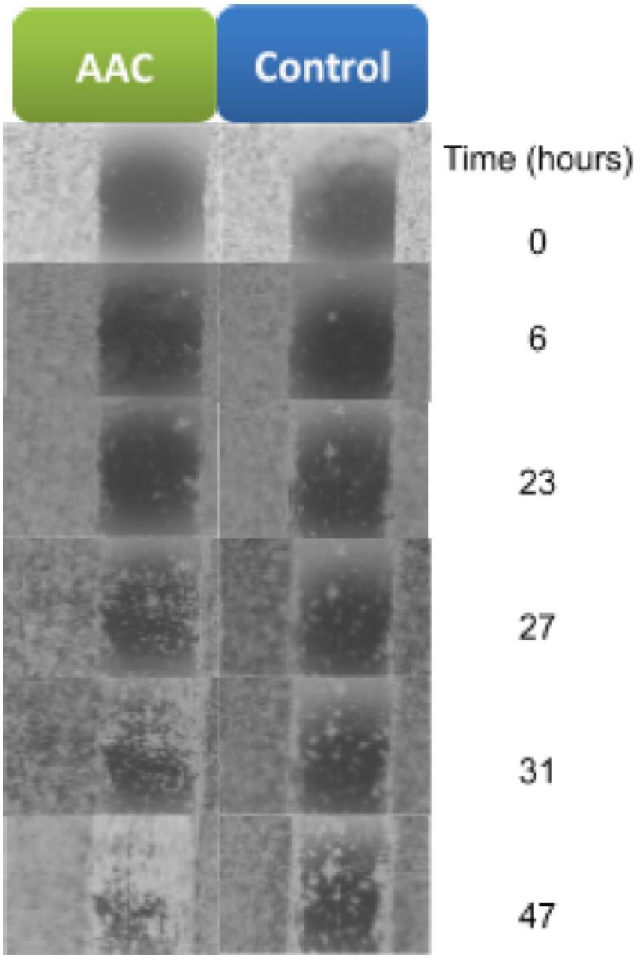


FIGURE 1 Scratch assay to compare the effect of AAComplex on cell renewal

4 | DISCUSSION

Skin is constantly exposed to external challenges, and a skincare product that can help to mitigate the damage caused by these challenges is desirable to consumers. Amino acids are building blocks of protein and make up 20% of the human body. While roughly 500 amino acids have been identified in nature, only 20 amino acids make up human bodies' 100 000 different proteins.[8] Amino acids are widely used in cosmetic skincare products,[9] but mainly to keep the skin hydrated. The EltaMD Skin Recovery System featuring the proprietary AAComplex technology with taurine, arginine, and glycine not only delivered a skin moisturization benefit but also significantly reduced skin irritation and helped to repair skin damage.

As demonstrated in the scratch assay, the AAComplex sped up the damage repair process by showing faster cell renewal which eventually led to a quick repair after the physical damage.

Interleukin-1 alpha (IL-1a) and Hsp27 were used as the biomarkers to evaluate irritation that was induced by SLES in the current *in vitro* human reconstructed tissue study. IL-1a is a cytokine of the interleukin-1 family that in humans is encoded by the IL-1 gene. In general, interleukin-1 is responsible for the production of inflammation, irritation, and has been used widely as the biomarker to evaluate skin irritation.[11–13] Hsp27 (also known as HSPB1) is a chaperone of the sHsp (small heat shock protein) group. The common functions of sHsps are chaperone activity, thermotolerance, inhibition of apoptosis, regulation of cell development, and cell differentiation.[14] HSP-27 plays a role in the regulation of final steps of keratinization[15] and in responding to stress caused by wound healing.[16] Both biomarkers were reduced significantly by the toner, serum, and lotion containing AAComplex versus their

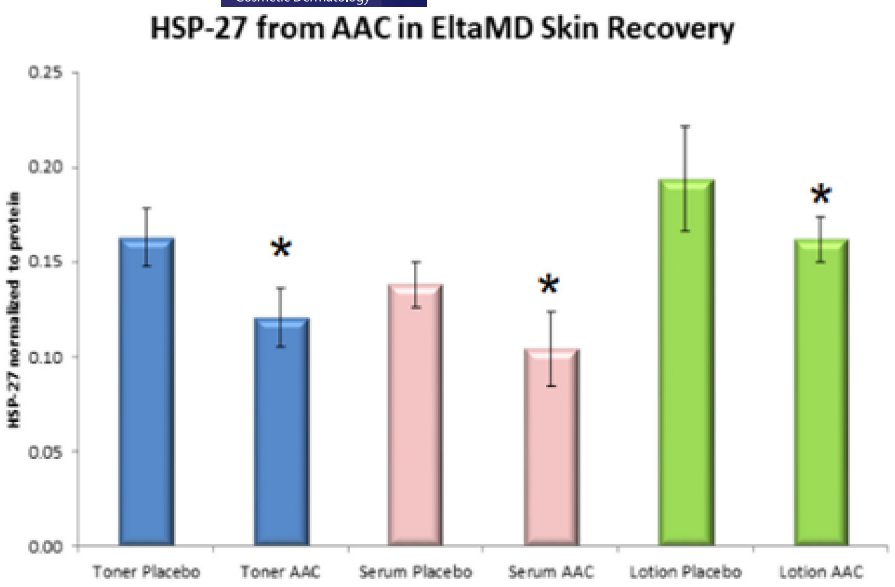


FIGURE 2 Levels of HSP-27 from Skin Recovery System with AAComplex (toner, serum, and lotion) vs. placebos

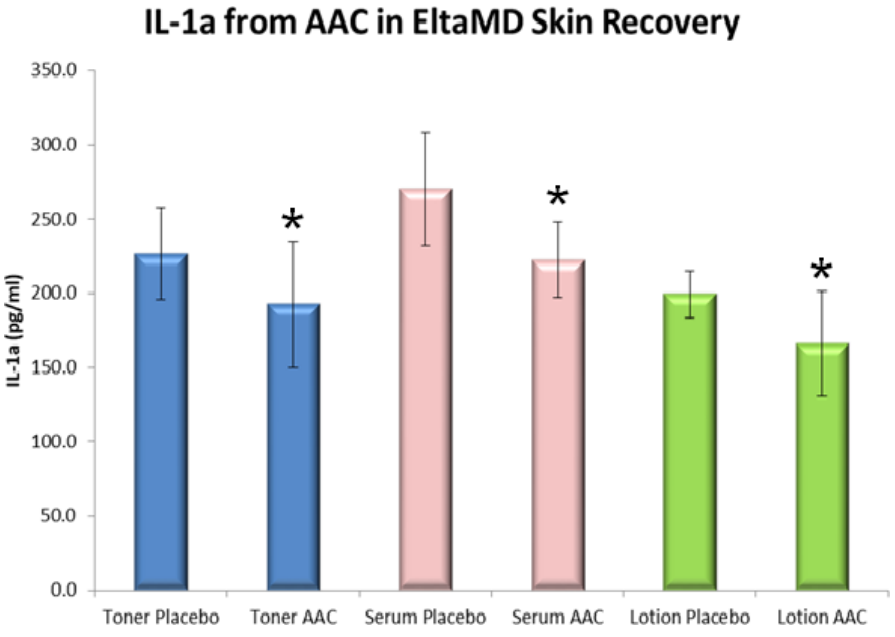


FIGURE 3 Levels of IL-1a from Skin Recovery System with AAComplex (toner, serum, and lotion) vs. placebos

respective placebo control, demonstrating the benefits from this technology.

In the clinical study, IL-1a was also used as the biomarker and was elevated after tape stripping. The serum containing AAComplex lowered IL-1a significantly versus the non-treated site (varying slightly from the *in vitro* work which included respective placebos). The redness reduction observed from the serum-treated site on the forehead as well as the cheek, treated with the three product regimen, was due to the reduction in skin irritation delivered by the test products containing the AAComplex. Skin hydration significantly increased 24 h after a single use of the regimen and doubled after 8 days demonstrating the comprehensive benefits delivered by the regimen of products containing this technology.

5 | CONCLUSION

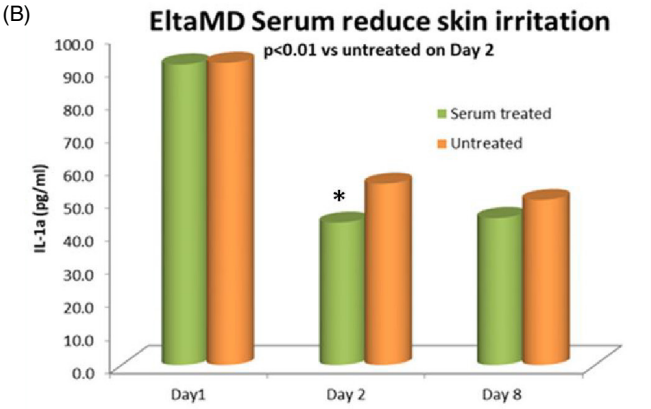
The *in vitro* and *in vivo* clinical studies complement one another in demonstrating that the EltaMD Skin Recovery System (toner, serum, and lotion) containing AAComplex technology effectively delivered a variety of benefits by reducing skin irritation and redness as well as accelerating the skin repair process.

FIGURE 4 A–C: *In vivo* data (VISIA Photographs, Facial Swabbing, and Corneometer)

(A) Forehead (EltaMD Skin Recovery serum only)

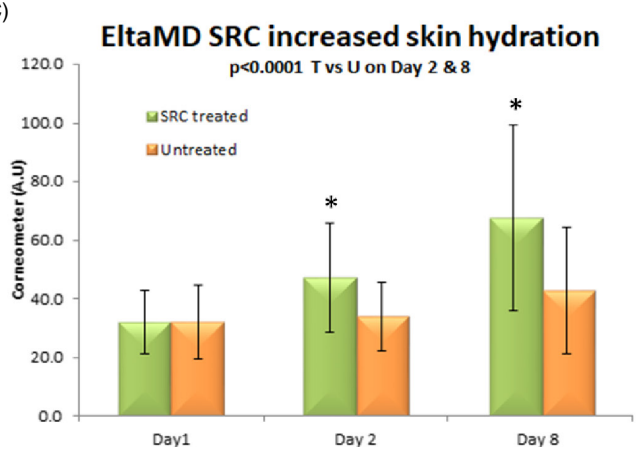
Cheek Area (EltaMD Skin Recovery System - toner, serum and lotion)

(B)



***significant differences p<0.05**

(C)



***significant differences p<0.05**

ACKNOWLEDGEMENTS

The authors would like to thank the Colgate-Palmolive Company and EltaMD for the funding of this research.

ETHICAL APPROVAL

All procedures performed in the study involving human participants were in accordance with the ethical standards of an IRB-approved informed consent (US Investigational Review Board) following Good Clinical Practices (GCPs).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Giorgiana Giancola https://orcid.org/0000-0002-7106-0903

REFERENCES

1. Lopez-Ojeda W, Pandey A, Alhajj M, Oakley AM. Anatomy, Skin (Integument).
2. Chuong CM, Nickloff BK, Elias PM, et al. What is the 'true' function of skin? *Exp Dermatol*. 2002;11(2):159-187.
3. Kappes UP, Luo D, Potter M, Schulmeister K, Rünger TM. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. *J Invest Dermatol*. 2006;126(3):667-675.
4. Pandel R, Poljšak B, Godic A, Dahmane R. Skin photoaging and the role of antioxidants in its prevention. *ISRN Dermatol*. 2013;2013:930164.
5. Koohgoli R, Hudson L, Naidoo K, Wilkinson S, Chavan B, Birch-Machin MA. Bad air gets under your skin. *Exp Dermatol*. 2017;26(5):384-387.
6. Aguilera SB, De La Pena I, Viera M, et al. The impact of COVID-19 on the faces of frontline healthcare workers. *J Drugs Dermatol*. 2020;19(9):858-864.
7. Li DTS, Samaranayake LP, Leung YY, Neelakantan P. Facial protection in the era of COVID-19: a narrative review. *Oral Dis*. 2020;27:10. Online ahead of print.
8. Wagner I, Musso H. New naturally occurring amino acids. *Angew Chem Int Ed Engl*. 1983;22(11):816-828.
9. Leuchtenberger W, Huthmacher K, Drauz K. Biotechnological production of amino acids and derivatives: current status and prospects. *Appl Microbiol Biotechnol*. 2005;69(1):1-8.
10. Ripps H, Shen W. Review: taurine: a "very essential" amino acid. *Mol vis*. 2012;18:2673-2686.
11. Corsini E, Bruccoleri A, Marinovich M, Galli CL. Endogenous interleukin-1 alpha associated with skin irritation induced by tributyltin. *Toxicol Appl Pharmacol*. 1996;138(2):268-274.
12. Bou-Dargham MJ, Khamis ZI, Cognetta AB, Sang QA. The role of interleukin-1 in inflammatory and malignant human skin diseases and the rationale for targeting interleukin-1 alpha. *Med Res Rev*. 2017;37(1):180-216.
13. Hu Y, Liang D, Li X, et al. The role of interleukin-1 in wound biology. Part II in vivo and human translational studies. *Anest Analg*. 2010;111(6):1534-1542.
14. Carper SW, Rocheleau TA, Kristian Storm F. cDNA sequence of a human heat shock protein HSP27. *Nucleic Acids Res*. 1990;18(21):6457.
15. Scieglinska D, Krawczyk Z, Sojka DR, et al. Heat shock proteins in the physiology and pathophysiology of epidermal keratinocytes. *Cell Stress Chaperones*. 2019;24:1027-1044.
16. Laplante AF, Moulin V, Auger FA, et al. Expression of heat shock proteins in mouse skin during wound healing. *Cytochem*. 1998;46(11):1291-1301.

How to cite this article: Diaz I, Namkoong J, Wu J, Giancola G. Amino acid complex (AAComplex) benefits in cosmetic products: *In vitro* and *in vivo* clinical studies. *J Cosmet Dermatol*. 2022;21:3046–3052. https://doi.org/10.1111/jocd.14544