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 ORIGINAL ARTICLE 

# Ultraviolet Protection From a Patented Amino Acid Complex Technology

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## ABSTRACT

**Objective:** This study aimed to investigate the ultraviolet (UV) protection/repair benefits of a patented Amino Acid Complex (AAComplex).
**Methods:** I) AAComplex was incubated with dermal fibroblasts, with/without UVA, and collagen I was measured with a GlasBoxPlus device. II) A lotion, with/without AAComplex (1%) was applied topically to skin explants, following UVA irradiation, and quantified for health-related biomarkers (TNFα, histamine, and MMP-1). III) A broad spectrum sunscreen with SPF 46 and a skincare serum containing AAComplex (2%) were assessed using epidermal equivalents, in the presence of UV irradiation, for effects on IL-1α, thymine dimers, Ki-67, filaggrin and Nrf2.
**Results:** I) Collagen I synthesis in dermal fibroblasts was significantly decreased after UVA compared to without UV. The presence of AAComplex prevented this decrease. II) UVA irradiation of skin explants increased histamine, TNFα, and MMP-1. Hydrocortisone aceponate cream significantly decreases all 3 biomarkers. AAComplex contained lotion also significantly decreased all 3 biomarkers, the no AAComplex control lotion only reduced histamine. III) With the regimen of sunscreen + AAComplex contained skincare serum, the significantly reduction in IL-1α was observed along with a complete recovery of Ki-67 and stimulation of filaggrin and Nrf2T. No thymine dimer positive cell was observed indicating the most positive skin impact from the regiment.
**Conclusion:** This research using different human skin models demonstrated that AAComplex can provide protection and damage repair caused by UV, at the ingredient level also when formulated in a serum or lotion formula. Skin may be best protected from UV damage when the regimen is used.

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## INTRODUCTION

Sunlight is critical for the maintenance of life on Earth; however, the ultraviolet (UV) wavelengths (100 nm - 400 nm) associated with sunlight can induce adverse reactions in human skin. The strong intensity ultraviolet C (UVC) (100 nm - 280 nm) is mostly blocked by the ozone layer of the Earth's atmosphere, while UVB radiation (280 nm - 320 nm) and UVA radiation (320 nm - 400 nm) reach the Earth's surface and ultimately the skin.[1-3] Ultraviolet B (UVB) radiation is absorbed in the epidermis, the uppermost layer of the skin, causing sunburn and DNA damage, such as thymine dimer formation. On the other hand, longer wavelength UVA radiation penetrates the skin deeper and into the dermis, causing harm through oxidation.[4]

The American Academy of Dermatology (AAD) recommends that everyone should use a sunscreen product on a daily basis when they are outdoors. They should choose products that offer broad-spectrum protection, ie. protect against UVA and UVB radiation, with a sun protection factor (SPF) ≥ 30.[5] In a consumer survey of attitudes and habits toward sunscreens, almost half of the participants indicated that the SPF factor was the top factor in their buying decision – which unfortunately misses the benefits of UVA protection. By contrast, having a "broad-spectrum sunscreen" was only the fifth-highest factor, and garnered support from only one-third of consumers[6]; suggesting that consumer education on sun protection is still needed.

Skin damage from UV radiation can take many different shapes and forms, such as erythema, photoaging, DNA damage, and carcinogenesis, among others.[7] In fact, UV radiation is classified as a Group I carcinogen (in the same category as arsenic and asbestos) by the World Health Organization.[8] An exposure of the skin to UV radiation can increase inflammatory cytokines, such as interleukin-1α.[9] UVB irradiation can also lead to the formation of thymine dimers,[10] which may ultimately lead to skin cancer, in the absence of repair.[11] Sub-lethal doses of UV radiation decrease cell proliferation – likely to allow cellular systems to check for damage incurred and correct mutations.[12]

In addition, UV radiation influences other skin functions. The expression of proteins involved in stratum corneum barrier function, filaggrin, and involucrin, is suppressed by UV radiation.[13,14] Reactive oxygen species (ROS) are formed upon exposure of the skin to UV radiation and lead to increases in skin damage and aging.[15] Nuclear factor erythroid 2- related factor 2 (Nrf2) is a transcription factor that binds to antioxidant-responsive elements (AREs), stimulating antioxidant enzymes such as catalase.[16] Nrf2 is one of the main defensive systems that act against ROS.[17]

Products that protect the skin from UV radiation can be applied proactively in the form of sunscreens prior to UV exposure. While the protective effects of sunscreen are well established, the skin recovery options after the incurrence of UV-induced damage, or the benefits of topical cosmetic products after UV exposure, are not well understood. In our previous research, we identified a proprietary amino acid complex (AAComplex) technology, which demonstrated suppression of skin irritation and an improved skin repair process.[18] The research outlined here further investigates the effects of AAComplex in vitro and ex vivo: it evaluates I) the impact of AAComplex, at the ingredient level, on fibroblast cells irradiated with UVA; II) the effect of AAComplex, formulated in a simple formula, on ex vivo skin exposed to UVA; III) combining a commercial serum containing AAComplex with a sunscreen (SPF 46) using epidermal equivalent models to assess the protective and beneficial effects on the skin of (1) a sunscreen (SPF 46) applied before UV irradiation; (2) an AAComplex containing serum applied after UV irradiation; and (3) the combination of sequential application of the sunscreen, exposure to UV irradiation, followed by application of the serum. These studies aimed to evaluate how this patented AAComplex technology can protect and repair UV-induced damage of different skin models (fibroblast cells, epidermal 3D, and ex-vivo skin explant), and any additional benefit this technology can provide when combined with a sunscreen.

## MATERIALS AND METHODS

**Experiment I: Fibroblast Cells Contractile Forces Evaluation of AAComplex With/Without UVA Exposure**

Fibroblast cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 40 mg/L of gentamicin, and 2 mg/l of fungizone (DMEMc), in an incubator at 37°C, 5% CO2, and 95% air. Fibroblast cells were treated in the following groups:

- Control: no UVA irradiation, no mixture of AAComplex
- UVA: UVA irradiation at 3 J/cm$^2$
- AAComplex: AAComplex at 0.1%
- AAComplex + UVA: AAComplex at 0.1%, UVA irradiation at 3 J/cm$^2$

Cytotoxicity was evaluated with MTT and was used to identify the proper concentration of AAComplex to be tested. A GlasBoxPlus device was used to measure collagen I synthesis through contractile forces of fibroblasts.

**Experiment II: Ex Vivo Cutaneous Microdialysis Study on Biomarkers With/Without UVA Exposure on Lotion Formulated With AAComplex**

*Topicals*

Three lotions were tested on ex vivo skin explants in this study: Lotion A (negative control), Lotion B (with 1% AAComplex), and a positive control (topical drug with 0.127% hydrocortisone).

*Skin Explants*

Triplicate samples of excised abdominal skin, obtained from surgery, were placed immediately in a phosphate buffer and incubated at 37°C. Six conditions were evaluated:

- Lotion A without UVA irradiation
- Lotion A with UVA irradiation
- Lotion B without UVA irradiation
- Lotion B with UVA irradiation
- Positive control without UVA irradiation
- Positive control with UVA irradiation

*Ex Vivo Microdialysis*

The microdialysis principle can be compared with an artificial blood vessel. Microdialysis sampling is performed by placing a tubular microdialysis membrane into the dermis, parallel to the skin's surface. The probe, which is permeable to water and small molecules, is continuously perfused with a physiological buffer solution at a low flow rate. Unbound substances present in the skin can cross the membrane and enter the lumen probe in proportion to a concentration gradient.

The microdialysis system consisted of a CMA/100 syringe pump and a CMA/140 microfraction collector, which collected samples. Six probes were inserted into the dermis of each fragment and perfused with Ringer solution at 3 µL/min. After 1 hour of stabilization, the microdialysis was started for 1 hour (T0). UVA irradiation was started using a UVA Bridge and performed for 4 hours, corresponding to 17 J/cm$^2$.

Immediately after the irradiation, 2 mg/cm$^2$ of Lotion A, Lotion B, or positive control were applied to the surface of the skin. Microdialysis samples were collected every hour for 24 hours. Microdialysates were pooled at 1h, 2h, 4h, 6h, 12h, and 24h, and frozen at -80°C until analysis.

*Biomarker Analysis*

Three biomarkers (TNFα, histamine, and MMP-1) were evaluated. Histamine was quantified by enzyme immunoassay (EIA); TNFα and MMP-1 were determined using ELISA kits.

*Statistical Analysis*

Data are expressed as mean sem. A variance analysis with one factor was performed followed if necessary by a Fisher test. A P value less than 0.05 is considered significant.

**Experiment III. 3D Reconstructed Epidermal Human Skin Model to Evaluate a Regimen Including AAComplex Serum and a Sunscreen**

*Topicals*

SunCare: Broad Spectrum Sunscreen with SPF46 containing zinc oxide and octinoxate.

SkinCare Serum: Serum with 2% AAComplex technology.

*Treatment of Epidermal Equivalents*

The epidermal equivalents (EpiDerm; MatTek, Ashland, MA) were used to assess topical formulas with or without UV exposure, and to evaluate skin benefits. The treatment plan is shown in Figure 1, with stepwise treatment visualization. When SunCare was a part of the treatment conditions, 10 µL SunCare was applied 1 hour prior to UV irradiation and removed with a sterile Q-tip after UV irradiation. When SkinCare Serum was a part of the treatment conditions, 10 µL SkinCare Serum was applied after UV irradiation and removed with a sterile Q-tip after a 24-hour treatment period. The epidermal equivalents were irradiated with a solar simulator (LS1000-6S-UV, Solar Light, Glenside, PA) for specified doses of 10 J/cm$^2$ UVA+UVB for the staining analysis, or 20 J/cm$^2$ UVA+UVB for the gene and protein expression analysis. These doses are similar to the dose needed to induce erythema in subjects with skin phototype II or III.[19] After UV irradiation with or without SkinCare Serum application, the media was replaced with fresh media. Following 24 hours of incubation, media was collected, and tissues were rinsed and harvested for different evaluations.

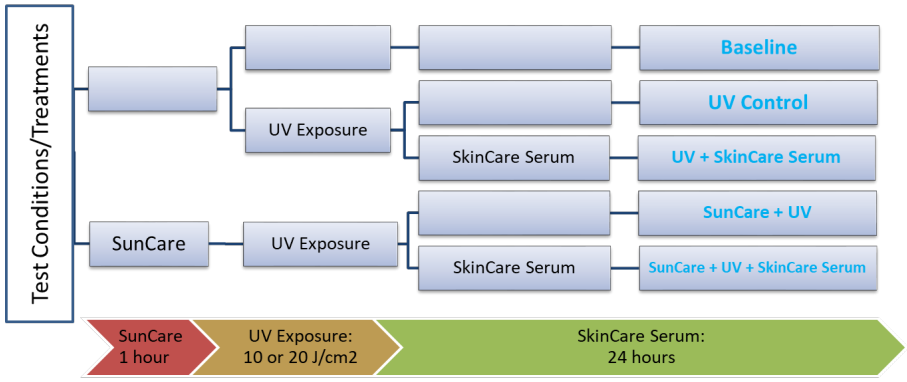
*Biomarker Analysis*

Tissues were lysed in RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO) with cOmplete™ ULTRA Tablets, Mini, EDTA-free (Roche, Pleasanton, CA) using TissueLyser II (Qiagen, Germantown, MD), following a standard lysing protocol. Tissue debris and beads were collected by centrifugation, and supernatants were used in the skin barrier ELISA (Filaggrin, Cusabio, Houston, TX), according to the manufacturer's protocol, and protein normalization was performed using Micro BCA assay kits (ThermoFisher Scientific, Rockford, IL) according to the manufacturer's protocol. Collected tissue culture media was used in skin irritation evaluation (IL-1α, R&D Systems, Minneapolis, MN). Student's t-test was used to evaluate statistical significance.

For immunofluorescence and immunohistochemistry, fixed tissues were embedded in paraffin and sectioned at 5 microns. For thymine dimer immunohistochemistry, tissue sections were rehydrated and heat-induced antigen retrieval was performed using a citrate buffer, followed by a permeabilization step using 0.25% Triton X-100 and the DNA denaturation step using 2N hydrochloric acid. The mouse monoclonal Thymine Dimer antibody was used at 1:1000 (T1192, Sigma-Aldrich), and visualized using Vectastain Universal Quick HRP Kit, Peroxidase, R.T.U and ImmPACT NovaRED (Vector Laboratories, Inc., Burlingame, CA). For Ki-67 immunofluorescence, anti-Ki67 (ab16667, 1:200, Abcam, Waltham, MA) was used without the DNA denaturation step, and the staining was visualized using goat anti-rabbit Alexa Fluor 488 (ThermoFisher Scientific) and Vectashield antifade with DAPI (Vector Laboratories, Inc.). All images were taken by EVOS FL (ThermoFisher Scientific) and visualized images were quantitated using ImageJ.

For reverse transcription-quantitative polymerase chain reaction (RT-QPCR), tissues were first submerged in RNAlater™ Stabilization Solution (ThermoFisher Scientific), and RNA was extracted from stabilized tissues using a homogenizer (Kinematica, Bohemia, NY), and RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's protocol. Extracted RNAs were

**FIGURE 1.** Test conditions and design. There were 5 experimental conditions. Baseline was without any topical or UV treatment, which served as the negative control. UV control was UV treated tissues, with either 10 J/cm$^2$ or 20 J/cm$^2$ UVA+UVB. SunCare treatment occurred prior to UV treatment in SunCare + UV, whereas SkinCare Serum was applied after UV exposure in UV + SkinCare Serum. When both SunCare and SkinCare Serum were applied with UV exposure in between, the sample is called SunCare+UV+SkinCare Serum.



quantitated using NanoDrop One (ThermoFisher Scientific). Based on the RNA concentrations, cDNA synthesis was performed using Maxima First Strand cDNA Synthesis kit for RT-qPCR with dsDNase (ThermoFisher Scientific), and gene expression analysis was performed using Taqman™ array cards using QuantStudio 7 Flex (ThermoFisher). Forty-eight skin-specific targets and endogenous controls were selected for analysis. Relative quantifications from the treatments were calculated and compared with UV control tissues or baseline control tissues. Student's t-test was used to evaluate the statistical significance and the P-value was corrected by Benjanmini-Hochberg false discovery rate using Thermo Fisher Cloud app.

## RESULTS

**Experiment I: Fibroblast Cells Contractile Forces Evaluation of AAComplex With/Without UVA Exposure**

Cytotoxicity of AAcomplex (0.00001 to 0.1%) in dermal fibroblast culture, after 24 and 48 hours, showed greater than 80% cell viability, and 0.1% was chosen as the concentration for further testing.

Collagen I synthesis was measured through contractile forces of fibroblasts, with a GlasBoxPlus device, in the presence or absence of UVA exposure (Figure 2). As expected, collagen I synthesis was significantly decreased after UVA irradiation, compared with the non-irradiated control.

The presence of AAComplex appeared to induce protection against the decrease in collagen I synthesis of fibroblasts irradiated with UVA. AAComplex-treated cells exhibited contractile forces close to the level before UV treatment (Figure 3a). A similar trend was observed when collagen I synthesis, quantified by ELISA assay, was significantly higher in cells incubated with AAComplex treatment compared with UVA alone (Figure 3b). Without UVA irradiation, AAComplex also demonstrated collagen-boosting benefits vs the control.

**Experiment II: Ex-Vivo Cutaneous Microdialysis Study on the Effects of an AAComplex Lotion on Biomarkers Modulated by UVA Exposure**

The expression of 3 biomarkers related to skin health – histamine (allergy), TNF-α (inflammation), and MMP-1 (breakdown collagen) – was evaluated, following the application of 3 lotions (with/without AAComplex and a positive control Hydrocortisone Aceponate Cream). UVA irradiation of skin explants increased histamine, TNFα, and MMP-1 production overall. Topically applied hydrocortisone Aceponate Cream, as the positive control, was able to decrease histamine (Figure 4), TNFα (Figure 5), and MMP-1 (Figure 6) significantly.

Topical treatment of skin explants with Lotion A (lotion base) significantly decreased histamine production after UVA irradiation. No modification was observed in the expression of TNFα and MMP-1. The treatment with Lotion B (containing AAComplex) significantly decreased histamine, TNFα, and MMP-1 expression, following UVA irradiation.

**Experiment III. 3D Reconstructed Epidermal Human Skin Model to Evaluate a Regimen of AAComplex-Containing Serum and a Sunscreen**

*Inflammation*

To detect inflammation, IL-1α was measured by ELISA from collected media under various experimental conditions (Figure

**FIGURE 2.** Fibroblasts cell contractile force (with/without) UV exposure measured by GlasBoxPlus.

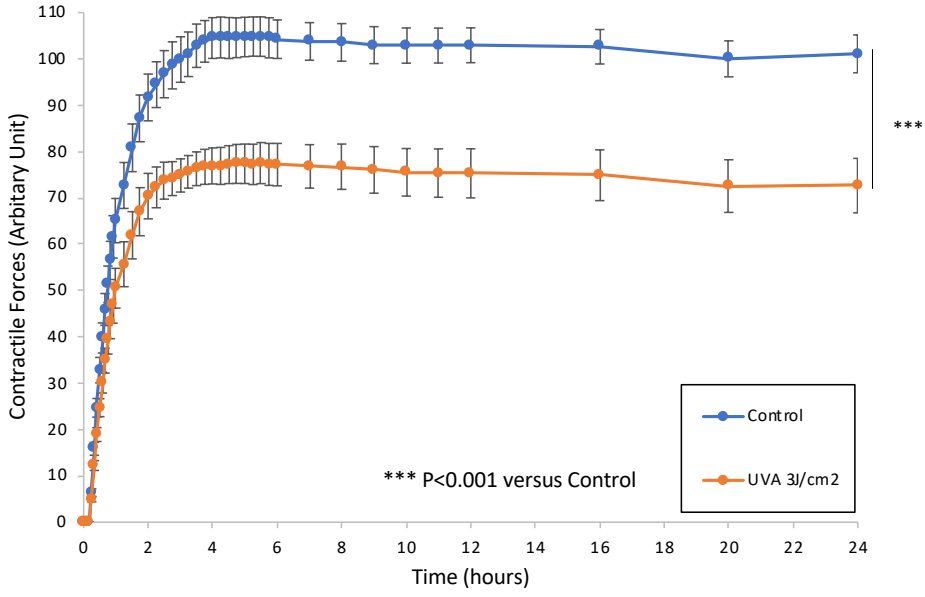
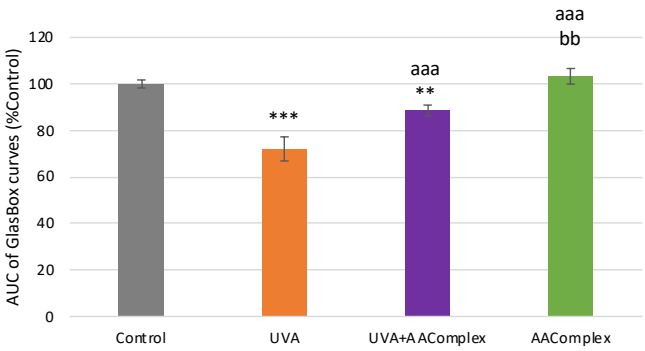


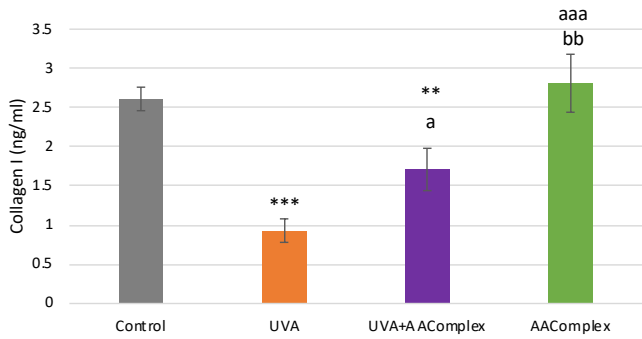
**FIGURE 3.** Collagen I synthesis measured through contractile forces of fibroblasts as quantified by GlasBoxPlus (A) and by ELISA assay (B) were compared with control (no UV exposure), UVA (with UVA exposure), UVA+ Mixture of amino acid (with AAComplex exposed to UVA) and Mixture of amino acid (with AAComplex without UVA exposure)

(A)




** P<0.01 and *** P<0 001 vs control
aaa P<0.001 vs UVA
bb P<0.01 vs UVA+AAComplex

(B)




** P<0.01 and *** P<0 001 versus Control
a P<0.05 and aaa P<0.001 versus UVA
bb P<0.01 versus UVA+AAComplex

7). Compared with baseline control, IL-1α was statistically significantly increased in UV control, suggesting the induction of inflammation. Application of SunCare prior to UV irradiation statistically significantly reduced IL-1α. An increase in IL-1α by UV exposure and suppression of IL-1α by SunCare was as expected for the nature of UV radiation and skin protection by sunscreen. Application of SkinCare Serum after UV irradiation also statistically significantly reduced inflammation as compared with the UV control. This topical product was previously shown to reduce skin irritation in the in vitro study and the clinical study, so this result was anticipated.[18] Finally, inflammation reduction was statistically significant, when the combination of SunCare application, followed by UV irradiation, and then followed by SkinCare Serum application was compared with the UV control or SunCare with UV, indicating that the combination of SunCare and SkinCare Serum can provide a benefit above and beyond SunCare alone.

*Thymine Dimer*

Cross-sections of the epidermal equivalents were stained for the thymine dimer immunohistochemically (Figure 8). Thymine dimers were visible in the UV control tissues, when compared with the baseline control tissues, and the baseline did not show any positive staining. When SunCare was applied prior to UV irradiation, the skin was protected, resulting in no positive thymine dimer staining, reinforcing the critical importance of sunscreen use. When SunCare was applied, followed by UV irradiation, followed by the application of SkinCare Serum, there were also no thymine dimer positive cells, since SunCare fully protected the epidermal equivalents. Surprisingly, there were lighter thymine dimer positive cells in tissue samples in which SkinCare Serum was applied after UV irradiation. This indicates that even though the SkinCare Serum cannot prevent DNA damage, it may play a role in the skin recovery and repair processes associated with UV damage.

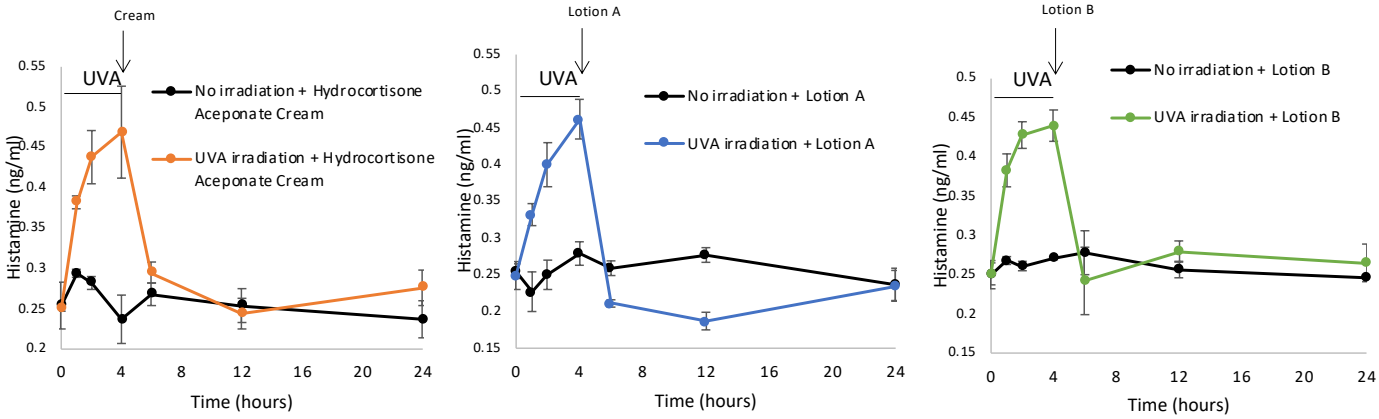
*Cell Proliferation*

To further assess the impact of UV radiation, Ki-67 expression was evaluated by immunofluorescence (Figures 9a and 9b) and QPCR (Figure 9c). Ki-67 is a keratinocyte cell proliferation biomarker, whose expression is restricted to the basal layer of the epidermis. Ki-67 is shown in green, while the DAPI nuclear counterstain is shown in blue; therefore, the Ki-67 positive nucleus is shown as aqua. Approximately 8.5% of the epidermis was Ki-67 positive in baseline control as shown in the quantitation graph (Figure 9b), but Ki-67 positive cells were eliminated in UV control, displaying a lack of cell proliferation in UV-induced skin. Application of SunCare prior to UV irradiation resulted in some recovery of Ki-67 positive cells, while application of SkinCare Serum following UV irradiation also helped with some recovery of Ki-67 positive cells. With the SunCare and SkinCare combination, Ki-67-positive cells were recovered back to the level of baseline control. From the gene expression analysis, UV irradiation suppressed Ki-67 gene expression very strongly, while application of SunCare, SkinCare Serum, or the combination of SunCare and SkinCare Serum resulted in Ki-67 expression back toward baseline control, which were not statistically different from baseline control.

*Skin Barrier*

The skin barrier biomarker, filaggrin, was assessed in this study by protein and gene expression. Filaggrin protein expression was first measured by ELISA as shown in Figure 10a. Filaggrin protein expression was not modulated in UV control tissues as

**FIGURE 4.** Histamine measured by ELISA assay.

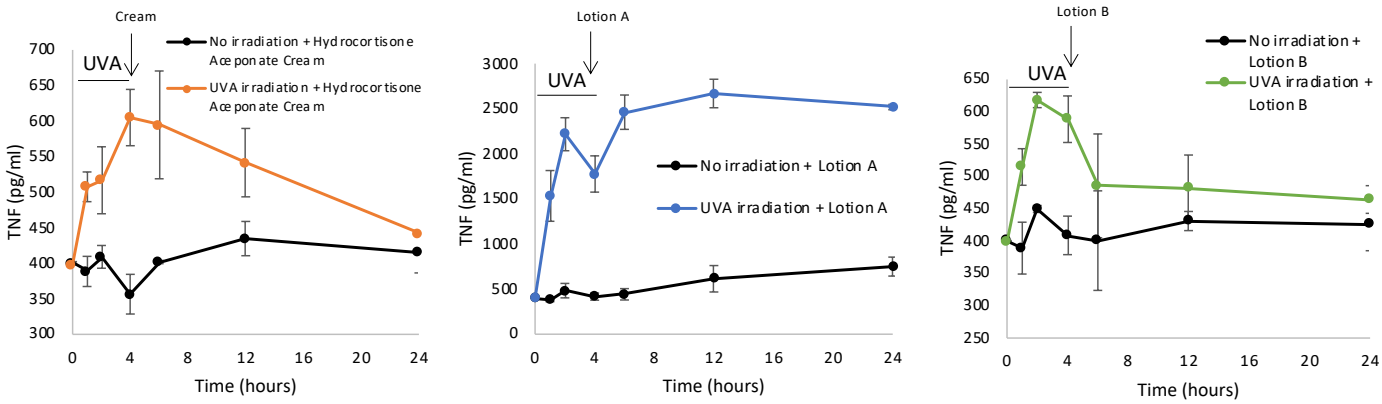



Positive Control
**Hydrocortisone Aceponate Cream**

Control Lotion

AAComplex Lotion

**FIGURE 5.** TNF-α measured by ELISA assay

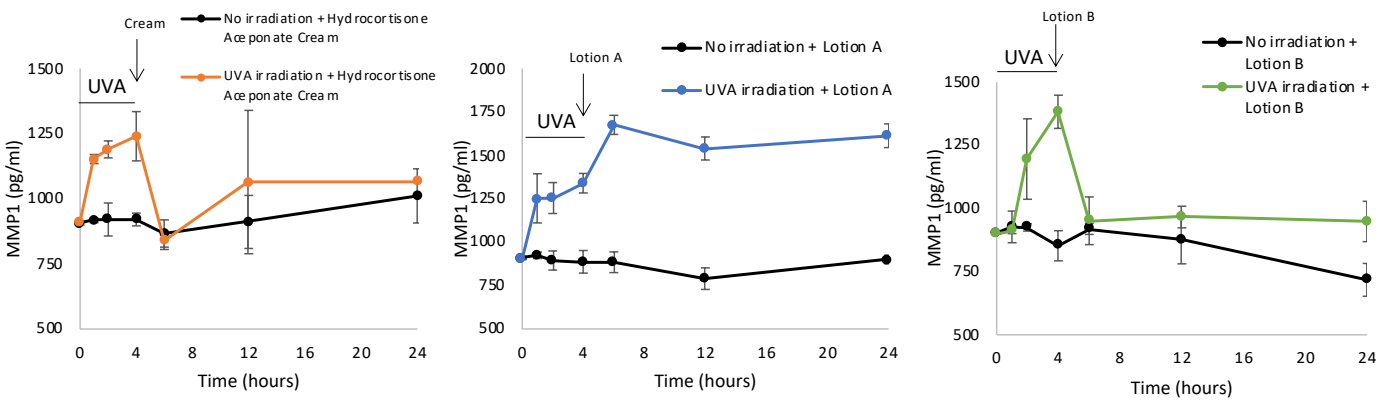



Positive Control
**Hydrocortisone Aceponate Cream**

Control Lotion

AAComplex Lotion

**FIGURE 6.** MMP-1 measured by ELISA assay.

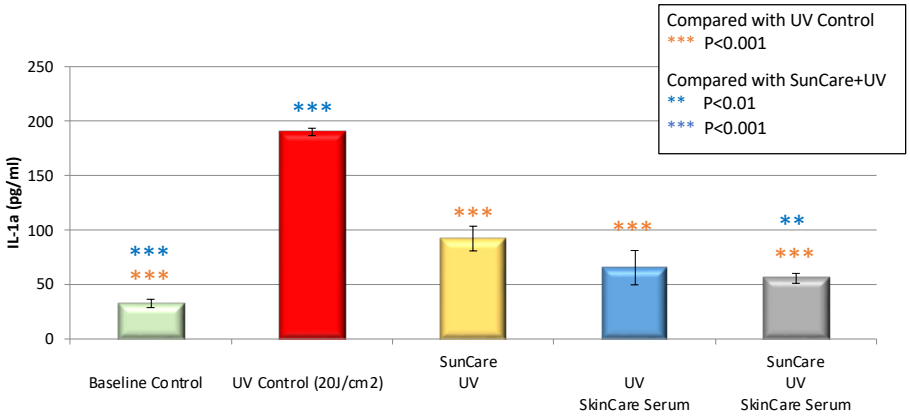



Positive Control
**Hydrocortisone Aceponate Cream**

Control Lotion

AAComplex Lotion

**FIGURE 7.** UV induced IL-1α secretion, measured by ELISA. UV radiation increased IL-1α release, whereas topical applications of SunCare, SkinCare Serum, or combined application suppressed IL-1α. Combined application suppressed IL-1α more than SunCare alone.




Red stars indicate statistical testing compared with the red bar (UV control) and blue stars indicate statistical testing compared with the purple bar (SunCare+UV) (**$P$<0.01, ***$P$<0.001).

compared with the baseline control tissues, likely due to only a 24-hour incubation, but downward trends were seen in the UV control. In contrast, application of SkinCare Serum following UV irradiation, statistically significantly increased filaggrin expression ($P$=0.033) as compared with baseline control, and application of SunCare prior to UV irradiation showed some stimulation tendency ($P$=0.083). This stimulation tendency cannot be attributed solely to the sunscreen, since SunCare contains additional ingredients known to stimulate filaggrin. However, there was no stimulation when both SunCare and SkinCare Serum were applied in combination with UV irradiation, which may suggest limited barrier protein production without impairment. Similar trends were observed from the filaggrin gene expression results, with clear differences from UV control (Figure 10b). UV irradiation statistically significantly suppressed filaggrin gene expression as compared with baseline control. Additionally, filaggrin gene expression was significantly stimulated in all topical treatment groups after UV irradiation compared with UV control, and the expression levels were similar to baseline control in all SunCare applied tissues, not different statistically.

*Antioxidant/Skin Protection*

The antioxidant modulator Nrf2 expression was evaluated by QPCR. As shown in Figure 11, Nrf2 expression was statistically significantly inhibited in UV control as compared with the baseline control tissues. Application of SkinCare Serum after UV irradiation or the application of SunCare prior to UV irradiation and followed by application of SkinCare Serum resulted in statistically significant stimulation of Nrf2 expression as compared with the UV control tissues. However, the application of SunCare prior to UV irradiation did not show statistically significant effects compared with UV control tissues, though the expression showed a stimulation tendency. One possible explanation might be that SunCare blocked the negative impacts of UV only partially, therefore still resulting in some suppression of Nrf2 expression.

**FIGURE 8.** UV-induced thymine dimer formation. After treatments, collected tissues were fixed and assessed for the presence of UV damage by measuring thymine dimers immunohistochemically. Thymine dimers are present in the nucleus, as shown by reddish brown staining in UV control and UV+SkinCare Serum. SunCare blocked UV-induced thymine dimers, therefore no positive staining is shown on baseline control, SunCare+UV, or SunCare+UV+SkinCare Serum.

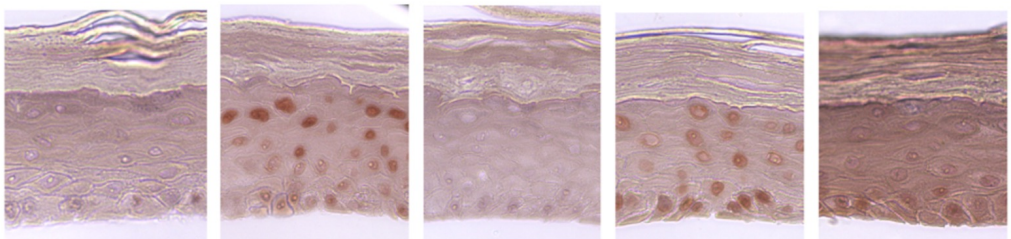
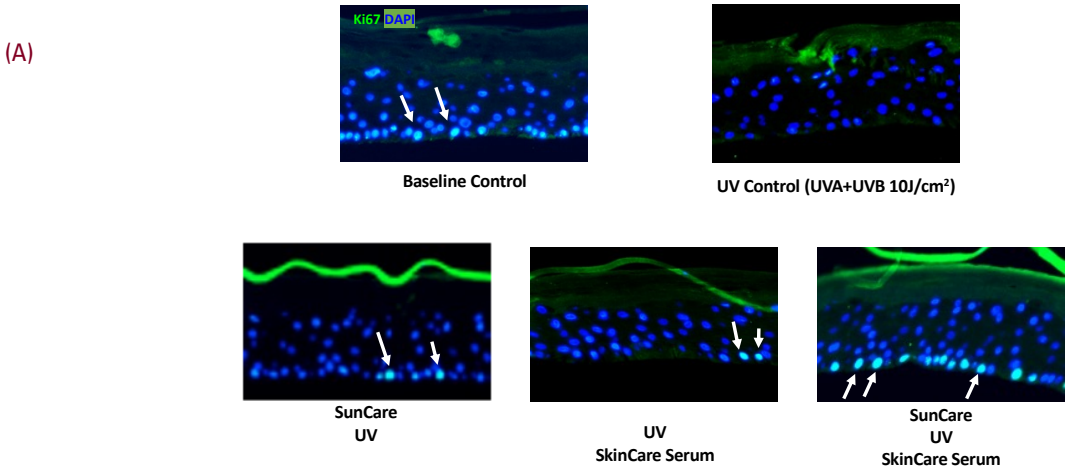
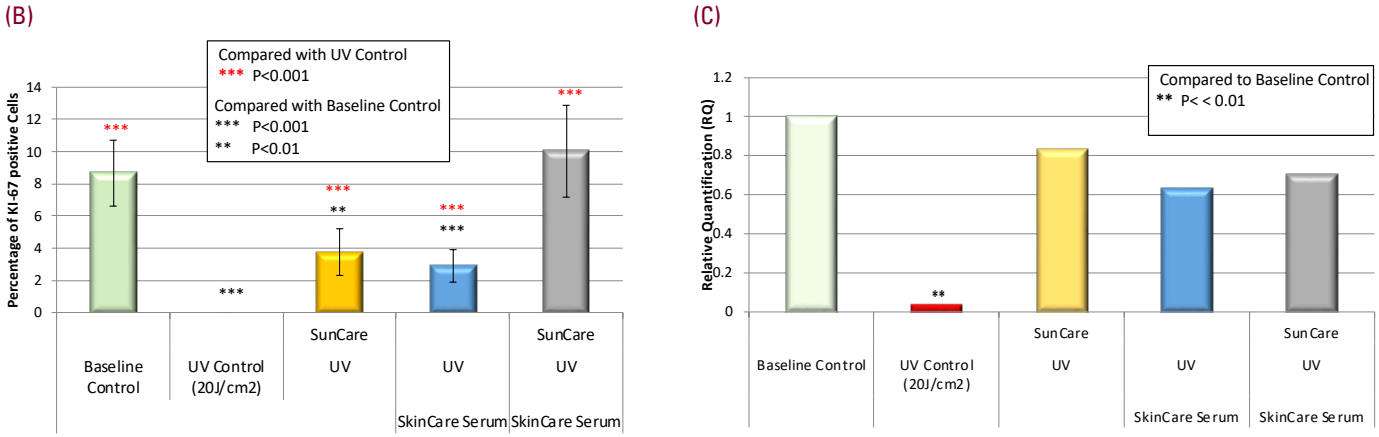


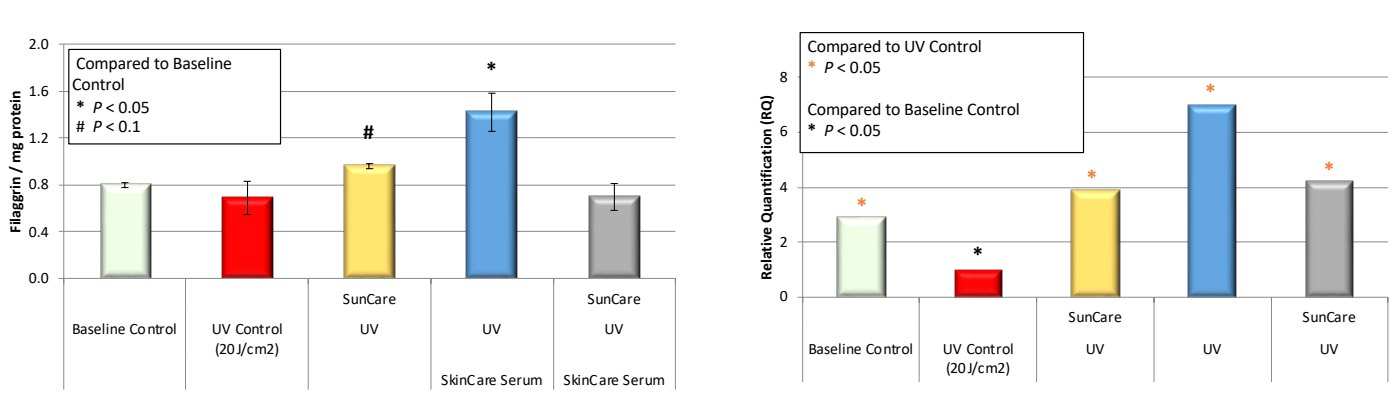
**FIGURE 9.** Ki-67 expression analysis. (A) Ki67 is shown in green with DAPI nucleus staining shown in blue. Ki67 is detected in the nucleus, therefore merged images show Ki67 positive cells in aqua, as some arrows are pointing. (B) Ki67 positive cells are counted and percent Ki67 cells over total cells are graphed for statistical testing. (C) MKI67 gene expression is shown in relative quantification (RQ).



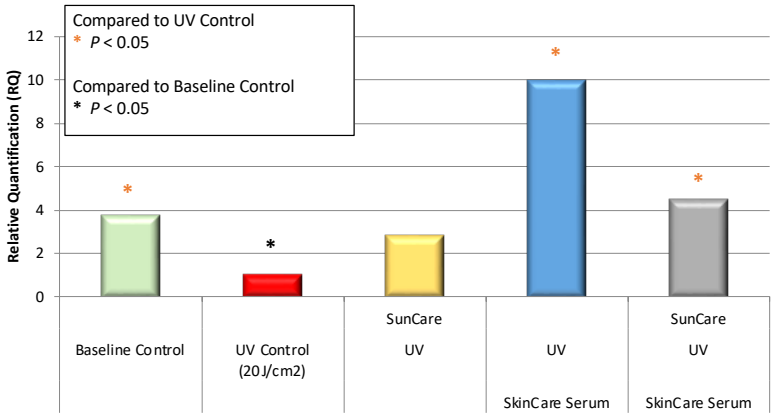

While UV Control is shown 27-fold reduction in MKI67 expression, SunCare and SkinCare Serum in UV treated recovered the MKI67 expression close to baseline Control (**$P$<0.01, ***$P$<0.001).

**FIGURE 10.** Filaggrin Skin Barrier Biomarker from ELISA (A) and Gene Expression (B). (A) Filaggrin protein expression is detected by ELISA. Compared to Baseline Control (beige), the purple bar (SunCare+UV) and blue bar (UV+SkinCare Serum) show an increase in filaggrin protein expression. (B) Filaggrin gene expression was measured by QPCR.




Compared to UV control (red), all treatment and Baseline Control show statistically higher filaggrin gene expression (#$P$<0.1, *$P$<0.05).

**FIGURE 11.** Gene Expression (Antioxidant: NRF2). NRF2 expression was measured by QPCR. Compared to UV control (red), Baseline Control (beige), UV+SkinCare Serum (blue) and SunCare+UV+SkinCare Serum (green) show statistically higher NRF2 gene expression.




Stimulation of NRF2 gene expression in SunCare+UV (purple) is not statistically significant (*$P < 0.05$).

## DISCUSSION

Skin is the part of the human body that is exposed to environmental insults on a daily basis, by UV radiation and pollution. The acute effects of UV radiation include sunburn and DNA damage as shown from the results of UV control samples, while the chronic effects can include skin cancer and photoaging.[7,20] The chronic effects accrue through many years and detrimental effects emerge later in life, deterring protection efforts. To be protected from UV radiation, the American Cancer Society (ACS) recommends that consumers avoid sun exposure during the middle of the day, wear sun protective clothing, and apply a broad spectrum sunscreen (SPF ≥ 30), plus re-apply every 2 hours.[21] Consumer usage of sunscreens is low despite the well-known protective benefits of sunscreens. The 2015 National Health Interview Survey-Cancer Control Supplement analysis determined that sunscreens were used by only 31.5% of adults in the United States.[22] This means that a majority of US adults are not following the recommendation from the AAD and the ACS with regards to sunscreen usage. Some wear sunscreen, but not often, or don't reapply, or don't look for broad-spectrum coverage. Thus, without the necessary sun protection during a typical summer day, sun exposure could result in the type of skin damage that was observed in the UV control epidermal equivalents that were exposed to UV radiation in our study. At the molecular level, UVA is known to decrease collagen synthesis.[23]

Furthermore, skin inflammation and sensitivity are increased, as measured by the skin inflammation markers interleukin-1 alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α), and skin sensitivity marker histamine.[24] DNA damage is detected, measured by thymine dimers. At the same time, UV irradiation suppressed the expression of Ki-67, filaggrin, and Nrf2. All of these changes indicate the negative impacts of UV irradiation on the skin. However, with the use of proper topical products, some damage can be minimized and the skin can be restored to a normal state, like baseline control in this study. Identification of technologies, such as AAComplex, which can prevent or mitigate the effects of UV damage, may also provide additional skin benefits. As an ingredient, AAComplex demonstrated a protective benefit on skin against the consequences of photodamage, by increasing collagen I expression significantly, from the decrease induced by UV exposure. Decreased collagen levels in photoaged skin may also arise from increased degradation of the protein by MMP1, whose expression levels increase following UV exposure. Topically applied AAComplex-containing lotion was able to lower MMP1 expression, whereas the control lotion, without the technology, could not. The lotion also reduced the levels of TNF-α and histamine, thus potentially addressing some aspects of inflammation and sensitivity or itch.

An interesting question is how a technology such as AAComplex would augment existing topical sun care formulations. For example, SunCare can protect the skin from UV-induced DNA damage and reduce inflammation. In addition, there was partial or full recovery of Ki-67 and stimulation of filaggrin. These results reinforce the critical importance of sunscreen use.

As was previously mentioned, most consumers are not applying sunscreens regularly. Additionally, the skin might be inadequately covered due to sweat, washing, or rub-off by clothes. In these instances, it is important to apply a skin care product that contains ingredients that can counteract the damage that UV irradiation can trigger and help the skin recover. This was observed with the use of the SkinCare Serum product after UV irradiation in our study, which decreases inflammation and slight suppression in thymine dimer formation, along with partial recovery of a cell proliferation biomarker, Ki-67,

stimulation of filaggrin and NRF2. Even though SkinCare Serum wouldn't be able to completely recover the skin from all UV-induced damages, it did demonstrate partial improvements.

Finally, the best of both worlds occurs with the synergistic benefits of the combination discussed here – ie, when sunscreen is applied before UV exposure and a skincare product is applied afterward. This regimen was simulated in our study by applying the SunCare product before UV irradiation and the SkinCare Serum product after UV irradiation. When the combination of these 2 products was applied, the most overall benefits were seen. While SunCare or SkinCare alone did not recover the skin to baseline control in Ki-67 protein expression, there was complete recovery of Ki-67, to the same level as the baseline control tissues without any UV exposure. The biggest reduction in IL-1α levels was also observed from combined topicals, and both filaggrin levels and Nrf2 levels significantly increased.

The 2 topical formulations evaluated in this research contain ingredients that are known to provide skin benefits. In particular, the SunCare product contains zinc oxide and octinoxate, 2 sunscreen ingredients that can reflect, scatter, and absorb both UVA and UVB radiation.[25,26] The SunCare product also contains niacinamide and tocopheryl acetate which may provide anti-inflammatory and antioxidant benefits. The SkinCare Serum contains the patented proprietary amino acid complex technology, which was shown to reduce skin irritation and redness along with accelerating the skin repair process.[18] Together, when used as a regimen, they provide the consumer with multiple opportunities to counteract the damaging effects of UV radiation.

## CONCLUSION

The current work demonstrated the benefits of AAComplex against UV irradiation from 3 aspects:

I. Fibroblast cells treated with AAComplex tended to exhibit restored contraction suppressed by UVA through increased collagen 1 synthesis capacity to a level significantly higher than without the AAComplex technology.
II. AAComplex formulated in a lotion appeared to lower the expression of factors contributing to inflammation, skin sensitivity, and collagen degradation, which confirmed the prevention of photo-aging benefit from the technology.
III. A regimen consisting of before-sun products with after-sun products can provide skin protection and aid in recovery from UV-induced damage. This research, using a 3D reconstructed human skin model, indicates that the most benefit to the skin occurs when a sunscreen (SPF 46) and a skin care product with skin recovery ingredients are used, by preventing and alleviating skin damage.

## DISCLOSURES

Joanna Wu, Jin Namkoong, Sayantani Goswami and Nadia Soliman are employees of the Colgate-Palmolive Company and EltaMD®. Joel L Cohen, MD, is a consultant of the Colgate-Palmolive Company and EltaMD®.

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