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ORIGINAL RESEARCH

Ex vivo Evaluation of a Liposome-Mediated Antioxidant Delivery System on Markers of Skin Photoaging and Skin Penetration

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Purpose: The topical application of antioxidants has been shown to augment the skin's innate antioxidant system and enhance photoprotection. A challenge of topical antioxidant formulation is stability and penetrability. The use of a targeted drug delivery system may improve the bioavailability and delivery of antioxidants. In this ex vivo study, we assessed the effects of the topical application of a liposome-encapsulated antioxidant complex versus a free antioxidant complex alone on skin photoaging parameters and penetrability in human skin explants.

Patients and Methods: Human organotypic skin explant cultures (hOSEC) were irradiated to mimic photoaging. The encapsulated antioxidant complex and free antioxidant complex were applied topically onto the irradiated hOSEC daily for 7 days. The two control groups were healthy untreated hOSEC and irradiated hOSEC. Photoprotective efficacy was measured with pro-inflammatory cytokine (IL-6 and IL-8) and matrix metalloproteinase 9 (MMP-9) secretion. Cell viability and metabolic activity were measured via resazurin assay. Tissue damage was evaluated via lactate dehydrogenase (LDH) cytotoxicity assay. Skin penetration of the encapsulated antioxidant complex was assessed via fluorescent dye and confocal microscopy.

Results: Compared to healthy skin, irradiated skin experienced increases in IL-6, IL-8 ($p < 0.05$), and MMP-9 ($p < 0.05$) secretion. After treatment with the encapsulated antioxidant complex, there was a 39.3% reduction in IL-6 secretion, 49.8% reduction in IL-8 ($p < 0.05$), and 38.5% reduction in MMP-9 ($p < 0.05$). After treatment with the free antioxidant complex, there were no significant differences in IL-6, IL-8, or MMP-9 secretion. Neither treatment group experienced significant LDH leakage or reductions in metabolic activity. Liposomes passed through the stratum corneum and into the epidermis.

Conclusion: The topical application of a liposome-encapsulated antioxidant complex containing ectoin, astaxanthin-rich microalgae *Haematococcus pluvialis* extract, and THDA improves penetrability and restored IL-6, IL-8, and MMP-9 levels in irradiated human skin explants, which was not seen in the comparator free antioxidant complex group.

Keywords: skin explants, ectoin, astaxanthin, THDA, tetrahexyldecyl ascorbate

Introduction

Oxidative damage induced by reactive oxygen species (ROS) and free radicals have been implicated in numerous dermatological conditions. Although the skin has a natural antioxidant system composed of various enzymes (eg, glutathione, catalase, isocitrate dehydrogenase) and non-enzymatic antioxidants (eg, ascorbic acid, alpha-tocopherol, uric acid). These antioxidants are readily depleted by ultraviolet (UV) exposure, and the use of supplementary antioxidants to scavenge excess free radicals have been reported to act as therapeutic agents against oxidative stress.[1–3] For example, oral supplementation of turmeric/curcumin products have been shown to improve disease severity in various dermatoses, partly via its antioxidative properties.[4] Carotenoids, such as astaxanthin-rich microalgae *Haematococcus pluvialis* extract, beta-carotene, and lycopene have also been shown to have antioxidative properties leading to photoprotective effects when taken orally.[5–7] The consumption of foods

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containing antioxidants such as almonds containing alpha-tocopherol[8] and carotenoid-rich mangos[9] have also been shown to improve measures of photoaging in postmenopausal women.

Moreover, the topical delivery of antioxidants has been shown to support the skin's innate antioxidant system. In one study, the topical application of tocopherol (vitamin E) in combination with L-ascorbic acid and other ingredients was shown to reduce signs of skin aging.[10] Topical formulations including ascorbic acid, otherwise known as vitamin C, have also been shown to reduce UV-induced expressed of MMP-1 and 9, which are implicated in photoprotection.[11] Acetyl zingerone is a novel antioxidant that, when applied topically, has been shown to improve the appearance of winkles, dyspigmentation, and redness intensity while maintaining photostability.[12]

A challenge of topical antioxidant formulation is stability and penetrability. Antioxidants that degrade rapidly may not have the bioavailability needed to exert their beneficial effects.[13] Furthermore, antioxidants that are more hydrophilic may not be permeable enough to bypass the stratum corneum and get into the deeper layers of the skin.[14] For example, ascorbic acid and vitamin E have been shown to degrade rapidly under UV exposure.[15,16] Ascorbic acid is also hydrophilic in its natural state, thereby hindering its ability to bypass the stratum corneum.[17] Alternatively, acetyl zingerone is an antioxidant which has been shown to be photostable and is able to stabilize the ascorbic acid precursor tetrahexyldecyl ascorbate (THDA).[18] Tocopherol is lipophilic, allowing it to easily penetrate the lipid-rich stratum corneum.[19] However, tocopherol in its pure form is poorly water soluble, photosensitive, and may be irritating to the skin.[20] To overcome obstacles such as these, recent approaches to topical antioxidant delivery design have focused on methods that preserve antioxidant stability and enhance the penetrability of the antioxidant to improve bioavailability.

Emerging approaches to modulate the delivery of antioxidants to the skin have utilized specialized delivery carriers such as nanoparticles or liposomes.[21,22] In these strategies, antioxidants are encapsulated in a variety of different vesicular, lipidic, and polymeric systems to prevent degradation and improve penetration.[22] For molecules to pass through the stratum corneum, there are physiochemical properties that must be met. For example, the partition coefficient (log P) must be high enough for the molecule to pass through the lipid-rich stratum corneum.[23] Moreover, the molecular weight would ideally be less than 500 Da and the melting point less than 200C to effectively bypass the stratum corneum.[23] Physiological factors such as age, anatomical location, race, gender, and environmental conditions also influence the rate of permeability.[24] Thus, the nanomaterials utilized may increase both the bioavailability and distribution of antioxidants to the skin.[25] Other benefits that nanocarriers may provide include passive delivery, increased surface area, controlled release, and decreased skin irritation.[26] These systems may include solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, polymer nanoparticles, and more.[27] For example, the topical application of solid lipid nanoparticles and nanostructured lipid carrier systems have been utilized with active compounds such as vitamin E (tocopherol),[28] retinoic acid,[29] and more.[30,31] Additionally, liposomes have been utilized to load anti-aging compounds such as type I collagen,[32] ferulic acid,[33] apigenin and doxycycline,[34] polyvinyl alcohol and gelatin,[35] and ligustrazine hydrochloride,[36] all of which have shown improved photoprotective effects with the utilization of the liposomal technology. Complexing lipids with polyphenols, termed lipophenols, have also been shown to be effective.[37,38] Moreover, nanocarriers have been utilized to stabilize vitamin C and its derivatives, thereby avoiding rapid oxidation and degradation.[39]

The topical administration of antioxidants to the skin is an important component of preventing oxidative stress that accelerates photoaging. However, some antioxidants are limited by their stability and permeability. Preliminary studies have shown that various drug delivery systems may enhance the bioavailability and delivery of these antioxidants.[22] Thus, the objective of this ex-vivo study was to assess the effects of a topical liposome-mediated drug delivery system with antioxidants (XOSM™ technology, Image Skincare, Lantana, FL, USA) versus free antioxidants alone on parameters of skin photoaging in human skin explants. Additionally, this study aims to test the penetrability of the liposome-mediated drug delivery system.

Materials and Methods

Human ex vivo Skin Model

Human organotypic skin explant cultures (hOSECs) were obtained with informed consent from healthy donors undergoing plastic surgery (authorization granted by French government ethical committee according to French law L.1245 CSP). Skin samples were obtained from a 42-year-old Caucasian female of Fitzpatrick skin type II from the abdomen with no marks or scars. The skin was cut into 0.8 cm^2 pieces and shipped in transport medium. Upon receipt, samples

were placed with dermis facing down and epidermis facing up in culture plates containing skin culture medium without animal components and supplemented with antibiotics (1% pen-strep). Tissue cultures were incubated for at least 48 hours at 37°C under 5% CO^2 for recovery prior to study initiation.

Materials

The two test items utilized in this study included a free antioxidant complex and an encapsulated antioxidant complex. The test items were stored in a dark at room temperature, according to NANOVEX (Nanovex Biotechnologies, Asturias, Spain) recommendations. In all experimental assays, the test items were applied topically over skin explant surface. In both cases, the ratio of components of the complex was as follows: ectoin, tetrahexyldecyl ascorbate, and *Haematococcus pluvialis* extract. The THDA utilized in this study is commercially produced in Parsippany, NJ, USA and has a purity of about 99%. The *Haematococcus pluvialis* extract utilized in this study is commercially produced in Frejus, France. The ectoin utilized in this study is commercially produced in Germany. They were provided as a final product (emulsion) and applied topically at 2 mg/cm² (10 µL).

Liposome Obtention

The Materials for the lipid mix are dissolved in ethanol (Phase A). This solution is then evaporated at low pressure to form a thin lipid layer on the inside of a round-bottomed flask or a glass vial. The purpose of this step is to create a lipid layer that will eventually shape the liposome structure.

Once the lipid layer is ready, it is rehydrated with an aqueous solution (Phase B) that has hydrophilic substances. This rehydration step involves adding the aqueous solution to the lipid layer while gently swirling or shaking the container with the obtention of large multilamellar vesicles (MLVs) To make small liposomes, the MLVs obtained in the rehydration step were homogenized to reduce their size. This method helps to break the larger vesicles into smaller, more uniform liposomes. The composition of liposomes is included in Table 1.

Treatment Groups and Regimen

In order to mimic skin photo-aging, sun-like light irradiation (5 J/cm²) was applied daily to the hOSEC for a total of 7 applications. Repeated irradiation at 5 J/cm^2 has been shown to act as a photoaging model for in vitro studies.[40,41] The wavelength range of the UV radiation applied was mainly in the ultraviolet, visible, and near infrared bands, with wavelengths between 0.2 and 3.0 micrometers (200 to 3000 nm) (Figure 1) and the instrument utilized was the Sol 500 Solar Simulator (Dr. Honle AG, Gilching, Germany). After the hOSEC was irradiated, the test products were administered topically at 2 mg/cm2, for a total of 7 applications as detailed in Figure 2. The test products were in contact with the hOSEC throughout the study. The four groups were as follows: 1) healthy control (untreated hOSEC); 2) photo-aged hOSEC (irradiated); 3) photo-aged skin + encapsulated antioxidant complex; and 4) photo-aged skin + free antioxidant complex complex.

Table 1 Composition of Liposomes

Component	%p/v	Phase
Phospatidylcholine	8.697	A
Cutin	0.850	A
Tetrahexyldecyl ascorbate	0.120	A
Haematococcus pluvialis extract	2.450	A
Glycerol	4.000	B
Ectoin	1.240	B
Panthenol	0.120	B

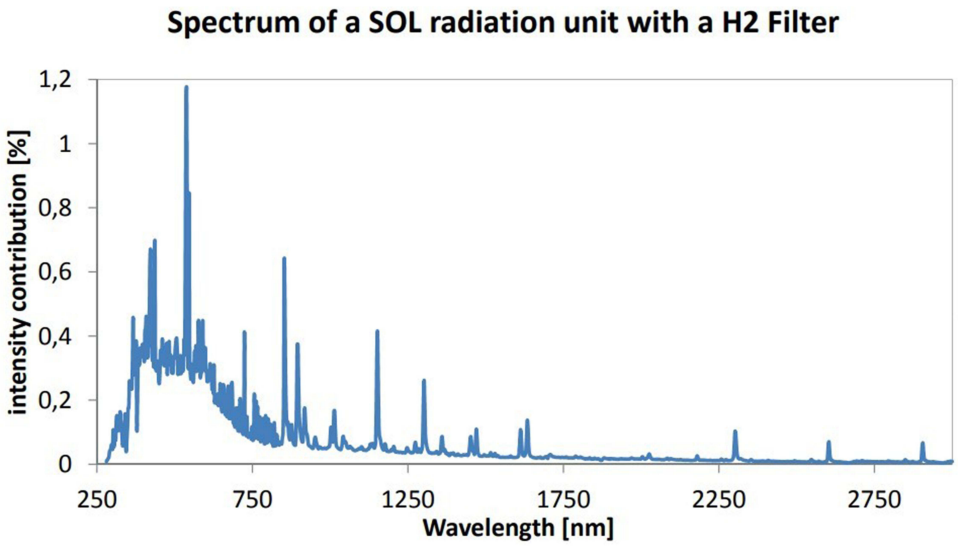

Figure 1 Emission spectra of a SOL Solar Simulator.

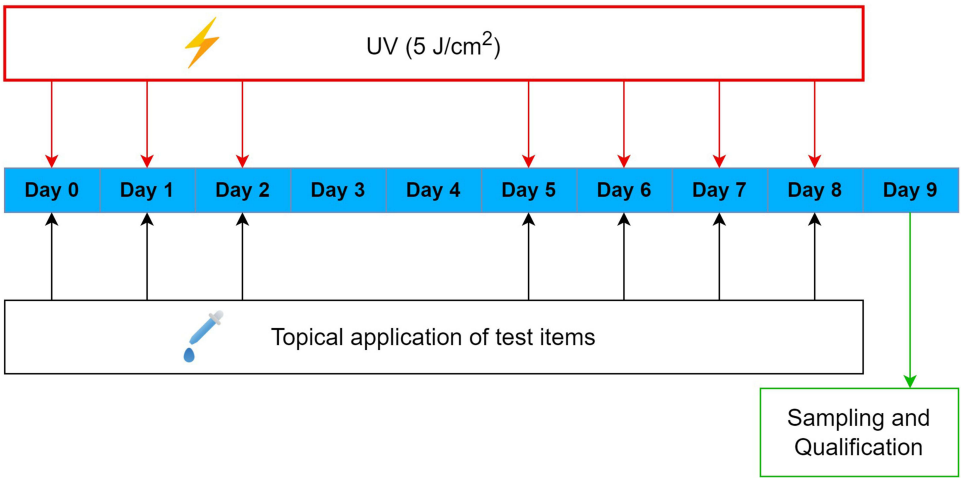

Figure 2 Graphical scheme of treatment regimen.

Points of Assay

The experimental design described above was replicated in order to obtain the following end points: LDH leakage, resazurin reduction activity, pro-inflammatory cytokines (IL-6 and IL-8) and matrix metalloproteinases (MMP-9) secretion.

Lactate Dehydrogenase Cytotoxicity Assay

A total of 50 µL of supernatant were removed from each sample and transferred into a 96-well microplate. Subsequently, 50 µL of formazan dye was added to each sample and after a 30-minute incubation period, absorbance was read using a standard ELISA plate reader at 490 nm. The released LDH in the culture medium supernatants was then measured. LDH oxidizes lactate to pyruvate which then reacts with the tetrazolium salt WST-1 to form formazan. The increase in the amount of formazan measured in the culture supernatant directly correlates to the increase in the number of lysed cells (damage) in the skin explant.

Resazurin Assay

The resazurin dye (7-hydroxy-3H-phenoxazin-3-one 10-oxide) was used in this study as an indicator of cell viability in proliferation and cytotoxicity. Prior to the topical application of the test items, the skin explants were treated with 6 µM

of resazurin solution for 1 hour. Subsequently, a volume of 100 µL sample was removed from each sample and transferred into a 96-well microplate. The resorufin formed was quantified in a fluorometer plate reader. The fluorescent signal was monitored using 530 nm excitation wavelength and 590 nm emission wavelength.

Pro-Inflammatory Cytokines Quantifications: IL-6 and IL-8

Quantification of the pro-inflammatory cytokines, interleukin (IL)-6 and IL-8, was conducted from tissue culture supernatant using commercial sandwich ELISA kits from R&D systems (Bio-Techne, Minneapolis, MN, USA) following manufacturer's instructions to measure UV-induced damage in the hOSEC.

Matrix Metalloproteinases Quantification: MMP-9

Quantification of matrix metalloproteinase 9 (MMP-9) was conducted from tissue culture supernatant using commercial sandwich ELISA kit from R&D Systems (Bio-Techne) following manufacturer's instructions. The assay measures the remodeling protein matrix metalloproteinase MMP-9.

Statistical Analysis

Values are given as mean ± standard deviation (SD). The homogeneity of variance was confirmed by the Brown-Forsythe test, and the normality was confirmed by the D'Agostino-Pearson omnibus test. One factor analysis of variance (ANOVA) with Fisher's LSD post-hoc tests were performed to assess differences among groups means. When homogeneity of variances could not be assumed, Welch's correction was used, and when parametric assumptions were not met Kruskal–Wallis with Dunn's post-hoc test was used. p-values < 0.05 were considered statistically significant. Values were compared against healthy control group values (*), against photo-aged skin group values (#) or between treatments ($).

Skin Penetration Study

Liposomes were synthesized including rhodamine-labelled phospholipids (18:1 PE CF) in the membrane of the liposomes. The full chemical name of the phospholipid(s) utilized is as follows: 1.2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(carboxyfluorescein)(ammonium salt). It is a phosphatidylethanolamine (PE) with an 18-carbon chain and one unsaturation which is labeled with the fluorescent dye (CF). The product was characterized prior to the analysis, to assure that it was in accordance with the specifications regarding size and polydispersity index. Frozen skin samples were hydrated and set to room temperature using a saline buffer. The skin was then mounted on the Franz diffusion cells, and the water bath was set at 37°C. A total of 100 µL of fluorescent vegan liposomes were added to the receptor compartment of the Franz diffusion cells, and the compound was let to diffuse for 16 hours. After that time, the skin sample was rinsed off with ultrapure water and fixed with paraformaldehyde (PFA) for 5 hours. Finally, the sample was embedded in OCT, cryopreserved, and sectioned using a cryostat, obtaining a cross section of the skin. These skin sections were dyed with DAPI, a fluorescent stain with specificity for cellular nuclei with an emission maximum at 461 nm (blue) and observed with a confocal microscope. The sample was also observed at the emission wavelength of rhodamine and contrasted with the transmission image of the sample, which was able to provide information about the penetrability of the liposomes.

Results

LDH Assay

Untreated healthy human skin explants (healthy) were set as 100% of the LDH concentration. Tissue damage is represented by increased leakage of LDH. Compared to healthy skin, there were no significant differences in the amount of LDH leaked in irradiated skin (control) or in either treatment group (Figure 3).

Resazurin Assay

The resazurin reduction assay was performed to measure cell viability and metabolic state. UV radiation reduced metabolic activity in irradiated skin (control) compared to healthy skin, however this difference was not statistically significant. When treated with the encapsulated antioxidant complex, metabolic activity was normalized (Figure 4); however, this effect was not seen in the free antioxidant complex group.

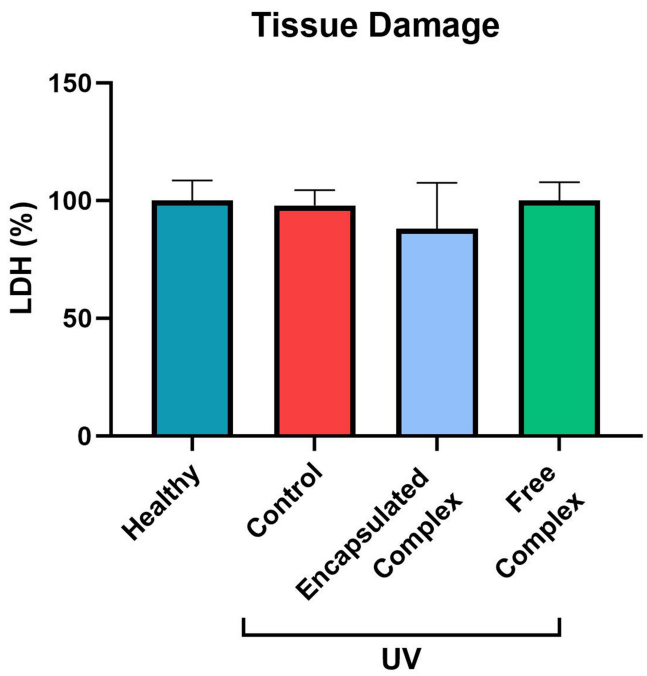

Figure 3 Tissue damage (LDH leakage) of non-irradiated hOSEC (Healthy), irradiated (UV Control) or irradiated and treated with either the free antioxidant complex or the encapsulated antioxidant complex.

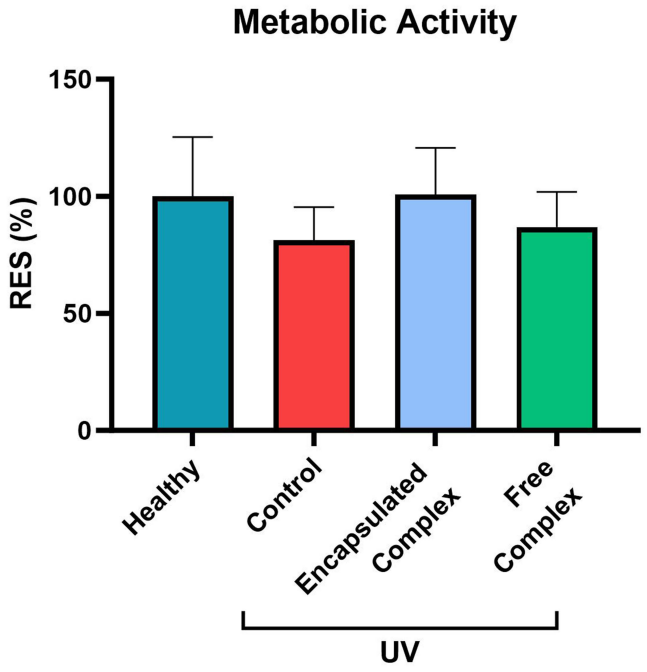

Figure 4 Metabolic activity (resazurin reduction) of non-irradiated hOSEC (Healthy), irradiated (UV Control) or irradiated and treated with either the free antioxidant or encapsulated antioxidant complex.

Pro-Inflammatory Cytokine Secretion

Compared to healthy skin, there was an increase in IL-6 secretion in irradiated skin (control). Treatment with the free antioxidant complex reduced IL-6 secretion by 9.9% compared to the control group. However, when treated with the encapsulated antioxidant complex, there was a 39.3% reduction in IL-6 compared to the control group (Figure 5).

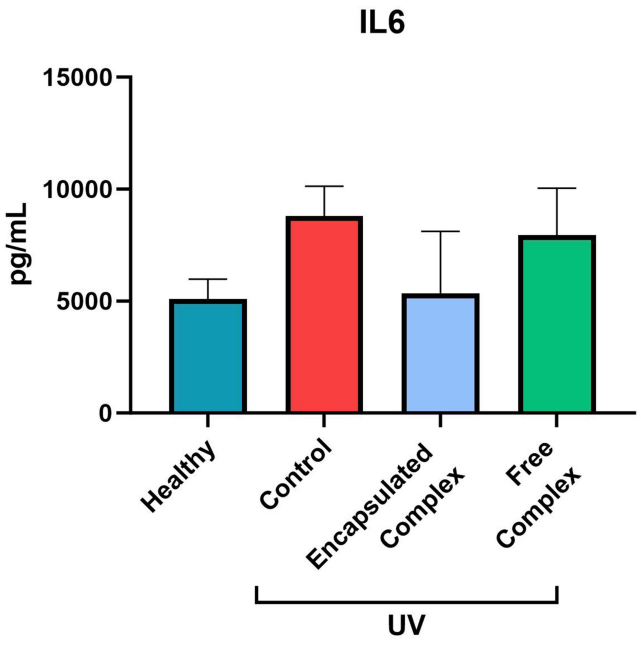

Figure 5 IL-6 secretion of non-irradiated hOSEC (Healthy), irradiated (UV Control) or irradiated and treated with either the free antioxidant complex or the encapsulated antioxidant complex.

Compared to healthy skin, there was a significant increase in IL-8 secretion in irradiated skin (control) ($p < 0.05$). When treated with the free antioxidant complex, there was a 0.9% reduction in IL-8 secretion compared to the control group. When treated with the encapsulated antioxidant complex, there was a significant decrease in IL-8 secretion by 49.8% compared to the control group ($p < 0.05$) (Figure 6).

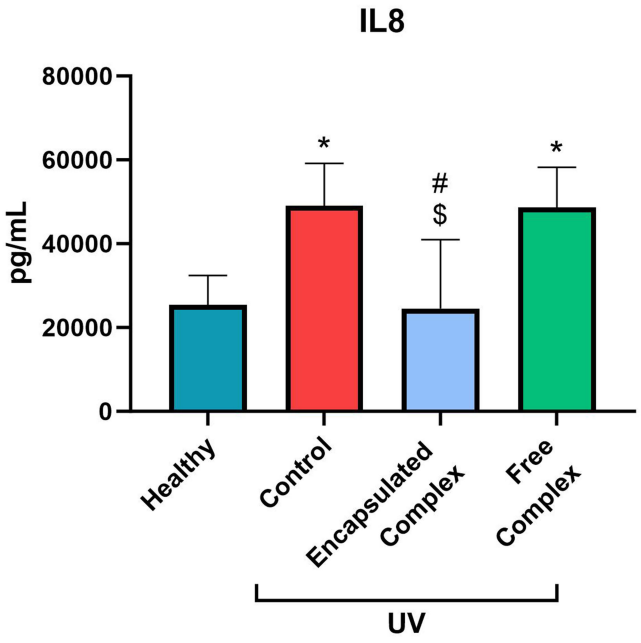

Figure 6 IL-8 secretion of non-irradiated hOSEC (Healthy), irradiated (UV Control) or irradiated and treated with either the free antioxidant complex or encapsulated antioxidant complex. Astericks *Indicate a statistically significant difference ($p<0.05$) compared to the Healthy group, hashes #Compared to the UV Control group, and dollar sign $Compared to the free antioxidant complex group.

MMP-9 Secretion

UV radiation increased the secretion of MMP-9 in irradiated skin (control) compared to healthy skin ($p < 0.05$). Treatment with Free antioxidant complex led to a 7.7% increase in MMP-9 levels, which was not statistically significant when compared to the control group. Treatment with Encapsulated antioxidant complex led to a 38.5% decrease in MMP-9 levels when compared to the control group ($p < 0.05$) (Figure 7).

Penetration Study

Fluorescent dye and confocal microscopy were utilized to visualize the penetrability of the encapsulated antioxidant complex. The liposomal distribution can be visualized in Figure 8, which shows that liposomes were able to pass through the stratum corneum and reach the epidermis.

Discussion

Our study found that the topical application of a targeted, encapsulated antioxidant complex containing ectoin, astaxanthin-rich microalgae *Haematococcus pluvialis*, and THDA may restore the photoprotective capabilities of photoaged skin whereas the topical application of free antioxidants alone may not provide any significant photoprotective effects. Furthermore, the encapsulated antioxidant complex was shown to be able to pass through the stratum corneum to exert its effects in the epidermal layer. Previous studies have shown that the use of targeted delivery systems may improve the photoprotective activity of antioxidants.[42–44] In our study, we visualized the penetrability of the delivery system utilized in our study to further validate our findings.

There were several notable findings in this study. Our study found that UV radiation increases the secretion of pro-inflammatory cytokines (IL-6 and IL-8) as well as MMP-9 in human skin explants. This agrees with studies showing that UVA-1 and UVB exposure in vivo significantly increases IL-6 levels,[45,46] thereby highlighting its role in skin photoaging pathology. UVA exposure in human keratinocytes have also been shown to up-regulate IL-8 mRNA expression,[47] which is also in agreement with our findings. MMP-9, a matrix metalloproteinase, also plays a crucial role in UV-induced skin inflammation and photoaging.[48] Other pro-inflammatory mediators including IL-1, epidermal growth factor (EGF), and tumor

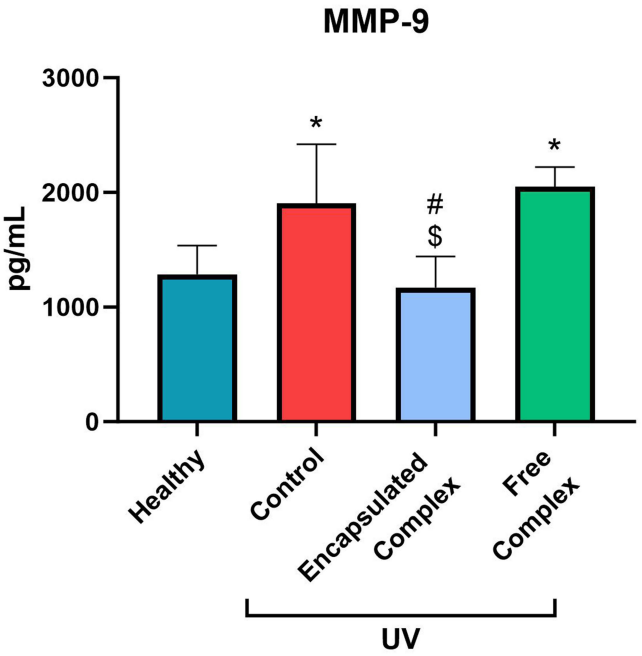

Figure 7 Matrix Metalloproteinase 9 (MMP-9) secretion of non-irradiated hOSEC (Healthy), irradiated (UV Control) or irradiated and treated with either the free antioxidant complex or encapsulated antioxidant complex. Astericks *Indicate a statistically significant difference ($p<0.05$) compared to the Healthy group, hashes #Compared to the UV Control group, and dollar sign $Compared to the free antioxidant complex group.

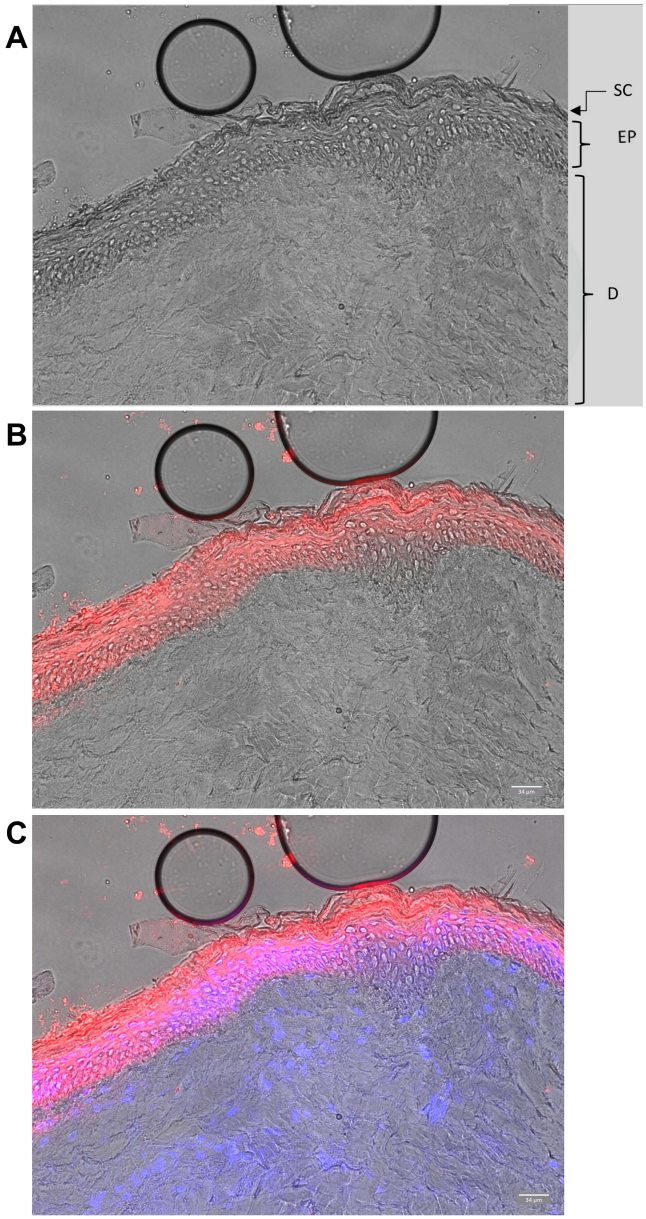

Figure 8 (**A**) Cross section of the skin sample. (**B**) Confocal image of encapsulated antioxidant complex liposomes (red) after penetration in a skin sample (grey) and (**C**) Confocal image of encapsulated antioxidant complex liposomes (red) after penetration in a skin sample (grey) stained with DAPI (blue).
Abbreviations: SC, stratum corneum; EP, epidermis; D, dermis.

necrosis factor receptors are also activated upon exposure to UVA and UVB and these pro-inflammatory markers may be interesting to investigate in future studies.

When irradiated skin was treated with the free antioxidant complex, there were no significant effects on IL-6, IL-8, or MMP-9 levels. Although the topical application of ectoin, astaxanthin, and THDA have been shown to improve skin photoprotection,[49–53] antioxidants such as these may have their respective Limitations in terms of stability and penetrability without the use of stabilizing ingredients or targeted delivery technology. For example, astaxanthin is difficult to apply topically to the skin due to its poor water solubility,[54] and the use of a nanostructure lipid carrier for astaxanthin was shown to improve the permeability and retention of astaxanthin.[55] Moreover, THDA degrades rapidly when exposed to singlet oxygen and upregulates inflammatory type I interferon signaling when applied by itself; however, these effects may be mitigated with the addition of stabilizing antioxidants such as acetyl zingerone.[16] Without the addition of targeted

delivery technology or stabilizing antioxidants, our findings are consistent with the established limitations of free antioxidants to the skin.

Treatment with the encapsulated antioxidant complex normalized IL-6, IL-8, and MMP-9 levels to basal levels like those found in healthy skin. Our penetration study showed that the encapsulated antioxidant complex (XOSM™ technology), which utilizes liposomes to encapsulate the antioxidants, was able to bypass the stratum corneum and deliver product to the epidermal layer of the skin. Liposomes are spherical vesicles composed of one or more concentric phospholipid bilayers that act as a delivery tool for therapeutic agents.[56] Liposomes have been shown to increase drug loading, prevent degradation of product, and improve the penetrability of agents with limited penetrability.[57] Furthermore, liposome composition may influence permeability and stability of the liposome. For example, liposomes formulated from unsaturated phosphatidylcholine, such as in our study, tend to have highly permeable and low stable properties, whereas liposomes formulated using saturated phospholipids, such as dipalmitoyl phosphatidylcholine, tend to be more impermeable.[56] Thus, the addition of the encapsulated delivery system for ectoin, astaxanthin-rich microalgae *Haematococcus pluvialis* extract, and THDA likely improved the bioavailability of these antioxidants, thereby improving the antioxidative and photoprotective capabilities of these ingredients on the skin. We chose these antioxidants due to their well-documented anti-photoaging effects, which have demonstrated that these antioxidants counteract UV radiation and oxidative stress on the skin. For example, ectoin has been used in anti-aging topicals due to its inhibitory effects on tyrosinase activity, anti-inflammatory effects, and ability to absorb UV radiation, thereby protecting DNA from photodamage.[58] The topical application of astaxanthin has been shown to protect against UV irradiation,[59,60] thereby improving wrinkle severity and skin hydration.[61] Furthermore, THDA has been shown to improve hyperpigmentation and photodamaged skin.[51] Therefore, the normalization of photoaging markers after treatment with an antioxidant complex with improved penetrability is consistent with expectations based on previous literature.

In addition to the role of IL-6 in photoaged skin, some studies have found that increased levels of IL-6 are implicated in atopic dermatitis,[62] psoriasis-like inflammation,[63] and basal cell carcinoma,[64] and that therapies that reduce IL-6 mediated signaling may improve these conditions. However, IL-6 is not always a symptom of inflammation. For example, IL-6 is involved in the regulation of the immune response, cell differentiation, and tissue repair and thus its effects can be context-dependent.[65] Similarly, IL-8 has been shown to be a key inflammatory cytokine involved in atopic dermatitis,[66] psoriatic disorders,[67–69] and acne vulgaris.[70] Furthermore, the overexpression of MMP-9 has been found to contribute to psoriatic disease,[71] atopic dermatitis,[72] connective tissue diseases,[73] and chronic wounds.[74] Thus, the downregulation of these markers by the encapsulated antioxidant complex also signifies the therapeutic potential of these antioxidants in numerous inflammatory dermatoses.

In our study, there was a reduction in metabolic activity in irradiated skin that was normalized with the encapsulated antioxidant complex but not with the free antioxidant complex alone. However, these differences were not statistically significant. Moreover, LDH leakage is an indicator of UV damage.[75] However, there were no significant changes in LDH release in either treatment group compared to the control group. Thus, neither treatment had significant cytotoxic effects on human skin explants. Although there were no noted cytotoxic effects on the human skin explants in our study, liposomal agents may impart toxicity to tissues and cause immunogenic responses depending on their chemical properties.[76] Cationic phospholipids, such as those utilized in our study, have been shown to influence macrophage uptake, have cytotoxic effects via ROS production, contribute to IL-6 and TNFα production, and activate the complement pathway.[76]

Limitations

The experimental system of this study was focused on ex vivo findings. The human skin explants utilized were derived from a single subject with replicates and the results may not be extrapolated to the general population without future clinical work in an expanded set of participants. Human skin explants have a lack of physiological processes such as circulation and desquamation that may influence the results. In vivo models allow studies that are close to reality over ex vivo models. Clinical studies should be completed to verify the results of the ex vivo model. Although our study visualized skin penetrability of the liposomes using staining and confocal microscopy, future studies may consider testing skin permeation by quantifying the bioactive compounds remaining in each Franz compartment. Furthermore, the skin has been shown to react differently to different UV wavelengths,[77] for example UVA versus UVB, and utilizing a different spectrum of irradiation may produce different results.

Conclusion

Sun-like light irradiation to human skin explants led to increases in pro-inflammatory cytokines IL-6 and IL-8 as well as MMP-9, therefore mimicking photoaged skin. The topical application of a liposome-encapsulated antioxidant complex (XOSM™ technology) containing ectoin, astaxanthin-rich microalgae *Haematococcus pluvialis* extract, and THDA to human skin explants restored IL-6, IL-8, and MMP-9 levels in irradiated human skin explants to basal levels, which was not seen in the comparator free antioxidant complex group. Neither treatment was found to be significantly cytotoxic based on LDH leakage or metabolic activity. Furthermore, the cationic liposomal technology in the delivery system utilized bypassed the stratum corneum to get into the epidermis, thereby emphasizing the potential of targeted delivery systems to improve the efficacy and safety of drugs and other therapeutic agents such as antioxidants. Future research on diversified skin samples and in vivo studies are warranted to confirm and expand on these results.

Abbreviations

hOSEC, human organotypic skin explant cultures; IL-, interleukin; MMP, matrix metalloproteinase; LDH, lactate dehydrogenase; THDA, tetrahexyldecyl ascorbate; ROS, reactive oxygen species; UV, ultraviolet.

Institutional Review Board Statement

This study complies with the Declaration of Helsinki. The study was conducted with skin explants that were obtained from a third-party supplier (Biopredic International, Saint-Gregoire, France) that has authorization granted by the French government ethics committee in accordance with the French law L.1245 CSP. Therefore, this study did not need formal IRB approval.

Data Sharing Statement

The data is not publicly available.

Informed Consent Statement

Human organotypic skin explant cultures (hOSECs) were obtained with informed consent from healthy donors undergoing plastic surgery (authorization granted by French government ethical committee according to French law L.1245 CSP).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

RKS serves as a scientific advisor for LearnHealth, Arbonne, and Codex Labs and has served as a consultant or speaker for Almirall, Burt's Bees, Novozymes, Novartis, Sanofi, Bristol Myers Squibb, Pfizer, Nutrafol, Galderma, Novartis, Abbvie, Lilly, Incyte, Image Skincare, Leo, UCB, Sun and Regeneron Pharmaceuticals. All other authors declare no conflicts of interest in this work.

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