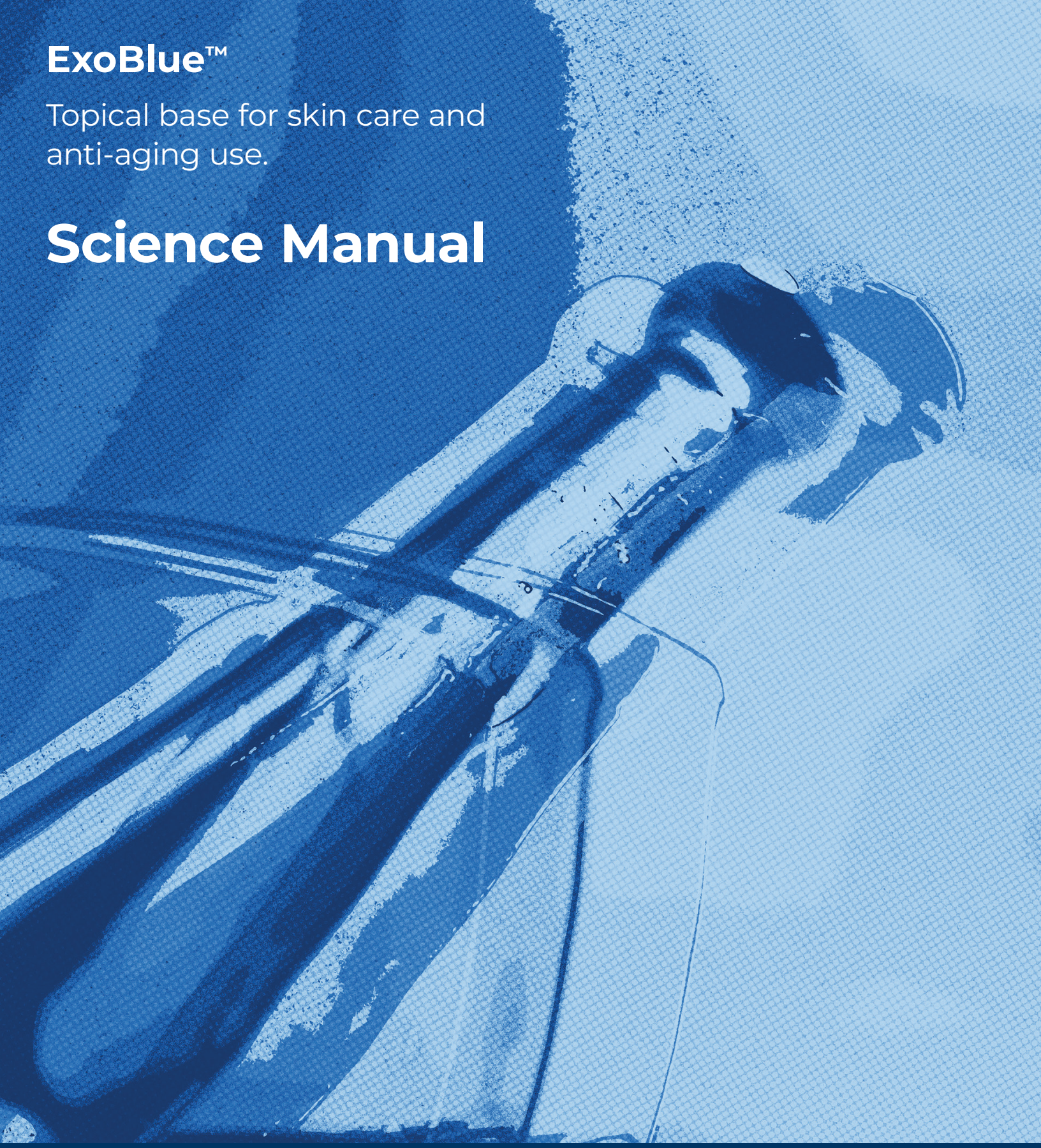
ExoBlue™

Topical base for skin care and anti-aging use.

Science Manual



PCCA® SCIENCE

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Technical Reports

A Clinical Evaluation of the Anti-Wrinkle Effects of PCCA ExoBlue™

SUMMARY: This study evaluated the effectiveness of PCCA ExoBlue in improving facial wrinkles and overall skin perception in 14 adult female subjects. Over an 8-week period, objective wrinkle measurements were obtained using a 3D imaging system, alongside subjective questionnaire assessments. Results demonstrated that 72% of subjects exhibited measurable wrinkle reduction by week 4, with 21% of subjects showing improvements by week 8. There are greater effects in reducing moderate and deep wrinkles compared to fine lines. Subject-reported outcomes supported instrumental findings, showing improvements in overall skin perception. These findings suggest that ExoBlue has the potential to provide anti-wrinkle benefits.

Introduction:

Fine lines and wrinkles are among the most visible signs of skin aging. Most topical anti-aging formulations primarily focus on hydration, barrier repair or targeting limited signaling pathways for cell proliferation. PCCA ExoBlue is a unique, multi-technology topical system designed not only to support hydration and barrier function, but also to synergistically stimulate cellular communication, promote dermal remodeling and synchronize facial muscle relaxation. This study evaluated both objective and subjective improvements following ExoBlue application over an 8-week period.

Methodology:

Study Design

This 8-week, single-arm pilot clinical study evaluated the anti-wrinkle effects of ExoBlue in healthy adult female subjects aged 40-60 years with facial fine lines and wrinkles. Subjects who met inclusion criteria were enrolled upon provision of informed consent and were instructed to apply ExoBlue twice daily (morning and evening) after cleansing. The use of AHAs, BHAs, vitamin C, other brightening acids and retinoids was not permitted throughout the study duration. Subjects were evaluated before, after 4 weeks and after 8 weeks of using ExoBlue.

Objective Measurements

Wrinkle assessments were performed using the EvaFACE S5-3D imaging system at baseline, week 4 and week 8. This non-contact optical system quantitatively measures skin topography and classifies wrinkles into three categories based on depth: fine lines (Class 1: −0.021 to −0.062 mm), moderate wrinkles (Class 2: −0.062 to −0.123 mm) and deep wrinkles (Class 3: −0.123 to −0.756 mm).

Subjective Measurements

Subjective effectiveness was evaluated using a structured questionnaire at week 4 and week 8, assessing multiple domains of skin appearance and feel using a 5-point Likert scale (strongly disagree to strongly agree).

Results and Discussion:

Fourteen subjects were enrolled and all completed the study without dropouts or reported adverse events.

The EvaFACE S5-3D imaging analysis showed measurable wrinkle reductions in 13 of 14 subjects, with the greatest improvement observed in moderate and deep wrinkles. By week 4, 72% of subjects demonstrated early response, with reductions in moderate wrinkles up to 55.7% and deep wrinkles up to 65.3% in individual cases (Figure 1). These early responses suggest a relatively rapid onset of action. Additional improvements were observed in 21% of subjects by week 8, while one subject showed no measurable response (Figure 2).

Across the cohort, the use of ExoBlue is associated with greater effect in reducing moderate and deep wrinkles compared to fine lines. This pattern implies that the formulation may exert effects beyond superficial hydration, potentially influencing dermal structure or skin elasticity. Variability in response magnitude and timing was observed among subjects, which may reflect differences in baseline skin condition, intrinsic aging processes or individual variability in product response.

Subject-reported outcomes were consistent with instrumental findings and showed a clear trend of progressive improvement in skin perceptions over time. At week 4, moderate improvements with a composite 80.2% positive responses were observed across questionnaire domains, and with a shift toward 87% positive responses by week 8. The overall questionnaire results at the end of the study are summarized in Figure 3.

Overall, the consistency between objective 3D imaging measurements and subject-reported outcomes supports the reliability and clinical relevance of the findings. The high responder rate and progressive improvement profile indicate that ExoBlue is a promising topical system for signs of skin aging.

A Clinical Evaluation of the Anti-Wrinkle Effects of PCCA ExoBlue™

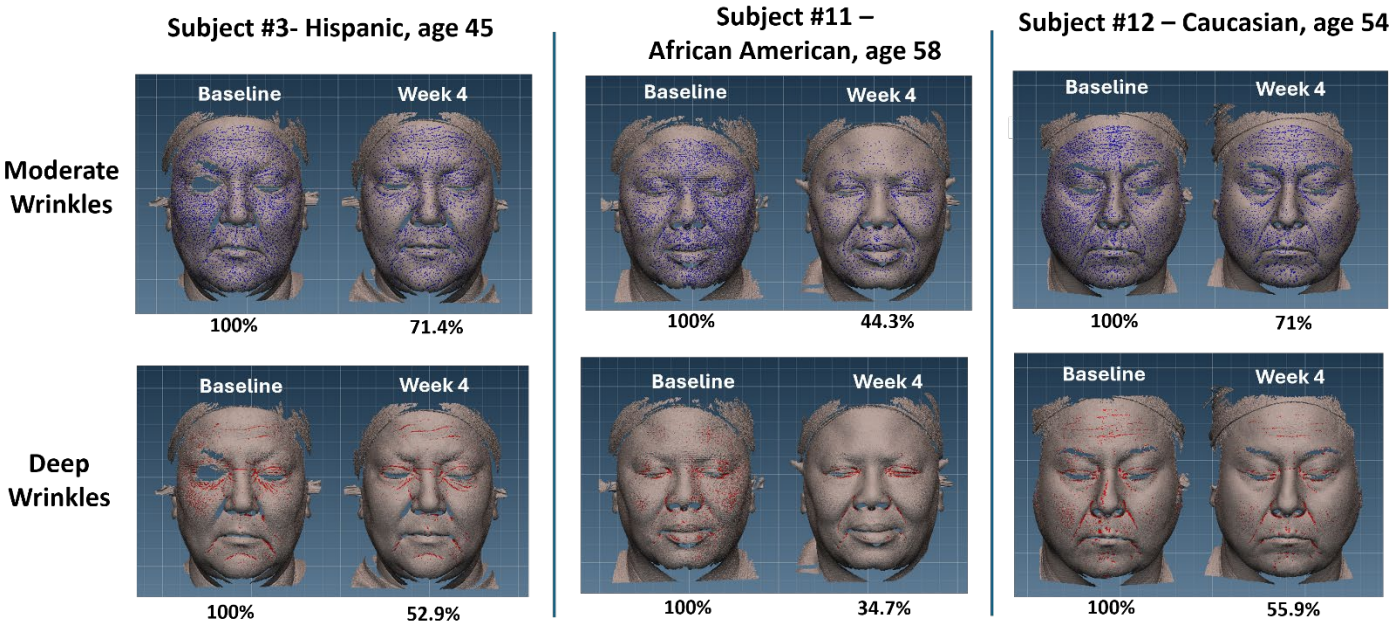

Figure 1. Representative 3D imaging of wrinkle reduction in 3 subjects following ExoBlue use for 4 weeks. In the 3D scans, moderate wrinkle areas are labeled with blue color and deep wrinkle areas are labeled with red color.

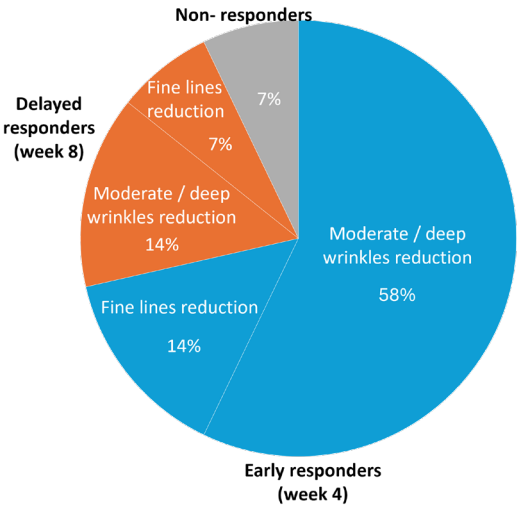

Figure 2. Distribution of treatment responses among subjects.

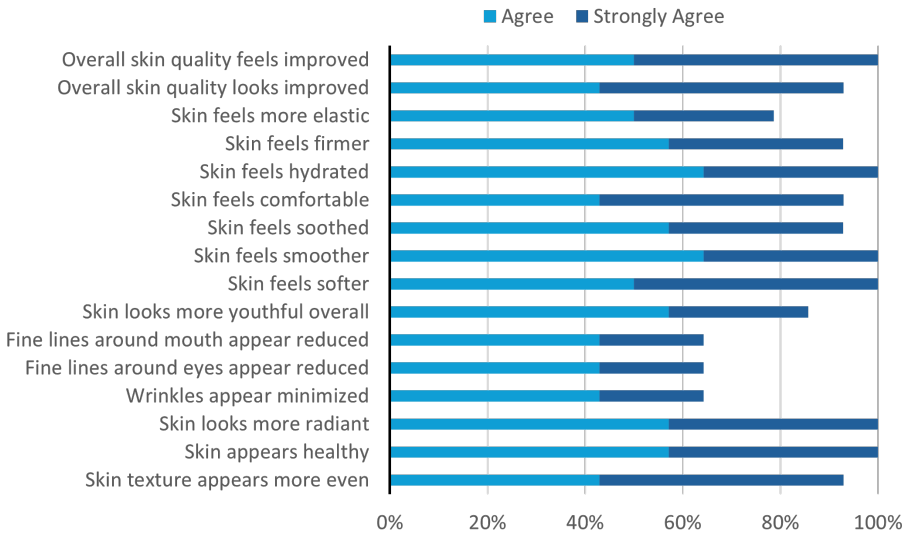

Figure 3. Subject-reported outcomes following 8 weeks' use of ExoBlue.

Evaluation of the Soothing Effects of E3 Topical Cream (ExoBlue™) in an SDS-Induced Irritation Model Using Reconstructed Full-Thickness Human Skin

SUMMARY: A topical formulation containing estriol (E3) 0.3% in ExoBlue was evaluated for its soothing effects using an SDS-induced irritation model in reconstructed full-thickness human skin. Histological analysis showed that treatment improved tissue morphology and restored epidermal integrity compared to irritated tissues. Additionally, IL-1α levels were reduced by 81%, demonstrating strong anti-inflammatory and soothing effects.

Introduction:

PCCA ExoBlue is a new, advanced dermatological base that combines exosomal signalling with precision peptide delivery and synergistic metal cofactors. This innovative blend includes *Centella asiatica* leaf extract exosomes, copper tripeptide-1, acetyl hexapeptide-8 and zinc hydrolyzed hyaluronate which work synergistically to enhance cellular communication, promote dermal remodelling, improve hydration and strengthen skin barrier function, offering a comprehensive approach to optimal skin performance beyond conventional topical products. Given these properties, a topical formulation including estriol (E3) 0.3% in ExoBlue (PCCA Formula #15843) was selected to assess its soothing effects on irritated skin and its ability to promote skin recovery.

Methodology:

The topical formulation was evaluated using a reconstructed full-thickness human skin model (EpiDerm™ FT) subjected to sodium dodecyl sulfate (SDS)-induced irritation. SDS is a widely known surfactant that disrupts skin barrier integrity by affecting lipids and proteins, leading to increased permeability and irritation characterized by dryness, erythema, scaling and inflammation, with effects increasing in a dose-dependent manner.

The skin tissues (EFT-400, MatTek) were divided into three groups, as follows: negative control tissues treated with 25 µL of Hanks' Balanced Salt Solution (HBSS); positive control (irritation) tissues treated with 25 µL of 0.2% SDS (L3771, Millipore Sigma); and treatment (test) tissues receiving a combination of 12.5 µL of 0.4% SDS and 12.5 µL of E3 0.3% in ExoBlue. Testing was done in duplicate, two tissues for each group. All tissues were incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2.

Following incubation, culture media were collected for quantification of the pro-inflammatory cytokine interleukin-1 alpha (IL-1α) using an ELISA assay kit (BMS243-2, ThermoFisher Scientific). In parallel, tissues were fixed, embedded, sectioned and stained with hematoxylin and eosin (H&E) to evaluate epidermal morphology, with emphasis on the integrity of the stratum corneum and viable cell layers.

Results and Discussion:

The histological evaluation (H&E staining) revealed distinct differences among the three groups of skin tissues, as shown in Figure 1. The negative control tissues (A) displayed normal architecture, characterized by a compact and intact stratum corneum. In contrast, tissues exposed to SDS (positive control) (B) showed clear signs of damage, including a loosened stratum corneum, disruption of viable epidermal layers and the presence of vacuoles, confirming the expected irritation. On the other hand, SDS-induced irritation and treatment with E3 0.3% in ExoBlue Cream (test tissues) (C) resulted in a marked improvement in tissue morphology, with restoration of epidermal structure and overall integrity.

These structural observations were supported by cytokine analysis (Figure 2). SDS exposure led to a significant increase in IL-1α secretion, indicating a strong inflammatory response. In contrast, tissues treated with the ExoBlue formulation showed a substantial reduction in IL-1α levels, with an 81% decrease compared to the irritation group. This finding demonstrates the formulation's pronounced anti-inflammatory and soothing effects. Together, these findings demonstrate that the ExoBlue formulation provides both protective and restorative benefits, addressing acute inflammation while promoting longer-term skin repair.

Evaluation of the Soothing Effects of E3 Topical Cream (ExoBlue™) in an SDS-Induced Irritation Model Using Reconstructed Full-Thickness Human Skin

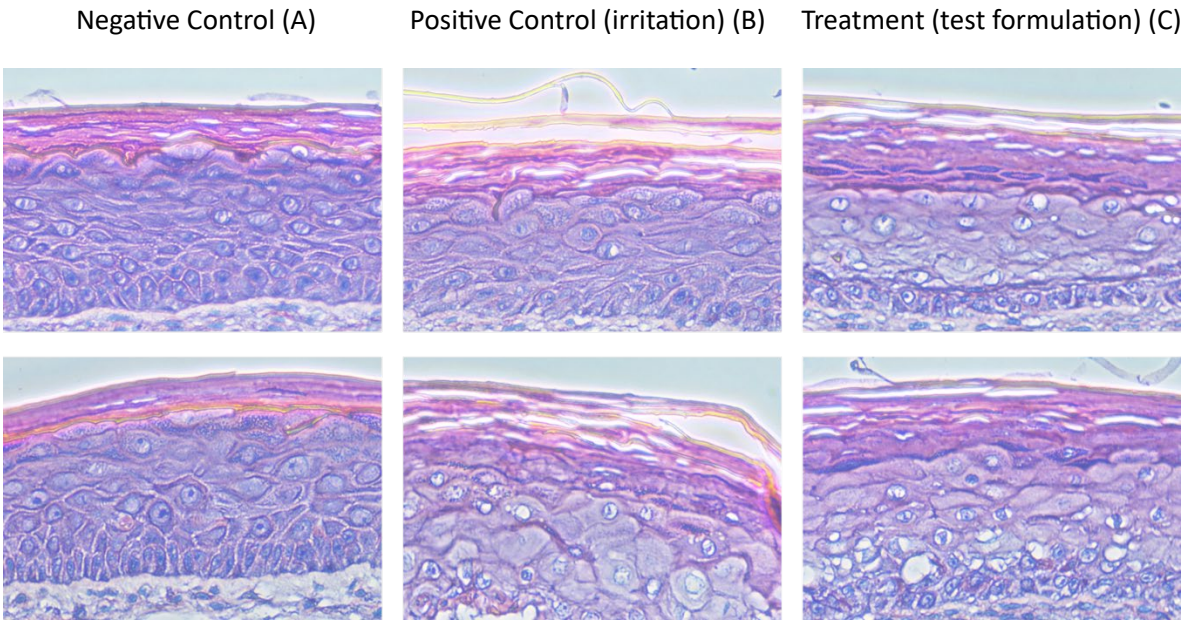

Figure 1. H&E-stained skin tissues (A, B, C) following SDS-induced damage (B,C) and treatment with E3 in ExoBlue Cream (C).

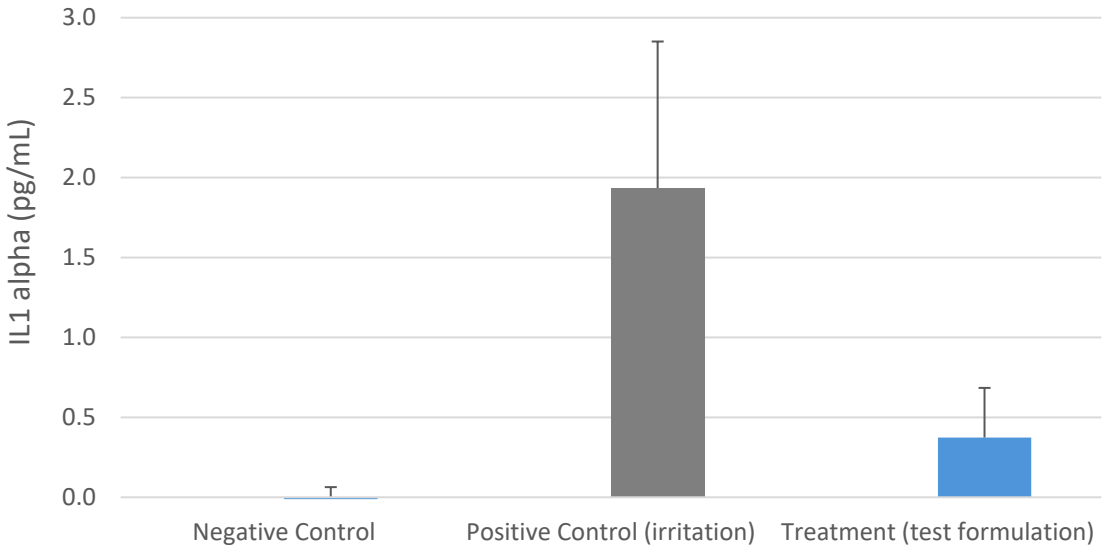

Figure 2. Quantification of the pro-inflammatory cytokine IL-1α across the three groups of skin tissues.

Evaluation of the Anti-Senescence Effects of E3 Topical Cream (ExoBlue™) on Human Dermal Fibroblasts: Hydrolase Enzyme (Part 1)

SUMMARY: E3 topical cream (ExoBlue) was evaluated in an oxidative stress-induced senescence model using human dermal fibroblasts, with senescence assessed via β-galactosidase activity and compared to E3 in a blinded Base B. Treatment with E3 in ExoBlue significantly reduced senescence, reversing ~64.8% of cells. In contrast, the comparator showed limited efficacy (~7% reversal), demonstrating the superior effect of the ExoBlue formulation.

Introduction:

PCCA ExoBlue is a next-generation dermatological base design to support skin function through a multi-mechanistic approach. It integrates advanced peptides, exosomes and essential metal cofactors, designed to influence key pathways involved in cellular signalling, structural integrity and moisture balance, contributing to improved skin resilience and overall performance. Given these properties, a topical formulation including estriol (E3) 0.3% in ExoBlue (PCCA Formula #15843) was selected to assess its potential to modulate cellular senescence and support skin cell recovery. Cellular senescence is a key contributor to skin aging, characterized by irreversible cell cycle arrest, altered protein expression and decreased regeneration capacity. β-gal is a hydrolase enzyme specifically overexpressed in senescent cells.

Methodology:

The ExoBlue topical formulation was evaluated in an oxidative stress-induced senescence model using human dermal fibroblast BJ cells (ATCC, CRL-2522). Senescence was assessed by the β-galactosidase (β-gal) assay (staining kit Cat #9860, Cell Signaling Technology). A proprietary compounding cream base, herein designated as Base B, was included as a blinded comparator, enabling comparison of E3 0.3% in ExoBlue versus E3 0.3% in Base B.

Cellular senescence was induced by exposure to hydrogen peroxide (H_2O_2), a well-established oxidative stressor. Following induction (0.2 mM H_2O_2 for 2 hours), cells were allowed to recover overnight and then treated for 5 days with either E3 0.3% in ExoBlue or E3 0.3% in Base B. Post-treatment, cells were washed with PBS, fixed at room temperature for 10 minutes, and incubated with staining solution at 37°C overnight.

β-gal density (blue signal) was quantified at 615 nm using a microplate reader, and representative images were acquired using a Nikon microscope.

Results and Discussion:

Human dermal fibroblasts exposed to H_2O_2 served as the positive control for senescence induction (Fig. 1B), whereas the fibroblasts exposed to H_2O_2 and treated with the test formulations (E3 in ExoBlue versus E3 in Base B) are shown in Fig. 1C. Untreated cells served as the negative control (Fig. 1A).

The positive control cells exhibited intense blue staining, indicative of elevated β-gal density and successful induction of senescence (Fig. 1B). When comparing the treated cells (Fig. 1C), E3 in ExoBlue resulted in a marked reduction in staining, with cells appearing more like untreated cells, suggesting substantial reversal of cellular senescence. In contrast, treatment with E3 0.3% in Base B produced only a modest decrease in β-gal density, with many cells retaining the senescent morphology and persistence blue staining. These observations are confirmed in Fig. 2, as the induction of senescence using the oxidative stressor resulted in a 65.1% increase in β-gal density (p=0.0014) compared to the negative control. Treatment with E3 in ExoBlue significantly reduced β-gal density, reversing 64.8% of senescent cells (p = 0.0019) to normal cells. In contrast, the comparator formulation (E3 in Base B) demonstrated only a modest effect, reversing approximately only 7% of senescent cells (p = 0.014), as shown in Fig. 2.

These findings indicate that E3 0.3% in ExoBlue provided a substantially greater protective and restorative effect against oxidative stress-induced senescence compared to the alternative base.

Evaluation of the Anti-Senescence Effects of E3 Cream (PCCA ExoBlue™) on Human Dermal Fibroblasts: Hydrolase Enzyme (Part 1)

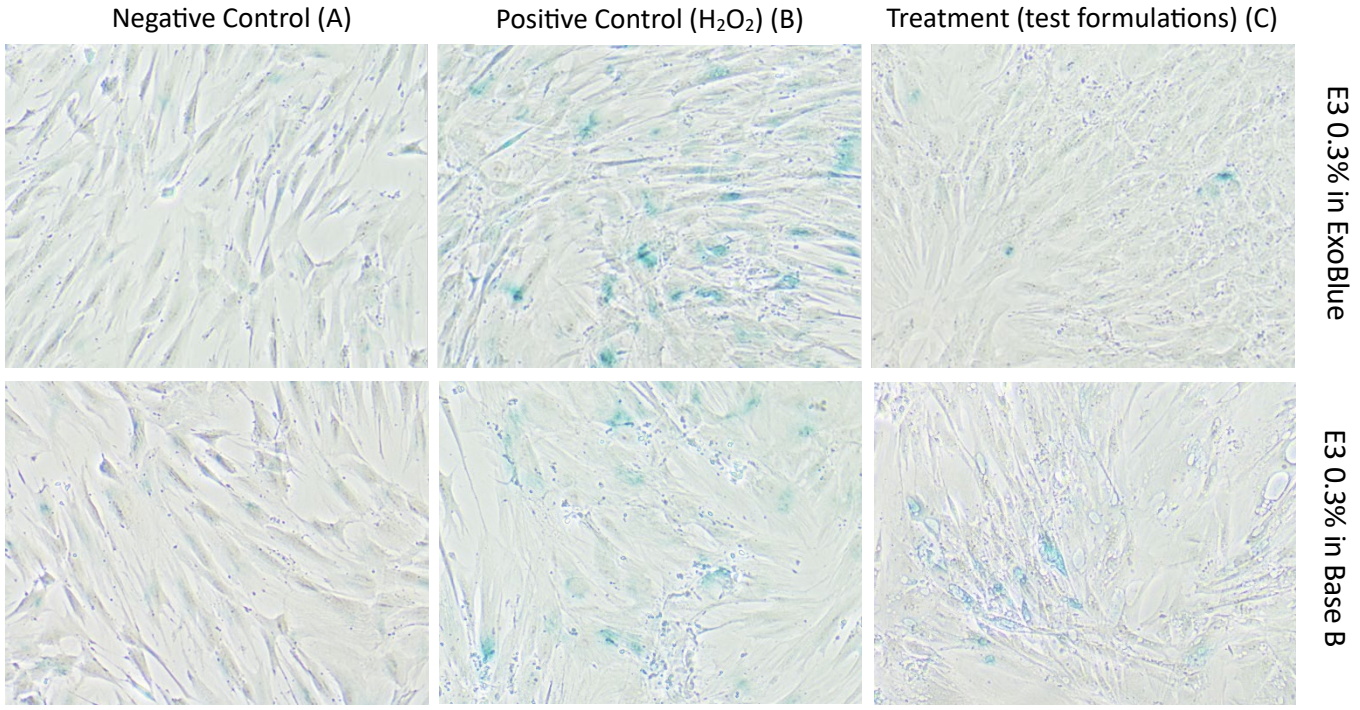

Figure 1. Microscope images of senescence-associated β-gal blue staining in human dermal fibroblast cells.

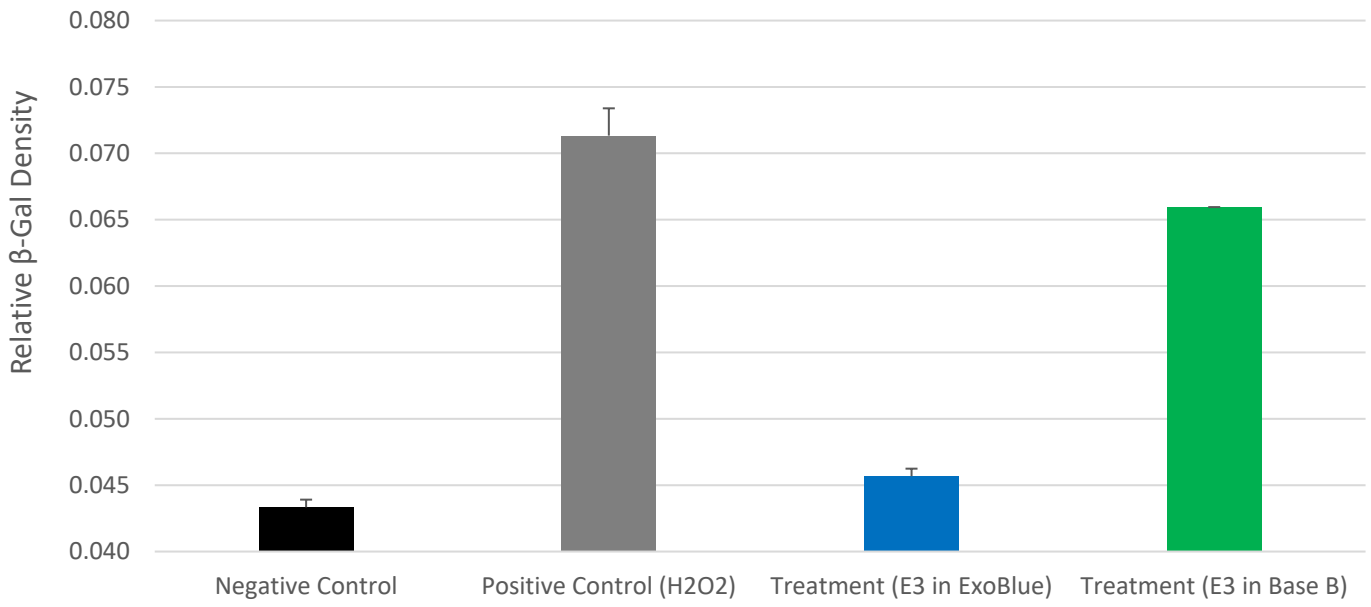

Figure 2. Quantification of β-gal density in human dermal fibroblasts across the two controls and the two treatment groups.

Evaluation of the Anti-Senescence Effects of E3 Topical Cream (ExoBlue™) on Human Dermal Fibroblasts: Protein Markers (Part 2)

SUMMARY: E3 topical cream (ExoBlue) was evaluated in an oxidative stress-induced senescence model using human dermal fibroblasts, with protein expression of key senescence markers analyzed by western blot to assess cell cycle regulation, nuclear integrity and DNA repair. The results of this study demonstrate that the E3 topical cream (PCCA Formula #15843) successfully modulated critical mechanisms associated with cellular aging.

Introduction:

PCCA ExoBlue is a next-generation dermatological base design to support skin function through a multi-mechanistic approach. It integrates advanced peptides, exosomes and essential metal cofactors, designed to influence key pathways involved in cellular signalling, structural integrity and moisture balance, contributing to improved skin resilience and overall performance. Given these properties, a topical formulation including estriol (E3) 0.3% in ExoBlue (PCCA Formula #15843) was selected to assess its potential to modulate cellular senescence and support skin cell recovery. Cellular senescence is a key contributor to skin aging, characterized by irreversible cell cycle arrest, altered protein expression and decreased regeneration capacity.

Methodology:

The ExoBlue topical formulation was evaluated in an oxidative stress-induced senescence model using human dermal fibroblast BJ cells (ATCC, CRL-2522). Cellular senescence was induced by exposure to hydrogen peroxide (H_2O_2), a well-established oxidative stressor. Following induction (0.2 mM H_2O_2 for 2 hours), cells were allowed to recover overnight and then treated for 5 days with E3 0.3% in ExoBlue. Post-treatment, cells were washed with ice-cold PBS and lysed with cell lysis buffer. Protein concentrations were determined using a BCA (bicinchoninic acid) Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL). Western blot analysis was performed by resolving proteins in SDS-PAGE gels, transferring them onto nitrocellulose membranes (Li-Cor Biotechnology, Lincoln, NE), and probing with primary antibodies. Signals were detected using IRDye secondary antibodies; images and band densities were analyzed using the Odyssey DLx imaging system (Li-Cor Biotechnology, Lincoln, NE).

Results and Discussion:

Protein expression of key senescence markers was analyzed by western blot to assess molecular pathways involved in cell cycle regulation, nuclear integrity and DNA repair. Phosphorylated p53 (p-p53) and p21 were evaluated as indicators of cell growth arrest, while Lamin B1 served as a marker of nuclear structure, and Poly ADP-Ribose Polymerase-1 (PARP-1) as a key regulator of DNA repair. Collectively, these markers provide insight into the cellular mechanisms underlying senescence and the impact of treatment on restoring normal cellular function. β-Actin served as loading control to validate the reliability and accuracy of the western blot results.

Human dermal fibroblasts exposed to H_2O_2 were the positive control for senescence induction, whereas the untreated cells were the negative control (Figure 1). The western blot analysis demonstrated that oxidative stress markedly altered the expression of key protein markers. Specifically, H_2O_2-exposure significantly increased the expression of p21 and p-p53, central mediators of stress-induced cell cycle arrest and an early driver of cellular senescence. Concurrently, a substantial reduction in Lamin B1 was observed, indicating disruption of nuclear architecture and chromatin organization, along with a marked decrease in PARP-1 expression, reflecting impaired DNA repair capacity and genomic instability.

Treatment with E3 0.3% in ExoBlue partially reversed these molecular alterations, as evidenced by downregulation of p21 and p-p53, suggesting attenuation of stress-induced cell cycle arrest signaling. In parallel, Lamin B1 expression was restored, indicating preservation of nuclear structure and chromatin integrity, while PARP-1 levels were increased, supporting improved DNA repair activity and maintenance of genomic stability.

Evaluation of the Anti-Senescence Effects of E3 Cream (PCCA ExoBlue™) on Human Dermal Fibroblasts: Protein Markers (Part 2)

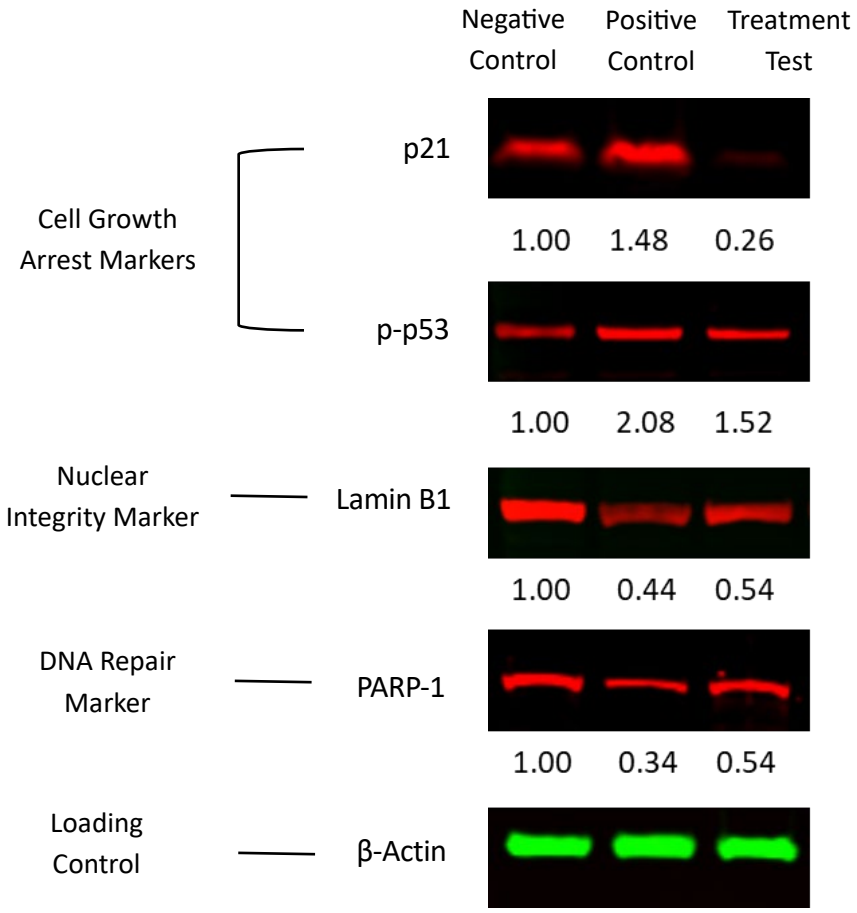

Figure 1. Western blot analysis of senescence-associated protein markers and β-actin (loading control) in human dermal fibroblasts under oxidative stress (H_2O_2) and treatment conditions (E3 in ExoBlue).

In this study, the human dermal fibroblast cells treated with E3 0.3% in ExoBlue (PCCA Formula #15843) supported the restoration of normal cellular function by reducing stress-induced growth arrest while preserving nuclear organization and enhancing the cell's ability to recover from damage. These effects suggest a coordinated modulation of pathways involved in cellular resilience and homeostasis under stress conditions. Collectively, these findings highlight the ability of the ExoBlue formulation to protect against senescence and promote recovery of normal cellular function.

In Vitro Assessment of PCCA ExoBlue™ Using Human Dermal Fibroblasts: Cell Proliferation and Viability

SUMMARY: This study evaluates the *in vitro* effects of PCCA ExoBlue on the proliferation and viability of human dermal fibroblasts using quantitative MTT analysis and qualitative Calcein AM fluorescence staining. Treatment with PCCA ExoBlue significantly increased fibroblast viability and proliferation compared with untreated control cells, while fluorescence imaging confirmed enhanced cellular activity and viability. These findings support the potential application of PCCA ExoBlue in skin regeneration and anti-aging topical formulations.

Introduction:

Copper peptide (GHK-Cu), a key component of PCCA ExoBlue, promotes skin regeneration by stimulating fibroblast proliferation, keratinocyte migration and basal stem cell activity. These effects enhance tissue repair, collagen synthesis and extracellular matrix organization, contributing to improved skin renewal and anti-aging benefits.

The objective of this study is to assess *in vitro* the cell proliferation and viability effects of PCCA ExoBlue on human dermal fibroblasts.

Methodology:

The cell proliferation and viability effects of PCCA ExoBlue on human dermal fibroblasts were evaluated by quantitative and qualitative *in vitro* assays, such as the MTT (quantitative) assay and the Calcein AM fluorescence staining (qualitative assay).

Cell Culture

Human dermal fibroblast BJ cells (CRL-2522) were obtained from ATCC and cultured in Eagle's Minimum Essential Medium (EMEM; 30-2003; ATCC) containing 10% fetal bovine serum (FBS; 30-2020; ATCC) and 1% penicillin/streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO_2.

MTT Assay

Confluent cells were treated with Trypsin-EDTA solution (30-2101; ATCC), and the resulting cell suspensions were seeded into 96-well plates at 7,000 cells per well. Plates were then incubated in a cell culture incubator for 16 hours. After incubation, the growth medium was replaced with 200 µL of fresh medium containing PCCA ExoBlue or with fresh medium alone, which served as the negative control. Cells were then incubated for an additional 24 hours.

Cell proliferation and viability were determined using the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) reagent. After treatment, cells were washed with phosphate-buffered saline (PBS), followed by the addition of growth medium and MTT reagent to each well. Plates were incubated for 4 hours to allow formation of formazan crystals. The reaction was terminated using a stop solution, and plates were further incubated overnight in the dark. Absorbance was measured at 570 nm using a CLARIOstar® plate reader.

Calcein AM Fluorescence Staining

Calcein AM is a non-fluorescent, cell-permeant dye that readily diffuses across cell membranes due to its lipophilic properties. Once inside viable cells, intracellular esterases hydrolyze Calcein AM to fluorescent Calcein, which is retained within the cytoplasm and emits bright green fluorescence upon binding to intracellular free calcium ions. In contrast, apoptotic or dead cells with compromised membrane integrity are unable to retain Calcein, resulting in reduced or absent fluorescence.

For proliferation and viability staining, Calcein AM (C3100MP) was obtained from Thermo Fisher Scientific, dissolved in dimethyl sulfoxide (DMSO) and diluted in PBS buffer to a final working concentration of 2 µM. After washing the treated cells (PCCA ExoBlue), the working solution was added and incubated for 45 min at 37°C. The staining solution was removed, and 200 µL of PBS was added to each well. Fluorescence images were captured using an ECLIPSE® TS100 fluorescence microscope (Nikon Corporation).

Results and Discussion:

The MTT assay demonstrated that PCCA ExoBlue significantly ($p<0.05$) enhanced the relative viability of fibroblast cells by 151%, as displayed in Figure 1.

In Vitro Assessment of PCCA ExoBlue™ using Human Dermal Fibroblasts: Cell Proliferation and Viability

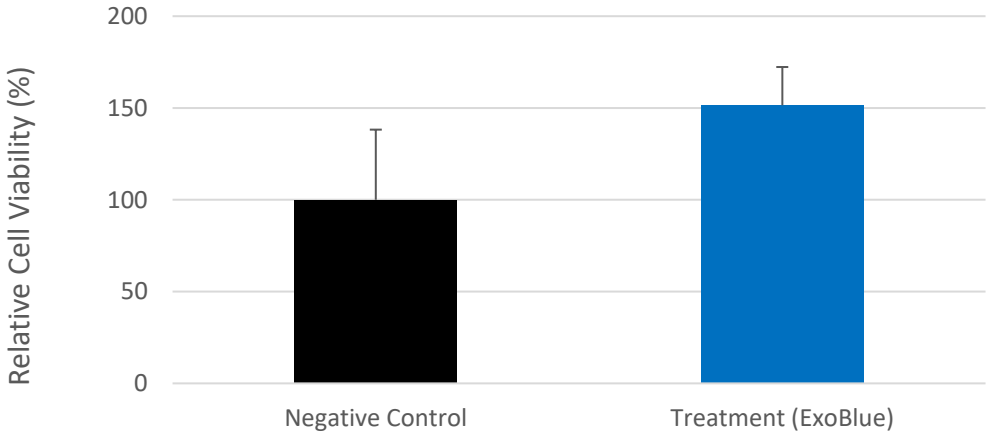

Figure 1. Quantification of the relative cell viability effects of PCCA ExoBlue on human dermal fibroblasts, in comparison with untreated cells (negative control).

The fluorescence microscopy images in Figure 2 indicate that treatment with PCCA ExoBlue enhanced the proliferation and viability of human dermal fibroblasts compared with the untreated negative control. The treated cells exhibited stronger and more widespread green fluorescence, consistent with a greater number of viable, metabolically active cells retaining intracellular Calcein. In contrast, the untreated control displayed lower fluorescence intensity and reduced cell density.

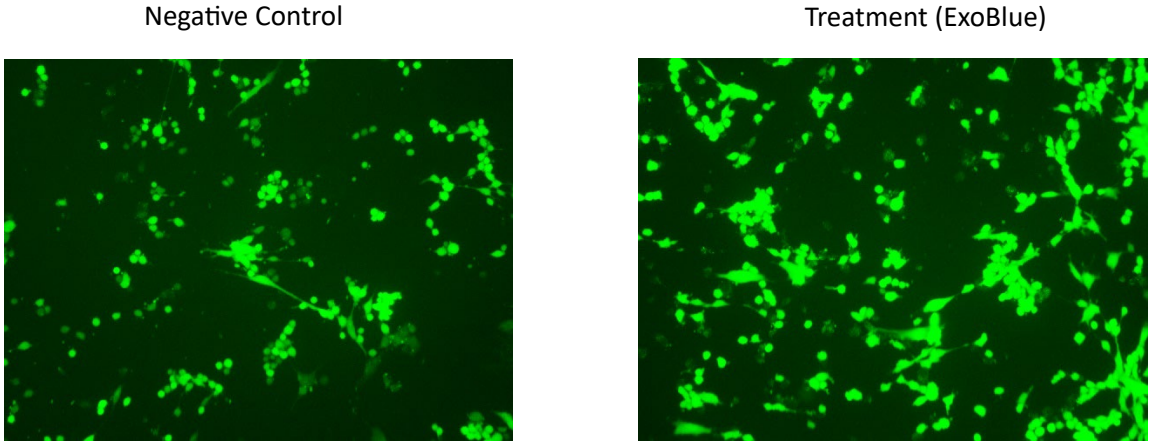

Figure 2. Representative Calcein AM fluorescence images showing the effects of PCCA ExoBlue on the proliferation and viability of human dermal fibroblasts (right), in comparison with untreated cells (negative control) (left).

These findings suggest that PCCA ExoBlue stimulates fibroblast growth and supports cellular activity without inducing cytotoxic effects. Because fibroblasts play a critical role in collagen production, extracellular matrix maintenance and tissue repair, the observed increase in proliferation supports the potential use of PCCA ExoBlue in skin regeneration and anti-aging topical formulations.

PCCA USA
9901 South Wilcrest Drive
Houston, Texas 77099
800.331.2498
800.874.5760 Fax
pccarx.com

PCCA CANADA
744 Baransway Drive
London, ON Canada N5V 5J2
800.668.9453
800.799.4537 Fax
pccarx.ca

PCCA AUSTRALIA
Unit 1, 73 Beauchamp Road
Matraville, NSW 2036 Australia
02.9316.1500
02.9316.7422 Fax
pccarx.com.au

PCCA UK
Unit 2 Regents Drive
Low Prudhoe Industrial Estate
Northumberland, UK NE42 6PX
0800.038.5095
0800.083.4222 Fax
pccarx.co.uk