PCCA ExoBlue™ **TECHNICAL REPORT**

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.

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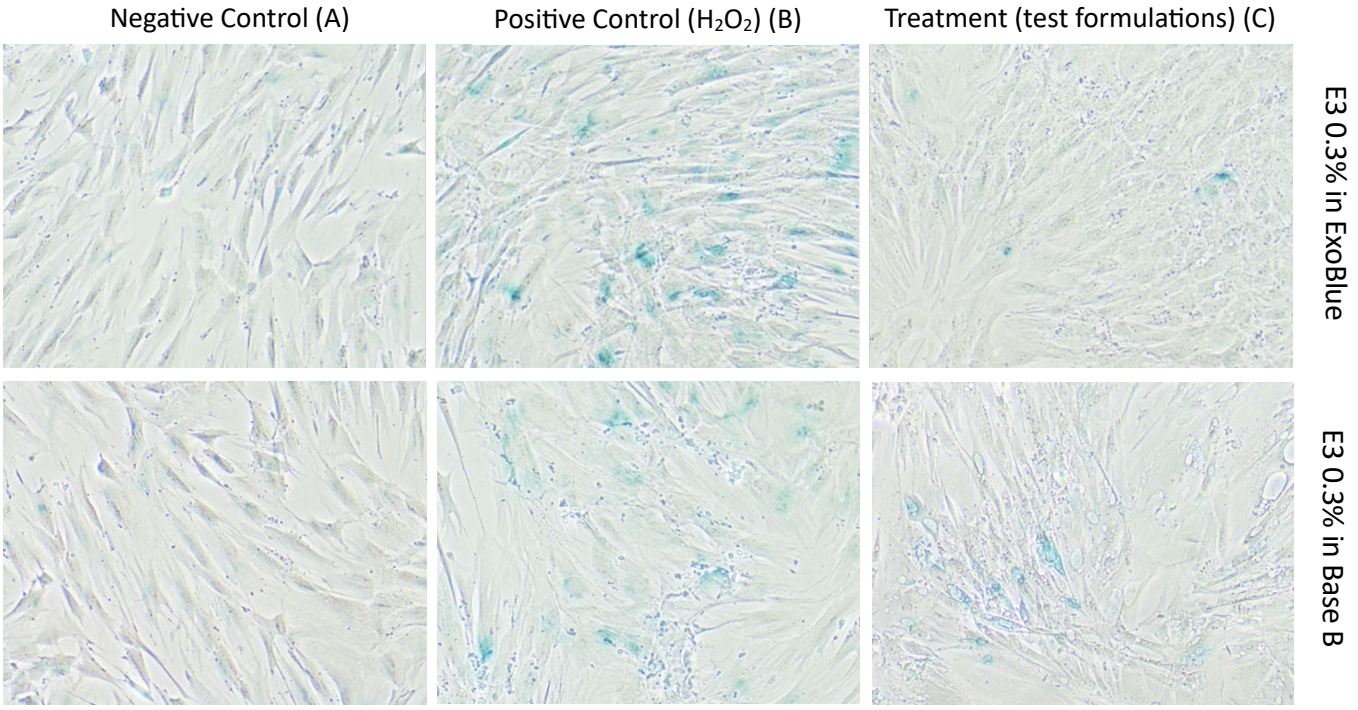

Figure 1. Microscope images of senescence-associated β-gal blue staining in human dermal fibroblast cells.

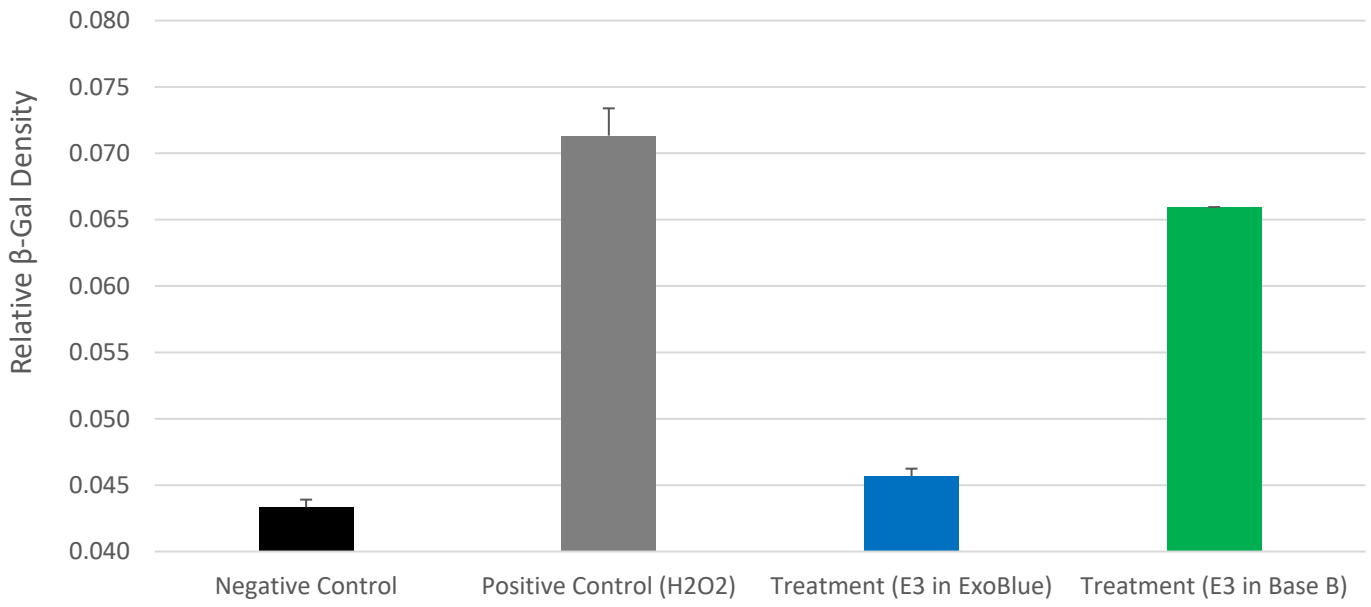

Figure 2. Quantification of β-gal density in human dermal fibroblasts across the two controls and the two treatment groups.