In Vitro Assessment of PCCA ExoBlue™ Using Human Dermal Fibroblasts: Cell Proliferation and Viability (Part 1)

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.

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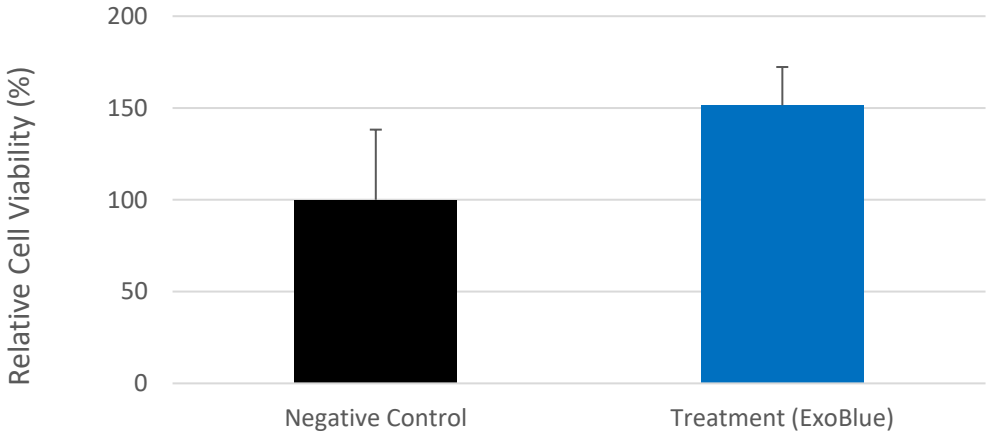

Figure 1. Quantification of the relative cell viability effects of PCCA ExoBlue on human dermal fibroblasts, in comparison with untreated cells (negative control).

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.

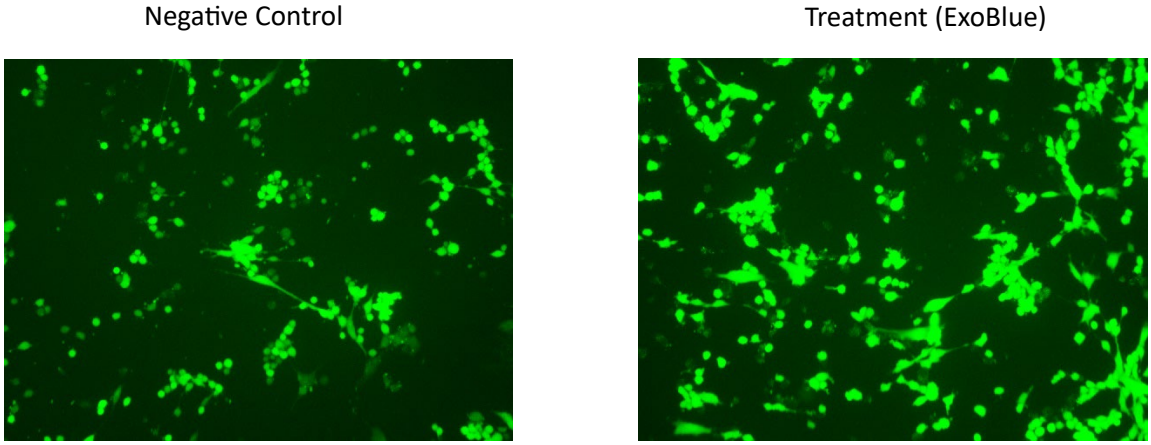

Figure 2. Representative Calcein AM fluorescence images showing the effects of PCCA ExoBlue on the proliferation and viability of human dermal fibroblasts (right), in comparison with untreated cells (negative control) (left).

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.

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