Elastin ELISA

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Tradename: AC ExoEternol

Code: 60200

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 90082-61-4 (or) 68132-21-8 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13041

Lot #: N250701O

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Elastin Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Elastic like fibers, such as elastin, comprise part of the extracellular matrix and confer elasticity to organs and tissues including the heart, skin, lungs, ligaments, and blood vessels. The encoded protein is rich in hydrophobic amino acids such as glycine and proline, which form mobile hydrophobic regions bounded by crosslinks between lysine residues. Degradation products of the encoded protein, known as elastin-derived peptides or elastokines, bind the elastin receptor complex resulting in the migration and proliferation of dermal fibroblasts. Deletions and mutations in this gene present as wrinkled or loose skin along with easy bruising and scaring. Increasing production of elastin is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Accordingly, an Elastin ELISA was conducted to assess the *in vitro* effect of **AC ExoEternol** on the extracellular release and intracellular synthesis of elastin from human dermal fibroblasts.

Assay Principle

The Elastin ELISA Kit operates by mixing an affinity tag labeled capture antibody with a reporter conjugated detector antibody that binds to elastin. After elastin is labeled, an immobilized complex is formed upon binding to anti-tag antibodies coating the wells. Unbound materials are removed during washing steps, and adding 3,3',5,5'-tetramethylbenzidine (TMB) Development Solution generates a blue color that is catalyzed by horseradish peroxidase (HRP). Adding Stop Solution to samples finalizes the color change from blue to yellow and absorbance is measured. The signal generated is proportional to the amount of bound elastin and concentrations are calculated. Cell culture supernatants (media) and solubilized fibroblast lysates from all conditions are collected to determine secreted and intracellular levels of elastin, respectively.

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Materials

A. Kit:	Human Elastin ELISA Kit (Abcam; ab239433)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes; Light microscope
D. Cell Line:	Normal Human Adult Dermal Primary Fibroblasts (ATCC; PCS-201-012)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Antibody Diluent CPI; 10X Wash Buffer PT; Sample Diluent NS; Sample Diluent 50BS
F. Reagents:	Dexamethasone (DEX) (10 µM); Elastin Recombinant Protein; 10X Elastin Capture Antibody; 10X Elastin Detector Antibody; TMB Development Solution; Stop Solution
G. Culture Plate:	Flat Bottom 12-Well Tissue Culture Treated Plate; Pre-Coated 96 Well Microplate (12 x 8 well strips)
H. Software:	Excel Analysis ToolPak (Microsoft)
I. Other:	Sterile disposable pipette tips; 15 mL Conical tubes; 1.7 mL Microcentrifuge tubes

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Human dermal fibroblasts were seeded into a 12-well tissue culture plate and allowed to grow to confluency in complete media (CM). 0.01% and 0.05% concentrations of **AC ExoEternol** in CM were added to cells and placed at 37°C. CM was used as the untreated control, while DEX (10 µM) was added to CM and utilized as a positive control. After 48 hours, media and fibroblast lysates were collected according to the manufacturer's instructions and utilized in the Human Elastin ELISA Kit (ab239433).

Elastin standards were prepared ranging in concentrations from 0 pg/mL to 12,000 pg/mL. After adding 50 µL of standards and samples to the appropriate wells, 50 µL of the Antibody Cocktail was added to all wells. Following a one-hour incubation at room temperature, all wells were washed three times with 350 µL of 1x Wash Buffer PT and 100 µL of TMB Development Solution was added to each well. After a 10-minute incubation in the dark, 100 µL of Stop Solution was added to each well. The optical density was read at 450 nm on a Synergy HT Microplate Reader.

Assays were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. A standard curve was created by reducing the data and generating a linear curve fit. The secreted and intracellular elastin concentrations of **AC ExoEternol**-treated fibroblasts were determined by extrapolation from the standard curve and expressed in pg/mL. Percent changes in elastin are relative to CM and were calculated with the following formula:

$$Percent\ Change = \frac{Elastin\ Concentration_{Sample} - Elastin\ Concentration_{CM}}{Elastin\ Concentration_{CM}} \times 100$$

Results

The data obtained met criteria for a valid assay and the positive control performed as anticipated. Compared to untreated fibroblasts, DEX increased secreted and intracellular elastin concentrations. Fibroblasts treated with **AC ExoEternol** at 0.01% and 0.05 enhanced the levels of secreted and intracellular elastin compared to untreated fibroblasts.

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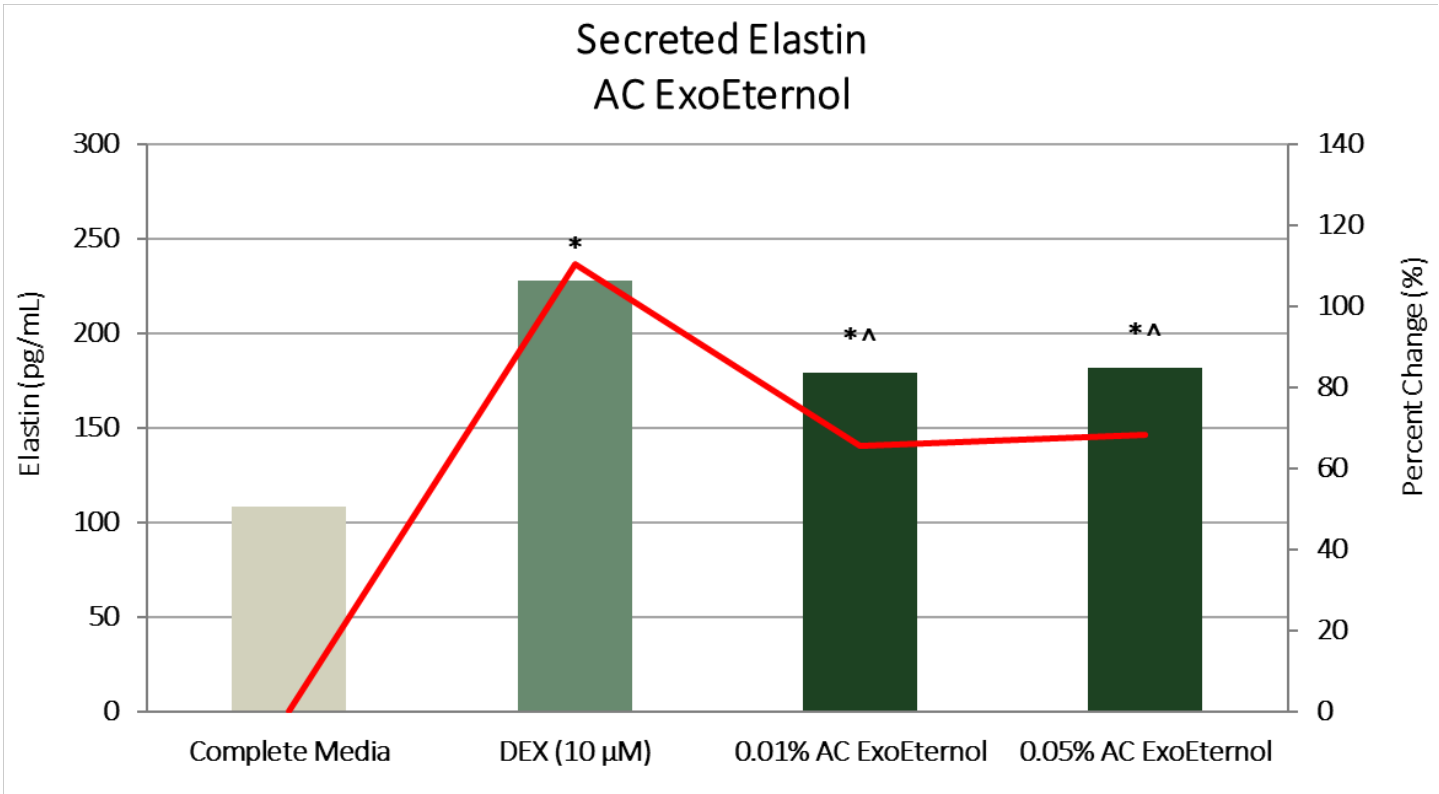

Figure 1. Concentrations of Elastin Secreted from **AC ExoEternol**-treated fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to DEX.

Table 1. Results from one-way ANOVA Statistical Analysis of Elastin Secreted Compared to untreated fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to DEX.

	Complete Media	0.01% **AC ExoEternol**	0.05% **AC ExoEternol**
Complete Media	-----	0.029*	0.017*
DEX	0.003*	< 0.001^	< 0.001^

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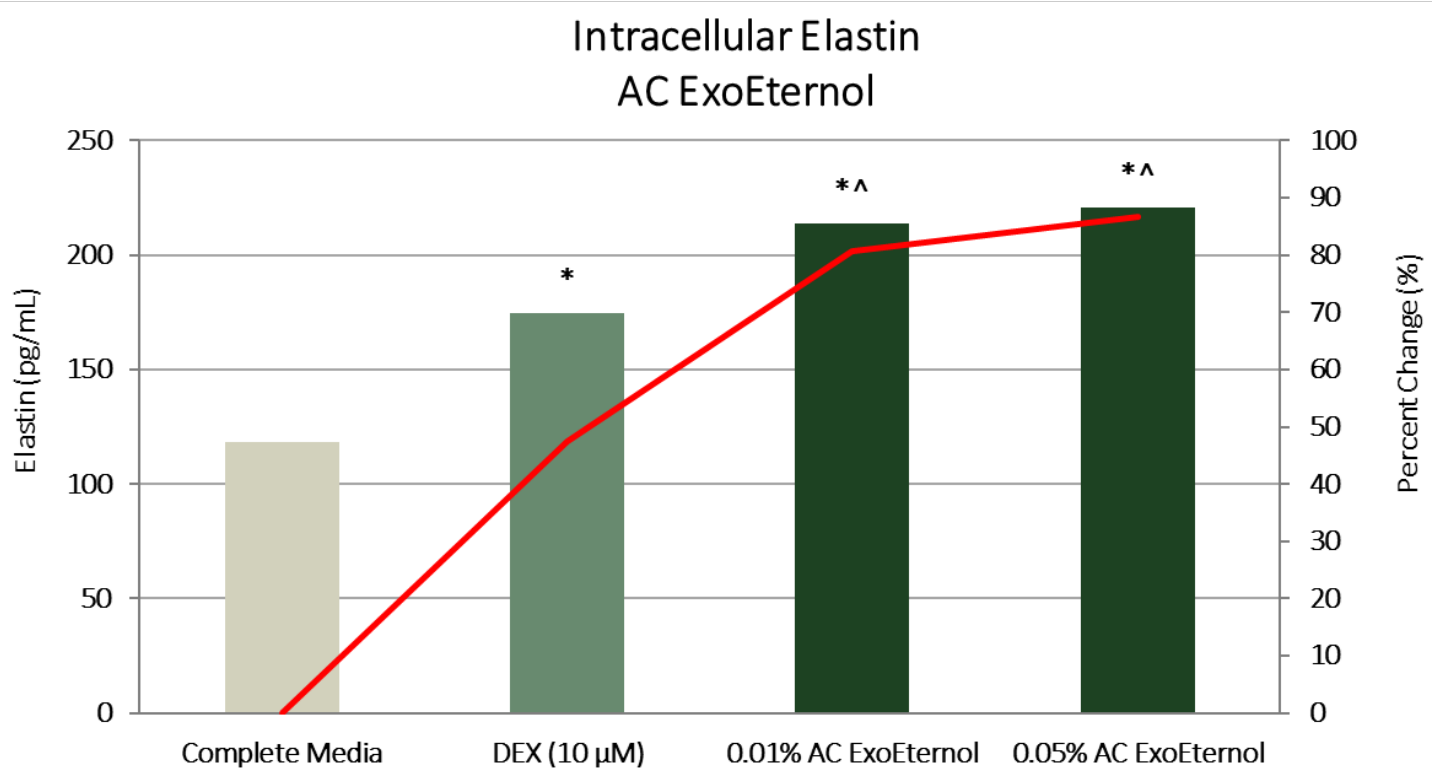

Figure 2. Intracellular concentrations of Elastin from **AC ExoEternol**-treated fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to DEX.

Table 2. Results from one-way ANOVA Statistical Analysis of Intracellular Elastin Compared to Untreated Fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to DEX.

	Complete Media	0.01% **AC ExoEternol**	0.05% **AC ExoEternol**
Complete Media	-----	0.003*	0.001*
DEX	0.006*	< 0.001^	< 0.001^

Discussion

As shown in Figure 1, fibroblasts exposed to DEX demonstrated a 110% increase in elastin secretion compared to untreated fibroblasts. This data demonstrates the levels of elastin released from fibroblasts can be augmented with compounds known to increase elastin synthesis. Similarly, treatment with **AC ExoEternol** at 0.01% and 0.05% demonstrated 66% and 68% increases in the levels of elastin released compared to untreated fibroblasts, respectively (Table 1). These data demonstrate **AC ExoEternol** augments fibroblast elastin secretion.

As shown in Figure 2, DEX exposure increased intracellular elastin by 47% compared to untreated fibroblasts. This data demonstrates intracellular elastin can be enhanced with compounds known to stimulate elastin deposition. Likewise, **AC ExoEternol** treatment at 0.01% and 0.05% augmented intracellular elastin concentrations by 81% and 87% compared to untreated fibroblasts, respectively. These data demonstrate **AC ExoEternol** increases elastin levels in fibroblasts.

Collectively, an increase in elastin synthesis indicates stimulation, migration, and proliferation of skin fibroblasts which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. These data indicate **AC ExoEternol** enhances skin matrix replenishment and anti-aging properties as well as slows skin matrix degradation when used at recommended use-levels.