In vitro Heavy Metal Protection Assay

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Tradename: AC Det'Ox Hair

Code: 21030

CAS #: 8013-01-2 & 68333-16-4 (or) 92128-79-5

Test Request Form #: 13170

Lot #: N250115A

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

Test Performed:
In vitro Heavy Metal Protection Assay

Introduction

Acute and chronic exposure to heavy metals is a threat to hair health as they disrupt natural biological processes. At the molecular level, exposure to heavy metals causes inflammation, physical damage, and accelerates characteristics of aging. As a result, hair appears dull and exhibits advanced aging qualities such as thinning and brittleness. Topical cosmetic products offering protection from heavy metals at the cellular level, by maintaining cellular viability and homeostasis, are becoming a critical component in limiting external factors detrimental to hair health and appearance.

Accordingly, an *in vitro* Heavy Metal Protection Assay was conducted to assess the ability of **AC Det'Ox Hair** to protect cellular homeostasis against exposure to heavy metals.

Assay Principle

Heavy Metal exposure was utilized as a model to exert deleterious effects on cellular viability. Human Hair Follicle Dermal Papilla Cells (HFDPCs) are incubated with test articles before exposure to Heavy Metals. After exposure to Heavy Metals, cellular viability is assessed. This assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction by the tricarboxylic acid cycle. Healthy cells easily convert resazurin into resorufin without harming the cells.

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Materials

A. Kit:	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy HT Microplate reader; Pipettes; Light microscope
D. Cell Line:	Human Hair Follicle Dermal Papilla Cells (HFDPCs) (Cell Applications Inc; 602K-05a)*
E. Media/Buffers:	Complete Follicle Dermal Papilla Cell Growth Medium (Cell Applications Inc.; C-26501)*; Collagen Coating Solution (Cell Applications Inc.; 125-100)*; Phosphate Buffered Saline (PBS)
F. Tissue Culture Plates:	Falcon flat bottom 96-well tissue culture treated plates*
G. Software:	Excel Analysis ToolPak (Microsoft)
H. Reagents:	PrestoBlue™ reagent (10X)*
I. Other:	Sterile disposable pipette tips

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

Methods

Human Hair Follicle Dermal Papilla Cells (HFDPCs) were seeded into a collagen coated 96-well tissue culture microplate and allowed to grow to confluency in Complete Media. 0.01%, 0.02%, and 0.04% concentrations of **AC Det'Ox Hair** in Complete Media were added to the cells and incubated at 37°C. Following a 24-hour incubation, the media in all wells was removed and cells were washed once with PBS. Heavy Metals (Table 1) were added to all wells, except control wells that received Complete Media, and incubated at 37°C. Following an 18-hour incubation, all media was removed, cells were rinsed once with PBS, and Complete Media was added to all wells. Viability reagent was added to Complete Media in culture wells and fluorometric measurements were taken at excitation/emission wavelengths of 560 nm / 590 nm.

Table 1. Heavy Metal Elements and Concentrations.

Element	Concentration (ppm)
Lead	0.1
Cadmium	0.05
Manganese	3.0
Chromium	1.0
Nickel	1.0
Zinc	50
Copper	13
Fluoride	40
Selenium	0.5
Thallium	0.02
Calcium	500

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 $p \leq 0.05$. Cellular viability results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

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$$Percent\ Change\ (\%) = \frac{MFU_{Sample} - MFU_{Heavy\ Metals}}{MFU_{Heavy\ Metals}} \times 100$$

Results

The data obtained met criteria for a valid assay and the control and Heavy Metals performed as anticipated. Compared to Complete Media alone, HFDPCs treated with Heavy Metals exhibited decreased cellular viability. HFDPCs treated with **AC Det'Ox Hair** prior to Heavy Metals exposure demonstrated improved viability compared to cells treated with Heavy Metals alone.

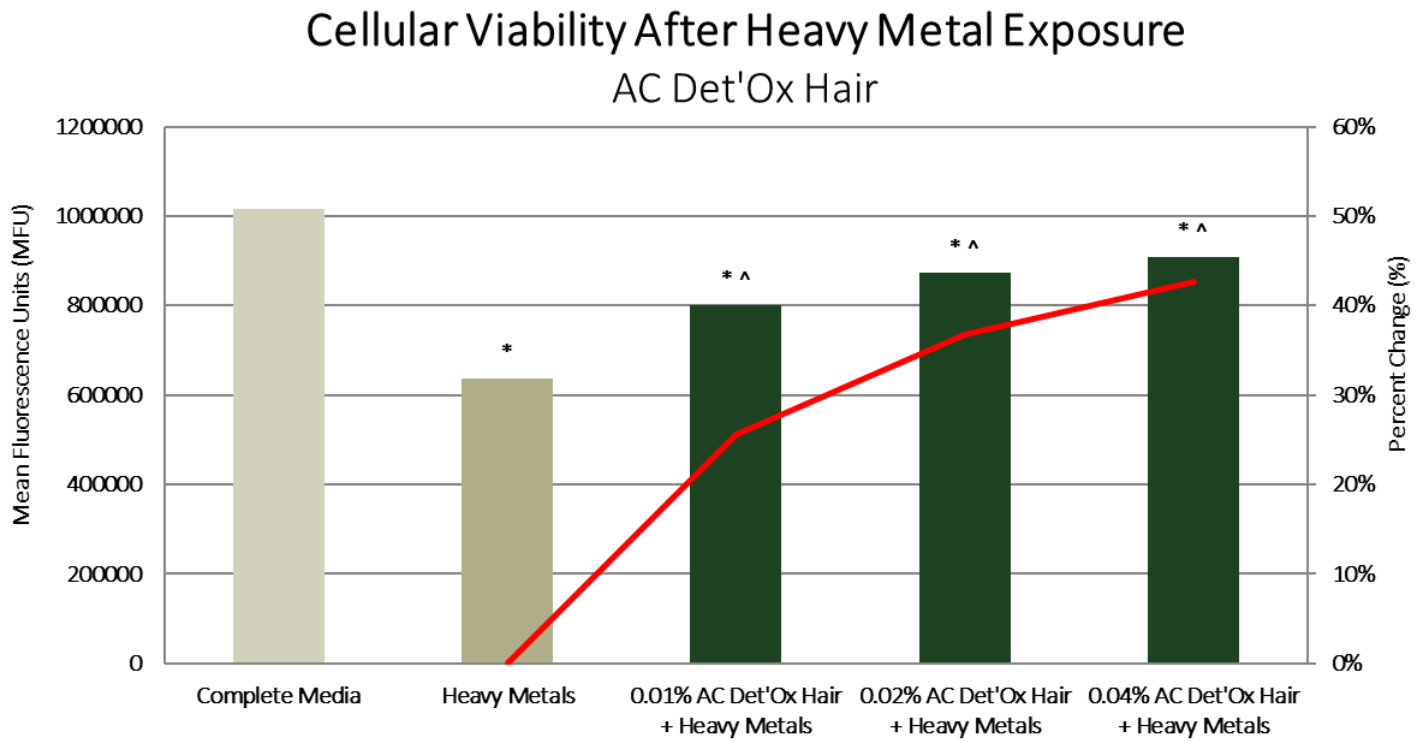

Figure 1. Cellular Viability of Human Hair Follicle Dermal Papilla Cells (HFDPCs). * indicates significance ($p \leq 0.05$) compared to untreated HFDPCs. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metals-treated HFDPCs.

Table 2. P-values from one-way ANOVA Statistical Analysis between the two conditions compared. * indicates significance ($p \leq 0.05$) compared to untreated HFDPCs. ^ indicates significance ($p \leq 0.05$) compared to Heavy Metal-treated HFDPCs.

	Complete Media	0.01% **AC Det'Ox Hair**	0.02% **AC Det'Ox Hair**	0.04% **AC Det'Ox Hair**
Complete Media	-----	< 0.05*	< 0.05*	< 0.05*
Heavy Metals	< 0.05*	< 0.05^	< 0.05^	< 0.05^

Discussion

As shown in Figure 1, HFDPCs exposed to Heavy Metals demonstrated a 37% reduction in viability compared to the untreated HFDPCs. Importantly, exposure to Heavy Metals induced significant decreases in cellular viability regardless of pre-treatments received (Table 2). These data demonstrated the detrimental effects of Heavy Metals on cellular viability and homeostasis.

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HFDPCs treated with **AC Det'Ox Hair** at 0.01%, 0.02%, and 0.04% prior to Heavy Metals exposure demonstrated 26%, 37%, and 43% increases in viability, respectively, compared to HFDPCs treated with Heavy Metals alone (Figure 1, Table 2). These data demonstrate **AC Det'Ox Hair** mitigates the deleterious effects of Heavy Metal exposure on cellular viability.

Exposure to Heavy Metals perturbs cellular homeostasis and accelerates characteristic signs of hair aging. Taken together, these data indicate **AC Det'Ox Hair** attenuates the negative impacts of Heavy Metal exposure on cellular viability. Collectively, **AC Det'Ox Hair** offers protection against Heavy Metals at a cellular level.