In vitro Airborne Pollution 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 #: 13098

Lot #: N250115A

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

Test Performed:
In vitro Airborne Pollution Protection Assay

Introduction

Airborne pollutants, such as cigarette smoke and secondhand smoke, are a threat to hair health as they disrupt natural biological processes. At the molecular level, exposure to airborne pollutants 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. Given the increasing amount of global pollution, topical cosmetic products offering protection from airborne pollutants at the cellular level are a critical component in limiting external factors detrimental to hair health and appearance. These topical cosmetic products combat air pollution and promote hair health by maintaining cellular viability and homeostasis.

Accordingly, an *in vitro* Airborne Pollution Protection Assay was conducted to assess the ability of **AC Det'Ox Hair** to protect cellular homeostasis against exposure to soluble cigarette smoke pollutants.

Assay Principle

Cigarette smoke was utilized as a model for airborne pollutants as it is not heavily influenced by environmental factors and exposure can be standardized. Cigarette Smoke Media is generated using an apparatus which bubbles cigarette smoke through cell culture medium using a manual syringe (Image 1). Cells are incubated with test articles before exposure to the Cigarette Smoke Media which contains soluble pollutants extracted from cigarette smoke. After exposure to Cigarette Smoke Media, 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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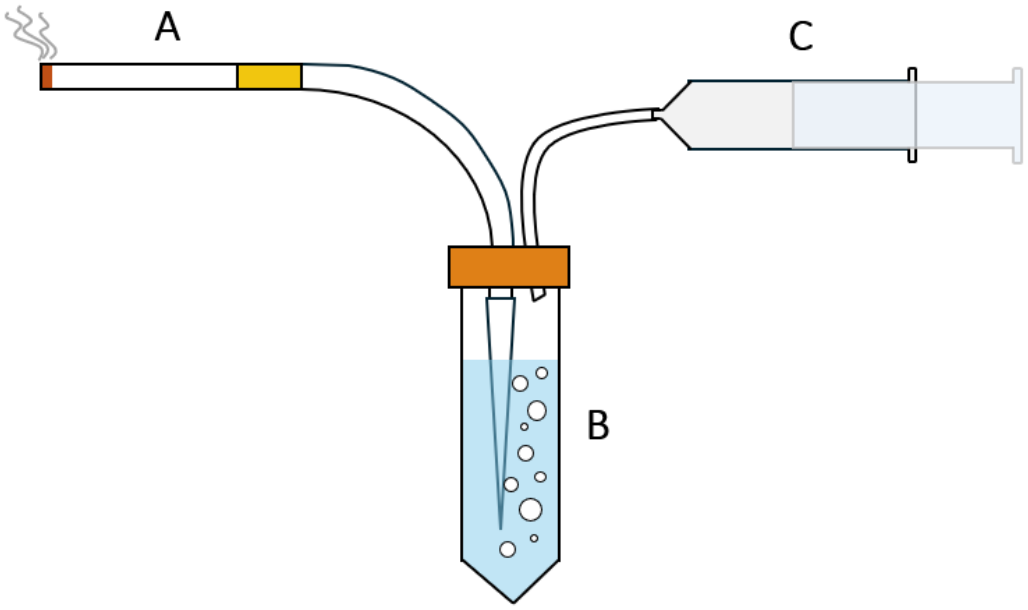

Image 1. Smoke Extraction Apparatus. Cigarette smoke (A) is bubbled through cell culture media (B) utilizing a manual syringe (C).

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; Smoke extraction apparatus
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); Cigarette Smoke Media
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 smoking was modeled as short two second puffs generated by the negative pressure of the syringe with 30 second delays between puffs. Aqueous constituents are consistent between batches by using the same cigarette to media ratio.

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. Cigarette Smoke Media was 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.

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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:

$$Percent\ Change\ (\%) = \frac{MFU_{Sample} - MFU_{Cigarette\ Smoke\ Media}}{MFU_{Cigarette\ Smoke\ Media}} \times 100$$

Results

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

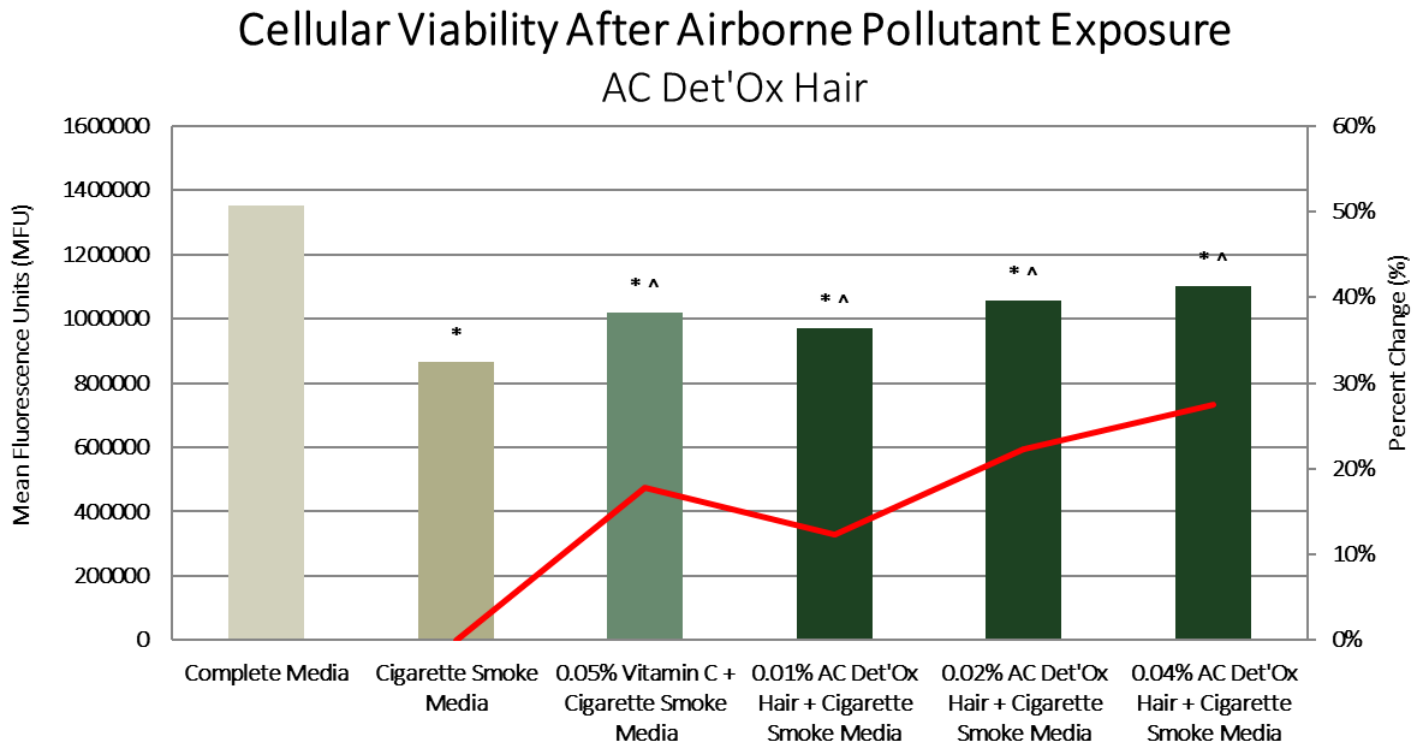

Figure 1. Cellular Viability of Human Hair Follicle Dermal Papilla Cells (HFDPCs). * indicates significance ($p \leq 0.05$) compared to untreated HFDPCs. ^ indicates significance ($p \leq 0.05$) compared to Cigarette Smoke Media-treated HFDPCs.

Table 1. 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 Cigarette Smoke Media-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*
Cigarette Smoke Media	< 0.05*	< 0.05^	< 0.05^	< 0.05^

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Discussion

As shown in Figure 1, HFDPCs exposed to Cigarette Smoke Media demonstrated a 36% reduction in viability compared to the untreated HFDPCs. However, cells treated with 0.05% Vitamin C exhibited an 18% increase in viability compared to HFDPCs treated with Cigarette Smoke Media alone (Figure 1). Importantly, exposure to Cigarette Smoke Media induced significant decreases in cellular viability regardless of pre-treatments received (Table 1). These data demonstrated the detrimental effects of airborne pollutants on cellular viability and homeostasis.

HFDPCs treated with **AC Det'Ox Hair** at 0.01%, 0.02%, and 0.04% prior to pollution exposure demonstrated 12%, 22%, and 28% increases in viability, respectively, compared to HFDPCs treated with Cigarette Smoke Media alone (Figure 1, Table 1). These data demonstrate **AC Det'Ox Hair** mitigates the deleterious effects of airborne pollutants on cellular viability.

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