Ex Vivo Hair Shaft Elongation Assay

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Tradename: AC ExoRoot

Code: 60202

CAS #: 7732-18-5 & 91079-57-1 (or) 223749-83-5 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13339

Lot #: N250520B

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

Test Performed:
Ex Vivo Hair Shaft Elongation Assay

Introduction

Hair consists of a visible structure, the hair shaft, and a component underneath the skin surface, the hair follicle. Hair shafts are thin, keratinized epithelial cells comprised of a central medulla, cortex, and cuticle cells that determine the aesthetics and mechanical properties of hair (i.e. color, texture, and strength). The hair follicle is the primary structure for hair shaft growth and is made up of inner and outer root sheaths and the hair bulb. The inner root sheath assists in hair shaft attachment to the follicle, while the outer root sheath is a reservoir of multipotent stem cells. Surrounding the dermal papilla, the hair blub actively produces hair as it contains nerve fibers and a capillary network.

The hair shaft undergoes rapid growth and elongation followed by stages of quiescence and regression that occur in distinct phases: anagen, catagen, and telogen. The anagen phase is characterized by active growth, the catagen phase marks a shift from growth to quiescence, and the telogen phase is defined by dormant hair follicles. Environmental factors such as topical personal care products, nutritional deficiencies, medications, and genetics can alter this tightly regulated growth cycle, which elicits excessive hair loss or growth in unwanted and/or abnormal locations.

Accordingly, an *Ex Vivo* Hair Shaft Elongation Assay was conducted to assess the effect of **AC ExoRoot** to augment the length of hair follicles. Additionally, minoxidil and a blend of **AC ExoRoot** with minoxidil were tested to elucidate any interactive effects.

Assay Principle

Hair follicles in the anagen phase are plucked from the scalps of male volunteers and cultured, after which hair follicle length is assessed. Provided plucked hair follicles contain viable follicular stem cells that exhibit proliferative and differentiation properties, culturing plucked hair follicles is a reproducible and quantifiable *ex vivo* method to assess hair follicle growth rate in a controlled system that mimics *in vivo* hair growth. Accordingly, increases in hair shaft length (hair follicle growth) are attributable to the production of a keratinized hair shaft.

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Materials

A. Tissue:	Plucked hair follicles from the scalp
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes; Watchmaker Forceps
D. Media/Buffers:	Fibroblast Basal Media (ATCC); L-glutamine (ATCC); Hydrocortisone Hemisuccinate (Millipore Sigma); Recombinant Human (rh) Insulin (ATCC); Penicillin-Streptomycin-Amphotericin B Solution (ATCC); Minoxidil (Millipore Sigma)
E. Culture Vessel:	Clear bottom tissue culture-treated 24-well microplate
F. Software:	ImageJ Analysis Software (National Institutes of Health); Excel Analysis ToolPak (Microsoft)

Methods

Plucked scalp hair was obtained from five healthy males, aged 18-40. Hair follicles were isolated with watchmaker's forceps, in the anagen phase, and were required to have an intact bulb. The plucked hair follicles were maintained free-floating in individual wells of a 24-well microplate in 500 µL of Complete Media containing 2 mM L-glutamine, 10 ng/mL Hydrocortisone Hemisuccinate, 10 µg/mL rh Insulin, 100 Units/mL Penicillin, 100 µg/mL Streptomycin, and 250 ng/mL Amphotericin B. Hair follicles were placed into Complete Media or 2.0% minoxidil, 2.0% **AC ExoRoot**, 5.0% **AC ExoRoot**, 2.0% **AC ExoRoot** + 2.0% minoxidil, or 5.0% **AC ExoRoot** + 2.0% minoxidil diluted with Complete Media. All follicles were cultured for 10 days at 37°C (5% CO2, 95% RH) and media was changed every 72 hours.

Images of each hair follicle were obtained under a light microscope with a 4x objective immediately after plucking (day 0) and after the 10-day culture period. Six hair follicles were utilized for each condition and any follicles exhibiting a deterioration in follicular architecture were excluded from analysis. Hair follicle length, defined as the distance from the base of the bulb to the end of the shaft, was assessed using ImageJ Analysis Software. Hair shaft growth, calculated by subtracting follicle length at isolation from follicle length at day 10, is expressed in millimeters by dividing the total shaft length (exported in pixels) by a conversion factor of a given number of pixels per millimeter.

Replicates for each condition are averaged and data is displayed as the average hair shaft length for all volunteers. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. Percent change is expressed relative to hair follicles in Complete Media and calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{Hair\ Shaft\ Length_{Condition} - Hair\ Shaft\ Length_{Complete\ Media}}{Hair\ Shaft\ Length_{Complete\ Media}} \times 100$$

Results

The data obtained met criteria for a valid assay as the production of a keratinized hair shaft was responsible for the increases in hair shaft length and the untreated control (Complete Media) performed as anticipated. Hair follicles treated with 2.0% minoxidil, 2.0% **AC ExoRoot**, and 5.0% **AC ExoRoot** grew more compared to untreated hair follicles. Similarly, both the 2.0% and 5.0% **AC ExoRoot** with 2.0% minoxidil outperformed all conditions.

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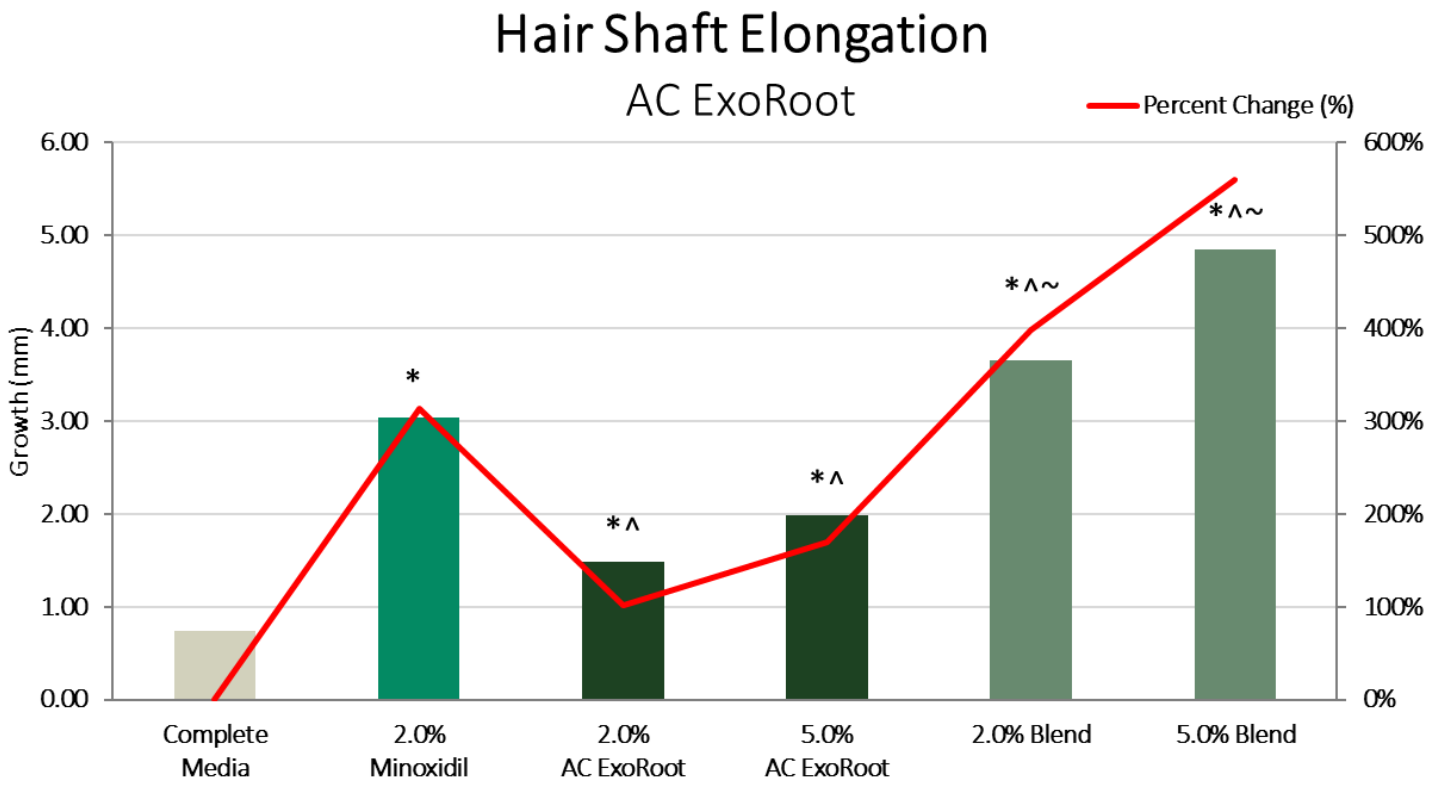

Figure 1: The Effect of **AC ExoRoot**, Minoxidil, and Blend on Plucked Hair Follicle Growth after 10 Days. Blend: % **AC ExoRoot** + 2.0% minoxidil. * indicates significance ($p < 0.05$) compared to Complete Media. ^ indicates significance ($p \leq 0.05$) compared to 2.0% minoxidil. ~ indicates significance ($p \leq 0.05$) compared to 2.0% and 5.0% **AC ExoRoot**.

Table 1. P-values from one-way ANOVA Statistical Analysis of Follicle Growth. Results represent p-values between the two conditions. Blend: % **AC ExoRoot** + 2.0% minoxidil. * indicates significance ($p < 0.05$) compared to Complete Media. ^ indicates significance ($p \leq 0.05$) compared to 2.0% minoxidil. ~ indicates significance ($p \leq 0.05$) compared to 2.0% and 5.0% **AC ExoRoot**.

	Complete Media	2.0% Minoxidil	2.0% **AC ExoRoot**	5.0% **AC ExoRoot**	2.0% Blend	5.0% Blend
Complete Media	---	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*
2.0% Minoxidil	< 0.05*	---	< 0.05^	< 0.05^	< 0.05^	< 0.05^
2.0% **AC ExoRoot**	< 0.05*	< 0.05^	---	< 0.05	< 0.05~	< 0.05~
5.0% **AC ExoRoot**	< 0.05*	< 0.05^	< 0.05	---	< 0.05~	< 0.05~
2.0% Blend	< 0.05*	< 0.05^	< 0.05~	< 0.05~	---	< 0.05
5.0% Blend	< 0.05*	< 0.05^	< 0.05~	< 0.05~	< 0.05	---

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Discussion

As shown in Figure 1, hair follicles incubated with Complete Media grew 0.73 mm after 10 days. However, 2.0% minoxidil elicited 3.04 mm of growth in hair follicles, augmenting growth by 314% compared to Completed Media. These data demonstrate hair shafts from plucked hair follicles are viable, continue to grow in culture via increases in a keratinized hair shaft, and can be manipulated by exogenous compounds.

Plucked hair follicles treated with 2.0% and 5.0% **AC ExoRoot** demonstrated hair shaft growths of 1.48 mm and 1.98 mm resulting in 102% and 170% increases in hair shaft length compared to untreated hair follicles, respectively. Similarly, 2.0% 5.0% **AC ExoRoot** with 2.0% minoxidil produced hair shaft growths of 3.66 mm and 4.85 mm resulting in 398% and 560% increases in hair shaft length compared to untreated hair follicles, respectively. These data demonstrate **AC ExoRoot** augments hair shaft length alone, and to greater extent when combined with minoxidil.

Active hair growth occurs during the anagen phase and takes place within the hair bulb of the hair follicle resulting in an increased length of the keratinized hair shaft. Despite the numerous factors contributing to hair growth and the highly regulated growth cycle, exogenous personal care products have the ability to alter growth rates. These data indicate **AC ExoRoot** increases hair shaft length, which may promote existing hair growth *in vivo* when added to personal care products at the recommended use-levels. Moreover, combining **AC ExoRoot** with minoxidil elicits a synergistic effect that produces greater hair follicle growth than either ingredient alone.