In Vitro Hair Shaft Elongation Assay

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Tradename: Active.Lite® Hair

Code: 22045

CAS #: N/A & 7732-18-5 & 222400-29-5 & 90106-73-3

Test Request Form #: 10078

Lot #: 9152500

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Study Director: *Maureen Drumwright*
Principle Investigator: *Daniel Shill*

Test Performed:
In Vitro 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 In Vitro Hair Shaft Elongation Assay was conducted to assess the effect of **Active.Lite® Hair** to augment the length of hair follicles.

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 *in vitro* 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)
E. Culture Vessel:	Clear bottom tissue culture-treated 24-well microplate
F. Software:	ImageJ Analysis Software (National Institutes of Health)

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 (CM) 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 CM or 0.01%, 0.1%, and 1.0% concentrations of **Active.Lite® Hair** diluted with CM. All follicles were cultured for 10 days at 37°C (5% CO_2, 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 growth for all volunteers.

Results

The data obtained from this study 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 control (CM) performed as anticipated. Hair follicles treated with **Active.Lite® Hair** at 0.01%, 0.1%, and 1.0% grew more compared to untreated hair follicles.

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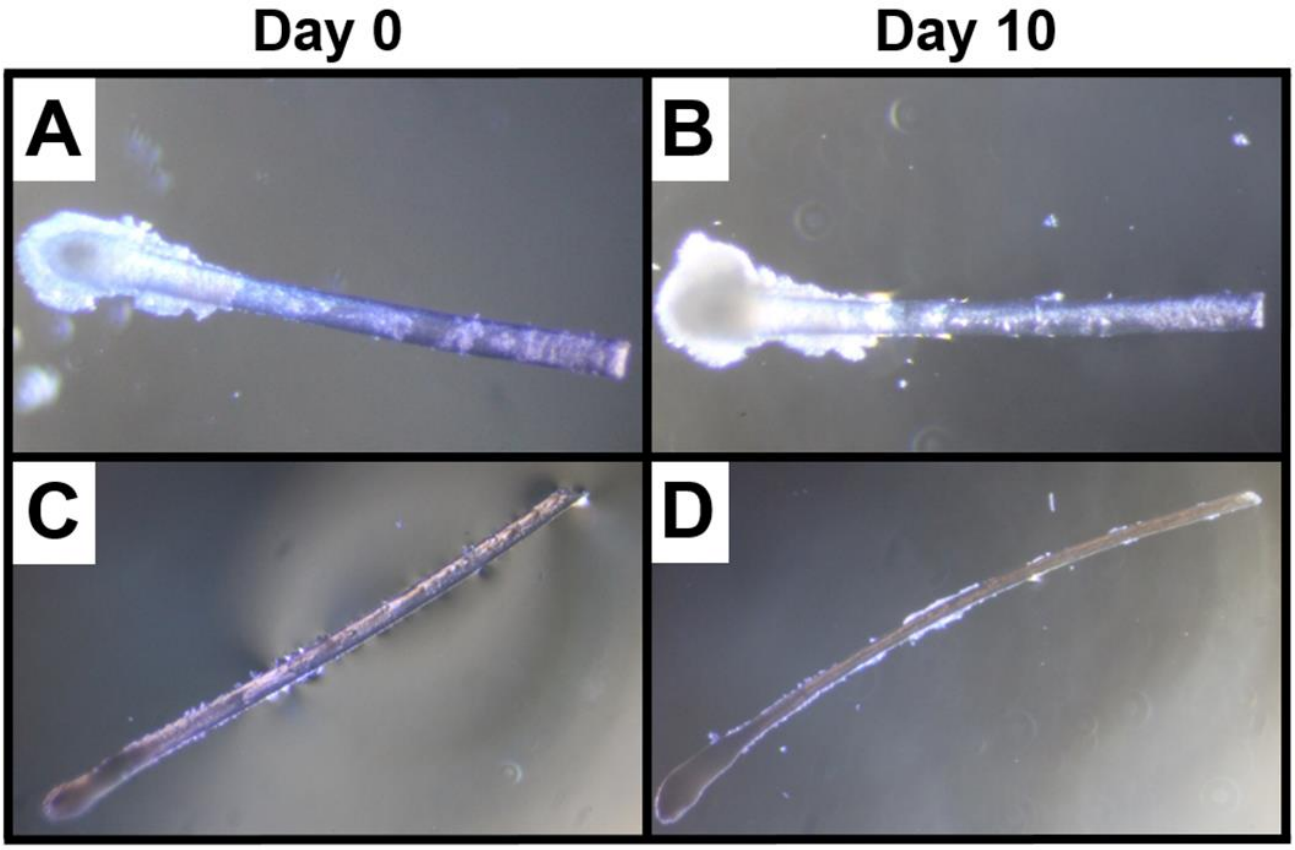

Figure 1: Representative images of plucked hair follicles incubated with complete media (A, B) and 0.1% **Active.Lite® Hair** (C, D) on Day 0 (A, C) and Day 10 (B, D).

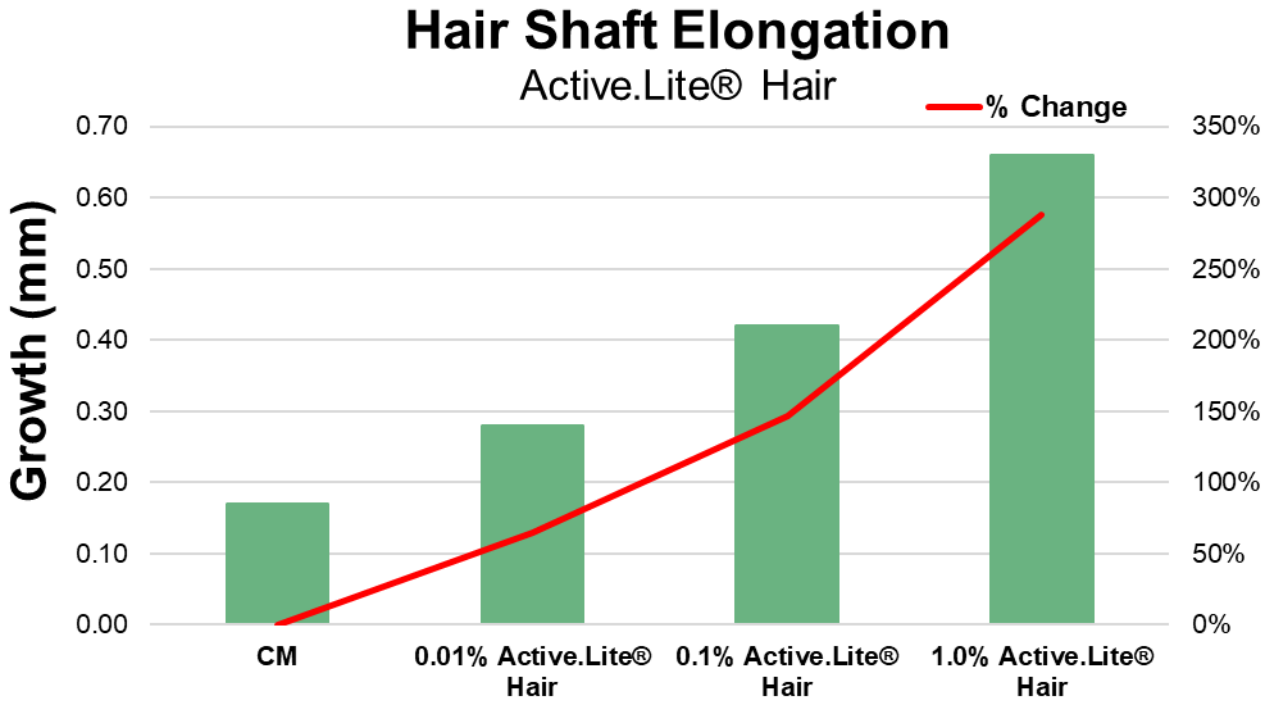

Figure 2: The effect of **Active.Lite® Hair** on plucked hair follicle growth after 10 days.

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Discussion

As shown in Figure 2, hair follicles incubated with CM grew 0.17 mm after 10 days. These data demonstrate hair shafts from plucked hair follicles are viable and continue to grow in culture via increases in a keratinized hair shaft.

Conversely, plucked hair follicles treated with **Active.Lite® Hair** at 0.01%, 0.1%, and 1.0% demonstrated hair shaft growths of 0.28 mm, 0.42 mm, and 0.66 mm resulting in 65%, 147%, and 288% increases in hair shaft length compared to untreated hair follicles, respectively. These data demonstrate **Active.Lite® Hair** augments hair shaft length.

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 **Active.Lite® Hair** increases hair shaft length in vitro, which may promote existing hair growth in vivo.