Scratch Assay

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Tradename: ACB Botanical Sugar Complex

Code: 20039

CAS #: 9005-25-8 & 68333-16-4

Test Request Form #: 1078

Lot #: NC150218-I

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principal Investigator: *Meghan Darley*

Test Performed:
Scratch Assay

Introduction

Wounded tissue begins a complex and structured series of events in order to repair the damaged region. Some of these events include upregulation of angiogenic factors causing increased vascularization, increased deposition of extracellular matrix, and increased cell proliferation. The wound healing process begins as cells polarize toward the wound, initiate protrusion, migrate, and close the wound area. These processes reflect the behavior of individual cells as well as the entire tissue complex.

A Scratch Assay was conducted to assess the *in vitro* wound healing properties of **ACB Botanical Sugar Complex** in dermal fibroblasts.

Assay Principle

The *in vitro* scratch assay is a well-known and widely used method to study cell migration and proliferation. This assay is based on the observation that when an artificial gap or scratch is made on a confluent cell monolayer, the cells will migrate towards the opening and close the scratch. The basic steps involve creating a scratch in a cell monolayer and capturing images throughout the healing or cell migration process. Through these images we can quantify the rate of cell migration.

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Materials

A. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
B. Equipment:	Forma Humidified Incubator; ESCO Biosafety Laminar Flow Hood; Camera; Pipettes; Light Microscope
C. Cell Line:	Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)*
D. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Phosphate Buffered Saline (PBS)
E. Reagents:	Epidermal Growth Factor-1 (100 ng/mL); Paraformaldehyde (3.7%); Crystal Violet Stain
F. Culture Plate:	6 Well Flat Bottom Tissue Culture Treated Plates
G. Analysis Software:	ImageJ (National Institutes of Health); Excel Analysis ToolPak (Microsoft)
H. Other:	Sterile disposable pipette tips

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

Methods

Human dermal fibroblasts were seeded into a 6-well tissue culture plate and allowed to grow to confluency in complete media. When cell growth reached confluency, scratches were made down the middle of the well in a straight line, generating an in vitro "wound" which is the area devoid of cells. The rate at which the fibroblasts migrate to fill the area devoid of cells indicates wound healing. All wells were washed with sterile PBS to remove cellular debris caused by the scratch. The positive control, Epidermal Growth Factor-1 (EGF-1) (100 ng/mL), was diluted with serum-free complete media. A 0.01% concentration of **ACB Botanical Sugar Complex** were diluted with serum-free complete media. Serum-free complete media was used as a negative control. Media was not changed throughout the duration of the experiment. Images were captured immediately after the scratch (t=0) and every 24-hours afterwards, up to 72 hours. Cells were fixed with 3.7% paraformaldehyde and stained with crystal violet for enhanced microscopy.

ImageJ software was used to analyze the images and calculate the area of the scratch and the closure rate. Percent scratch closure and migration rate were calculated by the following formulas:

$$\frac{Scratch\ Area_{\ t=x} - Scratch\ Area_{\ t=0}}{Scratch\ Area_{\ t=0}} \times 100 = \%\ Scratch\ Closure$$

$$\frac{Change\ in\ Area\ of\ Scratch\ (nm^2)}{Migration\ Time_{\ t=x}} = Migration\ Rate$$

$$x = time\ (hours)\ post\ scratch$$

Area under the curve was calculated for percent scratch closure with the following equation:

$$AUC = \frac{1}{2}\sum_{i=0}^{n-1}(t_{i+1} - t_i)\,(Percent\ Closure_i + Percent\ Closure_{i+1})$$

For percent scratch closure calculations *Percent Closure*$_1$ and *Percent Closure*$_2$ at times t_1 and t_2, the AUC between those two time points is equivalent to the product of difference in time and the average of the two percent scratch closure calculations. Three separate experiments were performed with conditions in duplicate and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

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Results

The data obtained met criteria for a valid assay and the positive and negative controls performed as anticipated. Fibroblasts treated with 0.01% **ACB Botanical Sugar Complex** increased cell migration and wound healing compared to the negative control (serum-free complete media).

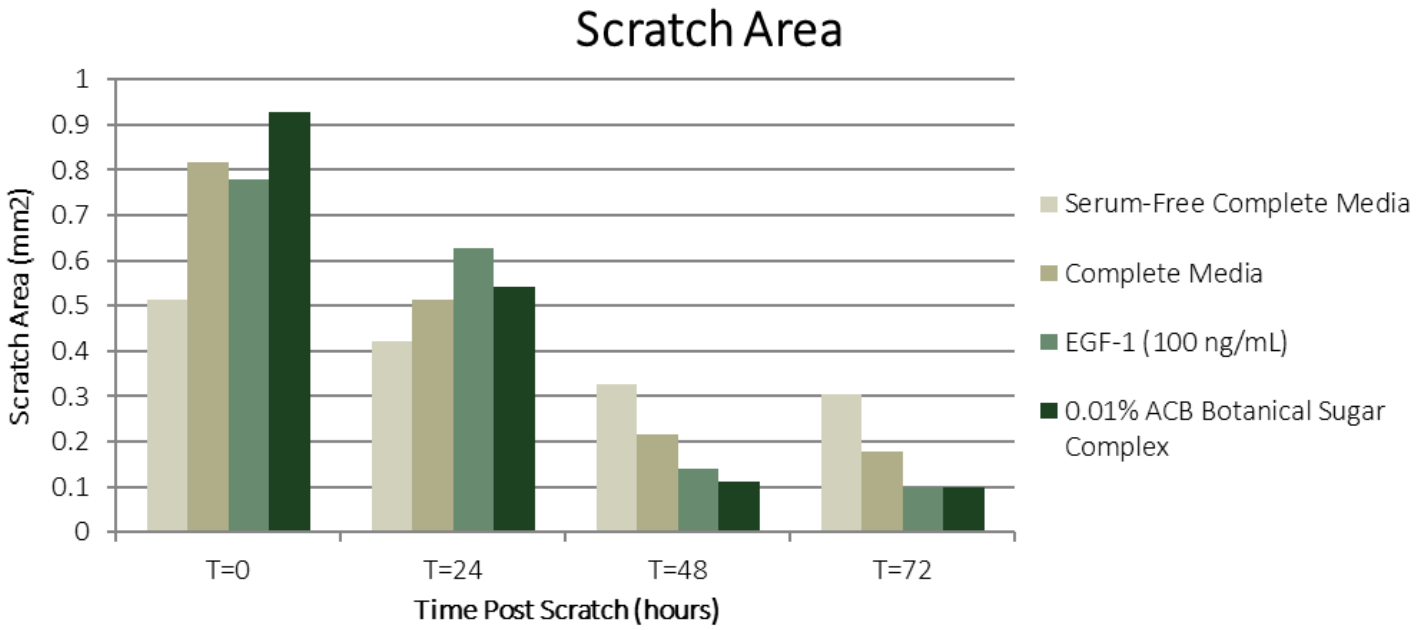

Figure 1. Effect of **ACB Botanical Sugar Complex** on the Area of Scratch over time.

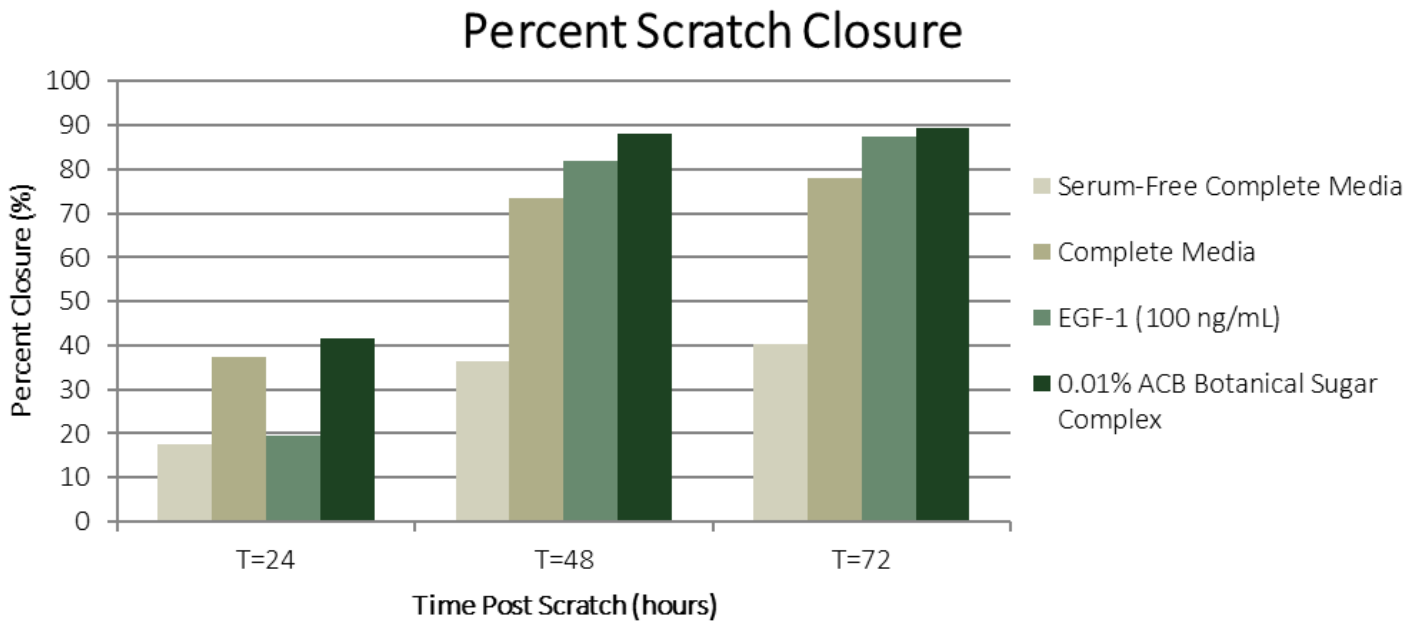

Figure 2. Effect of **ACB Botanical Sugar Complex** on the Percent of Scratch Closure over time.

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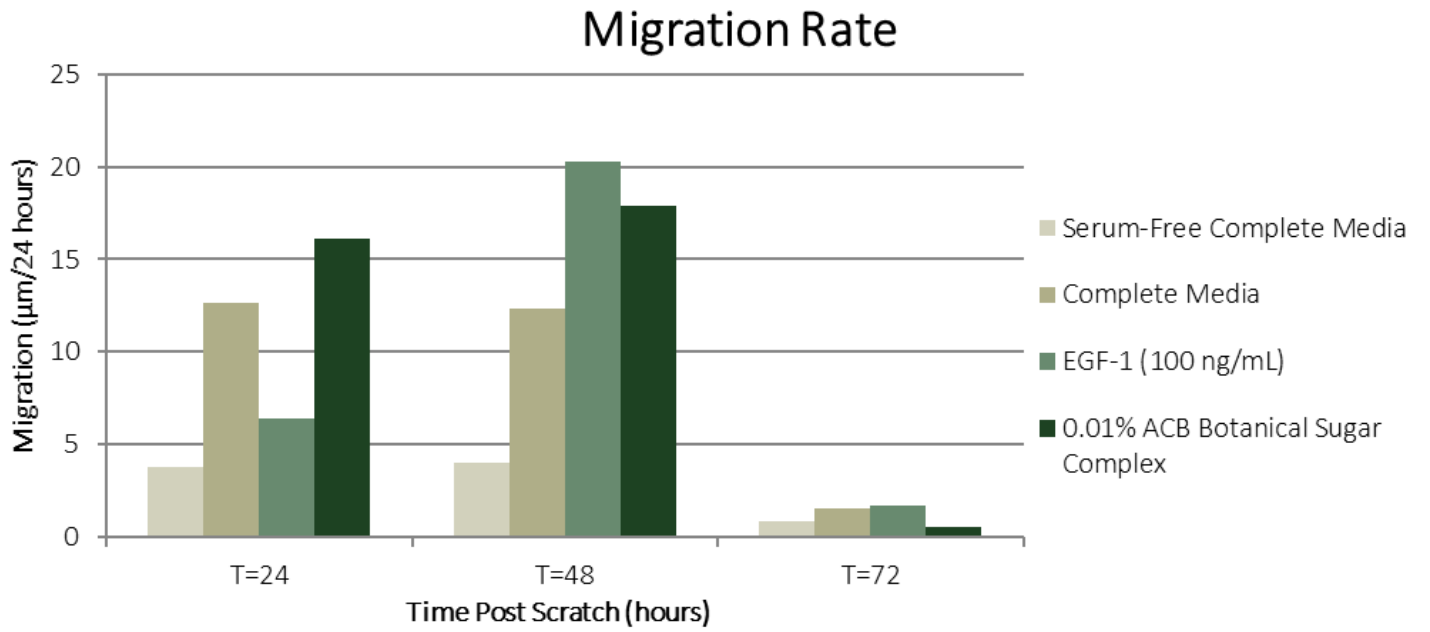

Figure 3. Effect of **ACB Botanical Sugar Complex** on the Rate of Migration.

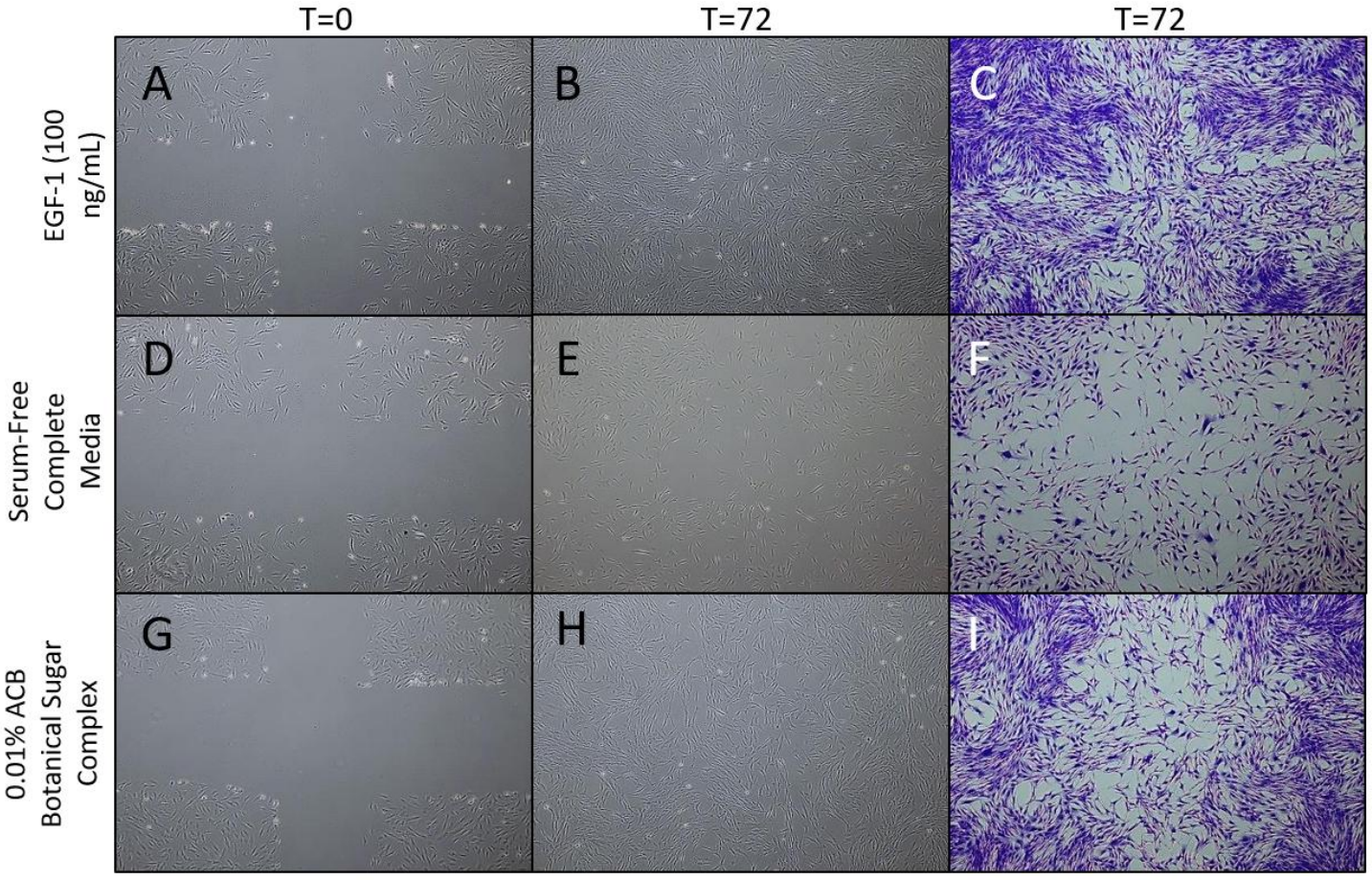

Figure 4. Images of EGF-1 (positive control), Serum Free Complete Media (negative control), and 0.01% **ACB Botanical Sugar Complex** at T=0 (A, D, G), T=72 (B, E, H), and stained at T=72 (C, F, I).

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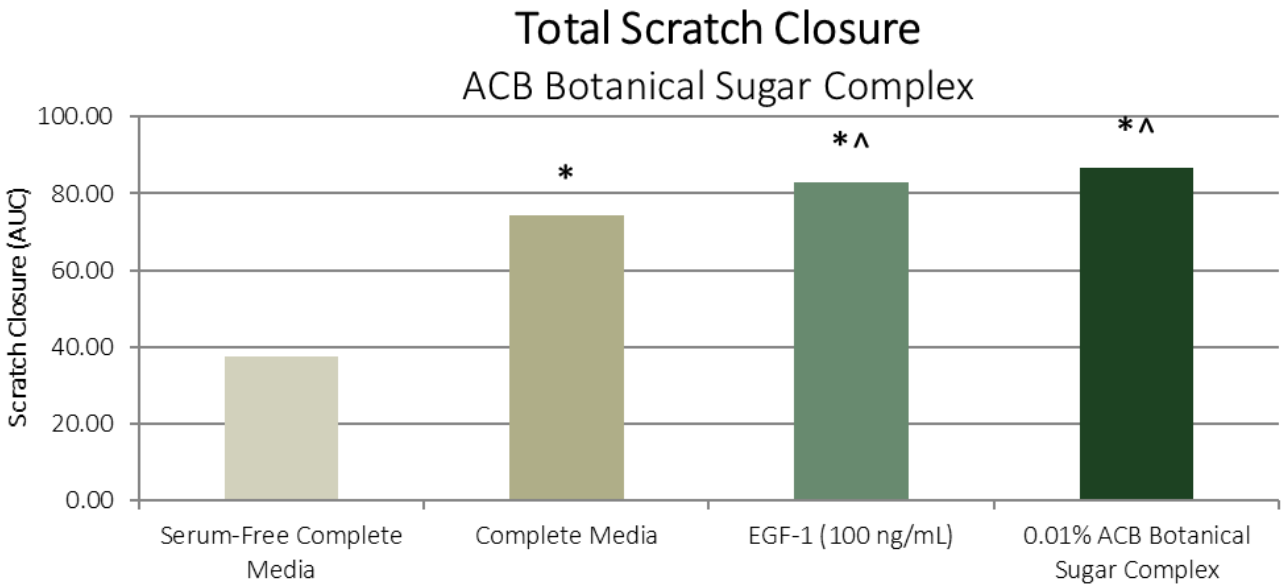

Figure 5. Area Under the Curve (AUC) of Percent Scratch Closure After 72 Hours of Treatment. * indicates significance ($p \leq 0.05$) compared to Serum Free Media. ^ indicates significance ($p \leq 0.05$) compared to Complete Media.

Table 1. Results from one-way ANOVA Statistical Analysis of Total Percent Scratch Closure (Percent AUC). Results represent p-values between the two conditions compared. * indicates significance ($p \leq 0.05$) compared to Serum Free Media. ^ indicates significance ($p \leq 0.05$) compared to Complete Media.

	Serum-Free Complete Media	Complete Media	EGF-1 (100 ng/mL)	0.01% **ACB Botanical Sugar Complex**
Serum-Free Complete Media	-------	< 0.05*	< 0.05*	< 0.05*
Complete Media	< 0.05*	-------	< 0.05^	< 0.05^
EGF-1 (100 ng/mL)	< 0.05*	< 0.05^	-------	> 0.05
0.01% **ACB Botanical Sugar Complex**	< 0.05*	< 0.05^	> 0.05	-------

Discussion

As shown in Figures 1-5, fibroblasts incubated with EGF-1, a molecule known to augment cellular migration, enhanced wound healing compared to untreated fibroblasts. Conversely, fibroblasts exposed to serum-free complete media, the negative control, elicited a reduction in cell migration compared to untreated fibroblasts. These data demonstrate cellular migration in fibroblasts is dynamic and can be manipulated with exogenous substances.

Similarly, fibroblasts treated with **0.1% ACB Botanical Sugar Complex** increased cell migration and closed the scratch at a rate comparable to the positive control (EGF-1) and greater than untreated fibroblasts (Table 1). These data demonstrate **ACB Botanical Sugar Complex** activates cellular migration and wound healing in fibroblasts.

Collectively, the mechanisms of *in vitro* scratch closure mimic the mechanisms seen in *in vivo* wound healing. These data indicate **ACB Botanical Sugar Complex** has wound healing properties and triggers cellular migration, which may assist in the wound healing process.