Scratch Assay

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Tradename: AC ExoRestore

Code: 60195

CAS #: 7732-18-5 & 93333-80-3 & 123465-35-0 (or) 8002-43-5

Test Request Form #: 10229

Lot #: N230727C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigator: *Hannah Duckett*

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 **AC ExoRestore** 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)
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), and a 0.01% concentration of **AC ExoRestore** were diluted with serum-free complete media. Serum-free complete media was used 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 48 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:

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

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

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

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Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated. Fibroblasts treated with 0.01% AC ExoRestore increased cell migration and wound healing compared to the negative control (serum-free complete media).

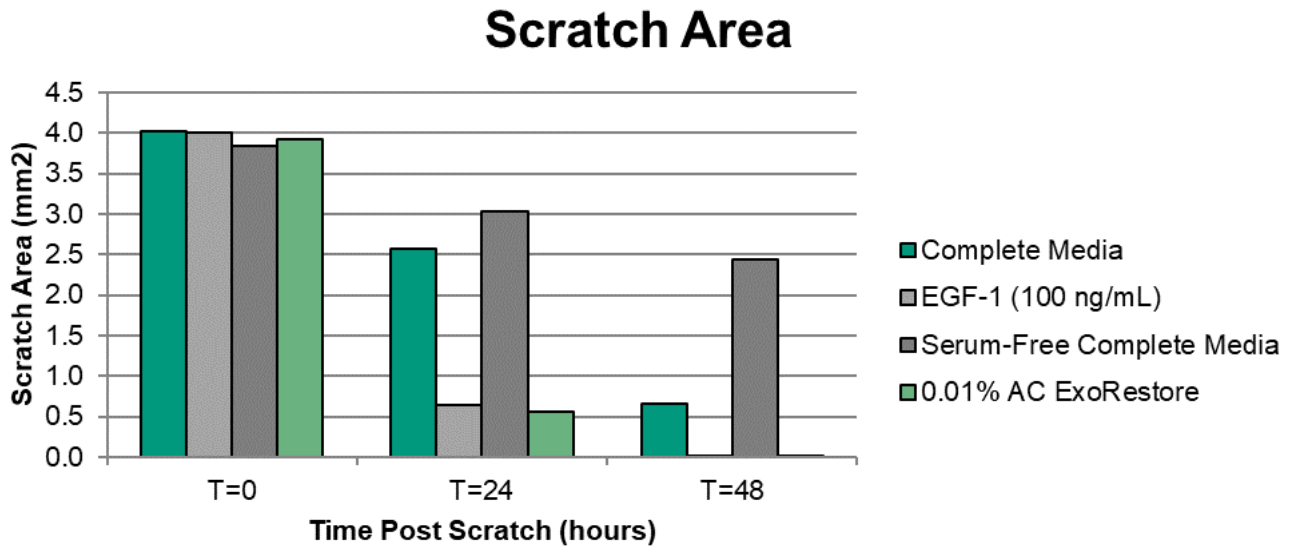

Figure 1. Effect of AC ExoRestore on the Area of Scratch over time.

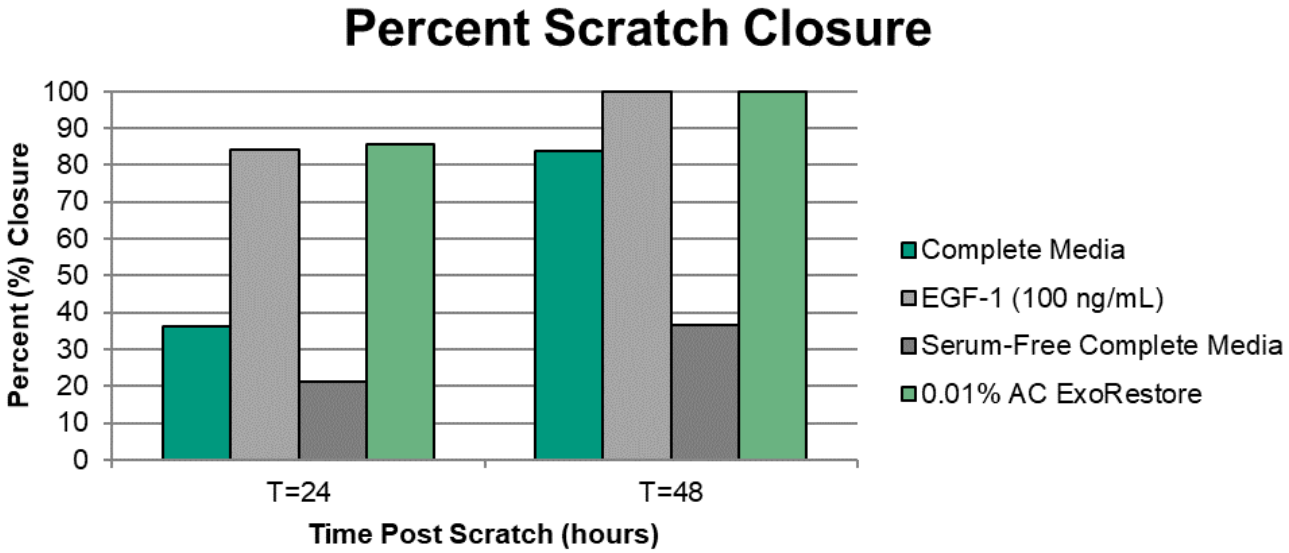

Figure 2. Effect of AC ExoRestore on the Percent of Scratch Closure over time.

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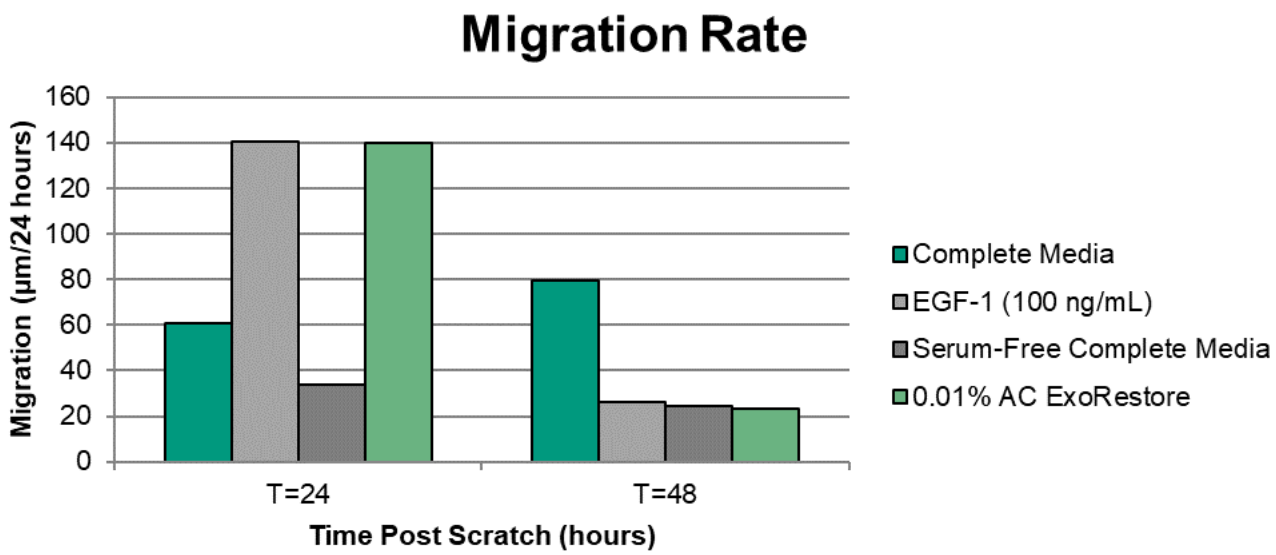

Figure 3. Effect of **AC ExoRestore** on the Rate of Migration.

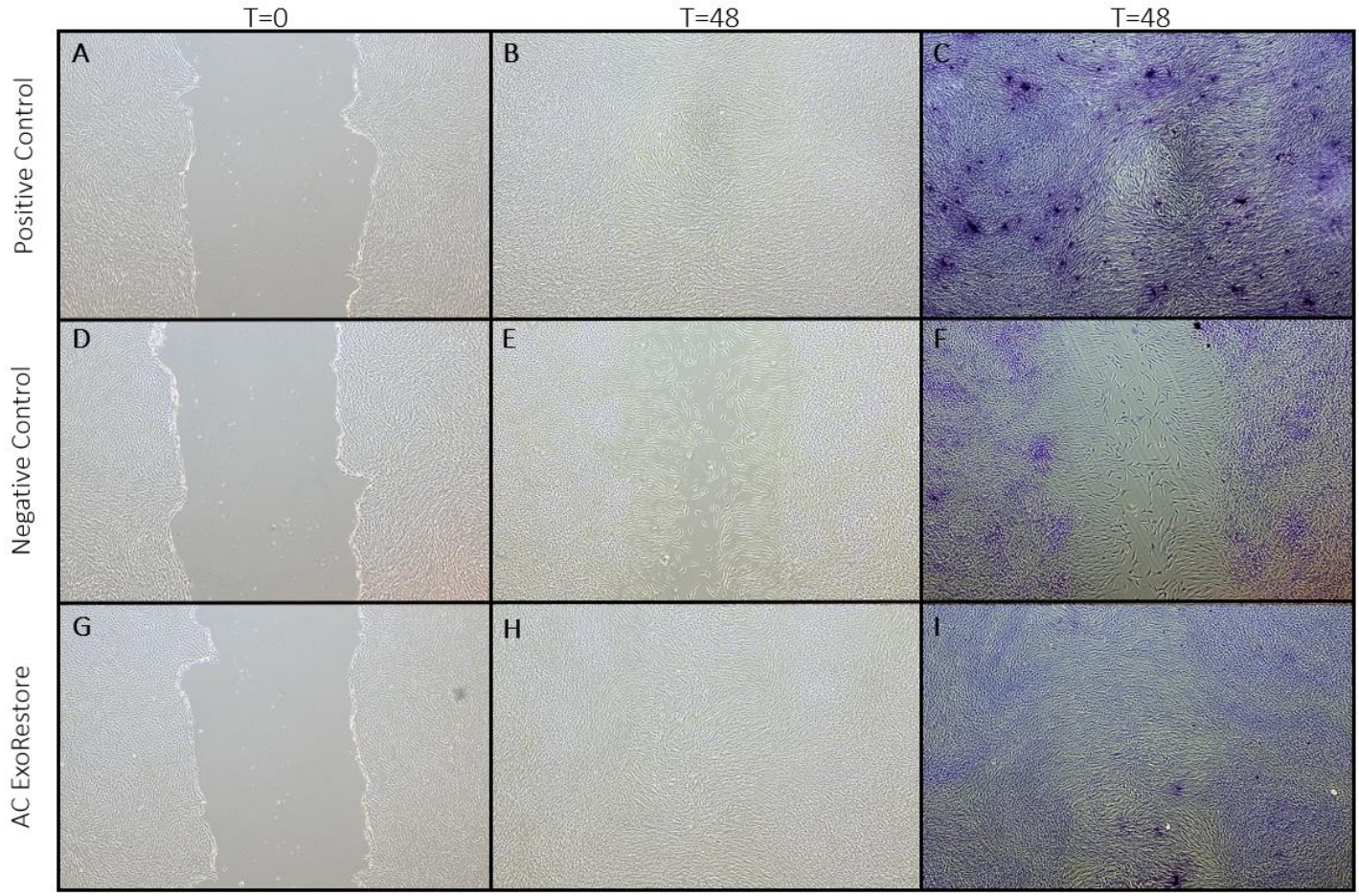

Figure 4. Images of EGF-1 (positive control), Serum Free Complete Media (negative control), and 0.01% **AC ExoRestore** at t=0 (A, D, G) and t=48 (B, E, H). At experiment completion (t=48), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

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Discussion

As shown in Figures 1-4, 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 0.01% AC ExoRestore increased cell migration and closed the scratch at a rate comparable to the positive control (EGF-1) and greater than untreated fibroblasts. Specifically, after 48 hours, AC ExoRestore achieved 100% wound closure, like the positive control, whereas the Complete Media and Serum-Free Complete Media only closed 84% and 37% wound closure. These data demonstrate AC ExoRestore activates cellular migration and wound healing in fibroblasts at a rate comparable to EGF-1.

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