Active Concepts

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Scratch Assay Analysis

Tradename: AC AmaraSense

Code: 12011

CAS #: 7732-18-5 & 84775-66-6 & 84082-82-6 & 84012-14-6 & & 1686112-36-6 (or) 68333-16-4 (or) 9015-54-7

Test Request Form #: 8732

Lot #: N211119A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Daniel Shill*

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.

The scratch assay was conducted to assess the wound healing properties of **AC AmaraSense** -treated *in vitro* cultured human 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 at 5% CO_2 and 95% Relative Humidity (RH)
B. Equipment:	Forma Humidified Incubator, ESCO Biosafety Laminar Flow Hood, Inverted Microscope; Camera; Pipettes
C. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
D. Media/Buffers:	Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004), Phosphate Buffered Saline (PBS)
E. Reagents:	Epidermal Growth Factor-1 (100ng/mL); Paraformaldehyde (3.7%); Crystal Violet Stain
F. Culture Plate:	Falcon Flat Bottom 6-Well Tissue Culture Treated Plates
G. Other:	Sterile Disposable Pipette Tips; Wash Bottles; 15mL Conical Tubes

Methods

Human dermal fibroblasts were seeded into 6-well tissue culture plates and allowed to grow to confluency in complete serum-free media. A 0.1% concentration of **AC AmaraSense** was added to the culture media and incubated with fibroblasts for the extent of the experiment. Epidermal Growth Factor-1 was utilized as the positive control and complete serum-free media was used a negative control.

When cell growth reached confluency scratches were made across the well in a cross or 'X' pattern. The wells were washed with sterile PBS and fresh media containing **AC AmaraSense** and the controls were added. Initial images were captured immediately after the scratch took place and every 24-hours afterwards, up to 96 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.

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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.

AC AmaraSense at a 0.1% concentration was able to increase cell migration and wound healing compared to our negative control.

Percent scratch closure and migration rate are expressed by the following formula:

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

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

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

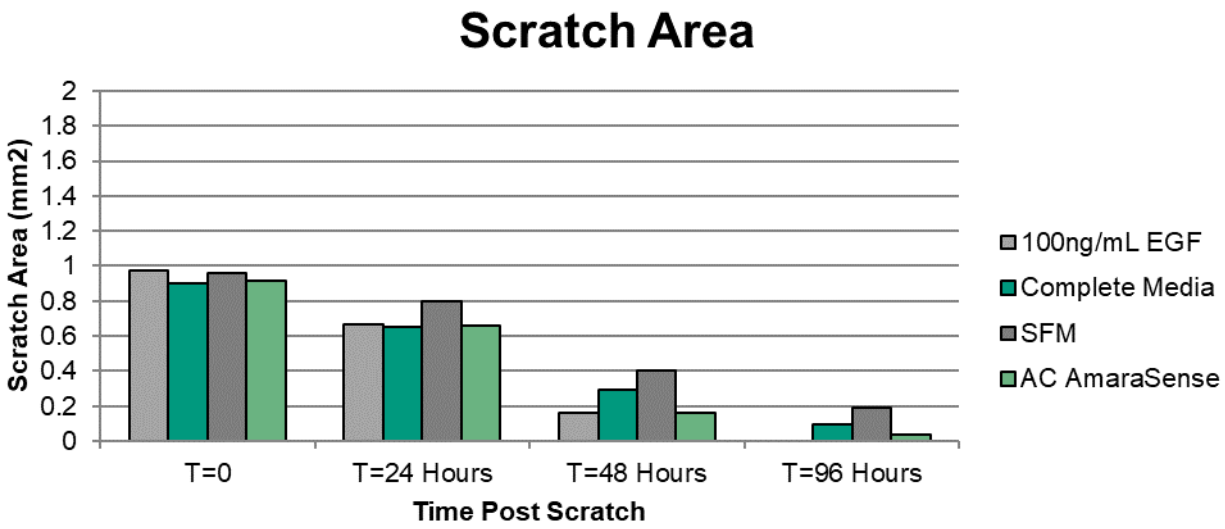

Figure 1: Area of scratch

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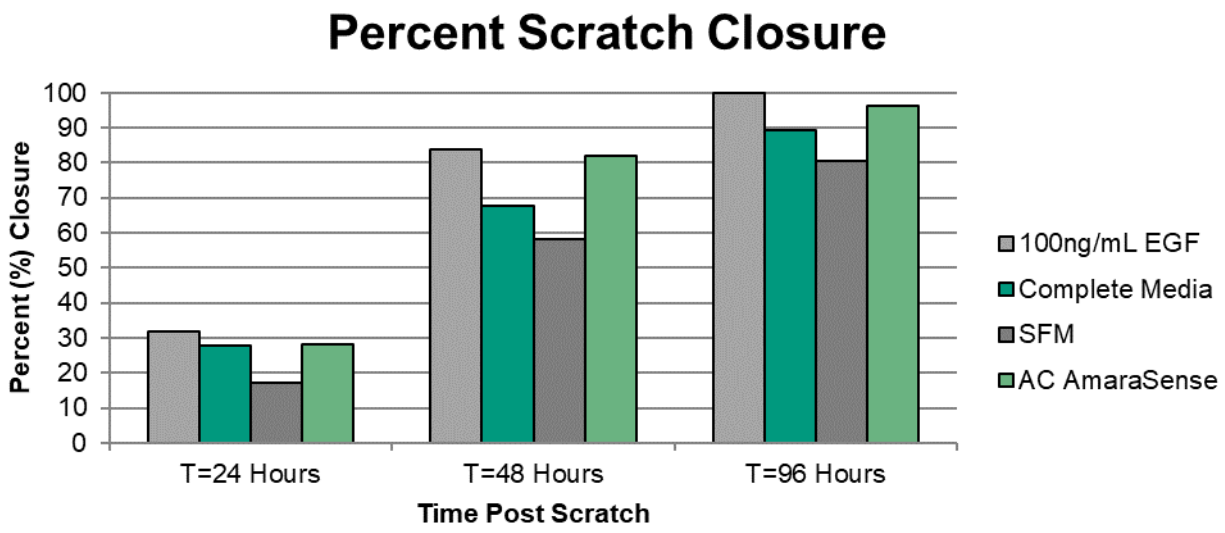

Figure 2: Percent scratch closure

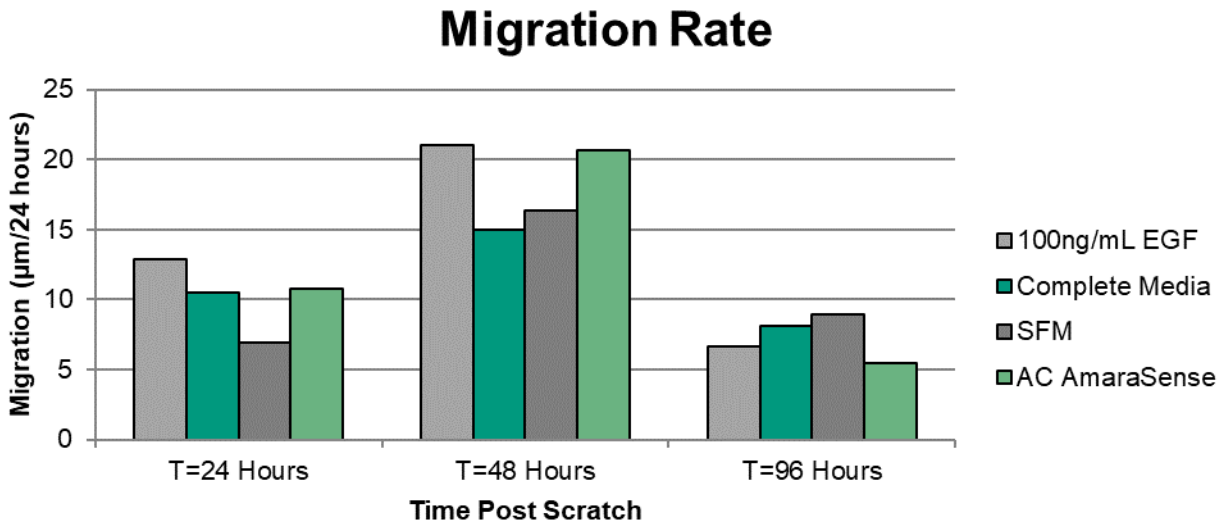

Figure 3: Cell migration rate

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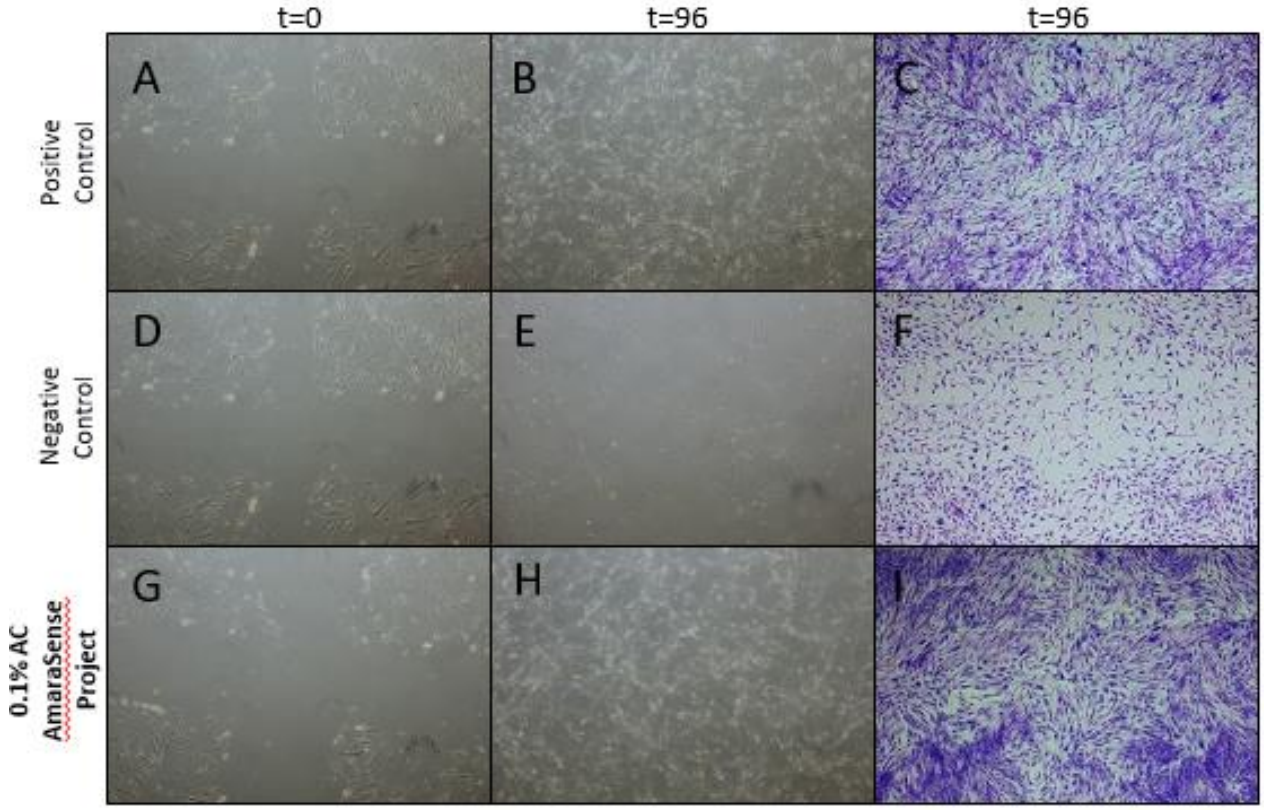

Figure 4: Images at t=0 hours (A, D, G) and t=96 hours (B, E, H) for **AC AmaraSense**, positive control (EGF-1), and negative control (SFM). At experiment completion (t=96 hours), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

Discussion

AC AmaraSense (12011) was able to increase cell migration and close the scratch at a rate comparable to the positive control. The mechanisms of the cells in the *in vitro* scratch assay mimic the mechanisms seen in *in vivo* wound healing therefore we can be assured that our results are translatable outside the laboratory. With the present study we can be confident that this product has healing abilities and cell proliferation properties.