Cellular Proliferation Assay

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Tradename: AcquaSeal® Algae

Code: 20852

CAS #: N/A

Test Request Form #: 3604

Lot #: NC170831-I

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

Test Performed:
In-vitro Keratinocyte Proliferation Assay

Introduction

During aging fibroblast growth (proliferation) rate decreases and this change is implicated in contributing to age related dermal changes. In the skin and in cell culture fibroblast growth is controlled by a host of specific growth factors including fibroblast growth factor and connective tissue growth factor. The purpose of this study was to determine whether or not **AcquaSeal® Algae** is capable of increasing keratinocyte proliferation.

Materials & Methods

In these studies human normal fibroblasts were obtained from an older donor (age 55) and grown in a minimally supplemented medium. Individual test flasks were supplemented with either a positive control FGF (fibroblast growth factor) or the test material **AcquaSeal® Algae** at various concentrations. Growth rates were calculated as the increase in the number of viable cells for a defined period, (twenty-four hours following supplementation), with an image processor based cell counter. Growth of control cultures was normalized to 1.0.

Tissue or cell samples (commercially sourced NHKE, fibroblasts or Skin Ethic synthetic skin model) are removed from the shipping tray and placed into a 6 or 12-well plate containing 2.5 - 5.0 ml of minimal growth medium (37±2°C). They are incubated for at least 24 hours at 37±2°C and 5±1% CO2. After this initial incubation, the growth medium is replaced with 2.5 - 5.0 ml of fresh medium (37±2°C), and 25-50 ul containing, (a) no additional factors as a control, (b) added FGF (Sigma) 1ug/ml (c), Test material (**AcquaSeal® Algae**) at various levels 0.001-0.1 ug/ml of test material or phosphate buffered saline (negative control) and applied directly onto the surface of the tissue. The 6-well plates are then incubated at 37±2°C and 5±1% CO2 for 24 hours. After 24 hours the contents of the individual wells including all tissue are removed, diluted into 5 ml of PBS and placed into glass tubes and cell count is determined with a Countless II FL cell counter.

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Results

As seen in Figure 1 below the positive control, FGF (1ug/ml supplementation), increased proliferation by about 70% after twenty fours. **AcquaSeal® Algae** also showed a positive effect on growth ranging from 13-56% concentration dependent.

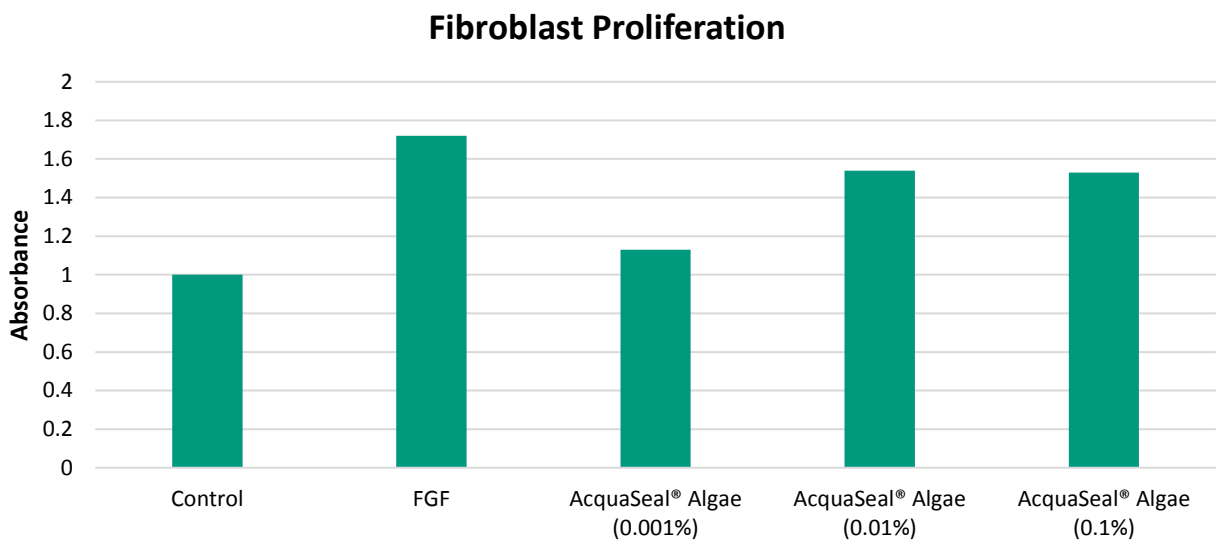

Figure 1. Improvements in fibroblast proliferation.

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