In-vitro Cellular Hydration 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 #: 3606

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 Cellular Hydration Assay

Introduction

An *in-vitro* study was conducted to determine if **AcquaSeal® Algae** could modify the ability of stratum corneum cells, harvested from test subjects via scalpel biopsy, to hold water. Results indicate that **AcquaSeal® Algae** is capable of significantly increasing water uptake compared to the control.

Materials & Methods

Harvesting of Cells

Superficial stratum corneum cells were isolated from the lower leg of 10 test subjects, age 60 or greater via scalpel shave biopsies. All subjects had dry and scaly, whitish looking skin prior to treatment. All treatments were done *in vivo* so the cells were harvested after subjects had been treated to reflect real in use conditions. No treatments were applied after the cells had been isolated.

Samples were taken before treatment started and after two weeks of treatment with 2.0% **AcquaSeal® Algae** twice per day approximately 3mg/ cm2 or a placebo. Sufficient squame samples were taken from each subject (about 50-100 mg) for accurate measurement on a Mettler Microbalance with a sensitivity of 0.01mg. The samples were pooled and thoroughly mixed to factor out differences between subjects, so all starting materials were identical. Samples were visual inspected with a microscope and generally were clumps of perhaps tens to hundreds of cells. Both untreated and treated samples look similar with respect to clump size under the microscope.

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

Sample Hydration

Cell samples (about 30-60 mg per sample) were equilibrated at 0% humidity (dry control) for twenty four hours, or soaked in water with mixing to ensure maximal hydration and equilibrated at 100% humidity (fully hydrated state). After equilibration and blotting off excess water, samples were weighed to determine water content. Water content was determined as dry weight subtracted from wet weight at various times.

Two different tests were conducted and treated and untreated samples compared. First, maximal water uptake (the % water cells were able to take up at 100% humidity) was determined. Second, the rate of loss of water when cells were transferred from 100% to 0% humidity was also assessed.

Placebo Composition Formulation

Phase A	
Deionized Water	62.600%
Magnesium Aluminum Silicate	0.400%
Xanthan Gum	0.150%
Acrylates/C10-30 AlkylAcrylate Crosspolymer	0.750%
Phase B	
Butylene Glycol	4.000%
Disodium EDTA	0.050%
Phase C	
Caprylic/Capric Triglyceride	8.500%
Octyl Palmitate	4.000%
Cetearyl Alcohol	2.000%
PEG-8 Stearate	1.000%
PEG-100 Stearate	0.800%
Phase D	
Triethanolamine 99%	0.100%
Phase E	
Water	14.000%
Phenoxyethanol	0.500%
Potassium Sorbate	0.100%
Methylisothiazolinone	0.050%
Butylene glycol	1.000%

Results

Maximal Water Uptake

Results are as described in the following Table 1. As can be seen below, the cells isolated from subjects treated with 2.0% **AcquaSeal® Algae** *in vivo* for two weeks, had an increased capacity for water uptake compared to the vehicle treated control. While control cells when fully hydrated, adsorbed about 4.7 mg of water, the cells from **AcquaSeal® Algae**-treated subjects absorbed about 80% more water 8.5 mg. This represents a significant increase in water holding capacity.

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

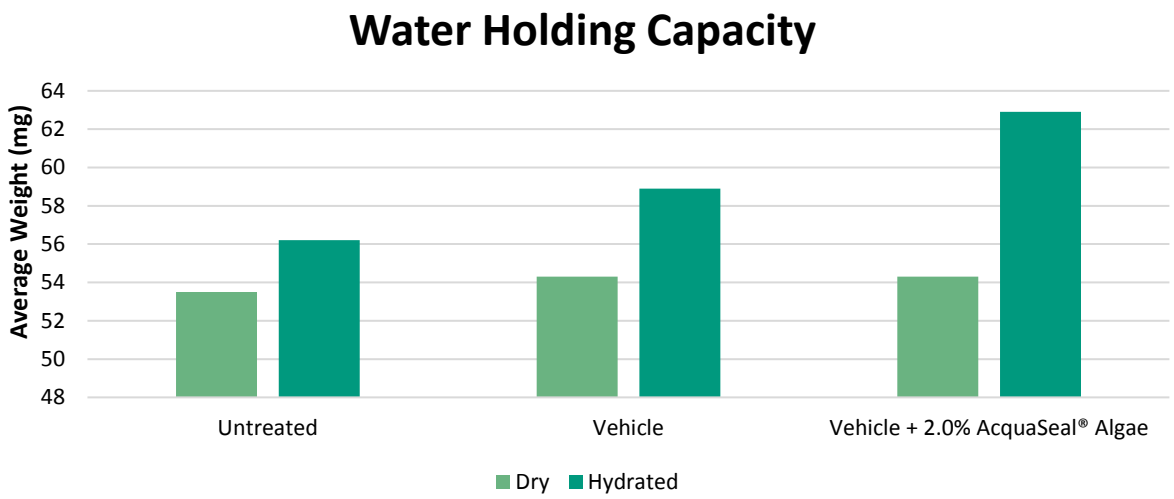

Figure 1. Average weight of skin cells for water holding capacity.

Cellular Water Loss Rates

Afterwards, the fully hydrated cell samples from the above experiment were relocated to an isolated chamber at a humidity of less than 5% and the weight of the samples was monitored over time. The total amount of water adsorbed by the cells and the rate of water loss would be indicative of the ability of the collected cells to maintain moisture. As can be seen below for both the untreated and the vehicle, more than 50% of the gained water was lost within the first 5 minutes. In contrast, the cells from the 2.0% **AcquaSeal® Algae** treated subjects only lost about 20% over that same time period. After two hours (test completion) both untreated and vehicle controls had lost all absorbed water while the 2.0% **AcquaSeal® Algae** samples still retained about 30% of the adsorbed water.

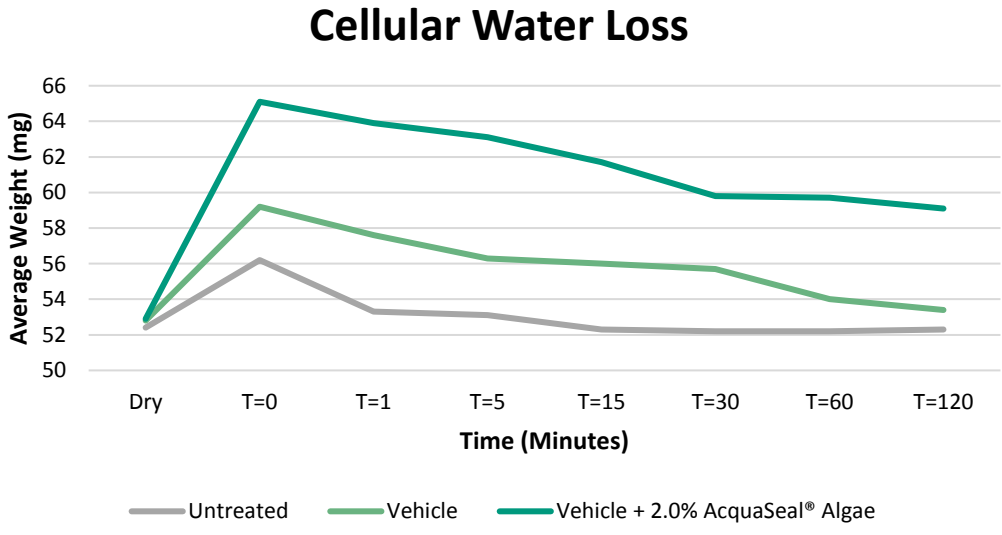

Figure 2. Average weight of skin cells for cellular water loss.

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

Discussion

AcquaSeal® Algae modifed the ability of stratum corneum cells to hold water, increasing both the maximal percent adsorbed, and reducing the rate of loss when cells were placed under low humidity conditions compared to untreated and a vehicle control (placebo). Since these tests were conducted on isolated cells and not structured layers of skin, the results reflect in a large part, the actual ability of the cells to hold water as opposed to an intact skin structure. This data indicates that **AcquaSeal® Algae** is capable of significantly increasing water uptake compared to the control.