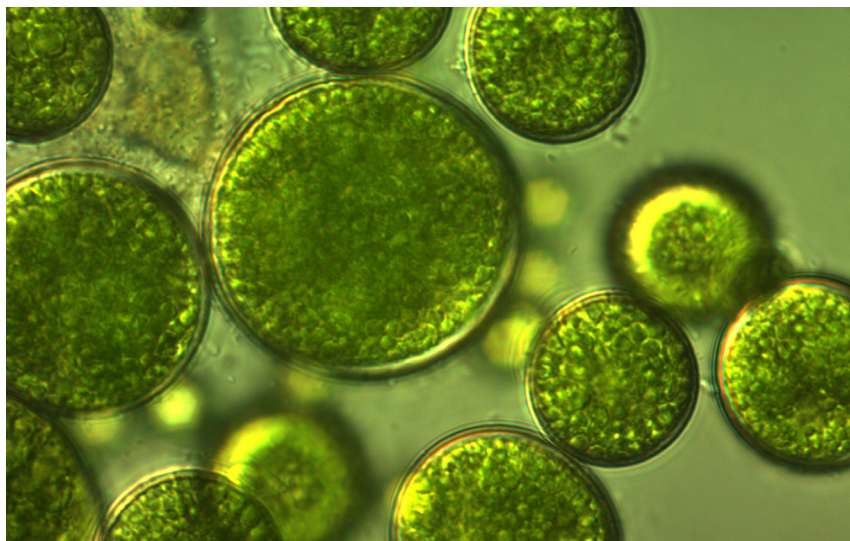


AcquaSeal® Algae



restorative
sustainable
marine trend
promote youthful looking skin
hydrating
oil soluble, moisturizing, protective
soothing, anti-inflammation

BACKGROUND

AcquaSeal® Algae is an oil soluble active designed to promote youthful skin, through several different mechanisms such as reducing ‘inflammaging’ and increasing cellular proliferation. Derived from *Chlamydomonas reinhardtii*, we use a proprietary technology to isolate and extract lipid fractions from this sustainable green algae source. Green algae inhabits a broad range of extreme habitats including fresh water, seawater, damp soil, and even snow. As a result of thriving in such diverse and extreme environments, green algae produce an array of unique bioactive complex lipids and fatty acids. The application of such lipids found in **AcquaSeal® Algae** can help to provide cellular renewal, cellular hydration, cellular proliferation, and anti-inflammation benefits to defend skin against the signs of aging.

Personal care and cosmetic ingredients that are sourced from nature, inspired by nature, bio-based, and sustainable are becoming more popular among consumers as natural beauty continues to gain traction in the global market. Active Concepts’ focus on sustainable technology and green chemistry piqued our interest in algae derivatives as a sustainable and natural-based source of cosmetic benefits. Green algae have a great potential to generate oils and other valuable byproducts in a sustainable fashion, that can be used in cosmetics to endow the dermis with restorative and protective properties, plumping the skin by rehydrating skin cells.

AcquaSeal® Algae contains algal lipid fractions derived from the green algae *Chlamydomonas reinhardtii*. These green algae when grown under defined high UV conditions produce a very high number of chloroplasts. After cell disruption, the chloroplasts are concentrated via density gradient separation. With proprietary technology, lipid fractions from the chloroplasts are isolated, providing cellular renewal, cellular hydration, cellular proliferation, and anti-inflammation benefits to defend against aging skin.

Code Number: 20852

INCI Name: Chlamydomonas Reinhardtii Extract

INCI Status: Conforms

REACH Status: Complies

CAS Number: N/A

EINECS Number: N/A

Origin: Plant

Processing:

GMO Free

No Ethoxylation

No Irradiation

No Sulphonation

Additives:

Preservatives: None

Antioxidants: None

Other additives: None

Solvents Used: N/A

Appearance: Semi-Solid Wax

Soluble/ Miscible: Oil Soluble

Microbial Count: <100 CFU/g,
No Pathogens

Suggested Use Levels: 1.0 – 10.0%

Suggested Applications:

Hydrating, Cellular Renewal, Cellular Proliferation, Reduce Water Loss

Benefits of **AcquaSeal® Algae**:

- Defends Against Aging Skin
- Sustainable
- Moisturizing

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SCIENCE

Skin aging is a complicated process. The aging we observe as individuals results from changes within the skin at the molecular, cellular and macroscopic level. The rate at which our appearance changes (deteriorates) is due to genetic factors, environmental factors, life style choices and of course time itself.

A youthful and healthy appearance is the goal of almost everyone. To achieve this goal requires an effort. Cosmetic products can both reverse and prevent some of the age associated changes in appearance. These "corrections" require intervention in molecular and cellular processes which have been changed over time. A greater understanding of how the skin functions has given the cosmetic scientist a greater arsenal of tools whereby to correct some of the changes that result in aged skin. Over time, and with UV damage and other external traumas, skin biology becomes reprogrammed. Changes from oxidative damage and DNA replication errors result in an altered cell metabolism. To effectively erase these errors, corrections must be implemented at the genomic level, the proteomic level, and the metabolomic level.

AcquaSeal® Algae has been designed to address damaged skin and prevent further damage through several specific actions. **AcquaSeal® Algae** is an activator of nuclear transcription factors (RxR and PPAR) resulting in a stimulation of epidermal and dermal cell proliferation. A hallmark sign of aging skin is the reduction cellular reproduction. Slower proliferation results in a slower repair capacity, diminished tissue function, an accumulation of cellular errors and an overall deterioration of skin appearance.

Skin inflammation has been shown to be a major causative agent of skin aging. Daily inflammation from, UV, and pollutants, and "invisible irritation" as a by-product of metabolism causes what has been termed by some, as "Inflamaging." As data will show, skin irritation is reduced dramatically whether induced from a variety of skin assaults. While we have focused on measuring the end results of reducing inflammation, the lipids in **AcquaSeal® Algae** work by reducing NF- κ B activation, as well as TNF- α (a pro-inflammatory cytokine).

BENEFITS

AcquaSeal® Algae is an oil soluble product designed to promote youthful skin, through several different mechanisms. Capable of providing cellular renewal, cellular hydration, cellular proliferation, and anti-inflammation benefits to defend against aging skin, **AcquaSeal® Algae** can be used in a wide variety of cosmetic and personal care applications. This unique ingredient can also be used to capitalize on the trends for sea-inspired cosmetics, while providing a young, refreshed look straight from the sea.

EFFICACY DATA

A series of *in-vitro* and *in-vivo* studies were conducted to evaluate the ability of **AcquaSeal® Algae** to provide active benefits to the skin. 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. Superficial stratum corneum cells were isolated from the lower leg of test subjects (age >60) via superficial scalpel biopsies. All subjects had dry and scaly whitish looking skin. Samples were taken before treatment started and after one week of treatment with 2.0% **AcquaSeal® Algae** in a cosmetic base or the base alone. Products were applied twice per day for the one week test period. Enough squame samples were taken for accurate measurement on a Mettler Microbalance with a sensitivity of 0.01mg. Cell samples (about 50-100 mg) were treated as follows: a) equilibrated at close to 0% (less than 5%) humidity or b) soaked in water with mixing to ensure maximal hydration, briefly blotted, and equilibrated at 100% humidity. After either treatment samples were weighed to determine water content. Initial weighing was done and then continually monitored in a small humidity controlled chamber. Low humidity (less than 5%) was ensured by a constant flux of heated and desiccated dry air passing thru the chamber. The total amount of water capable of being adsorbed by the cells and the rate of water loss would be indicative of the ability of the collected cells to maintain moisture.

As shown in Figure 1, 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.

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Water Holding Capacity

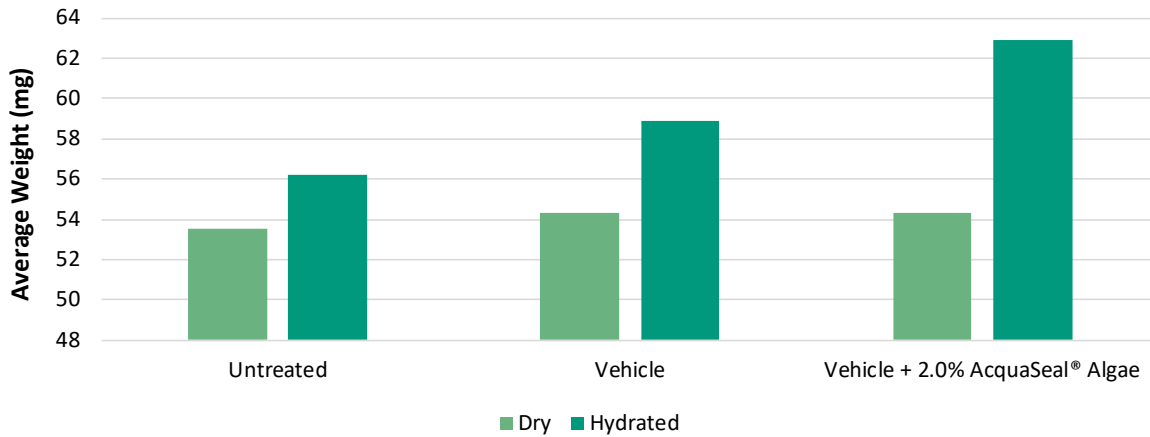


Figure 1. Average weight of skin cells for water holding capacity.

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, both untreated and vehicle controls had lost all adsorbed water, while the **2.0% AcquaSeal® Algae** treated samples retained about 30% of the adsorbed water.

Water Loss by Weight

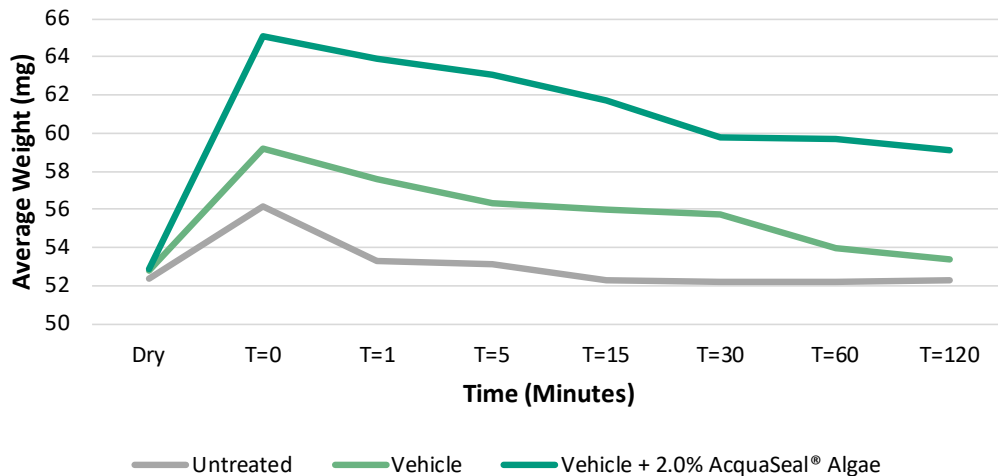


Figure 2. Average weight of skin cells for cellular water loss.

AcquaSeal® Algae modified 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. 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, suggesting **AcquaSeal® Algae** has superior moisturizing ability.

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An *in-vitro* cellular proliferation assay was performed to determine the effects of **AcquaSeal® Algae** on fibroblast cellular proliferation. Fibroblasts are connective tissue cells that secrete collagen, glycoproteins, and other macromolecules for skin health and maintenance. During the aging process, fibroblast proliferation rate decreases and this change is implicated in contributing to age related dermal changes. By increasing fibroblast proliferation, the visible effects of skin aging can be prevented, promoting youthful looking skin. As seen in Figure 3 below, the positive control, Fibroblast Growth Factor (1ug/ml supplementation), increased proliferation by about 70% after twenty fours and **AcquaSeal® Algae** showed a positive effect on fibroblast growth ranging from 13.0-56.0% concentration dependent.

Fibroblast Proliferation

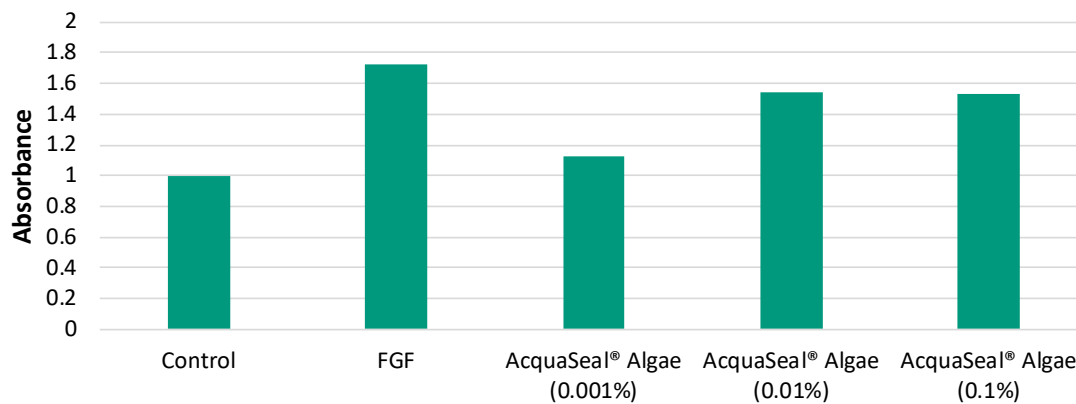


Figure 3. Improvements in fibroblast proliferation.

A Skin Genomics Assay was performed to evaluate mediation of gene expression in cultured keratinocytes or commercial skin equivalents. Both the RxR and PPAR gamma gene code for nuclear regulatory factors and work together in modulating a number of other key genes involved in proliferation and inflammation. **AcquaSeal® Algae** increased expression of both PPAR gamma and RxR, indicating that the use of **AcquaSeal® Algae** would likely result in increased epidermal proliferation. As shown in Figure 4, three genes important in the skin inflammatory process were down-regulated; these include NF-κB, TNFα and COX 2 (cyclooxygenase). CD 44, involved in cell adhesion, are usually down regulated during differentiation was up-regulated, suggesting a shift from differentiation to proliferation.

Expression of Epidermal Genes

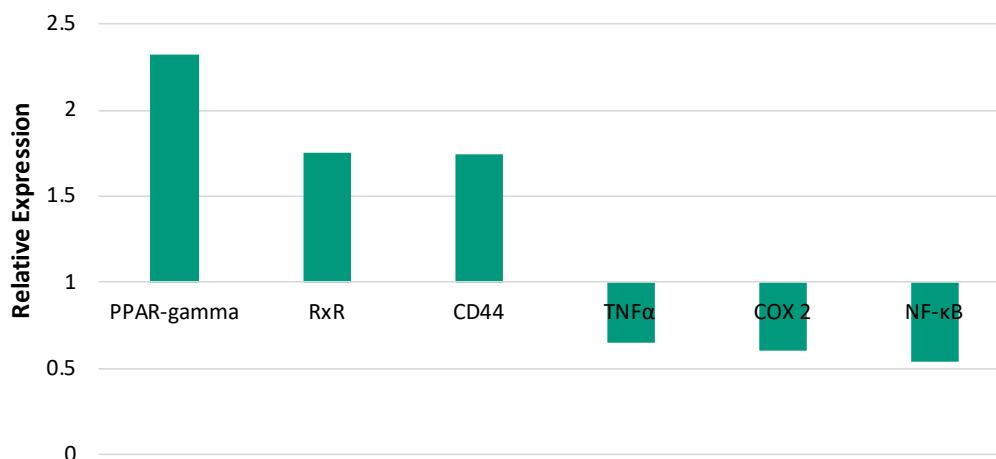


Figure 4. Expression of epidermal genes.

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A Dansyl Chloride assay was performed to determine the ability of **AcquaSeal® Algae** to modify the rate of skin turnover. Skin cell turnover or renewal rates can be easily measured via a dansyl chloride staining method. Transit time varies but is typically 15-20 days for people about twenty-five years old and 25-30 days for people sixty and older. It is believed that this decrease during aging is responsible for some of the age associated changes observed. It is also been demonstrated that increasing rates or reducing turnover times results in a rejuvenation effect to varying degrees. To assess skin turnover time, eight subjects, average age fifty three, were treated with 5.0% Dansyl chloride as per published methods. A second group (10) subjects, average age 27 was treated similarly. To assess effects on turnover time a placebo and 2.0% **AcquaSeal® Algae** were applied to the volar forearm untreated site serving as a control. Removal of the stain, reflecting loss of stratum corneum is monitored daily. As indicated in Figure 5, **AcquaSeal® Algae** reduced turnover time in both age groups. The results indicate that **2.0% AcquaSeal® Algae** is capable of increasing cellular renewal by approximately 20.0% per age group when compared to the untreated control.

Cell Turnover Time

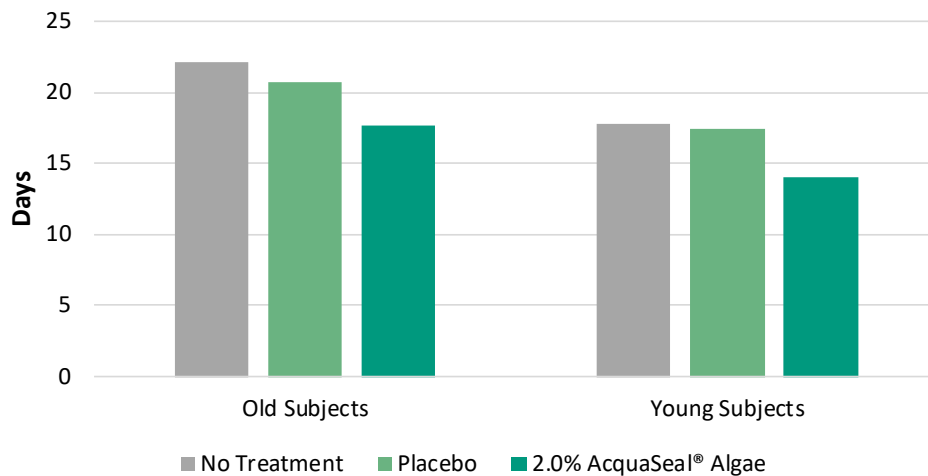


Figure 5. Average number of days for stain removal.

An *in-vivo* immediate and long term analysis was performed to determine the ability of **AcquaSeal® Algae** to modify the skin cosmetic properties after a single use and with repeated use. A single use fifteen subject study evaluated the immediate impact of **AcquaSeal® Algae** on skin properties. **AcquaSeal® Algae** was formulated into a lotion base at 2.0% and applied to the face (one side) of test panelists. To the other side the control lotion was applied. A number of skin parameters were assessed after one hour. Measurements included skin feel, skin friction and skin hydration. As shown in Figure 6, the placebo did not significantly alter skin hydration or feel or hydration characteristics after one hour. **AcquaSeal® Algae** on the other hand, significantly improved skin hydration, reduced skin friction and provided a positive skin feel.

For the long term clinical study, fifteen subjects evaluated the effects of **AcquaSeal® Algae** on skin properties related to cosmetic benefits and skin health over a four week period. Each of the fifteen subjects applied 2.0% **AcquaSeal® Algae** in a base lotion to their half of their face twice-a-day for four weeks. The placebo (base lotion alone) was applied to the other half. Evaluations were made prior to the study start and after four weeks. Summarized below in Figure 7 are the results after one month product usage. **AcquaSeal® Algae** produced considerable positive changes in skin quality after both four weeks. Statistically significant improvements in skin hydration, clarity, lines and wrinkles, firmness, flakiness and overall appearance were observed with continuing use of **AcquaSeal® Algae**. The placebo showed no such changes.

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Relative Change in Skin Properties

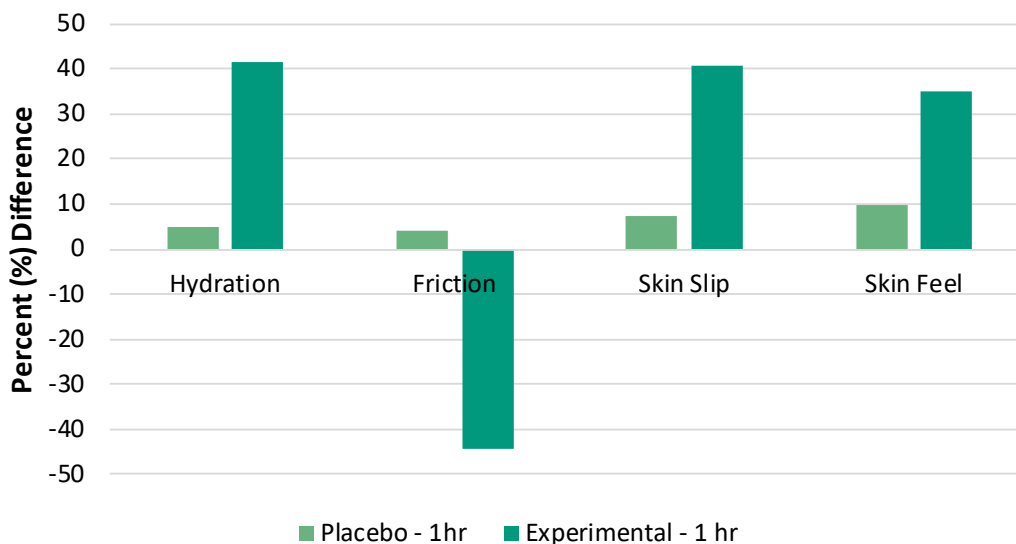


Figure 6. Relative change in skin properties after one hour.

Relative Change in Skin Properties

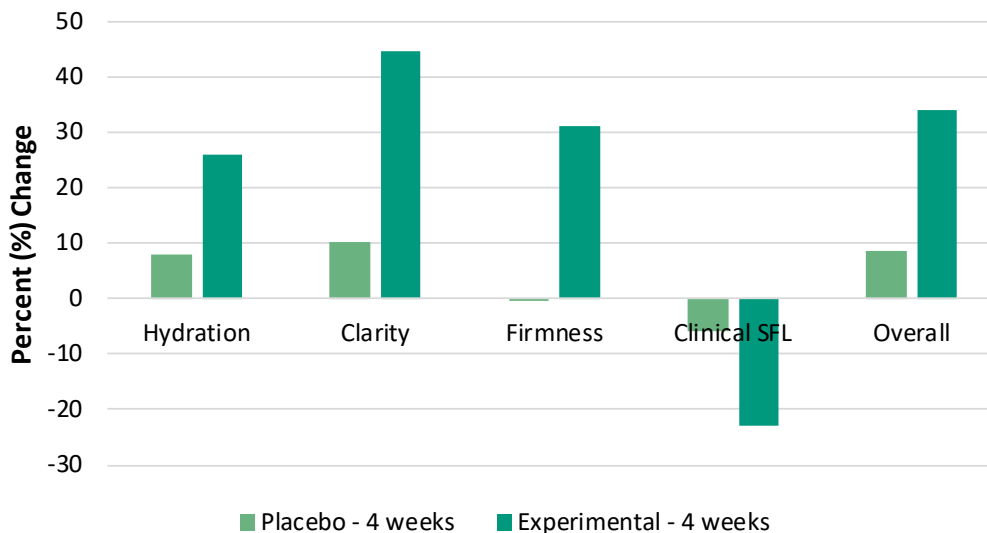


Figure 7. Percent change in skin properties after four weeks.

An *in-vivo* Reduction and Prevention of Skin Irritation assay was performed to determine the ability of **AcquaSeal® Algae** to reduce Balsam of Peru-mediated skin inflammation/irritation. A known contact irritant Balsam of Peru was used to induce skin irritation on the volar forearm: redness, and was evaluated with Minolta Chroma Meter. 2.0% Balsam of Peru was applied to the skin in squalane, for thirty minutes. After application the site was wiped free of excess product and skin irritation was monitored by measuring redness using the (a*) value of the Minolta Chroma Meter. Immediately prior to application, sites were treated with 2.0% **AcquaSeal® Algae** or a control vehicle. In a second test, the forearm was treated for one week (twice a day) with either the control vehicle or 2.0% **AcquaSeal® Algae**. Balsam of Peru induced irritation was evaluated as above.

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As seen below in Figure 8, Balsam of Peru increased skin redness (a*) value dramatically of skin treated simply with the control vehicle. After a single application of the base lotion before application of the irritant (preventative effect), skin redness increased from about 16 to 25, an almost 60% increase in skin redness (a* value). In contrast a single application of 2.0% **AcquaSeal® Algae** in base lotion (before induction of irritation) lessened this increase to 19 (a* units) and increase of only 17% compared to 60%.

Average Skin Redness

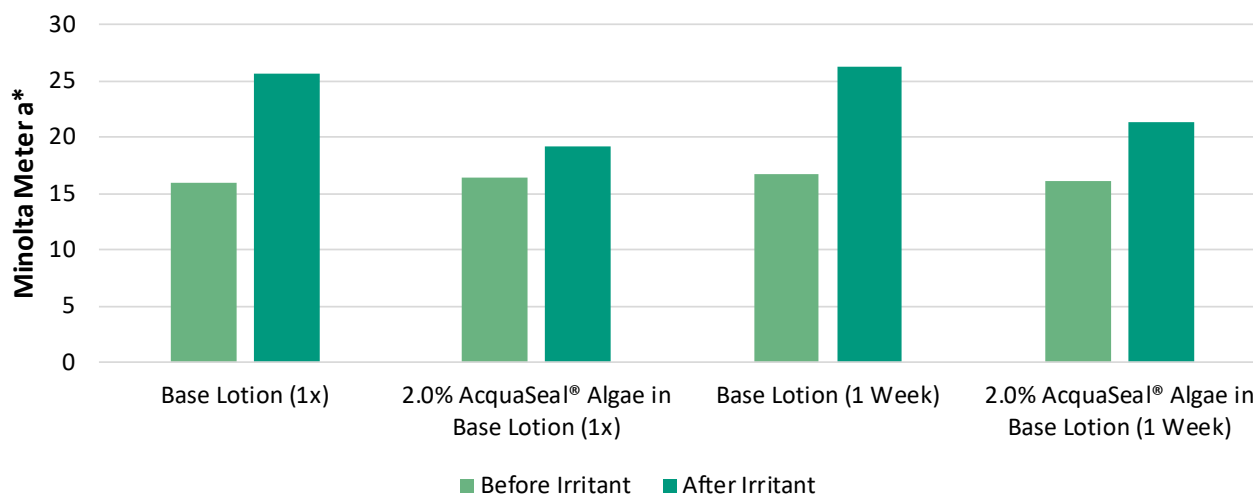


Figure 8. Average skin redness before and after Balsam of Peru application.