The information contained in this technical bulletin is, to the best of our knowledge, true and accurate. No warranty, expressed or implied is made or intended. The use should be based upon the customer’s own investigations and appraisal. No recommendation should be construed as an inducement to use a material in infringement of patents or applicable government regulations.
WHO CREATED THE WORD CORNEOTHERAPY?

- First used by the American dermatologist Pr. Albert Kligman in the mid 1960’s.
- Treatment centred on renovation of the corneal layer to correct the entire skin: “OUTSIDE-IN THERAPY”.

“Long term effects of therapeutic treatment of the corneal layer could assist repair of the underlying structures of the skin such as the epidermis and dermis”.

Dr. Kligman understood that cells could communicate.

Barnet adopted this word and approach in December 2013.
Whether it be from the aging process or some other factor, occasionally the stratum corneum fails in places. When weakened, viruses and bacteria penetrate the skin, and water escapes the skin at a faster rate. Like a dam that's sprung a leak, a failing stratum corneum demands immediate attention.

Corneotherapy aims at the maintenance or the recovery of the stratum corneum to improve the function of the skin barrier homeostasis (balance) of the skin.
However, the undesirable corneocyte was not necessarily seen as a key cell.

No nucleus = no brain!
Today, the star corneocytes are protected and considered for:

- Reducing dullness
- Reducing fine lines
- Reducing visible pores
- Reducing stinginess
- Reducing yellowing
- Reducing dryness
GLM-DS - Time release technology of AHAs to gently remove dead cells. A glycolic/lactic/malic acid in a mineral delivery system.

Furcellaran—To bind water on the skin, to increase lipids and filaggrin in the top layers of the skin, and fence off ROS

AWL Complex — To double water in corneocytes in 2 weeks. An apple/watermelon/lentil extract complex

Silver vine extract—To reduce the grey color of the skin

Ume extract—To reduce the yellow color of the skin

Saccharide Isomerate—To do a perfect differentiation for a resistant stratum corneum to stingers with less visible pores and fence off ROS and bacteria

Lysophosphatidic acid—To improve differentiation with less visible pores for skin using biochemistry

Lavanda oil- To bind corneocytes and to restore lipids
THE REMOVAL OF DEAD CELLS

Alpha Hydroxy Acids in skin care are back. AHAs were one of the earlier and broadly embraced approaches for a visible clinical effect. In the 1990’s though, “irritation potential” was a concern. The use level of AHAs was a numbers game: 5% to 15% or even more. The cosmetic industry moved toward “exfoliation at the skin’s pH” with a glucosamine-based complex.

In 2016, we offer GLM-DS. It is designed to diffuse AHAs in a progressive and controlled way. Exfoliation performance equals conventional AHAs within 2 weeks without any stinging sensation.

A new tool for gently resurfacing the stratum corneum, GLM-DS is a lamellar gel acting as a cutaneous reservoir of AHA.
EFFECT OF 2% GLM-DS ON ACNE SCARS

4 weeks, 24 people

DAY 0

DAY 14

DAY 28

25% less visible scars, 80% less blackheads
GLM-DS (Lamellar Water Gel) was designed to diffuse AHAs in a progressive and controlled way into the skin and to be easy to incorporate into formulations with a non-acid pH.
CONTROLLED DIFFUSION OF AHA

To demonstrate the benefits of Lamellar Water Gel (LWG) technology, we compared its diffusion properties with a conventional Carboxy Methyl Cellulose (CMC) gel.

CMC does not have any special reservoir properties, similar to a formulation containing free AHAs.

The LWG and CMC structures both contain 28% of the same AHAs (same ratios of lactic, glycolic and citric). They were applied to the surface of an agarose gel in which AHA diffusion is measured with infrared spectroscopy. This approach enables the measure of AHA diffusion kinetics.
GLM-DS: CONTROLLED DIFFUSION OF AHA

The AHAs of GLM-DS are released in a more progressive and continuous manner.

We can therefore expect 2 types of benefits:
- Extended contact time giving high performance exfoliation of the skin.
- A more progressive supply of AHA which is therefore less aggressive for the skin.

Kinetics of AHA release (time to reach a plateau):
- 5h 25 min for the CMC technology.
- 9h 12 min for the LWG technology.
PERFORMANCE INDEX (P.I.)
DEFINITION AND CALCULATION METHOD

AHAs are evaluated with two major parameters: exfoliation and inflammation.

The P.I. of an AHA or combination of AHAs is the ratio of its exfoliating performance to the inflammation it generates (1).

A P.I. greater than 1 is characteristic of a good exfoliation with a minimum of inflammation.

It is important to maintain a balance between both effects. A cocktail of AHAs cannot be used for powerful exfoliation if it is going to generate severe irritation or even burns.


<table>
<thead>
<tr>
<th>Creams</th>
<th>pH</th>
<th>% Exfoliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLM-DS 2% (=0.56 AHA)</td>
<td>3.68</td>
<td>+ 50%</td>
</tr>
<tr>
<td>AHA 8%</td>
<td>1.90</td>
<td>+ 59%</td>
</tr>
<tr>
<td>AHA 15%</td>
<td>1.62</td>
<td>+ 75%</td>
</tr>
</tbody>
</table>

Protocol
Human skin explants (39 year old donor). Application of creams at Day 0, Day 1 and Day 2. Analysis of desquaming effectiveness at Day 5 by stripping and counting of scales obtained on the strip.
EVALUATION OF INFLAMMATION LEVEL

The quantification of the enzyme COX2 (green band), responsible for the production of Prostaglandin mediators, is first performed in the epidermis. An additional analysis showed that the 8% and 15% AHA formulations also caused significant inflammation of the dermis, characteristic of an aggressive effect.

While GLM-DS did not generate any significant inflammation, the AHA 8 and 15 formulations activate a significant inflammatory response of more than 60%.

**CREAM GLM-DS 2%**
(0.56% AHA)
Good epidermal cohesion.
15% inflammation

**CREAM AHA-8**
(8% AHA)
Damaged cohesion
68%***inflammation

**CREAM AHA-15**
(15% AHA)
Damaged cohesion
82%***inflammation

*** p<0.001 Student t Test

Protocol
Human skin explants (39 year old donor). Application of creams at D0, D1 and D2 after stripping. Analysis of inflammation-inducing effect by quantification of COX2 (purple-pink) by immunohistochemistry.
EXFOLIATION BENEFIT / INFLAMMATION

EXFOLIATING PERFORMANCE

CREAM
GLM-DS 2% (0.56% AHA)
+50%* of scales

CREAM AHA-8 (8% AHA)
+59% of scales

CREAM AHA-15 (15% AHA)
+75%* of scales

INFLAMMATION LEVEL

15%
Non Aggressive Inflammation

68%***
Aggressive Inflammation

82%***
Aggressive Inflammation

PERFORMANCE INDEX

3.33
0.86
0.91

The P.I. index of GLM-DS is 3 or 4 times higher than the P.I. of AHA formulas.

**p<0.05; ***p<0.001 Student t Test
QUALITY OF THE EXFOLIATION

An aggressive treatment causes deep desquamation and eliminates a clump of epidermal cells. This phenomenon is visible on the strips with the AHA 8 and 15 creams. The exfoliation then damages the cohesion of the skin below the stratum corneum and may be accompanied by burns.

Only GLM-DS provides uniform exfoliation which means its action is more targeted and optimized than the AHA 8 and AHA 15 formulations.

A higher number is a sign of aggressive and anarchic exfoliation.

Protocol
Human skin explants (39 year old donor). Application of preparations at D0, D1 and D2. Analysis of desquamating effectiveness at D5 by stripping and counting of scales obtained on the strip.
TEST ON SENSITIVE SKIN

- Ten volunteers with sensitive skin
- A solution of water with 10% GLM-DS is applied under an occlusive patch for 48 hours.
- Skin is observed 30 minutes and 24 hours after removing the patch.
- The Median Irritation Index (MII) is 0.00 out of 4.00.
INCI Name: Water (and) Glycolic Acid (and) Lactic Acid (and) Sodium Magnesium Silicate (and) Citric Acid (and) Xanthan Gum

REACH: All components are pre-registered or exempt.

Canada: Low volume exemption.

China: All components are listed in the Inventory of Existing Cosmetic Ingredients in China (IECIC).

Use Level: 2%

Sunburn Alert: This product contains an alpha hydroxy acid (AHA) that may increase your skin’s sensitivity to the sun and particularly the possibility of sunburn. Use a sunscreen, wear protective clothing and limit sun exposure while using this product and for a week afterwards.
Application: Topical application of 1.5% Furcellaran for one week on human skin explants.

Explants treated with Furcellaran have an homogenous and smoothed surface with only a few desquaming cells.
HYALURONIC-LIKE ACTION – HYDROSCOPY COMPARATIVE STUDY

Subjects: 5 volunteers

Samples: 100% Furcellaran and 100% Hyaluronic Acid (HA)

Application: Put in cells with a relative humidity that will vary from 0 to 95%

Measurement: For each condition of relative humidity, the polymers capture water molecules to reach a maximum called “gain mass sorption equilibrium”. The value of gain mass reflects the ability of each polymer to trap water molecules.

Polysaccharide obtained via an original depolymerization process. Economic Process.
Results

Furcellaran is able to trap atmospheric water molecules following a kinetic comparable to that of hyaluronic acid.

Furcellaran is able to trap nearly its own weight in water, and to maintain a moisture film on the surface of the skin.

Furcellaran is HA-like.
Furcellaran vs. HA – IN VIVO TEST

Subjects: 30 volunteers

Samples: solution containing 1.5% Furcellaran (sol.)
(equivalent to 0.009% Furcellaran)
solution containing 0.025% Hyaluronic Acid (MW = 1.3 to 1.8 MD)

Application: 15 volunteers apply Furcellaran solution
15 volunteers apply Hyaluronic Acid
Applied twice daily for 2 weeks
Skin hydration level measured using corneometer

HA is a high molecular weight: 2,000,000 D
Furcellaran is 200,000 D
Furcellaran increases the skin hydration level 4 hours after only a single application. Its hydrating action is faster and more efficient than Hyaluronic Acid.
FURCELLARAN AND STRATUM CORNEUM STRENGTH

Effect of Furcellaran on Gene Expression of Proteins Involved in Corneocyte Formation, Adhesion and Integrity

- **Corneodesmosin (CDSN)** – adhesion protein of the extracellular portion of corneodesmosomes, involved in the cohesion of corneocytes
- **Calmodulin-like 5 (CALML5)** – forming part of the family of calcium-binding protein, a key enzyme in the terminal differentiation of keratinocytes
- **Cornuline (CRNN)** – protein which plays a role in epidermal differentiation
- **Small proline-rich protein 2A (SPRR2A)** – protein important for the barrier function of the epidermis
- **Loricrine (LOR)** – protein which plays an important role in the structure of corneocytes in cornified envelope and junctions between corneocytes formation

**SHORT TERM EFFECT**

Furcellaran increases by 41% the expression of CDSN after one application (24H). This protein is located at the superficial layer of the epidermis.

Furcellaran increases the expression of CALM5, CRNN, SPRR2A and LOR by respectively 86%, 54%, 48% and 69% after 3 applications (72H). These proteins are located deeper.

**LONG TERM EFFECT**
<table>
<thead>
<tr>
<th><strong>Primary Use:</strong></th>
<th>Moisturizer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INCI Name:</strong></td>
<td>Water (and) Sodium Carrageenan (and) Sea Salt</td>
</tr>
<tr>
<td><strong>REACH:</strong></td>
<td>All components are pre-registered or exempt.</td>
</tr>
<tr>
<td><strong>Canada DSL:</strong></td>
<td>All components are listed or sold in commerce</td>
</tr>
<tr>
<td><strong>China Registration:</strong></td>
<td>All components are listed on the Inventory of Existing Cosmetic Ingredients in China (IECIC).</td>
</tr>
<tr>
<td><strong>Suggested Use Level:</strong></td>
<td>1% - 3%</td>
</tr>
</tbody>
</table>
Before AWL Complex  

After 2 hours, with 3% AWL Complex: 
Fine lines are significantly diminished.
AWL Complex is a natural complex combining watermelon rind extract, Lens Esculenta (Lentil) Fruit Extract and unripened apple and apple skin in an optimized delivery system.

Watermelon rind is one of nature’s few materials to contain citrulline. Citrulline is essential to the functioning of filaggrin which forms a critical part of the skin’s own water based moisturizing complex.

The lentil extract contains vitamin B5 and trisaccharides.

The apple starch is a source of polysaccharides, sodium lactate and sodium PCA.
AWL COMPLEX– IMMEDIATE SKIN HYDRATION (15 MIN.)

AWL Complex is shown to hydrate the skin after 15 minutes in a dose dependent manner.

Protocol
A gel containing 3% AWL Complex was applied to the skin. Measurements taken at various time periods.
AWL COMPLEX – SKIN HYDRATION (24 HOURS)
SINGLE APPLICATION

AWL Complex continues to hydrate the skin after 24 hours.

Protocol
A gel containing 3% AWL Complex was applied to the skin. Measurements taken at various time periods.
After 2 weeks of AWL Complex use at 3%, cells were recovered by biopsies and weighed. There was 85% more water in cells treated by AWL Complex than those not treated. The cells were then applied onto paper to see how long it would take to lose 90% of water.

Control | 30 Minutes
AWL Complex Treated | 2 Hours X4

It was more difficult for cells treated with AWL Complex to lose their water. It took 4 times longer than the control cells.

Protocol
20 subjects, 60 or older with dry and scaly, whitish looking skin used 3% AWL Complex twice daily for two weeks on legs. Cells were recovered from shave biopsies.
AWL COMPLEX – MOISTURIZING FOR 10 DAYS

Dermatologist Assessment of Dryness on Face

1 - Slight Flaking
2 – Moderate Flaking
3 – Marked Scaling
4 – Severe Scaling

*Data presented are averages of at least 20 subjects and based on a 0-4 point scale described above. Data highlighted in yellow are statistically significant

<table>
<thead>
<tr>
<th>Test material</th>
<th>Conc</th>
<th>BL</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo gel</td>
<td>0%</td>
<td>2.7</td>
<td>2.74</td>
<td>2.71</td>
<td>2.6</td>
<td>2.77</td>
<td>2.66</td>
</tr>
<tr>
<td>AWL Complex</td>
<td>3%</td>
<td>2.74</td>
<td>2.42</td>
<td>2.37</td>
<td>1.75</td>
<td>1.4</td>
<td>1.05</td>
</tr>
<tr>
<td>% Improvement</td>
<td></td>
<td></td>
<td>12%</td>
<td>13%</td>
<td>35%</td>
<td>50%</td>
<td>61%</td>
</tr>
</tbody>
</table>

Results show a complete reduction of excessive scaling in 10 days, while the placebo has no effect.
AWL COMPLEX – REDUCING IRRITATION FOR 10 DAYS

Dermatologist Assessment of Redness, Skin Irritation on Face

1 - Minimal
2 – Moderate
3 – Severe
4 – Fiery Red

*Data presented are averages of at least 20 subjects and based on a 0-4 point scale described above. Data highlighted in yellow are statistically significant

<table>
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<tr>
<th>Test material</th>
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<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo gel</td>
<td>0%</td>
<td>1.8</td>
<td>1.78</td>
<td>1.82</td>
<td>1.87</td>
<td>1.92</td>
<td>1.89</td>
</tr>
<tr>
<td>AWL Complex</td>
<td>3%</td>
<td>1.82</td>
<td>1.7</td>
<td>1.64</td>
<td>1.58</td>
<td>1.47</td>
<td>1.32</td>
</tr>
<tr>
<td>% Improvement</td>
<td></td>
<td></td>
<td>5%</td>
<td>10%</td>
<td>16%</td>
<td>25%</td>
<td>30%</td>
</tr>
</tbody>
</table>

Results show a gradual improvement during the 10 days of treatment with AWL Complex, while the placebo has no effect.
Before AWL Complex

Cells are not cohesive, they are easy to remove.

After 2 weeks with AWL Complex

Tape strips fewer cells as they are more cohesive.
AWL COMPLEX

INCI Name: Water (and) Glycerin (and) Citrullus lanatus (Watermelon) Fruit Extract (and) Pyrus malus (Apple) Fruit Extract (and) Lens Esculenta (Lentil) Fruit Extract (and) Sodium PCA (and) Sodium Lactate

REACH Status: Low volume exemption

Canada DSL: Components are listed on the DSL, RICL or exempt

ECOCERT Status: In compliance with the ECOCERT Standard for natural ingredients

China Registration: All components are listed in the Inventory of Existing Cosmetic Ingredients in China (IECIC).

Suggested Use Level: 3%
CONCEPT: TRANSLUCENT SKIN

In all age groups in Japan, “skin translucency” is a top priority.

If we average all of the age groups, 55% of 1077 women interviewed that “ideal skin” is translucent skin – more than any other criteria (pore size, dryness, etc.)

Ref. JMA Research Institute.Inc.
http://www.jmar.biz/hot/html/w_dai07_2.html
Before carbonylation

Stratum corneum collected from the upper arm

Carbonylation treatment for 16 hours

20µmol/L sodium hypochlorite & Silver Vine.

Sodium hypochlorite induces carbonylation.
SILVER VINE EXT. (1%) BLOCKS CARBONYLATION INDUCED BY A CHEMICAL

SILVER VINE EXTRACT showed a very strong inhibition effect on protein carbonylation in the stratum corneum.
EXHAUST GAS INCREASES CARBONYLATION

Collection of stratum corneum by tape stripping

↓ React with car exhaust gas or tobacco smoke in sealed plastic containers for 24 hrs

Fluorescence labeling with carbonyl group

Control (Stored in normal air)

Reacted with car exhaust gas 24 Hours

Protein carbonylation in the stratum corneum increased when exposed to car exhaust gas.
**SILVER VINE EXTRACT (1%) DECREASES CAR EXHAUST CARBONYLATION**

**Protocol**
1) Stratum corneum was collected from volunteer’s upper arm.
2) The stratum corneum was soaked in solution containing 50 µg/mL of Silver Vine Extract for 24hrs and dried.
3) The stratum corneum were placed in a plastic bag.
4) Car exhaust gas was put into the bag and sealed and stored for 24hrs.
5) Evaluation

*SILVER VINE EXTRACT* at 1% inhibited protein carbonylation in the stratum corneum induced by the exposure to car exhaust gas by 100%.
Silver Vine Extract at 2% totally inhibited protein carbonylation in the SC induced by the exposure to tobacco smoke.

**Protocol**
1) Stratum corneum was collected from volunteer’s upper arm.
2) The stratum corneum was soaked in solution containing 100 µg/mL of Silver Vine Extract for 24hrs and dried.
3) The stratum corneum were placed in a plastic bottle.
4) Tobacco smoke (mainstream smoke) was put into the bottle and sealed and stored for 24hrs.
5) Evaluation
SILVER VINE EXTRACT (1%) REVERSES POLLUTION-INDUCED CARBONYLATION

**Protocol**

1) Stratum corneum was collected from volunteer’s upper arm.
2) The stratum corneum were placed in a plastic bag.
3) Car exhaust gas was put into the bag and sealed and stored for 24hrs.
4) The carbonylated stratum corneum was soaked in solution containing 50 µg/mL of Silver Vine Extract for 24hrs.
5) Evaluation

---

**Graphical Data**

- **Carbonylation level (AU)**
  - **0**
  - **20**
  - **40**
  - **60**
  - **80**
  - **100**
  - **120**
  - **140**
  - **160**

- **Silver Vine solid**
  - **-**
  - **+**
  - **+**

- **(µg/mL)**
  - **0**
  - **50**

- **Silver Vine solid**
  - **0**
  - **50**

- **50 µg/mL = 1% Silver Vine Extract**

---

**Evaluation**

- **Silver Vine solid**
  - **05 00**
  - **+**
  - **05 00**
  - **+**

- **SILVER VINE EXTRACT (1%)**
  - **REVERSES POLLUTION-INDUCED CARBONYLATION**

- **38.8% degradation**

- **n=3**
  - ***: p<0.05**
  - *****: p<0.001**
<table>
<thead>
<tr>
<th><strong>INCI Name:</strong></th>
<th>Water (and) Butylene Glycol (and) Actinidia polygama Fruit Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REACH Status:</strong></td>
<td>Natural / Low Volume Exemption</td>
</tr>
<tr>
<td><strong>Canada DSL:</strong></td>
<td>Natural / Low Volume Exemption</td>
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<td><strong>China Registration:</strong></td>
<td>All components are listed in the Inventory of Existing Cosmetic Ingredients in China (IECIC).</td>
</tr>
<tr>
<td><strong>Japan:</strong></td>
<td>Approved as a QD additive</td>
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<tr>
<td><strong>Suggested Use Level:</strong></td>
<td>0.2% - 1.0%</td>
</tr>
<tr>
<td><strong>Solubility:</strong></td>
<td>Water</td>
</tr>
</tbody>
</table>
WHEN THE COLOR OF THE SKIN REVEALS YOUR AGE

Apparent age: 38.9. This picture was artificially darkened (at right).

Apparent age: 42.4. People interviewed then guess that this person is older than 42.
WHY THE SKIN GETS DARKER

A combination of GLYCATION

The accumulation of AGEs and melanin make the skin look darker.


AGE = Advanced Glycated Elements and MELANIZATION
1. INHIBITORY EFFECT ON CROSS-LINK REACTION

Protocol
Mix ribose (reduction sugar), lysozyme (protein) and test sample. Keep at 37°C for one week. Electrophorese the mixture, then measure the amount of cross-linked protein by Coomassie dye.

The chart shows that 1% Ume Extract is good at reducing the cross-link.

Ume Extract Freeze Dried (µg/ml)*
*: 1000 µg/ml = 5% liquid product
2. CUTOFF EFFECT ON CROSS-LINK REACTION

Protocol
Mix α-diketone and test sample. Keep at 37°C for 10 hours. Measure decomposition (benzoic acid) produced by HPLC.

The chart shows that 1% Ume Extract is good at cutting the cross-link.

Ume Extract Freeze Dried (µg/ml)*
*: 2000 µg/ml = 10% liquid product
3. INHIBITORY EFFECT ON AGEs FORMATION

**Protocol**
Mix ribose, type 1 collagen and test sample. Keep at 37° for 2 weeks. Measure the amount of produced AGEs using AGEs antibody.

The chart shows that 1% Ume Extract is good at reducing AGE.

**Ume Extract Freeze Dried (µg/ml)**
*2000 µg/ml = 10% liquid product*
4. ACCELERATION OF AGEs DECOMPOSITION

Protocol
Mix ribose, type 1 collagen. Keep at 37° for 2 weeks. Add test sample. Measure the amount of remaining AGEs using AGEs antibody.

The chart shows that 1% Ume Extract has a slight effect on AGE decomposition.

Ume Extract Freeze Dried (µg/ml)
1000 µg/ml = 5% liquid product

* p<0.05
**UME EXTRACT**

<table>
<thead>
<tr>
<th><strong>INCI Name:</strong></th>
<th>Water (and) Butylene Glycol (and) Prunus mume Fruit Extract</th>
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<td>All components are listed in the Inventory of Existing Cosmetic Ingredients in China (IECIC).</td>
</tr>
<tr>
<td><strong>Suggested Use Level:</strong></td>
<td>3%</td>
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TWO SOLUTIONS FOR PORE MINIMIZATION

- Acting on the critical genes for perfect differentiation
- Acting on the biological pathway for perfect differentiation
CLINICAL TEST: OVERALL PERFECTING ACTION OF SACCHARIDE ISOMERATE ON ONE VOLUNTEER (OF 20)

After 4 weeks using Saccharide Isomerate, this woman founds her skin smoother, softer, healthier, and regenerated.
CONCEPT

BENEFITS
Saccharide Isomerate is produced by culture in bioreactor to obtain a pure, natural and characterized molecule (GALACTOSE and N-ACETYL-GLUCOSAMIN), which has no land-based equivalent.

Saccharide Isomerate moisturizes and exfoliates the skin. Benoiderm helps new corneocytes to be healthy, which results in less visible pores and more resistance to stingers.

Saccharide Isomerate stimulates the skin’s defense against P. acnes.
Glucosamine HCl: Alternative to AHA in Skin Care

Glucosamine competes with a lectin called CD44. This CD44 is a cell adhesion molecule. Glucosamine competitively binds to CD44 and reduces cell adhesion. Glucosamine disrupts corneocyte bonds.

J. Cosmet. Sci., 60, 423-428 (July/August 2009)
SACCHARIDE ISOMERATE IMPROVES SKIN RENEWAL

Cell renewal rate is doubled in just 1 week. By improving the skin’s natural renewal process, Saccharide Isomerate will help to eliminate dead cells on the skin’s surface and avoid the formation of a too thick and rough stratum corneum.

Protocol:
- 17 volunteers aged between 18 and 59
- Application of a colored cream containing 5% DHA, 1 day before start of treatment.
- From Day 0, lotion containing 1% Saccharide Isomerate applied twice a day for 2 weeks.

Skin pigmentation by the DHA enables monitoring of cell renewal; pigmentation will be eliminated faster if cell renewal activated.
Improving skin renewal and reactivating water memory assists in obtaining an increase in skin smoothness.

Protocol:
• 20 volunteers aged between 35 and 45
• Lotion containing 1% Saccharide Isomerate applied twice a day for 28 days
• Use of the VISIA tool to visualize skin texture smoothing effect
The primary function of the epidermis is to produce the stratum corneum. It is formed through the differentiation of the keratinocytes from the basal layer to the skin’s surface layer. Many proteins are involved at each stage of this differentiation process.

**Differentiation proteins:**

**INVOLUCRIN (INV):** involved in forming the cornified envelop

**TRANSGLUTAMINASE 1 (TGM1):** ensures assembling of proteins that make up the cornified envelope

**SMALL PROLINE RICH PROTEIN (SPRP):** precursor proteins in the formation of the cornified envelope

**LATE CORNIFIED ENVELOP (LCE):** precursor proteins in the formation of the cornified envelope

**CORNEODESMOSINE (CDSN):** major role in cohesion of the cornified layer

**NICE 1:** involved in terminal differentiation of keratinocytes properly turn into corneocytes

An ingredient upregulating the gene coding for these different proteins would be a prime candidate in corneotherapy to create healthy corneocytes. Saccharide Isomerate is tested on these genes.
SACCHARIDE ISOMERATE INCREASES THE SYNTHESIS OF DIFFERENTIATION PROTEINS ACTING ON 9 GENES

The increase in differentiation proteins will contribute to the formation of a higher quality physical barrier, and activation of stratum corneum renewal. The gene NICE is especially increased.
**SACCHARIDE ISOMERATE DECREASES SKIN SENSITIVITY**

*Principle of stinging test:* the test consists of applying lactic acid at 10% (pH 2) on the nasal fold, and physiologic serum on the other one. The stinging sensation triggered by lactic acid is evaluated by volunteers themselves, on a scale from 0 to 3 (no severe sensation to severe sensation). The soothing effect of a product is assessed according to the variation of stinging sensation generated by lactic acid.

![Graph showing average and maximum variation](image)

After 1 week treatment only, 76% of volunteers observed a decrease in their skin’s sensitivity due to a stronger stratum corneum.

**Protocol:**
- 30 volunteers aged 30 +/- 2
- Application of a gel containing 1% Saccharide Isomerate, twice daily for 1 week.

\[ p<0.01 \text{ student test} \]

\[ \text{Average variation} \]
\[ -37\%^{**} \]

\[ \text{Maximum variation} \]
\[ -100\% \]
OVERALL PERFECTING ACTION OF SACCHARIDE ISOMERATE AT 1%
PORES ARE LESS VISIBLE

Variation of number and total surface area of visible pores after 28 days.

- Number of pores: -7% (-41% vs. T0)
- Total surface area of pores: -11% (-58% vs. T0)

p<0.01 student test

Astringent effect on pores

BEFORE

AFTER

Pores are less visible
THE MEDIATORS OF INNATE IMMUNITY AND INFLAMMATORY REACTION

Peptides of innate immunity:
Defensin beta (DEFB103): deconstructs the membrane of exogenous bacteria; known for its efficacy against *Staphylococcus aureus* Secretory Leucocyte Peptidase Inhibitor (SLPI): inhibits proteases, mainly elastases activated by bacteria to better penetrate the tissues Ribonuclease Rnase 7 (RB RNASE 7): destroys bacterial and viral RNA
S100 Calcium Binding Protein A10 (S100A10): inhibits bacterial growth by interacting with their cellular cycle

Mediators of inflammation:
S100 Calcium Binding Protein A7 (S100A7): also called psoriasin, promotes the activity of collagenases and elastases during inflammation Toll-like Receptor 2 (TLR2) recognizes bacterial LipoPolySaccharides (high allergen potential) and activates TNFa
TNFa: activates the chemokins CXCL5 and CXCL10
CXC Ligand 5 et 10 (CXCL5 et CXCL10): chemokins involved in the migration of neutrophils
By acting on both innate immunity and inflammatory mediators, Saccharide Isomerate improves skin health while decreasing its reactivity.

Protocol: assessment of gene expression by human reconstituted epidermis treated with 1% Saccharide Isomerate applied topically.
P. Acnes have bacterial excretions, known as porphyrins, that lodge in the pores and are a contributing factor in acne. It is possible to visualize and quantify the amount of porphyrin on the surface of the skin since this molecule is fluorescent under UV light.

Protocol:
20 volunteers age 35 to 45 applied a lotion with 1% Saccharide Isomerate, twice daily for 4 weeks. Effect of bacterial growth evaluated by quantification of porphyrin thanks to UV light.

On average, the porphyrin amount is decreased by 54%. This means that the P. acnes population is reduced.
## SACCHARIDE ISOMERATE

<table>
<thead>
<tr>
<th><strong>INCI Name:</strong></th>
<th>Water (and) Saccharide Isomerate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REACH Status:</strong></td>
<td>Exempt</td>
</tr>
<tr>
<td><strong>Canada DSL:</strong></td>
<td>Listed RICL (Revised In Commerce List)</td>
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<td><strong>China Registration:</strong></td>
<td>All components are listed in the Inventory of Existing Cosmetic Ingredients in China (IECIC).</td>
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<tr>
<td><strong>Suggested Use Level:</strong></td>
<td>1.0% - 2.0%</td>
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<tr>
<td><strong>Solubility:</strong></td>
<td>Water</td>
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* Saccharide Isomerate is available in two versions: with phenoxyethanol (as shown in this presentation) and with phenethyl alcohol. The phenethyl alcohol version is in compliance with the ECOCERT Standard for natural ingredients, and is available on a special order basis.
LYSOPHOSPHATIDIC ACID (LPA) MINIMIZES PORE SIZE

0.1% Lysophosphatidic Acid reduces by 20% the visible size and number of pores (tested on 11 volunteers, twice daily).
Presence of nucleated cells (parakeratosis) around pores (in cheek area).

Large pore surrounded by nucleated cells. Photo by Nikko Chemicals.
RELATIONSHIP BETWEEN DIFFERENTIATION AND PORE SIZE

Abstract: Conspicuous pores are one of the most frequent skin problems for women of various ages. We examined the characteristics of the pore and found that it formed a cone shaped hollow, which had many nucleated cells, a sign of parakeratosis.

“Effect of sebum component on skin condition around facial pore and improvement of conspicuous pores.”
Toshii Iida, Shinji Inomata (Life Science Research Center, Shiseido).

Presence of nucleated cells (parakeratosis) around pores (cheek area).
(Life Science Research Center, Shiseido).
(1) Calcium helps for activation of PKC and CaMK.

When Lysophosphatidic Acid attaches to the receptors, Ca2+ intake channels become wider letting in more Ca2+. Ca2+ acts as a major differentiation regulator with DG.
LYSOPHOSPHATIDIC ACID TRIGGERS A CALCIUM FLASH

An influx of calcium initiates differentiation.
LPA induces phosphorylation of PKCα and increases CA\(^{2+}\) intake.

A Larger spot means increased phosphorylated PKCα.
IMPROVEMENT OF STRATUM CORNEUM: IN VIVO @ 0.2%

0.2% LPA

Before Treatment

After 6 weeks

IMPROVEMENT

Corneocytes are packed in thin layers, show hexagonal shape and no nucleated cells are observed: stratum corneum is healthy.

Placebo

Before Treatment

After 6 weeks

No change
PORE SIZE REDUCTION: IN VIVO @ 0.1%

Number of visible pores

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>0.1% LPA</th>
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<tbody>
<tr>
<td>Before</td>
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<td>0</td>
</tr>
<tr>
<td>After 4 weeks</td>
<td>0</td>
<td>-20%</td>
</tr>
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Size of visible pores

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<th>0.1% LPA</th>
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</thead>
<tbody>
<tr>
<td>Before</td>
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<tr>
<td>After 4 weeks</td>
<td>0</td>
<td>-20%</td>
</tr>
</tbody>
</table>

0.1% LPA reduced visible size and number of pores on human skin by 20%.

Protocol
Subjects: 11 people.
Cream with 0.1% LPA and placebo were applied twice daily for 4 weeks (split face).
LYSOPHOSPHATIDIC ACID (LPA)

INCI Name: Lecithin (and) Lysophosphatidic Acid (and) Lysolecithin

REACH Status: Low volume exemption

Canada DSL: Listed DSL as BARPORE 42*

China Registration: All components are listed in the Inventory of Existing Chemical Substances in China (IECSC) and the Inventory of Existing Cosmetic Ingredients in China (IECIC) as BARPORE 42*

ECOCERT Status: Compliant with the ECOCERT Standard for natural ingredients

Suggested Use Level: 0.1% - 0.2%%

Solubility: Water

* BARPORE 42 is the same material as Disapore 20, but with a China-friendly INCI name (Lecithin).
SMOOTHING EFFECT OF 1% LAVANDA OIL

Analysis of the “micro-depression network” (MDN) in the epidermis

Lavanda Oil significantly decreases (p<0.10) the micro-depression index and smooths the skin.

Protocol
• 10 volunteers
• Twice daily application for 28 days
• Lavanda Oil 1%
WHAT IS A CORNEODESMOSOME?

1. Cytoskeleton filaments
2. Desmosome
3. Hemidesmosome
4. Basement membrane

Elements of Corneodesmosomes

- **Desmoplakin**: linked to cytokeratin and catenin
- **Catenin**: bridge between desmoplakin and desmocollin
- **Desmocollin**: transmembranous protein, linked together thanks to calcium; proteolysis during desquamation.
WHAT IS A KERATINOSOME (AKA LAMELLAR BODIES)?

Lamellar Bodies = Odland Bodies = Keratinosomes

Organelles of storage and secretion in the extracellular domain of lipids (ceramide, cholesterol)

Their synthesis occurs in the cornified layer and is responsible for barrier properties.
ROLE OF EXTRACELLULAR LIPIDS IN STRATUM CORNEUM HEALTH

- **Normal stratum corneum**

- **Presence of extracellular lipids**

- **Lipids removed by acetone**

- **Stripped stratum corneum**
IN VITRO TEST: IMPROVING CORNIFIED LAYER COHESION

Effect on the number of keratinosomes responsible for the synthesis of lipids involved in the formation of lipidic cement

Lavanda Oil 1%

By increasing the number of keratinosomes Lavanda Oil 1% increases the synthesis of lipidic cement and therefore improves the sealing of stratum corneum and retains water in the stratum corneum.
IN VITRO TEST: IMPROVING CORNIFIED LAYER COHESION

Effect on the synthesis of adhesion proteins responsible for corneocytes adhesion

Lavanda Oil 1%

Formation of inter-corneocytes bonds in a culture of corneocytes

Lavanda Oil at 1% increases the expression of the adhesion proteins and improves the cohesion between corneocytes
<table>
<thead>
<tr>
<th><strong>INCI Name:</strong></th>
<th>Caprylic/Capric triglyceride (and) Hydrogenated vegetable oil (and) Lavandula stoechas extract</th>
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ROS QUENCHING POTENTIAL OF THE EPIDERMAL CORNIFIED CELL ENVELOPE

Wilbert P. Vermeij1,3, A. Alia2 and Claude Backendorf1

The cornified cell envelope (CE) is a specialized structure assembled beneath the plasma membrane of keratinocytes in the outermost layers of the epidermis. It is essential for the physical and permeability properties of the barrier function of the skin. Our skin is continuously exposed to atmospheric oxygen and threatened by reactive oxygen species (ROS). Here, we identify the CE as a first line of antioxidant defense and show that the small proline-rich (SPRR) family of CE precursor proteins have a major role in ROS detoxification. Cysteine residues within these proteins are responsible for ROS quenching, resulting in inter- and intramolecular S–S bond formation, both in isolated proteins and purified CEs. The related keratinocyte proline-rich protein is also oxidized on several cysteine residues within the CE. Differences in antioxidant potential between various SPRR family members are likely determined by structural differences rather than by the amount of cysteine residues per protein. Loricrin, a major component of the CE with a higher cysteine content than SPRRs, is a weak ROS quencher and oxidized on a single cysteine residue within the CE. It is inferred that SPRR proteins provide the outermost layer of our skin with a highly adaptive and protective antioxidant shield.

Journal of Investigative Dermatology (2011) 131, 1435–1441; doi:10.1038/jid.2010.433; published online 20 January 2011
ROS ARE ALL OVER
CORNEOTHERAPY: “A BARRIER AGAINST UNWANTED INFLUENCES FROM THE ENVIRONMENT”

- Pore Minimization
- Saccharide Isomerate
- Carbonylation
- Silver Vine
- Glycation
- Ume Extract
- Bacteria
- Differentiation Gene NICE1
- Saccharide Isomerate
- Cornified Envelope
- Saccharide Isomerate
- Furcellaran
- Corneodesmosome
- Lavanda Oil
- Saccharide Isomerate
- Beta Defensine
- Saccharide Isomerate
- LPA
- Dead Cell
- Exfoliation
- GLM-DS

N = Nucleus
R = Receptors

Hygroscopy
NMF
Furcellaran
Water
AWL Complex
Lipids

Epidermis
Stratum Corneum

Exfoliation
Detergent (strips out lipids)