GENENCARE™ OSMS unlocking the power of Nature

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GENENCARE™ OSMS protects your skin

Agenda

- The Science of Osmolytes
  - Protection of cells from dehydration
  - Protection of proteins from denaturation

- *In-vitro* studies

- Clinical studies
Protection: a global perspective

In USA

34% of women worry about effects of environment on their skin

Source: Mintel Reports, Facial Skincare-US, May 2014

In China

41% of consumers of 20-49 concern about pollution

Source: Mintel Report, Regional Lifestyles-China, December 2013
Europe: what consumer wants from facial skincare

1. To protect my skin from the environment/pollution - Facial moisturiser
   - France: 21%
   - Germany: 32%
   - Italy: 31%
   - Spain: 29%

2. To protect my skin from the environment/pollution - Specialty or treatment products (i.e., acne treatments, anti-aging products, face masks or peels)
   - France: 12%
   - Germany: 19%
   - Italy: 17%
   - Spain: 15%

3. To protect my skin from the environment/pollution - Cleansing products (i.e., facial cleansing, makeup removers, toners, wipes)
   - France: 27%
   - Germany: 34%
   - Italy: 39%
   - Spain: 28%

Source: Mintel Consumer Data Charts Facial Skincare 2014
GENENCARE™ OSMS product line

GENENCARE™ OSMS BA
Betaine anhydrous

GENENCARE™ OSMS CC
Betaine powder

GENENCARE™ OSMS MI
Myo-Inositol
## GENENCARE™ OSMS BA Benefit Matrix

<table>
<thead>
<tr>
<th></th>
<th>SKIN CARE</th>
<th>HAIR CARE</th>
<th>COLOR COSMETICS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moisturization</strong></td>
<td>Maintains skin moisture balance</td>
<td>Increases water retention capacity of hair fibers</td>
<td>Improves moisturization of lips</td>
</tr>
<tr>
<td><strong>Sensoriality</strong></td>
<td>Reduces stickiness</td>
<td>Improves hair shine and soft feel</td>
<td>Brings soft touch color intensity, strength and homogeneity</td>
</tr>
<tr>
<td></td>
<td>Provides smoother, softer skin feel</td>
<td>More luxurious and richer foam</td>
<td></td>
</tr>
<tr>
<td><strong>Protection</strong></td>
<td>Strengthens skin barrier</td>
<td>Strengthens the hair</td>
<td>Limits drying out of the formula</td>
</tr>
<tr>
<td></td>
<td>Mitigates irritation of surfactants</td>
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</tbody>
</table>

More luxurious and richer foam

Strengthens the hair

Limits drying out of the formula

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The Science of Osmolytes

- Osmolytes
  - Involved in osmosis
  - Osmolytes actively regulate water traffic by binding and transporting water
  - Control of water balance in living cells
  - Protein stabilizing effect

- Betaine and Inositol
  - Naturally occurring osmolytes, also present in the skin
Osmolytes: Control of water balance in living cells

HYPERTONIC ENVIRONMENT
High solute (salt) content outside cells
Water leakage
Increase of osmolytes intake to transport water in and re-inflate the cell

ISOTONIC ENVIRONMENT
Water balance
No water exchange needed

HYPOTONIC ENVIRONMENT
High solute (sugar) content inside cells
Water intake
Release of osmolytes to get the water out
Osmolytes & Proteins

Osmolytes

- Osmolytes are naturally occurring small organic molecules
- Glycine betaine, sorbitol, taurine, inositol, trehalose, trimethylamine oxide (TMAO)
- Osmolytes = “chemical chaperone”
- Osmolytes are typically accumulated in the intracellular environment
- Osmolytes are compatible in the intracellular environment at high concentrations without perturbing cellular processes

Proteins

- Proteins need to maintain their natively folded structures for proper functions under physiological conditions
- Proteins are sensitive to change in cellular and environmental conditions (stress) :
  - Temperature
  - Pressure
  - Presence of salts and other solutes (hypertonicity)
  ➔ Instability ➔ Denaturation
  ➔ Or Adaptation
Osmolytes: Protein stabilizing effect

- Protein/peptide backbone is “osmophobic”.
- In the unfolded state, the protein backbone interacts directly with surrounding water (high enthalpy state).
- In osmolyte solution, the unfolded state is very unfavorable.
- Osmolytes, by attracting water, increase thermodynamic stability of the native folded state of the proteins ($\Delta G<0$).

$\rightarrow$ Osmolytes cause proteins to fold and stabilize their native, functional 3-D conformation, without direct interaction with the protein.
Osmolyte interaction in protein stabilization is additive

- Cells may contain different kinds of osmolytes
- Osmolytes have interchangeable complementary protective effects
- INCI name: **Betaine**
- Chemical Description: **Trimethylglycine**
- Small amino acid derivative: stable **zwitterion** (dipolar ion) in neutral pH
- Naturally present in the cells of living organism (plant, animals)
- Very hydrophilic, readily soluble in water
- Actively transported in/out (skin) cells
GENENCARE™ OSMS BA functionalities

- Organic osmolyte: control of water balance
- Protein stabilizing effect of (macro) molecules
- Skin moisturizer (immediate and longer term)
  - Humectant (upper layers of epidermis)
  - Component of the NMF
  - Manage water balance
  - Reinforce skin barrier
- Stimulation of various biosynthetic processes
Improves Tight Junctions integrity in stratum granulosum (🌟)

⇒ **Strenghtens** skin barrier and prevents water loss

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Water binding properties

⇒ **Humectancy** at the skin surface

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Regulates cell water balance

⇒ **Osmoprotection**
Different types of Betaine

- **Trimethylglycine : GENENCARE™ OSMS BA**
  - Natural origin from sugar beet roots
  - Found in most plants and animals, also in our human body and especially in skin.
  - White crystalline powder (99% pure)

- **Surfactant : Betaine derivatives**
  - Also a zwitterionic compounds
  - Synthetic origin and derived from trimethylglycine
  - Used as surfactants for cleaning
  - The most common is cocoamidopropylbetaine
  - Clear viscous foaming liquid, diluted in water
GENENCARE™ OSMS MI (Myo-Inositol)

- Inositol = Isomer of glucose
- Nine stereoisomers, most important = Myo-inositol
- White odorless crystalline powder
- Natural occurring osmolyte (animals, plants)
- Other cosmetic uses: moisturizer, sebum control, anti-oxidant, foam booster
- Involved in many biological processes (IP3; PDGF↑)
In-vitro studies

Control of water balance:
- Osmolyte strategy of keratinocytes

Protein stabilization
- Increased integrity of Tight Junctions
- Increased Zein protein stability
Osmolytes: Control of water balance in keratinocytes

Hyperosmotic stress – Dry environment

» Water efflux $\rightarrow$ cell shrinkage
» Increased production of betaine, taurine and myo-inositol transporters (BGT-1, TAUT, SMIT)
» Increased uptake of osmolytes $\rightarrow$ cell hydration retained

Oxidative stress caused by UVA and UVB radiation

» Opening of K+ channels $\rightarrow$ K+ and water efflux $\rightarrow$ cell shrinkage
» Increased production of betaine, taurine and myo-inositol transporters (BGT-1, TAUT, SMIT)
» Increased uptake of the osmolytes $\rightarrow$ cell hydration retained


$\rightarrow$ GENENECARE™OSMS protects keratinocytes from dehydration
Differentiated keratinocytes were treated with GENENCARE™ OSMS BA (0-500 µM) from the apical side and the strength of Tight Junctions was measured using chopstick electrodes at different time points.

**Osmolytes: Strengthening effect on Tight Junctions**

- **GENENCARE™ OSMS improves Tight Junctions integrity and strengthens skin barrier**

Source: Poster presentation 40th Annual Meeting of the European Society for Dermatological Research; Helsinki, Finland, September 8th to 10th 2010.
Osmolytes: Protein stabilizing effect in Zein test

- There is a correlation between irritancy potential of a surfactant and its ability to dissolve the Zein protein.

- GENENCARE™ OSMS BA does not dissolve Zein, which correlates with \textit{in vivo} non-irritancy.

- The addition of 3.5 \% GENENCARE™ to mixed surfactant solution decreases the Zein number.

\textbf{\rightarrow GENENCARE™ OSMS BA reduces the solubilization of the Zein protein, which correlates with \textit{in vivo} reduction of skin irritation}

\begin{itemize}
  \item DSLS : dissodium laurylsulfosuccinate
  \item SLES : Sodium laurylsulfate
\end{itemize}

Clinical studies

- Four-week *in vivo* application study
- Clinical patch test with surfactant/betaine blends
4-week *in vivo* application study
GENENCARE™ BA and GENENCARE™ BA + MI versus control

![Graph showing TEWL changes over time](image)

**TEWL** = TransEpidermal Water Loss

- **Baseline**
- **Day 3**
- **Day 29**
- **Day 31**
- **Day 35**

**Treatment period**
- Control (=O/W polymeric emulsion) as baseline
- 3% GENENCARE™ OSMS BA
- 3% GENENCARE™ OSMS BA + GENENCARE OSMS MI

**Regression period**

![Graph showing TEWL changes over time](image)

**Significant differences**
1. Genencare™ OSMS BA versus Control
2. Genencare™ OSMS BA + MI versus Control

based on Fixed Effect LSMeans Tukey HSD
4-week *in vivo* application study

**Conclusions**

→ **GENENCARE™ OSMS BA** and **GENENCARE™ OSMS BA + MI blend** significantly decrease the Transepidermal Water Loss
  » during and after 4 weeks of treatment (29 days)
  » 2 days after the treatment stopped (regression period)

→ It confirms that **GENENCARE™ OSMS BA** and **GENENCARE™ OSMS BA + MI** have a significant positive effect on the skin barrier integrity

→ The results demonstrate that **GENENCARE™ OSMS BA** and **GENENCARE™ OSMS BA + MI** have complementary protection benefits
Addition of GENENCARE™ OSMS at level over 3.5% produced a significant reduction in the irritation potential of SLES. This effect is dose dependent.

**Clinical evaluations:** Observation of the irritation score

**Clinical patch test SLES/GENENCARE™ OSMS BA**

**Test conditions:**
- 25 volunteers
- 24 H and 48 h occlusive patch
- SLES (10 % solution) + GENENCARE™ OSMS at several percentages 0, 2, 3.5, 5, 7%

**Graph:**
- Irritation score vs. % GENENCARE(TM) OSMS BA in SLES solution
- Significant differences compared to SLES 10% solution without betaine based on Fixed Effect LSMeans Tukey HSD

**Conclusion:** GENENCARE™ OSMS BA reduces the irritating effect of detergents.
GENENCARE™ OSMS unlocks the power of Nature

Conclusions

- GENENCARE™ OSMS contributes to control the water balance in living cells
- GENENCARE™ OSMS strengthens the Tight Junctions
- GENENCARE™ OSMS protects proteins from denaturation
- Strengthening the tight junctions protects the skin against harmful substances such as pollutants
- Protection of proteins from denaturation can contribute to mitigate the irritation effect of detergents
Thank you!

For more information,

To discover more GENENCARE™ products, the natural step is to visit

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or email

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