CELLULAR COMMUNICATION BEHIND A YOUNG COMPLEXION
CONTENTS

- Intercellular communication in mature and menopausal skin
  - CELLYNKAGE™ marine ingredient
  - Efficacy
  - Conclusions
  - Applications and claims
  - Technical data
MENOPAUSE, A DEMANDING PERIOD OF TIME

- Menopausal skin:
  - Hormonal deficiency
  - Low signaling exchange between cells
  - Decreased skin homeostasis

Accelerated skin aging:
- Decline in extracellular matrix (ECM) components
- Disorganization of microrelief structure

A good understanding of menopausal skin aging is necessary to meet its specific requirements.

Dryness
Thinness
Roughness
Wrinkles
Age spots
**Intercellular Communication**

Exchange of signals (ions, second messengers, small metabolites, etc.)

**Direct**

Between adjacent keratinocytes through cell-cell junctions.
- Communicating gap junction channels are formed by connexin (Cx) proteins

**Crosstalk**

Between epidermal and dermal cells.
- Exosome vesicles are information carriers that use connexins to deliver signals to dermal cells (e.g. Cx43).

Both direct communication and crosstalk through connexins are key in maintaining skin homeostasis.
EPIDERMAL GAP JUNCTIONS

- Gap junctional intercellular communication (GJIC) is key in cell growth, differentiation, maintenance of homeostasis and morphogenesis.

**Connexin arrangements:**

- Homeric
- Heteromeric
- Homotypic
- Heterotypic

**Cx distribution in the epidermis:**

- With age and also with a decrease in certain hormones, such as estrogens in menopause, an alteration in connexin expression occurs.

Intrinsic and hormonal aging involve a reduction in connexins, leading to a less attractive skin.
Microbial communities exchange signaling molecules to coordinate responses as a group. One of these communication mechanisms is known as quorum sensing.

The luminescence behavior in the Hawaiian squid *Euprymna scolopes*:

Hawaiian squid $\xrightarrow{\text{symbiosis}}$ *Vibrio fischeri* bacterium (living in community) $\xrightarrow{\text{quorum sensing}}$ Bioluminescence

Optimizing community performance through intercellular communication
The Agua Amarga salt marsh in Costa Blanca, Alicante (Spain), is the habitat of the halophilic bacteria *Halomonas eurihalina*, which:

- uses the quorum sensing phenomena as a communication tool to adapt to hypersaline concentrations.

- under this environmental conditions, the microorganism is able to secrete certain exopolysaccharides (EPS).
**CELLULAR COMMUNICATION BEHIND A YOUNG COMPLEXION**

EPS obtained through biotechnology that improves cellular communication among keratinocytes and also between epidermal and dermal cells for a rejuvenated menopausal skin.

**Enhancement in intercellular communication**

**IN VITRO**
- Expression of connexin genes
- Connexin protein levels on reconstructed epidermis
- Better communication through gap junctions
- Epidermal-dermal exchange

**Menopausal skin rejuvenation**

**IN VIVO**
- Epidermal thickness, collagen density, microrelief structure and surface smoothness.
AUGMENTED CONNEXIN GENE EXPRESSION (I)

IN VITRO EFFICACY

• AGED KERATINOCYTES

54 years-old (y.o.) human epidermal keratinocytes (HEKa) treated with 0.02 mg/mL CELLYNKAGE™ marine ingredient concentrate, for 24 h.

Connexin gene expression through RT-PCR.

Restored connexin gene expression to similar values of those in younger cells
AUGMENTED CONNEXIN GENE EXPRESSION (II)

**MENOPAUSAL KERATINOCYTES**

HEKa cells incubated in menopausal medium conditions with 0.02 mg/mL CELLYNKAGE™ marine ingredient concentrate, for 24 h.

Connexin gene expression through RT-PCR.

Controls: non-treated HEKas in menopausal and non-menopausal medium conditions

higher connexin expression despite a menopausal hormonal deficit
**CONNEXIN PROTEIN LEVELS INCREASE (I)**

**IN VITRO EFFICACY**

- **RECONSTRUCTED HUMAN EPIDERMIS (RHE)**

  RHE treated with 0.02 mg/mL CELLYNKAGE™ marine ingredient concentrate, for 24 h.

  Connexin protein expression through immunohistochemistry.

- **Increased connexin proteins throughout the different layers of the epidermis**

  Connexin protein expression through immunohistochemistry.

  Green color: connexins expression; Blue color: cell nuclei

- **Levels of protein expression (%)**

  *p<0.05; ***p<0.001

  Control: non-treated RHE. Each Cx is normalized respect to its corresponding control.
CONNEXIN PROTEIN LEVELS INCREASE (II)

IN VITRO EFFICACY

- MENOPAUSAL RECONSTRUCTED HUMAN EPIDERMIS (RHE)

RHE treated with 0.02 mg/mL CELLYNKAGE™ marine ingredient concentrate under menopausal conditions, for 24 h.

Connexin protein expression through immunohistochemistry.

Cx37 and Cx59 reached comparable levels to those under non-menopausal conditions

Green color: connexins expression; Blue color: cell nuclei
0.5 mg/mL CELLYNKAGE™ marine ingredient concentrate on keratinocytes, for 24 h.

Scrape loading dye transfer technique to evaluate activation of GJIC.

Dye diffusion across a monolayer of keratinocytes, indicating GJIC levels.

Control: non-treated keratinocytes
Positive control: cAMP (0.05mM)

Expanded communication between cells via gap junction.
EFFECT ON EPIDERMAL-DERMAL EXCHANGE

IN VITRO EFFICACY

54 y.o. human dermal fibroblasts (HDFa) incubated for 24 h with the supernatants of 54 y.o. HEKa previously treated with 0.02 mg/mL CELLYNKAGE™ marine ingredient concentrate.

Extracellular matrix (ECM) genes expression through RT-PCR.

Preserved ECM through paracrine communication

Upregulation of genes involved in the synthesis of the ECM and downregulation of those that degrade it.

COL3A1 (collagen, type III, alpha 1)
ITGA1 (integrin, alpha 1)
ADAMTS1 (metalloproteinase with thrombospondin type I, motif 1)
HYAL1 (hyaluronoglucosaminidase)
MMP9 (matrix metallopeptidase 9)
MENOPAUSAL SKIN REJUVENATION (I)

IN VIVO EFFICACY

20 menopausal women
50-60 years old

Cream containing 2% CELLYNKAGE™ marine ingredient and placebo cream, half face
Twice a day
56 days

Epidermal thickness
Collagen density
Microrelief structure
Smoothness of the skin surface
MENOPAUSAL SKIN REJUVENATION (II)

**EPIDERMAL THICKNESS**

- Evaluated through skin **echography**.

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<table>
<thead>
<tr>
<th>Variation in epidermal thickness (%)</th>
<th>Placebo</th>
<th>2% CELLYNKAGE™ marine ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>15%</td>
<td>15%</td>
</tr>
<tr>
<td>56 days</td>
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</tbody>
</table>

* *p<0.05

15% thicker menopausal epidermis
MENOPAUSAL SKIN REJUVENATION (III)

IN VIVO EFFICACY

- **COLLAGEN DENSITY**
  
  Evaluated through a spectrophotometric analyzer.

Increased collagen density, for an improved dermal support
MENOPAUSAL SKIN REJUVENATION (IV)

**IN VIVO EFFICACY**

- **MICRORELIEF STRUCTURE**
  - Evaluated through *microscopic images* of surface samples.

The dark coloration corresponds to the area of lines that define polygons called plateaus, which provide a well-structured microrelief.

Better **organized skin through a more regular meshing**.

**Improvement in microrelief arrangement for a rejuvenated skin complexion**
MENOPAUSAL SKIN REJUVENATION (V)

**IN VIVO EFFICACY**

- **SMOOTHNESS OF THE SKIN SURFACE**

  Analysis of facial roughness from digital photographs.
  
  Improved surface homogeneity, also compared to placebo (p<0.01).

Homogeneity of skin surface is obtained from the distribution in the histogram of intensities of grayscale-transformed and filtered images.

**Decreased irregularities for a smoother skin**
CONCLUSIONS

↑ connexin genes in aged and menopausal epidermal cells

↑ epidermal connexin proteins (also in menopausal conditions)

↑ epidermal thickness 15%

↑ cell communication via gap junction

↑ epidermal-dermal crosstalk, favoring the ECM structure

1.9 fold

↑ collagen density 2.4%

↑ skin surface homogeneity 7.9%

↑ microrelief structure 113%*

* compared to placebo.
APPLICATIONS AND CLAIMS

**Application ideas**

- Cosmetic formulations to rejuvenate the skin complexion.
- Anti-aging products intended to delay the specific aging process experienced by menopausal skin.

**Claim ideas**

- The cellular network behind a young complexion
- Communicating youth from within
- Biotechnological ingredient to enhance menopausal complexion
- Improving menopausal skin through cell communication
- Defying the main aging signs of menopause
### TECHNICAL INFORMATION

**cellynkage™**  
*marine ingredient*

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Translucent solution containing 0.10% Saccharide Isomerate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INCI</strong></td>
<td>Propanediol, Water (Aqua), Saccharide Isomerate</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Soluble in glycerin</td>
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<tr>
<td><strong>Dosage</strong></td>
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<tr>
<td><strong>Recommended pH</strong></td>
<td>5.0 - 8.5</td>
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</tbody>
</table>
CELLULAR COMMUNICATION BEHIND A YOUNG COMPLEXION

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