STRATUM CORNEUM CARE
Pore Size Reduction

Active phospholipid for balanced skin barrier, better cell differentiation, improvement of tight junction and pore size reduction.

LPA

Enzyme-degraded soybean lecithin that contains high concentration (20-30%) of Lysophosphatidic acid (LPA). LPA exists in human skin and is an essential element of cell membrane.

LPA promotes the development of healthy epidermis by stimulating cell differentiation. Keratinocyte differentiation is stimulated through the Ca2+ influx mediated cascade. LPA also accelerates ceramide synthesis in skin.

LPA stimulates uptake of Ca2+ in the cells. Ca2+ is a major factor of cell differentiation. LPA up-regulates uptake of Ca2+ from both Ca2+ channels on cell membrane and endoplasmic reticulum.

Cell membrane has receptors specifically for LPA. When LPA attaches to the receptors, Ca2+ uptake channels become wider letting more Ca2+ inside the cells.

Effect of LPA on Ca2+ influx (in-vitro)

Phase contrast microscope

Increase in intracellular uptake of Ca2+ in keratinocytes (% of control)

Fluorescence microscope

LPA increases synthesis of key four proteins of tight junction (TJ)

Occludin

Claudin-1

Top: NHEKs treated with control sample. Bottom: NHEKs treated with LPA (10 mg/mL).
In-vivo data: LPA improves corneocyte condition and suppresses TEWL

Photographs of stripped stratum corneum treated with 0.2% LPA and control emulsion. The improvement of stratum corneum condition (due to stimulation of cell differentiation) can be observed when 0.2% LPA applied: corneocytes are packed in thin layers and have hexagonal shape.

In-vivo data: LPA diminishes visible size of pores by 20% due to stimulation of cell differentiation and improvement of corneocyte condition
Reduction of size of facial pores is a very popular new trend in skin care development in Japan. Diminishing of visual appearance of pores (especially in T-zone) is an important advantage of modern treatment product.

Rose Fruit Extract BG-01

INCI: ROSA MULTIFLORA FRUIT EXTRACT, WATER, BUTYLENE GLYCOL

Rose Fruit Extract BG-01 exhibits several properties very important for diminishing of appearance of skin pores:

1. Reduction of sebum secretion
2. Astringent effect
3. Regulation of keratinization

Regulation of keratinization

Cornified envelope (CE) is a strong membrane covering with outside of corneocyte. This membrane is produced from a special protein: involucrin. Therefore, low amount of involucrin causes incomplete CE production and results in misbalance of desquamation. It is recognized that stimulation of involucrin production helps to promote better desquamation and keep stratum corneum in fine condition.

Promotion of involucrin synthesis

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Involucrin synthesis (%) vs placebo</th>
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</thead>
<tbody>
<tr>
<td>Rose Fruit Extract BG-01 0.6%</td>
<td>+ 54.5%</td>
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</table>

Rose Fruit Extract BG-01 enhanced involucrin synthesis effectively.
Rose Fruit Extract BG-01

In-vivo evaluation: reduction of visual appearance of pores.
After 4 weeks of application (twice daily).

**Picture of replicas**

Before testing

After testing

1% Rose Fruit Extract BG-01

**Microscopic picture**
(after 4 weeks of application)

1% Rose Fruit Extract BG-01

Control

**Close up pictures**

1% Rose Fruit Extract BG-01

Control

**Change in volume of pores**

The volume of pores was significantly decreased by 1% Rose Fruit Extract BG-01 in all age groups.

![Graph showing the change in volume of pores](image)

- **Before test**
- **After test**

*Rose Fruit 2007.04*
Marjoram Extract BG

INCI Name: ORIGANUM MAJORANA LEAF EXTRACT; WATER; BUTYLENE GLYCOL.

Marjoram grows in many parts of the world, especially in dry areas. The leaves of marjoram are used in traditional medicine in anti-inflammatory and pain relieving preparations. It is also famous as a spice under the popular name "oregano".

Efficacy of Marjoram Extract BG
1. Synthesis of hyaluronic acid (moisturization)
2. Synthesis of Aquaporin-3 (moisture flow and cell nutrition)
3. Stimulation of transglutaminase-1 expression (improvement of corneocytes’ condition and skin barrier)

Synthesis of hyaluronic acid (Ex-vivo, 3D skin model)

After 9 days after application of Marjoram Extract BG (1%)

Transglutaminase-1 (TGase-1) is an enzyme expressed during differentiation of epidermal keratinocytes. It is essential in the process of formation of corneofied envelopes (CE). TGase-1 is a catalyst for cross linking of CE-related proteins (involucrin and loricrin), which is an important step in formation of CE.

Marjoram Extract BG significantly increased expression of TGase-1 in human keratinocytes (in vitro).

2010-08
New mechanism of moisturization: stimulation of synthesis of PCA (Pyrrolidone carboxylic acid) in skin

Mallow Moisture

Mallow Moisture is a natural extract from the flowers of mallow (Malva sylvestris). In skin care, it demonstrates two important effects: anti-inflammation (through inhibition of the inflammatory cytokine Prostaglandin E2) and moisturization (hydration) of the skin through increased PCA synthesis.

Pyrrolidone carboxylic acid (PCA) is one of the major natural components of skin moisture, as it makes up approximately 15% of NMF. Mallow Moisture increases the synthesis of PCA in the skin (+12%) and promotes the restoration of the damaged skin barrier (tested in vivo).

Subjects: 7.
Method: Skin was dried and damaged with surfactant solution. Amount of PCA in corneocytes was measured by HPLC. Test sample and control were applied for 14 days.

Mallow Moisture significantly reduced TEWL in damaged skin already after 1 week.

Subjects: 10.
Method: Skin was dried and damaged with surfactant solution.
Oil-soluble derivative of vitamin B6: promotion of filaggrin and NMF synthesis in skin. “True natural moisturizing” from inside the skin.

**VB6-IP**

INCI Name: Pyridoxine Tris-Hexyldecanoate

VB6-IP is an oil-soluble liquid ester of vitamin B6. It provides effective natural moisturization through increase of NMF production in the skin. VB6-IP stimulates production of filaggrin, the precursor protein for amino acids, which are an essential part of skin’s NMF. VB6-IP is extremely stable against heat and oxidation and due to its lipophilic nature it exhibits much higher skin penetration than hydrophilic pure vitamin B6.

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**Mechanism of action of VB6-IP**

**Quantification of Intracellular Filaggrin Content**

Normal human keratinocytes were treated with 50, 100 μM of VB6IP for 72 h. The content of intracellular filaggrin was determined by dot-blot (Immunoblot) analysis.

<table>
<thead>
<tr>
<th>Dot-Blot Profile VB6IP (μM)</th>
<th>Densitometric Analysis</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>150</td>
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Membrane: Nitrocellulose (BioRad)

1st antibody: Anti-Filaggrin (Argene)

2nd antibody: HRP-labeled mouse anti-IgG (Nichirei)

VB6IP significantly increased intracellular filaggrin content.
**True Moisturizing / NMF**

**VB6-IP**

**Immunohistochemical Staining for Filaggrin**

VB6 or VB6-IP (1.5 mM) was applied to the upper side of reconstructed 3D skin model (Toyobo). After 1 week cultivation, the frozen section was prepared and filaggrin was detected by immunohistochemical approach. First antibody: Anti-filaggrin (Argene). Second antibody: FITC-labeled mouse anti-IgG (Zymed).

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**Moisturizing effect (in-vivo)**

The gel with VB6-IP and placebo gel were applied to each cheek of human volunteers (n=20) twice a day.

After 7, 14, 28 day-application, the water content was estimated by the measurement of skin-surface conductance (SKICON).

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[Graph showing moisturizing effect]
Silver Vine Extract BG30

Brightening for Stratum Corneum:

Recently it was found that decrease in the skin transparency is induced by protein carbonylation in the stratum corneum. Carbonylation makes corneocytes opaque and enhances their color resulting in “non-transparent skin” and yellowish or grayish unhealthy appearance.

Silver Vine Extract BG30 effectively inhibits carbonylation of proteins in corneocytes (stratum corneum cells). The in vivo evaluation demonstrated that “skin transparency index” can be improved by one point with 1% of Silver Vine Extract BG30.

In vitro: Inhibition of protein carbonylation in corneocytes (carbonylation level is evaluated by intensity of green fluorescent dye)

In vivo: Improvement of skin transparency
Period: 28 days.
Dosage of Silver Vine Extract BG30: 1%.
Results: Skin transparency index (evaluated by dermatologist) increased by approximately one point. Color of the skin became brighter and skin roughness was improved.

Transparency

Translucent skin: Light is reflected from inside of the skin
Opaque skin: Decrease in the reflected light from inside of the skin
Lecinol S-10


Prevention of corneocytes’ damage caused by Sodium Lauryl Sulfate (SLS). Closed patch (SLS: 5%); after 72 hours. Microscope photograph of corneocytes.

Lecinol S-10 protects stratum corneum from damage by strong detergents.

Anti-stinging effect (in vivo)

Application of Lecinol S-10 at 1.0% significantly reduces average stinging score against methylparaben solution (after 1 week; n=3). Lecinol S-10 suppresses stinging reaction in humans.

Anti-inflammatory effect: inhibition of Prostaglandin E2 (in vitro)

Inhibition of UV induced Prostaglandin E2 (PGE2)

Application of Lecinol S-10 at 1.0% significantly reduces average stinging score against methylparaben solution (after 1 week; n=3). Lecinol S-10 suppresses stinging reaction in humans.
Skin Turnover Promotion

Promotion of skin turnover is an important feature of a moisturizing / skin balancing personal care line.

Jujube Extract

Extract from Zizyphus jujuba fruit. Demonstrates c-AMP activation and turnover promotion effect.

Clinical trial (in-vivo): promotion of skin turnover. Surface area of corneocytes was evaluated by digital planimeter.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Jujube Extract BG-J</th>
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<tbody>
<tr>
<td>Before application</td>
<td></td>
<td></td>
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<tr>
<td>After 27 days</td>
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Surface area of corneocytes (μm²)

- Placebo
- Jujube Extract BG-J

Updated: 2006-04
**Citrus Extract Complex BG**

Blend of three types of citrus extracts from Japan (Citrus Unshiu, Citrus Junos and Citrus Aurantium). Improves turnover speed reduced by UV and internal factors. Strong keratinocyte proliferation.

Clinical trial (in-vivo): promotion of skin turnover. Surface area of corneocytes was evaluated by digital planimeter. After 7 days of application, 1% solution of Citrus Extract Complex BG showed 8% increase of turnover compared to butylene glycolic solution.

- 0.5% Butylene Glycol
- 1% Citrus Extract Complex BG

**Alpinia Leaf Extract BG**


Clinical trial (in-vivo): promotion of skin turnover. Number of subjects: 5. Lotion with 2% Alpinia Leaf Extract BG reduced turnover time by 50 hours (2 days) compared to placebo lotion.

![Graph showing surface area (mm²) over days for 0.5% Butylene Glycol and 1% Citrus Extract Complex BG](image)

![Graph showing average turnover (days) for Alpinia Leaf Extract BG (2%) and Placebo](image)
Agar Treated Powders

Agar Sericite E-4
Agar Talc F-4

New concept in treated powders: sericite and talc treated with agar. Agar coating provides unique control over moisture; it absorbs moisture in wet conditions, and releases it when air becomes dry. Contributes to the long-lasting effect of makeup product.

Powders with agar treated surface provide original concept of “self moisture control conditions of makeup formula in different conditions”. This concept is based upon the fact that agar film is able to absorb excess sweat or water and release it in dry condition.

Agar treated powders improve stability and adherence of makeup to skin due to formation of agar gel. Superior application texture. Highly recommended for water and sweat resistant formulations.

Pore concealing effect of foundation with 20% agar treated powders

Before application
Immediately after application
4 hours after application
This emulsifying blend creates extremely stable oil-in-liquid crystal-in-water (O/LC/W) emulsions. Multilayer liquid crystal network, which is formed around each oil droplet, mimics structure of human skin and consists of similar components (phospholipids, fatty acids, triglycerides etc.) The content of water bound in LC network in the Nikkomulese LC emulsion is approximately equal to bound water in human skin: 13~19%.

O / LC / W (liquid crystal emulsion) can be made with almost any type of oils. Nikkomulese LC provides long-lasting moisturizing effect due to high amount of bound water. It allows to make low viscous formulas (lotions). Liquid crystal structure remains stable even at over 40 degrees Celsius.

Instructions for application of Nikkomulese LC:

1. Add Nikkomulese LC in oil phase and disperse well before emulsification.
2. During emulsification use homogenizer (over 3000 rpm) and emulsify by adding oil into water phase gradually (by little portions).
3. Both water and oil phases must be heated to 80 degrees Celsius while homogenizing.
4. Add actives when the formulation is cooled below 45 degrees Celsius.
5. Use level: 3 – 8%.

Updated: 2006-06
**Emulsifier / Liquid Crystal**

### Additional data

**Nikkomulese LC**

The optical micrographs of O/NC/W and O/W emulsions, observed by polarized light microscopy. A: O/W. B: O/NC/W (with Nikkomulese LC). Liquid crystal network can be observed only when Nikkomulese LC is used as an emulsifier.

Micrographs of O/NC/W emulsion applied on inside forearm for 3 and 6 hours. Liquid crystal network remains visible on the skin even 6 hours after application.

Photographs of stripped stratum corneum treated with O/NC/W (Nikkomulese LC) and regular O/W emulsion. The improvement of stratum corneum condition can be observed when O/NC/W emulsion is applied.

**In-vivo trial: changes of TEWL on the inner side thighs (12 subjects).**

O/NC/W system shows significant reduction of TEWL in human subjects.

Updated: 2006-08