Rona

RonaCare® VTA

Protector of the Extra Cellular Matrix

EMD

The skin, it's composition and modification during aging

The human skin is a highly specialized organ, covering an area of almost two square meters in adults. Skin is composed of different tissues, the epidermis, dermis and subdermal tissues.

Main cell types in the epidermis are keratinocytes, melanocytes and immunocompetent cells (Langerhans cells). Keratinocytes proliferate constantly in order to replace the desquamated corneal layer. There is a steady state of proliferation, migration and differentiation that is regulated by growth factors and cell-to-cell communication between the epidermal and dermal layers, physically separated from each other by the basement membrane.

On the other side, keratinocytes perceive environmental modifications which induce them to transmit intercellular signals to other functional cell types in the deeper skin layers.

The dermis forms a complex combination of different structures. The cellular components, the fibroblasts, are embedded in the Extra Cellular Matrix (ECM) which is composed of fibers (collagens and elastins) and non-fibrous macromolecules. Those can be grouped into proteoglycans (decorin, biglycan), glycosaminoglycanes (hyaluronic acid, chondroitin sulfate, heparan sulfate, dermatan sulfate) and structural glycoproteins (fibronectin, laminin).

In the course of ageing modifications of cell functions occur, resulting in modification of the composition and in the structure of the extra cellular matrix:

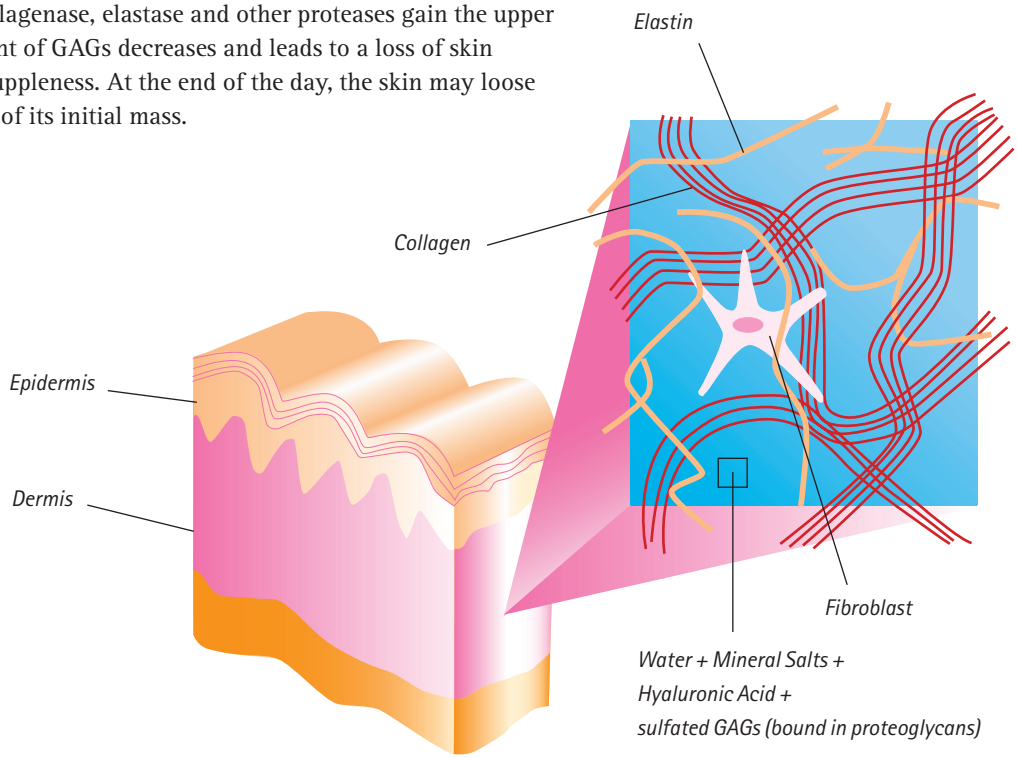
Fibers: The various types of collagen, in sum representing 70% of the extra cellular matrix, change their relative amount. For example, the ratio of collagen III/I decreases with age.
Elastin fibers form about 1-3% of the total dermis. In young dermis, they are found mainly in a vertical position. During ageing, they are replaced more and more by horizontally orientated fibers, leading to a loss of elasticity.

Glycosaminoglycans (GAGs) play a key role in the extra cellular matrix. They are a family of macromolecules with extraordinary water-binding properties, the most prominent being hyaluronic acid. Sulfated glycosaminoglycanes comprise heparan sulfate, chondroitin sulfate, keratan sulfate and dermatan sulfate. Filled with water like a sponge, GAGs form a colloidal gel whose turgescence pressure gives the skin resilience and hence firmness and tone.
Another important function of GAGs is the structural arrangement of the matrix components and orientation of the fibers in the dermis, preventing intermolecular cross-links.
Last not least, sulfated GAGs interact with all skin cells and the base membrane. They bind, store and release cellular messengers. For example, fibroblast growth factor has a high affinity to heparan sulfate. Thus, the bio-regulation of the dermis is influenced by the different members of the sulfated GAG family.

To summarize:
Macromolecules as proteoglycans, glycosaminoglycans and fibers in the extra cellular matrix are of most crucial importance for skin moisture, firmness and wrinkle prevention.

Degradation of the extra cellular matrix is mainly due to two mechanisms: enzymatic activity and oxidative stress.

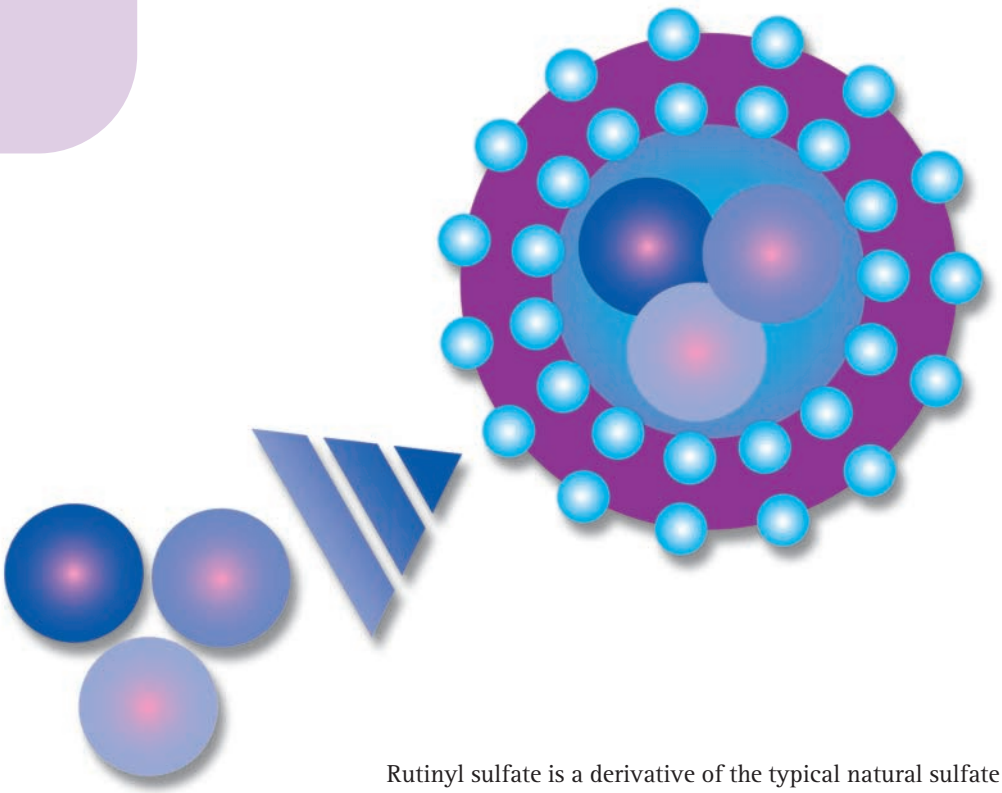
During lifetime the balance between the synthesis and degradation of the extra cellular matrix constituents becomes disrupted. Degrading enzymes like collagenase, elastase and other proteases gain the upper hand. The amount of GAGs decreases and leads to a loss of skin hydration and suppleness. At the end of the day, the skin may loose as much as 60% of its initial mass.



Major constituents of the dermis

What is RonaCare® VTA?

- is Vecteur Tri-Actif
- is a new age defying concept
- is a liposom containing Rutinyl sulfate.



Rutinyl sulfate

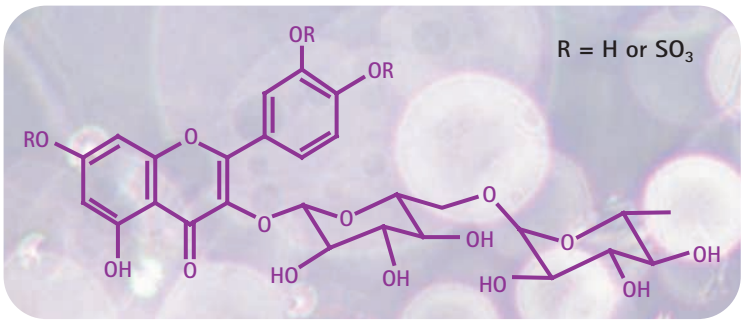
MAP

L-HP

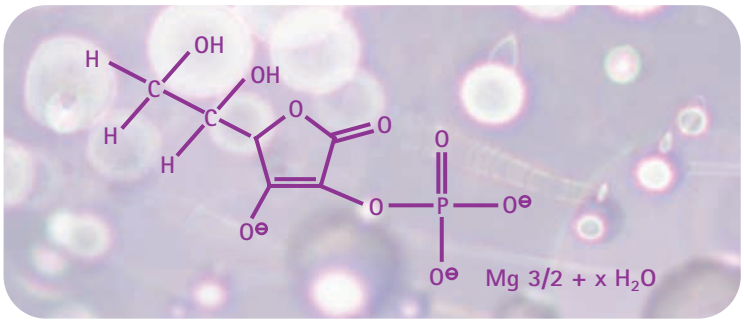
Rutinyl sulfate is a derivative of the typical natural sulfate metabolite of Rutin. Rutin, a natural bioflavonoid extracted from the plant Fava d'Anta, is known for a long time for its anti-oxidant, anti-allergic and anti-inflammatory properties. Its use in cosmetic has been limited until now due to its poor solubility. Sulfation of insoluble molecules provides solubility and is a common reaction in human tissues. In case of Rutin these properties are expressed by its derivative Rutinyl sulfate.

The cosmetic efficacy of Rutinyl sulfate is enhanced by Magnesium Ascorbyl Phosphate (MAP), the stable form of vitamin C, and L-Hydroxyproline (LHP), an important constituent of the dermal collagen and known to stimulate collagen production.

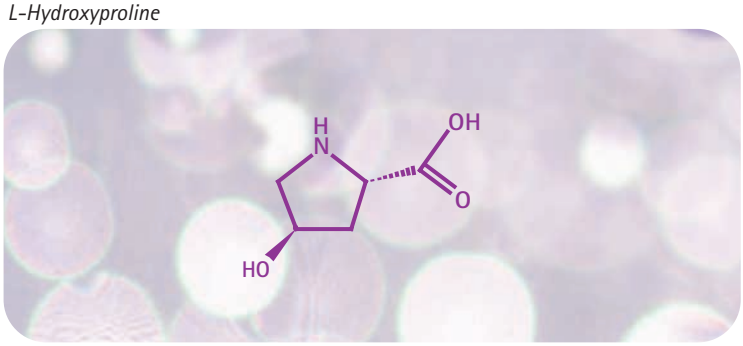
Rutinyl sulfate



Magnesium Ascorbyl Phosphate



L-Hydroxyproline



Encapsulated in and delivered through the RonaCare® VTA liposom the active ingredients show complementary anti-enzymatic, anti-oxidant, and protecting activities resulting in substantial protection of hyaluronic acid and other essential compounds of the extra cellular matrix.

RonaCare® VTA supports the extra cellular matrix to fulfill its most crucial tasks:

- Secure skin moisture, turgescence and tonus
- Regulate the bioavailability of growth factors

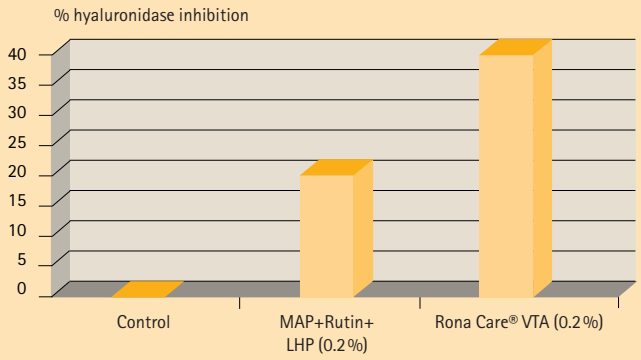
Technical data

INCI: Water (Aqua), Alcohol, Lecithin, Disodium Rutinyl Disulfate, Hydroxyproline, Sorbitol, Magnesium Ascorbyl Phosphate, Tocopherol, Ascorbyl palmitate, Glyceryl Stearate, Glyceryl Oleate, Citric Acid
CAS No.: 7732-18-5, 64-7-5, 8030-76-0, 12768-44-4, 51-35-4, 50-70-4, 113170-55-1, 10191-41-0, 137-66-6, 31566-31-1, 25496-72-4, 77-92-9
Appearance: Yellow to beige opalescent liquid
Solubility: miscible with water
Storage: store tightly closed between +5 and 30°C; protect from freezing and from sun light. Storage in refridgerator is recommended.
Use immediately after opening.

Efficacy tests in-vitro

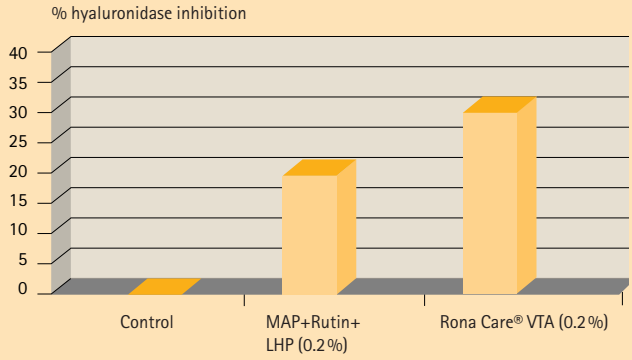
1. Anti-hyaluronidase activity of RonaCare® VTA

Assay on fibroblasts



Fibroblasts and 0.2% of RonaCare® VTA were incubated for 48 hrs. After a washing step to remove the active ingredients, fibroblasts were again incubated for 48 hrs. The supernatant was then collected, freeze-dried and resolubilized in a 10fold concentration. The anti-hyaluronidase activity in the supernatant was determined by turbidimetry after addition of hyaluronic acid and hyaluronidase.

Assay on fibroblasts via keratinocytes

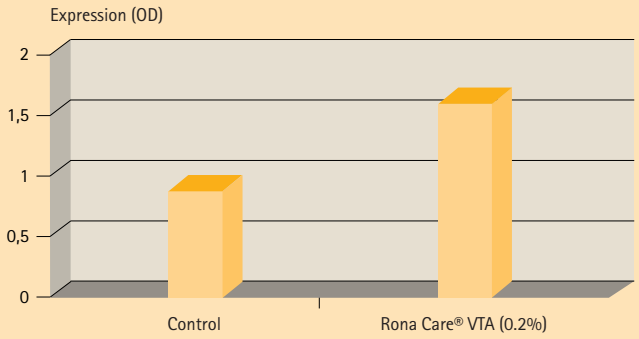


Keratinocytes and 0.2% of RonaCare® VTA were incubated for 48 hrs. After a washing step to remove the active ingredients, keratinocytes were again incubated for 48 hrs. The supernatant was then collected and transferred to a fibroblast culture. The fibroblasts were incubated with the supernatant for 48 hrs, then washed and again incubated for 48 hrs. The fibroblast supernatant was then collected, and anti-hyaluronidase activity was determined as in the first assay.

RonaCare® VTA inhibits hyaluronidase activity by 40% when directly applied on fibroblasts, and by 30% after keratinocyte stimulation. The unformulated mixture also shows activity, but to a significant lower degree.

2. Expression of sulfated glycosaminoglycanes in the presence of RonaCare® VTA

Assay on fibroblasts

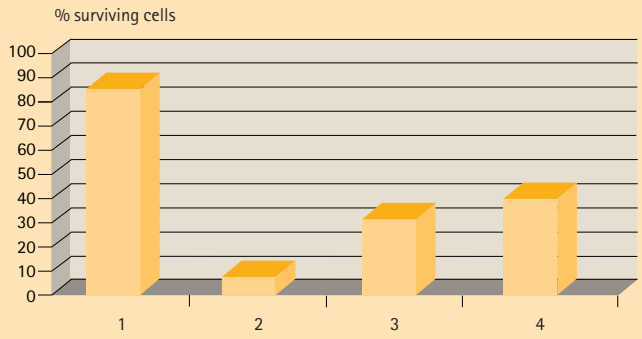


Fibroblasts and 0.2% of RonaCare® VTA were incubated for 96 hrs. The amount of sulfated GAGs in the supernatant was determined by quantitative spectroscopic measurement at 656 nm.

Cells treated with RonaCare® VTA show an 80% higher amount of GAGs than untreated cells.

3. Protective effect of RonaCare® VTA against oxidative stress

HX-XO test with RonaCare® VTA on fibroblasts



1: Control (neg.) 2: Control + HX-XO (pos.)
3: Vit.E + HX-XO 4: RonaCare® VTA (0.2%)+ HX-XO

Extrinsic aging or photo-aging is due to environmental stress. UV radiation produces oxygen free radicals (OFR) in the skin. Those OFR attack GAGs and especially hyaluronic acid and are able to destroy them.

Fibroblasts were incubated with the radical producing hypoxanthin-xanthin oxidase system in the presence of protecting agents as Vit.E and RonaCare® VTA. After a washing step, the number of living cells was determined via cytotoxicity test (XTT).

In the HX-XO test the anti-oxidative activity of RonaCare® VTA is approx. 25% higher than that of Vitamin E.

Ex-vivo studies

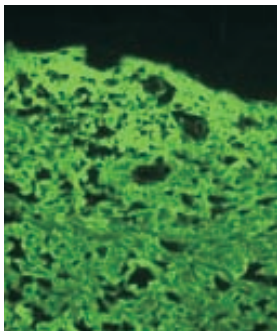
GAG protection and restoration by RonaCare® VTA

Results achieved in the in-vitro tests with regard to hyaluronic acid and sulfated GAGs were confirmed by the following ex-vivo study. Skin explants originated from a 44-year-old woman were incubated with an emulsion containing RonaCare® VTA for 10 days. The negative control was incubated with a placebo. For the direct immunofluorescence assay, 5 µm sections were obtained by cryostat cuts. Heparan sulfate and hyaluronic acid were specifically determined using monoclonal antibodies.

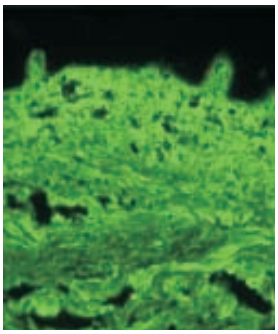
During cultivation of the skin explants without special treatment, the amount of heparan sulfate and of hyaluronic acid decreased (see control t=0 and negative control (placebo) t=10 days). Addition of RonaCare® VTA stopped or even inverted this trend.

Hyaluronic acid

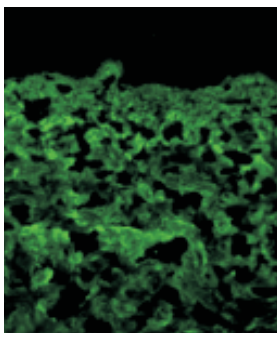
Control (t = 0)



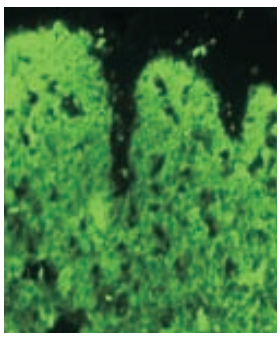
3 % RonaCare® VTA / t = 10 days



Negative control (placebo) / t = 10 days



5 % RonaCare® VTA / t = 10 days



Hyaluronic acid degradation is significantly reduced when skin is treated with 3 to 5 % RonaCare® VTA.

Heparan sulfate

Control (t = 0)

Negative control (placebo) / t = 10 days

3 % RonaCare® VTA / t = 10 days

5 % RonaCare® VTA / t = 10 days

Heparan sulfate appears denser and more regular after treatment with RonaCare® VTA.

Clinical studies

Improvement of skin roughness and of wrinkle depth

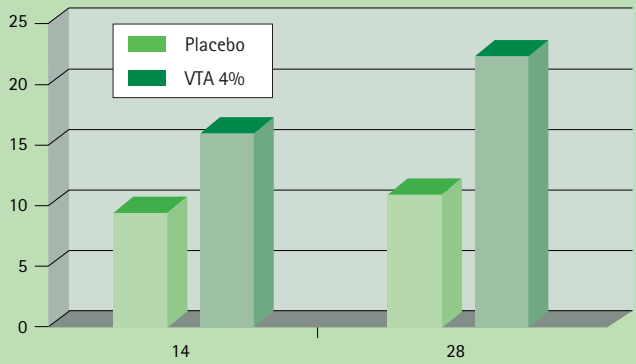
Study protocol

- Test persons: 20 human female volunteers, aged from 41 to 59 years
- Study:
  1) treatment of inner forearms (determination of skin roughness, R_z)
  2) treatment of crow's feet (determination of wrinkle depth, R_{max})
- Formulation: o/w emulsion with 4% RonaCare® VTA, placebo formulation
- Application: twice daily at indicated testing areas for 4 weeks. Measurements were done at day 0, 14, and 28.
- Evaluation: with a digital micromirror device technology (PRIMOS)
- Testing institute: Derma Consult, Germany

1. Skin roughness

At the beginning of the study (day 0, before treatment), all the test areas showed similar skin roughness values.

Treatment with placebo and with RonaCare® VTA decreased the skin roughness R_z significantly, compared to the untreated control area.



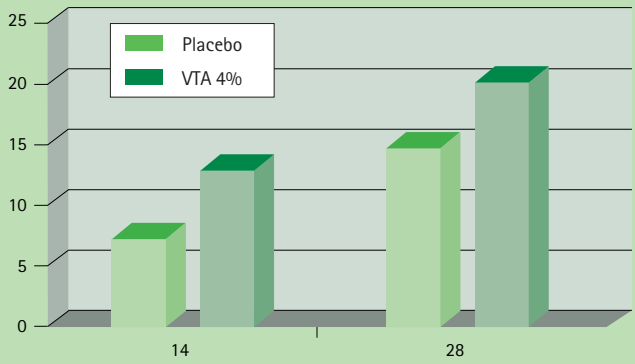
Compared to untreated skin, RonaCare® VTA increased the skin smoothness by more than 20% after 4 weeks treatment.

The improving effect of RonaCare® VTA was double the placebo effect.

2. Depth of wrinkles

At the beginning of the study (day 0, before treatment), the crow's feet areas showed similar wrinkle depth values (mean R_{max} values).

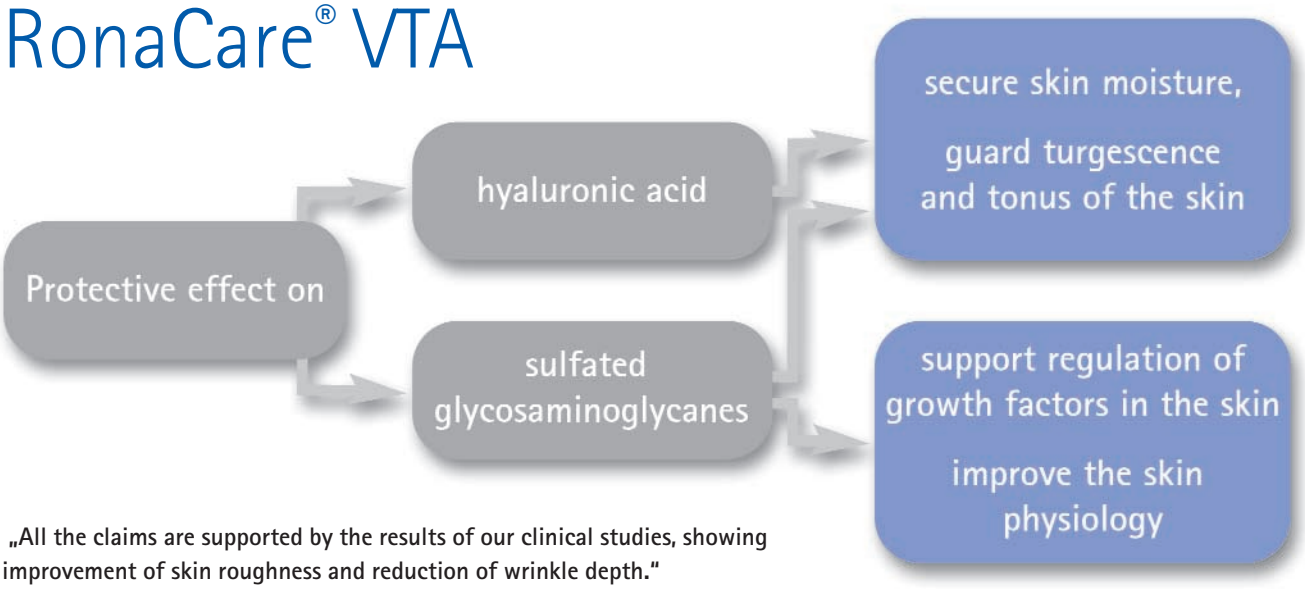
Treatment with placebo and with RonaCare® VTA both decreased the depth of wrinkles. The effect of a formulation with 4% RonaCare® VTA was significantly higher than that of the placebo formulation.



RonaCare® VTA decreased the depth of wrinkles by 20% within 4 weeks treatment.

Compared to placebo, the treatment with RonaCare® VTA achieved a 37% better result after 4 weeks.

Features and benefits of RonaCare® VTA



„All the claims are supported by the results of our clinical studies, showing improvement of skin roughness and reduction of wrinkle depth."

RonaCare® VTA: Protector of the Extra Cellular Matrix

Suggested application

Daily skin care
Age-defying products

Formulation Guidelines

- RonaCare® VTA can easily be incorporated into lotions, creams and gels
- Use level about 3 – 5 %
- Incompatibilities: Ionic surfactants and bivalent cations should be avoided because they may disrupt the liposome structure over time. Water-soluble film formers and silicone polymers should also be avoided because they hinder the reception of external signals from keratinocytes
- pH requirements: neutral to slightly acidic
- RonaCare® VTA should be added to the formulation after cooling down to 35°C. Homogenization is possible but should be kept to a minimum.
- In formulated products, RonaCare® VTA is stable over a broad temperature range from 40 °C to -10°C.

Ordering information

Name	*Item No.*	*Pack Size*
RonaCare® VTA	**130201**	1 kg, 2.5 kg

Safety profile

All components of RonaCare® VTA have already been used as single ingredients in cosmetic formulations for a long time. None of them have shown any adverse effects neither in toxicological studies nor under use conditions.

RonaCare® VTA itself has been tested in a human patch test for 48 hours under occlusive conditions. No primary irritancy was observed.

References

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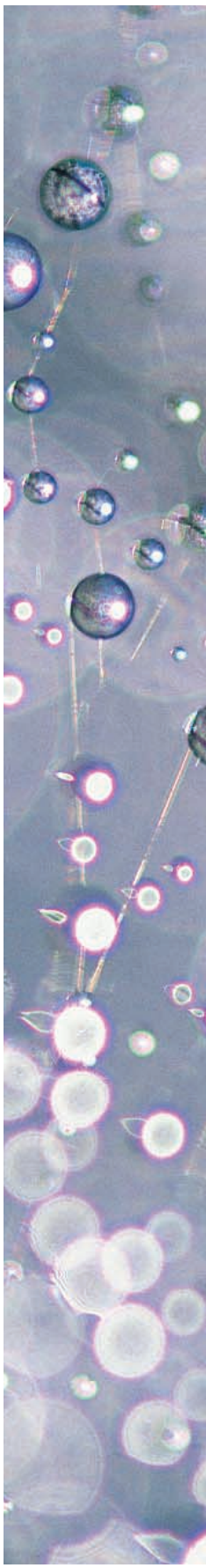

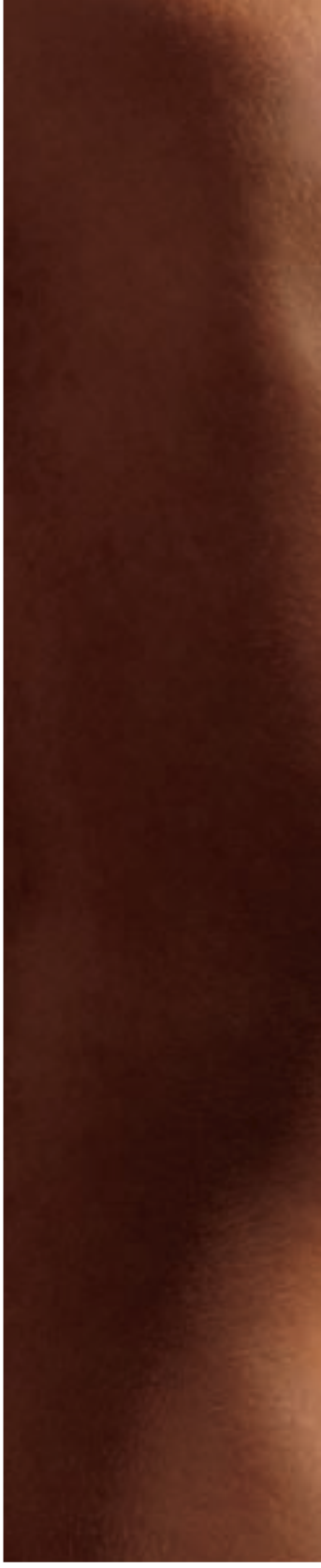

For more information please contact:

The United States
EMD Chemicals Inc.
Chemical and Pigments Division
Hawthorne, NY
USA
Phone: +1-914-592-46 60
Fax: +1-914-785-58 94
Internet: www.emdchemicals.com
E-mail: rona@emdchemicals.com

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