IMPROVING THE DERMAL STRUCTURE

Active ingredients | Dr. Stefan Hettwer from Rahn explains how tannins and silicon – old acquaintances renewed – can tighten the collagen network.

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Recent research has enabled us to gain a deep insight into the molecular mechanisms by which astringent molecules, such as the complex mixture of gallotannic acid derivatives from tannins, can strengthen the dermal structure.

Another bioactive compound, orthosilicic acid from millet, is supposed to act synergistically by providing additional hydrogen bond donors to stabilise collagen fibres. Importantly, these interactions with the collagen fibres are non-covalent, giving the dermal network the opportunity to react and rearrange upon mechanical stress. With this mode of action, the active ingredient Liftonin can tighten the dermal network. This significantly improves the skin's microstructure, which was confirmed in vitro and in vivo. The ingredient proved to be suitable for both Caucasian and Asian skin

TANNINS

Astringent molecules from tannins can strengthen the dermal structure

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and gives it a youthful appearance.

It is the network of fine lines that constitutes the skin's texture. In the ageing process, some of these lines become aggravated and appear as furrows and wrinkles. The main triggers are repeated mechanical stress, intrinsic and extrinsic ROS and UV exposure. All these stresses cause the skin to decrease in thickness[1].

Wrinkles occur in the upper regions of the skin. They are first and foremost expressions of a general ageing of the connective tissue of the dermis. The connective tissue fibres mainly consist of proteins from the collagen family. The differently-oriented collagen fibre bundles provide tension within the connective tissue. In young skin, the network has a dense, intersecting arrangement of these collagen fibres, which gives rise to the skin's firmness. Gradual losses in density, interconnectivity, strength and orientation of this network occur increasingly with age[2]. At the bottom of the wrinkle, the epidermis becomes significantly thinner as the dermal-epidermal junction loses its strong connection to the dermis. The reason is a reduction in collagen IV and VII connecting the dermis to the basal lamina[3]. Beneath the wrinkles in particular, the dermis' collagen network is significantly reduced as well and the collagen bundles become disorganised[4]. This opens the door to further aggravation of the wrinkles (Fig. 1 left). Our active ingredient **Liftonin**, featuring orthosilicic acid, clamps loose collagen connections and tightens the entire fibre network. The tannins are capable of interlinking larger areas of the dermal matrix and filling the gaps beneath wrinkles. They establish an environment for the infiltration of collagen-producing fibroblasts[5]. Taken together, this results in a cosmetic lifting and anti-ageing effect: skin flaws such as fine lines, wrinkles and unevenness are reduced in the short term and their reformation is prevented in the long term (Fig. 1 right).

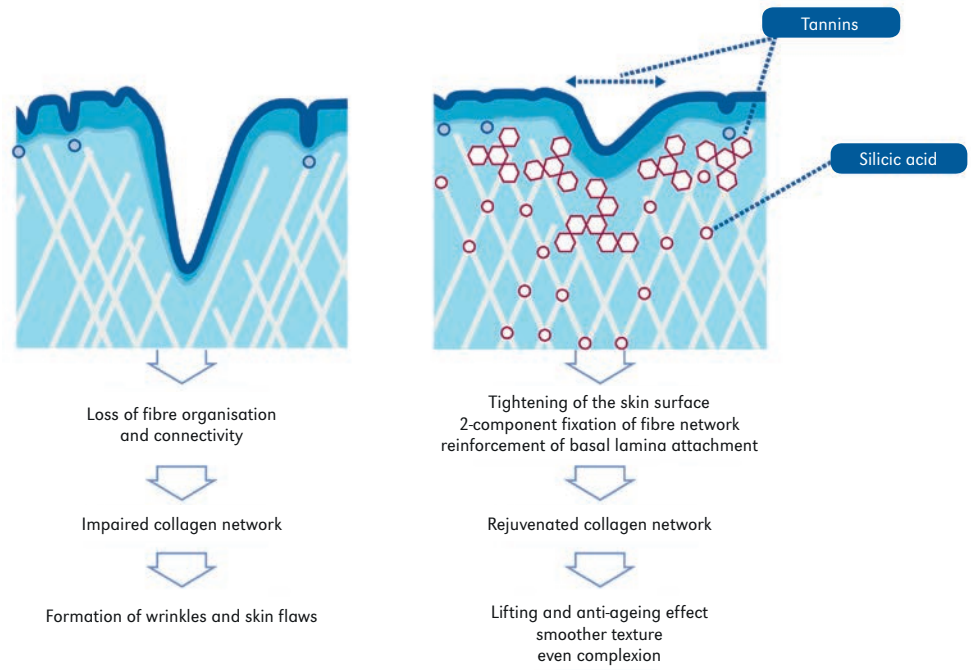

Fig. 1: The skin ageing process (left) and how Liftonin tightens the dermal network (right)

The mode of action for silicon and tannic acid

For decades, tannic acid and silicon have been widely used in cosmetic products for anti-ageing purposes (Fig. 2a and 2b). Tannic acid is known as an astringent that levels out wrinkles and silicon is said to strengthen the connective tissue and thus the dermis. However, the mechanisms behind these effects remain unclear. Here, we try to elucidate the mode of action of these botanical ingredients for Liftonin*.

"THE ACTIVE CAN TIGHTEN THE DERMAL NETWORK"

Silicon in our body

Silicon is an essential component in all human connective tissues; our body contains a total of 1.4 grams of silicon[6]. Several reports indicate beneficial effects of silicon for scavenging poisonous heavy metals and for promoting collagen synthesis[7, 8]. Fingernails benefit from a diet supplemented by silicon, as shown in a placebo-controlled study[9]. However, it is still unclear how silicon drives these effects. To date, there is no known enzyme that uses silicon as a co-factor and no biochemical reaction has been identified using e.g. orthosilicic acid as a catalyst. With age, the silicon content in the connective tissues decreases by a factor of 2–6[10]. A silicon deficiency is typically visible through symptoms such as slack connective tissue, dull hair with split ends and brittle fingernails.

Silicon in the skin

The elasticity and resilience of the skin decreases with age, and the skin's mechanical properties lose their uniformity. A lack of silicon also contributes to a breakdown in collagen and glycosamino-

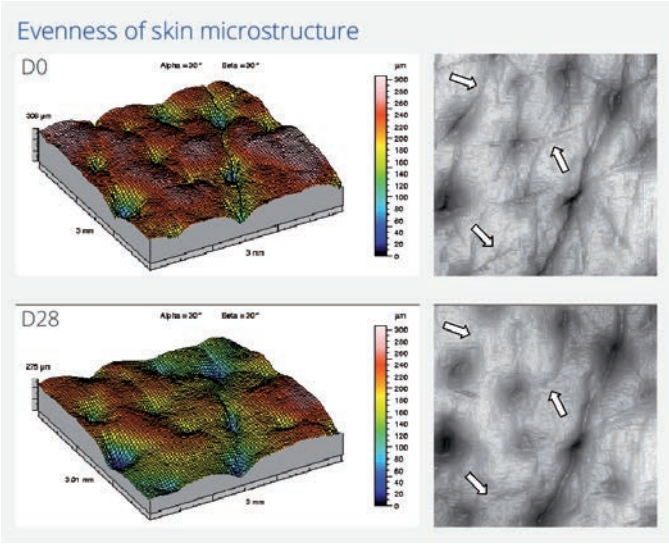

Fig. 6 a: Improvement in the complexity of microstructure and micro-relief after treatment
Left panels: Microstructure of a test area before treatment (top panel) and after 28 days' treatment with 2% Liftonin (right panel) (arrows)

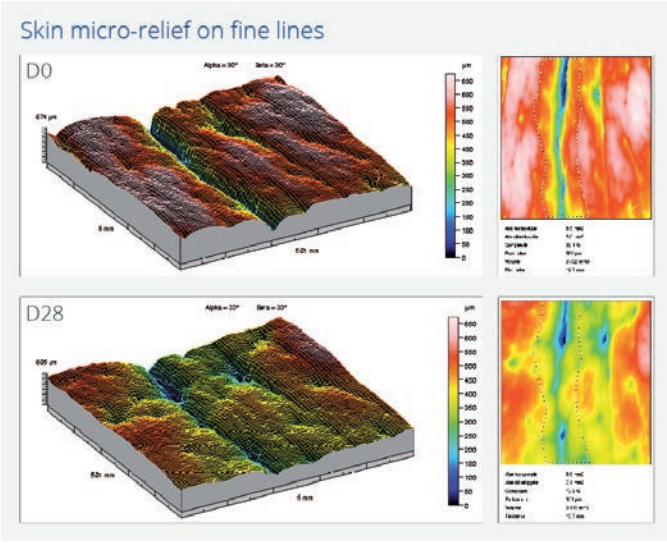

Fig. 6 b: Improvement of the micro-relief
The right panels show the levelling of fine lines, correspondingly

"REPEATED MECHANICAL STRESS, INTRINSIC AND EXTRINSIC ROS AND UV EXPOSURE ALL TRIGGER AGEING"

glycans. Scientific studies have shown that collagen type I is only formed properly in the presence of silicon. In vitro experiments on skin fibroblasts confirm these findings: in the presence of silicon, the collagen synthesis of the cells is significantly increased[11].

Bioavailability through botanical silicon

Besides the poorly informed supportive functions of silicon, silicon derivatives have another structural mode of action. The biological activity of silicon is highly dependent on its chemical form. Even the finest silicon dioxide is biologically inactive: it cannot be absorbed by the body. Orthosilicic acid ($Si(OH)_4$), on the other hand, is easily assimilated by the body (see below). This is the biologically active form of silicon. Botanical sources of silicon are to be found in numerous types of cereal and millet in particular (Fig. 2a)[12]. In the body, orthosilicic acid can bind collagen and glycosaminoglycans and in this way stabilise the dermal networks. Several investigations in the field of material and medical science prove a very high binding capacity of orthosilicic acid to collagen. Due to its four hydroxyl groups, orthosilicic acid can very easily interlink different collagen fibres into the required bundles, which strengthens an existing collagen network[13].

SILICON

Orthosilicic acid from millet provides hydrogen bond donors to stabilise collagen fibres

Silicon strengthens the connective tissue

Natural astringents from oak galls

Tannins are quite common in the plant kingdom, where they serve as protectants against herbivores. Oak galls, the source of tannic acid for the active ingredient Liftonin, contain 55–65 % tannins (Fig. 2b). The correct chemical designation for these tannins is tannic acid. Admittedly, tannic acid does not stand for a single compound but resembles a mixture of mainly poly-galloyl glucoses, i.e. sugar molecules with different amounts of gallic acid moieties[14].

Tannins are excellent astringents: they are extremely rich in hydrogen donors and acceptors, which allows them to form multiple hydrogen-bindings and a tight bonding with proteins. Recent research points to a structural benefit for the skin: tannins contribute to a tight crosslinking and stabilisation of the fibre network of the dermis' extracellular matrix and reorganise the collagen bundles in a way that supports the infiltration of fibroblasts[5], the construction engineers of the dermal collagen.

Moreover, tannic acid has also been shown to inhibit collagenases, elastase and hyaluronidase[15-18]. Thus, tannins not only improve the quality of the fibre network, but also protect it from degradation. Finally, tannins were also shown to inhibit tyrosinase; this tyrosinase inhibition may contribute to a more even complexion[15-18]. In conclusion, botanical silicon and tannic acid act

synergistically to support and reorganise the dermal collagen network (Fig. 1 right).

Reinforcing the collagen matrix

In a gel contraction assay, we investigated the ability of Liftonin to strengthen an existing collagen network. Primary human fibroblasts were seeded on a pre-formed collagen gel matrix, which was pre-incubated for 30 minutes with a maintenance medium with or without our active ingredient. Fibroblasts seeded on a collagen gel matrix will apply tensile forces in the attempt to reorganise the loosely associated collagen fibres. As a result, the gel matrix will be contracted and its area reduced. As such, the tensile forces can be detected by measuring the area of the gel matrix. The smaller the gel's area, the higher the tensile force. In the event that the collagen matrix is stabilised, the time to contract the gel will be increased as the fibroblasts have to expand more force for contraction. The gel contraction was measured during a time frame of 10 days.

The blank collagen gel without cells and the active ingredient remained stable throughout the entire experiment (Fig. 3, top panel*). In the control without the active ingredient, the gel size was reduced by 50% (100mm^2) within three days, while the fibroblasts on the collagen gel matrix with the active ingredient took until day seven to achieve this result. The application of 0.2% of the active ingredient led to a two- to three-day

Fig. 2 a: Millet has one of the highest yields of bioavailable silicon

Fig. 2 b: Tannic acid is isolated from oak galls

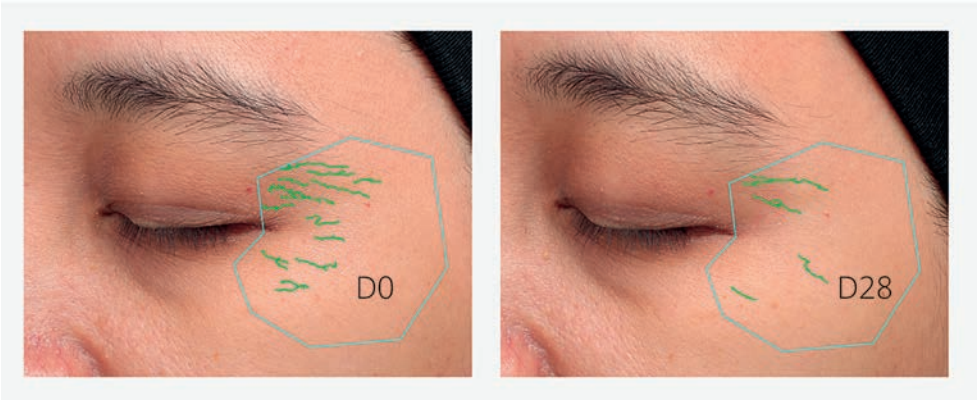

Fig. 7: Reduction of fine lines and wrinkles

delay of collagen gel contraction (Fig. 3*, bottom). The final gel contraction after 10 days was not different from the control without the active ingredient. This means that the cells remained their vitality. As such, the observed result clearly demonstrates that Liftonin stabilises the collagen matrix. We expect this is due to the hydrogen bonding of orthosilicic acid and tannic acid to the collagen.

In aged skin, fibroblasts have to produce collagen to fill the gaps in the dermis' extracellular matrix to gain a youthful appearance. Collagen I is of particular importance as it is responsible for the firmness and resilience of the dermis. To test for collagen production, primary human dermal fibroblasts were seeded in a culture medium and supplemented with our active ingredient. After 48 hours, the cultures were analysed for collagen I amounts with an Enzyme Linked Immunosorbent Assay (ELISA). The formulation with Liftonin significantly increased collagen I production (Fig. 4*). Fibroblasts produced 42% more collagen than the untreated control when stimulated with 0.2% of the active ingredient. An increased concentration of Liftonin led to a dose-dependent increase of collagen I.

The ingredient stabilises the dermal collagen network

It strengthens the dermal-epidermal junction

It gives Caucasian and Asian skin a **youthful appearance**

To prove that the silicon in our active ingredient is bioavailable, we applied a formulation containing 3% of the active to the fingernails of one hand while on the other hand, a formulation without the active was applied. Before application, the fingernails were cut and stored. After four months, the nails grew from the lunulae to the tips and were cut again. Silicon content was analysed and revealed an almost threefold significant increase in the silicon content of the fingernails treated with the active compared to the placebo (Fig. 5*). The increase of the silicon content in the placebo control can be explained by the 1.5% dimethicone present in the base formulation, a polysiloxane commonly used in cosmetic formulations with low bioavailability. Although the additional silicon in the Liftonin formulation is marginal (Fig. 5*, right panel), it increased the silicon content dramatically by 37.7 ppm compared to the placebo. This shows that all silicon in Liftonin is bioavailable, and that a high concentration of polysilanes is not able to perform as well as a botanical cosmetic active.

The above-mentioned mechanistic in vitro and in vivo findings were confirmed in a double-blind, placebo-controlled, randomised in vivo study: 31 females with healthy Caucasian skin, 40–55 years (average 48 years), were divided into two groups. One group applied a placebo emulsion; the other group applied the same emulsion with 2% of the active for 28 days twice daily on the face, especially in the crow's feet area. The skin's microstructure was evaluated with laser profilometry. Liftonin significantly improved the skin's microstructure with a difference of 23% compared to the placebo after 28 days of application (not shown). Fine lines and furrows were markedly reduced leading to a smoother skin texture (Fig. 2 a and b).

Liftonin is also effective on an Asian panel: 21 females with healthy skin, aged 22–45 years (average 34 years) and showing the first signs of ageing, applied an emulsion with or without 3% of the active twice daily for 28 days on the face. The wrinkle count and wrinkle score was evaluated in the crow's feet area using a Visia Complexion Analysis System. The active ingredient significantly reduced the number of wrinkles by 28%, and performed 2.5-fold better than the placebo, which did not cause a significant change of wrinkle appearance (Fig. 7).

This observation is in line with the active ingredient's dermal structure-supporting mode of action. By tightening the collagen network, the skin is firmed, wrinkles are levelled out and the overall skin texture is improved. □

"THE ACTIVE HELPS THE DERMAL NETWORK TO REARRANGE UPON MECHANICAL STRESS"

* INCI: Water, Glycerin, Panicum Milliaceum (Millet) Seed Extract, Citric Acid, Tannic Acid, Potassium Sorbate

* These figures, the reference list as well as additional product information can be found on the Internet – see download panel