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Epidermal hyaluronic acid: a new look at hydration

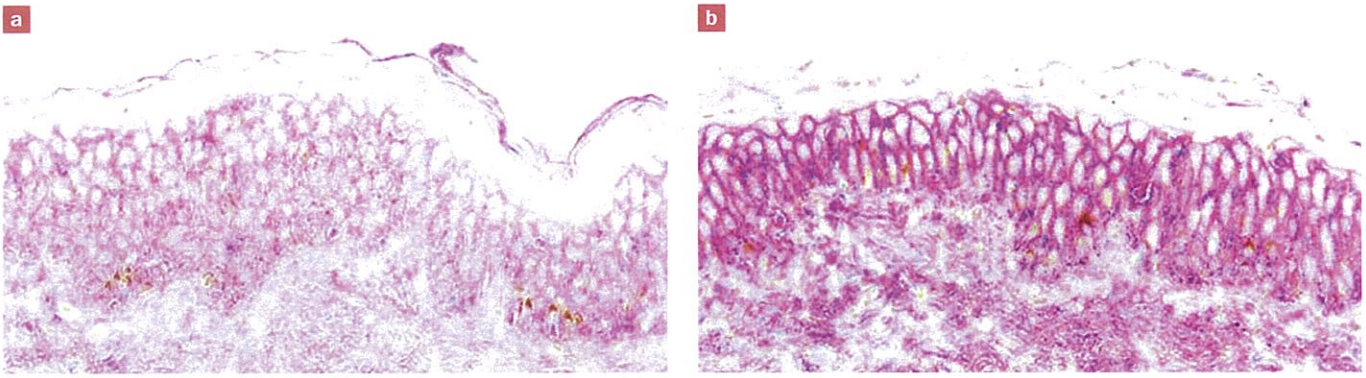

Figure 1: *View of epidermal hyaluronic acid synthesis in a human skin explant,* **a)** *untreated, and* **b)** *treated with 1.5% Hydranov. Epidermal hyaluronic acid is highlighted with immunolabelling (fuchsia colour).*

Even though it is famous for its hydrating and skin-filling properties, hyaluronic acid is not as well known as it may appear. Although present to a higher degree in the extracellular matrix of the dermis, it is also found in the epidermis where its function presents an unused potential for hydration and overall skin restructuring strategies. By developing Hydranov, a high technological furcellaran concentrate, Codif Recherche et Nature is targeting epidermal hyaluronic acid to generate an overall hydra-restructuring effect and a greatly enhanced hyaluronic-like result.

Epidermal hyaluronic acid: a still overlooked potential

The rate of hyaluronic synthesis is higher in the epidermis than in the dermis. Since the dermis is much thicker than the epidermis, it contains four to nine times more hyaluronic acid, but in 1991 Tammy *et al*[1] showed that for equivalent tissue quantities, the epidermis synthesises four times more hyaluronic acid than the dermis. In the epidermis, hyaluronic acid is localised in the intercellular space of the basal and spinous layers. In the same way as in the dermis the hygroscopic properties of hyaluronic acid are of great importance in hydrating the deep layers of the epidermis, but its function goes further than conventional hydration. It has been shown that the presence of high concentrations of hyaluronic acid in the intercellular space tends to destabilise desmosomes and the adherens junctions, obliging them to renew regularly and so reorganise the keratinocytes not only within the basal layer, but also throughout the process of differentiation as far as the *stratum corneum*.[2] It might be thought then that an excessive accumulation of hyaluronic acid in the intercellular spaces would lead to complete destructuring of the epidermis but as opposed to the dermis, in the epidermis, hyaluronic acid is quickly metabolised with a half life of 24 hours thus preventing any excess build-up.[2] Hyaluronic acid acts therefore like an overall epidermal restructuring agent by initiating and maintaining constant cellular remodelling from the basal layer to the cornified layer.

At the present time, natural moisturising factors (NMF) are the molecules which are the most targeted in epidermal hydration strategies but their localisation in the

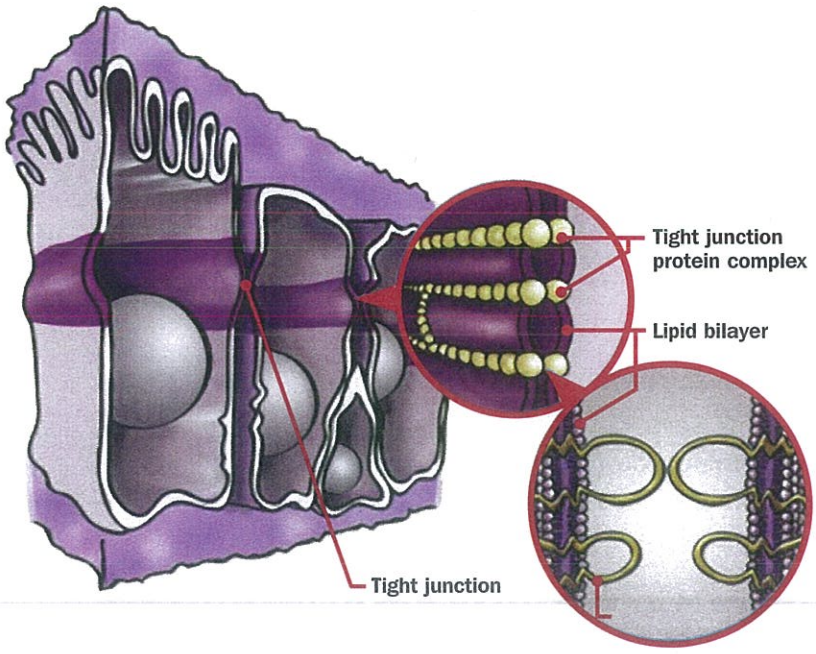

Figure 2: *Schematic view of a tight junction and role of claudin 1.*[9]

stratum corneum means that hydration of the entire epidermis cannot be assured. On the other hand, epidermal hyaluronic acid possesses a potential which has been underused up to now. It is a fully hydra-restructuring agent and one of the only molecules able to generate global hydration of the epidermis. In view of these factors, Codif Recherche et Nature has developed a concentrate of sodium oligofurcellaran with hyaluronic acid-like and hyaluronic acid activator properties, called Hydranov. The oligofurcellaran is obtained using a unique depolymerisation process: subcritical CO_2 depolymerisation in anhydrous conditions. In the same way as hyaluronic acid, Hydranov has hygroscopic properties which enable water molecules to be captured at the surface of the skin but it also stimulates epidermal hyaluronic acid synthesis for total restructuring and hydration of the epidermis. Finally, a clinical study shows superior and faster hydrating action by Hydranov than from hyaluronic acid.

Comparative study of hygroscopic properties

The oligofurcellaran concentrated in Hydranov (now referred to as 'the furcellaran concentrate') has a molecular weight of roughly 200 kDa. Therefore in the same way as hyaluronic acid it acts as a macromolecule with surface properties. The ability of the furcellaran concentrate to capture water molecules was compared to that of hyaluronic acid using sorption and desorption measurements in water saturated atmospheres. Placed in a cell saturated with 75% of water, hyaluronic acid adsorbs a quantity of water equivalent to 34% of its initial weight, while the furcellaran concentrate adsorbs 30% weight equivalent. Therefore the results obtained for the two polymers are almost identical. These results indicate that the furcellaran concentrate will generate a surface hydration action at least equivalent to that of hyaluronic acid.

Effect on epidermal hyaluronic acid synthesis

Given the size of the oligofurcellaran and the low probability that it can penetrate into the epidermis, the effect of the furcellaran concentrate on epidermal hyaluronic acid synthesis was evaluated by topical application on human skin explants. A gene expression analysis after application of the furcellaran concentrate at 1% for 72 hours showed a 64% increase in synthesis of hyaluronan synthase 3. Combined use of immuno labelling methods was used to visualise (Fig. 1) and quantify a significant increase ($p<0.01$, Student's *t*-test) in epidermal hyaluronic acid synthesis of 211% in human skin explant treated with 1.5% furcellaran concentrate. The total surface area occupied by hyaluronic acid in the epidermis was increased by a factor of 3. This increase in the level of epidermal hyaluronic acid should be accompanied by cellular remodelling and epidermal restructuring.

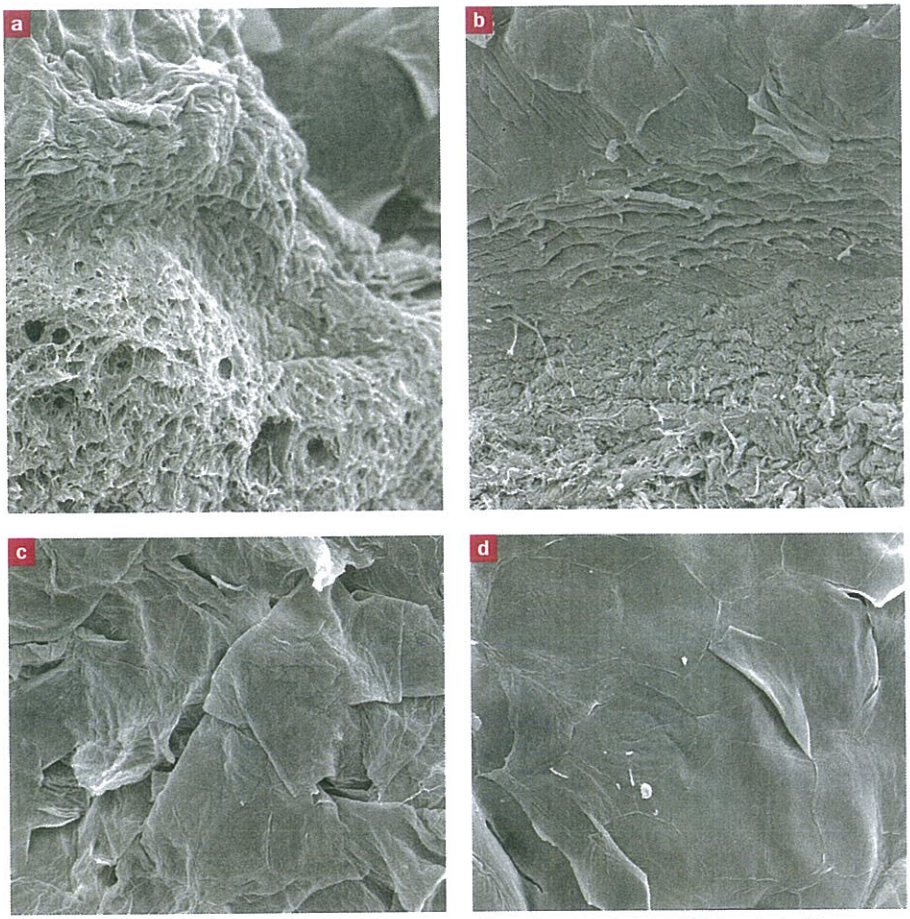

Figure 3: *SEM view of a section of human skin explant* **a)** *untreated, and* **b)** *treated with Hydranov at x400 magnification. Observation of the surface of a human skin explant* **c)** *untreated, and* **d)** *treated with Hydranov at x1000 magnification.*

Action on claudin 1: new target for restructuring

The restructuring effect of the furcellaran concentrate was evaluated on a not very well known molecule which is however essential to the formation of tight junctions – claudin 1. The function of tight junctions is to block circulation of fluids between cells and to ensure in this way that there is a tight seal between two tissue compartments. They were identified in human skin explants in the medium layers (granular and sub-basal layers).[3] The main factor contributing to the formation of these tight junctions is a protein called claudin 1 (Fig. 2). In 2002, Furuse *et al.* showed that mice which did not express claudin 1 died of massive trans-epidermal water loss (TEWL) due to a deficient barrier function in the granular layer.[4] Topical application of 1.5% furcelleran concentrate on human skin explant stimulates claudin 1 synthesis by 45%. In parallel, a significant 56% increase ($p<0.01$, Student's *t*-test) in the synthesis of epidermal ceramides was measured.

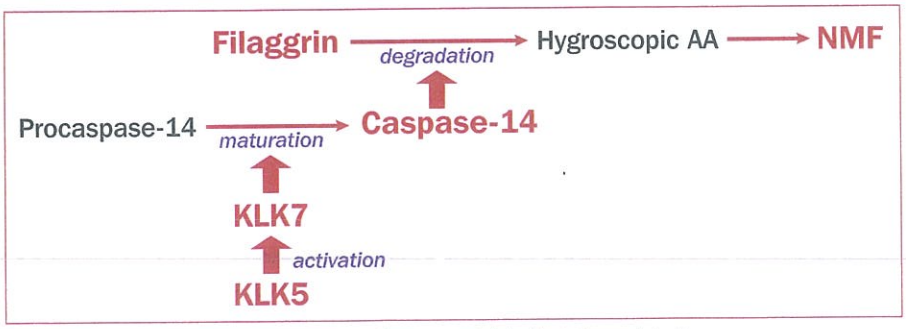

Figure 4: *Schematic view of activation of caspase 14 in filaggrin metabolism.*

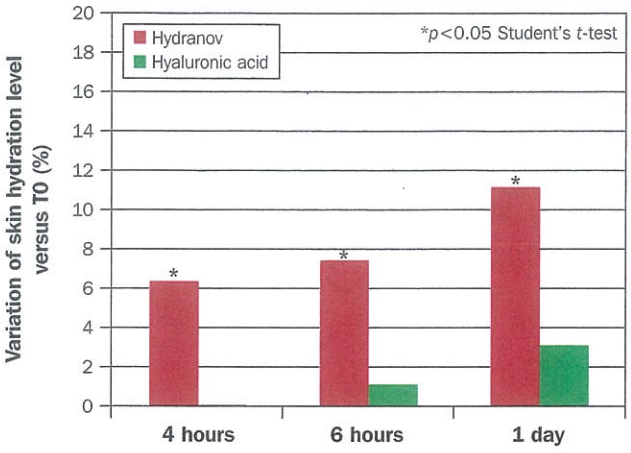

Figure 5: *Comparative study of hydrating properties of Hydranov and hyaluronic acid after a single application.*

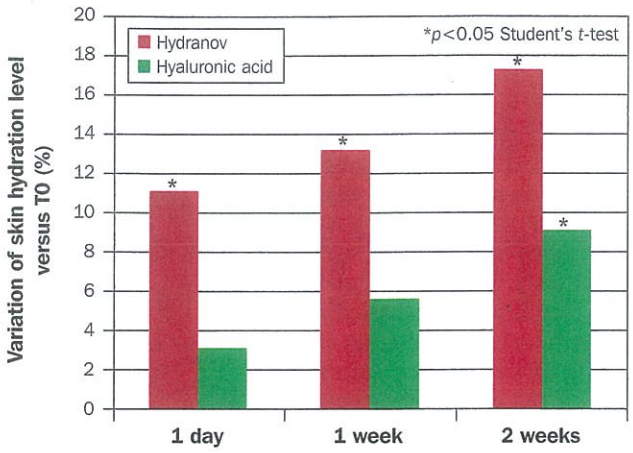

Figure 6: *Comparative study of hydrating properties of Hydranov and hyaluronic acid after two weeks application, twice daily.*

By stimulating hyaluronic acid synthesis, the furcellaran concentrate causes renewed and increased claudin 1 synthesis, the main factor in the formation of tight junctions. The increase in ceramide synthesis reinforces the water-loss prevention capability of the epidermis. This restructuring effect of the furcellaran concentrate was observed on human skin explants under a scanning electron microscope (Fig. 3). Observation in section enables the effect of the furcellaran concentrate on the leaved structure of the epidermis to be observed; whereas surface observation highlights the effect of the furcellaran concentrate on corneocytes cohesion at the surface of the skin.

Activation of filaggrin metabolism to reinforce NMFs

Numerous studies report the essential role of filaggrin which, when it is broken down in the *stratum corneum*, adds hygroscopic amino acids to the NMF.[5] An absence of filaggrin expression leads to an almost complete lack of *stratum corneum* (ichtyosis) and major skin dehydration (xerosis).[6] The precursor to filaggrin, profilaggrin, is synthesised in the granular layer under the control of gene FLG. It is then cleaved into filaggrin which then becomes biologically active and enables aggregation of keratin filaments. Filaggrin is then broken down in the *stratum corneum* by caspase 147 (Fig. 4) which is itself matured (clivage of procaspase into active caspase) under the control of a serine protease called kalikkrein-related peptidase 7 (KLK7).[8] Activation of KLK7 depends on a second kalikkrein coded by the gene KLK5. Topically applied at 1% on reconstituted human epidermes, the furcelleran concentrate increases filaggrin synthesis by 43% and synthesis of KLK5, KLK7 and caspase-14 by 60%, 39% and 65% respectively. The increase in filaggrin synthesis as well as synthesis of molecules involved in its breakdown leads to increased amounts of hygroscopic amino acids in NMF.

Comparative hydrating properties

The hydrating properties of the furcellaran concentrate were compared to those of hyaluronic acid in a clinical trial involving 30 volunteers. 15 volunteers applied a solution containing 1.5% furcellaran concentrate (equivalent to 0.009% pure oligofurcellaran) on the forearm twice-a-day for 2 weeks. A second group of 15 volunteers followed the same protocol by applying a 0.025% solution of pure hyaluronic acid (concentration recommended by the supplier). Hydration measures were taken using corneometer.

Short timescale hydration measurements at 4, 6 and 24 hours show that the furcellaran concentrate has a hydrating action greatly superior to that of hyaluronic acid (Fig. 5). This difference is still observed after 2 weeks of application. The hyaluronic acid solution must be applied for more than two weeks to achieve a level of hydration similar to that obtained after only 24 hours with the furcelleran concentrate (Fig. 6).

Conclusion

Hydranov reinvents hydration by targeting new molecules with unexploited potential. By increasing the synthesis of epidermal hyaluronic acid, Hydranov promotes not only hydration of the deepest layers of the epidermis, but also renewal of tight junctions to reorganise the keratinocytes in all the layers of the epidermis. In the corneal layer, Hydranov activates metabolism of filaggrin to enrich NMF with hygroscopic amino acids while on the surface, in the same way as hyaluronic acid, it captures water molecules to maintain a hydrating film. Due to this overall hydra-restructuring effect, Hydranov provides the skin with a tenfold hyaluronic acid like action. The hydrating effect of hyaluronic acid after two weeks of use is obtained in only 24 hours with Hydranov. PC

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