Topical Action of a Cross-Linked Hyaluronic Acid on Skin Hydration and Barrier Function

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OBJECTIVE

The objective of the present study is to assess the properties of RHA (Resilient Hyaluronic Acid®, Teoxane Laboratories, Switzerland), an injectable cross-linked HA, to preserve the skin barrier function when applied topically.

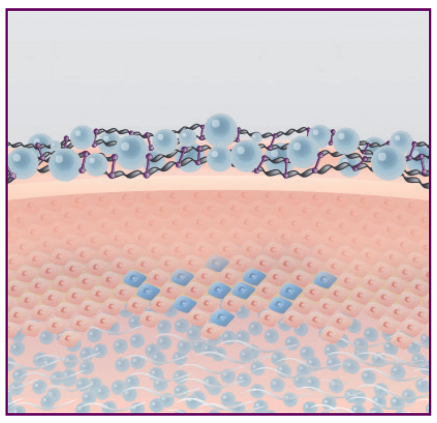


Figure 1: RHA on skin. Structured for resilience and better water retention

INTRODUCTION

Water is of very high importance for the good working property of the skin and its different involved layers.[1] It is now generally accepted that adequate skin care is regarded as a major strategy for maintaining skin barrier, skin hydration and health.[2] Because of its water attracting capacity and its excellent water solubility, HA is often used in cosmetics under the form of HA oligomers or HA of low molecular weight (LMW HA) to high molecular weight (HMW HA). However, reticulated HA, processed with a cross-linked material, very popular as dermal filler in aesthetic medicine, has been, to our knowledge, rarely used in cosmetics. Teoxane Laboratories has developed a new technology called RHA benefiting from 10 years of research and international patents.

The strategy was to comparatively characterize the effect of topical application of RHA over linear HA (LMW and HMW) on the *stratum corneum* (SC) and the epidermis of living human skin explants studying its morphology, water content, Trans-Epidermal Water Loss (TEWL) and microrelief.

MATERIAL AND METHODS

Sample Preparation and Application on Skin Explants

Teoxane's proprietary Cross-linked Hyaluronic Acid (RHA), High Molecular Weight (1.5 MDa) NaHA and Low Molecular Weight (50 kDa) NaHA were prepared and sterilized at 25 mg/g and diluted to a final concentration of 3% of in an aqueous carboxymethyl cellulose (CMC).

The products were applied topically on D0, D1, D2, D3, D5, D6, D7 and D8 on the explants (triplicate) of the concerned batch on the basis of 2 µL/explants and spread using a small spatula. Half of the culture media were refreshed (1 mL) on D2, D5 and D7.

Microscopic Studies

For morphological analysis, the skin explants were fixed, dehydrated and impregnated in paraffin. The samples were then embedded in paraffin and sections (5 µm) were cut on a microtome, mounted on histological glass slides and stained. Sections were observed with a Leica DMLB or a BX43 Olympus microscope. The microscopic observations were realized with a MEB Quanta 200 microscope under high vacuum pressure (50 Pa).

Raman Spectroscopy (Water Content)

Raman spectra were realized with a confocal Raman microscopy Xplora (Horiba, Jobin Yvon) on 10 µm thick frozen skin sections and using a 532 nm laser. Spectra were realized on 60 different points along the explants on the *stratum corneum* (SC) and the epidermis.

Measurement of TEWL

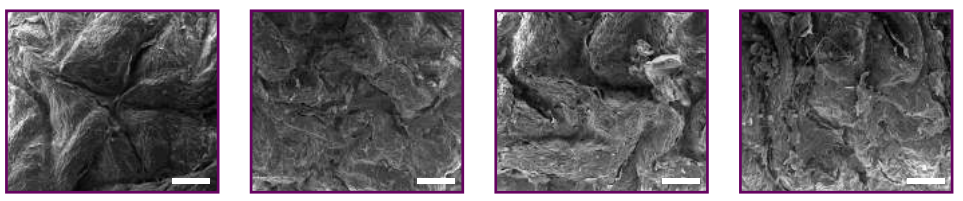
TEWL was measured using the Tewameter® TM300 probe. The measures were realized before the application of the products. 40 consecutive measures (1 measure each second) were realized on the same zone and averaged after stabilization of the measured values.

RESULTS

Electronic Microscopy

The surface of skin explants was characterized by the presence of numerous creases of various depth, forming the primary lines of the microrelief. The cutaneous microrelief of treated explants was assessed as very similar to the non-treated reference at D9 for linear HA, HMW or LMW. Only explants treated with cross-linked HA showed an improvement in the depth of the primary lines (rated as moderately deep).

Only cross-linked HA topical treatment afforded the two following benefits : the flattening of the microrelief while keeping a good cohesion of corneocytes.



D9, non-treated *D9, cross-linked HA* *D9, HMW HA* *D9, LMW HA*

Skin surface by electronic microscopy at D9 of a reference non-treated explant and explants after treatment with Cross-linked HA, HMW HA and LMW HA. (Scale: 200 µm)

Raman Spectroscopy

The water content in superficial layers of the skin was compared to the reference non-treated explants as well as on treated explants after 9 days of treatment. Integrations of the RAMAN spectral domain (3200-3400 cm^{-1}), associated with water, in the treated explants were compared to the data recorded on the reference samples (Table I).

	Cross-linked HA	HMW HA	LMW HA
Stratum Corneum	- 10.7% **	- 9.1% *	- 2.3% ^{ns}
Epidermis	+ 7.6% $^{\#}$	- 3.2% ^{ns}	+ 4.1% ^{ns}

^{ns} (non-significant),
** significant with p<0.1,
* significant with p<0.05,
$^{\#}$ significant with p<0.01

Table I: Variations of the water content measured by RAMAN microscopy at D9, from the non-treated explants at D9.

As compared to water contents measured on the reference non-treated explants at D9, statistically significant decreases of the water content was measured on the SC of the explants topically treated by cross-linked HA and HMW HA (- 10.7% and - 9.1% respectively, Table I).

More interestingly, a statistically significant increase of water content was observed in the epidermis of explants treated with cross-linked HA as compared to non-treated explants (+7.6%, Table I) whereas no significant differences were observed between the reference and the LMW and HMW treated explants.

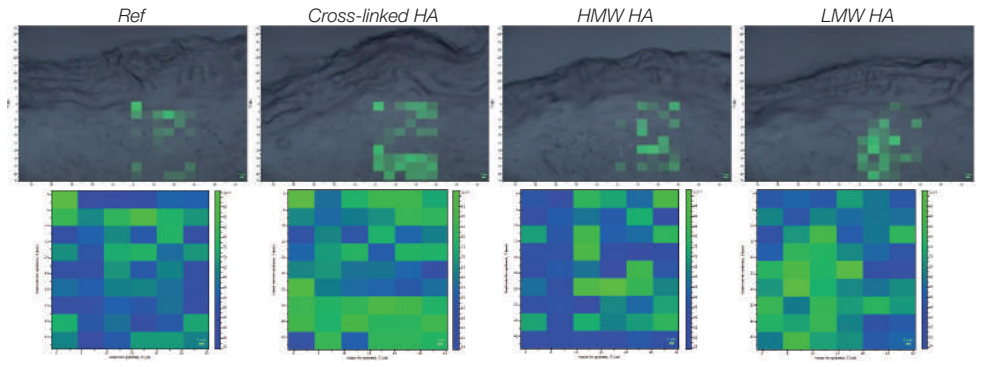


Figure 3: Spectral cartography of the water content of the living epidermis layer at D9. Deep blue means no water and bright green means high water content.

Cartographies visually confirmed the global higher water content of the epidermis of the explant treated with cross-linked HA as compared to the reference non-treated explant at D9 as well as the explants treated with HMW and LMW HA at D9.

Trans-Epidermal Water Loss

TEWL is a measurement of water vapor flux density ($kg.m^{-2}.s^{-1}$) and is therefore in a different unit range to water content measurement. After 2 days of treatment, the TEWL was significantly increased (+59.4%) when LMW HA was applied compare to the skin without treatment at D2. Only topical treatment with cross-linked HA and HMW HA, after 2 days, diminished TEWL (-23.8% and -11.7% respectively) compare to the skin without treatment.

This TEWL decrease, usually associated with an increase of the skin barrier function, was observed after only 2 days of treatment and was stronger with cross-linked HA.

DISCUSSION

The SC is an interactive, dynamic structure, and maintenance of hydration can impact its barrier function.[3] An ideal moisturizer would perform four functions: repair the skin barrier, maintain skin integrity and appearance, reduce TEWL, restore the lipid barrier's ability to attract, hold and redistribute water.[4]

Moisturizers are generally classified based on their mechanism of action as occlusive, humectants and emollient. Results obtained herein with LMW HA confirm the description of natural HA as a humectant.[5,6] Nonetheless, increasing the molecular weight of HA (HMW HA) apparently results in an occlusive type of action, while losing the capacity of penetration to act in the deeper levels of the skin.

Results obtained with cross-linked HA are different and of higher magnitude than those observed with LMW and HMW HA. The results reveal that when cross-linked HA is applied topically, the increase of the water content in the epidermis (+7.6%, Table I) reflects the feature of a humectant, whereas the drop in the TEWL (-23.8 %) is expected for an occlusive moisturizer.

One could hypothesize that cross-linked HA has the effect to reduce water-miscibility, improving the surface retention and a film forming capacity that are in favor of an occlusive action. The cross-linking process may also have the effect to generate a minority of smaller fragments of HA material (smaller than in a plain sample of HMW HA) that would be able to penetrate and act as humectant by attracting water from deeper skin layers.

Interestingly, cross-linked HA applied topically at the surface of skin explants also displays strengthening and smoothering capacities while keeping a good cohesion of corneocytes (as shown by electronic microscopy). Those surface observations could result from a strong film forming capacity of cross-linked HA.

CONCLUSION

The topical application of RHA (Resilient Hyaluronic Acid)® has several advantages :

- **The microrelief is smoother**
- **The TEWL is reduced by 23.8%**
- **Water content in the epidermis is enhanced by 7.6%**

The obtained results, give good confidence that RHA (Resilient Hyaluronic Acid®), Teoxane Laboratories, Switzerland) in a cosmetic preparation would perform several highly valuable functions: to reduce TEWL, hold and redistribute water, maintain skin integrity and appearance while strengthening the skin barrier.

REFERENCES

1 - Blank, I.H., Moloney, J., III, Emslie, A.G., Simon, I. and Apt, C. J Invest Dermatol. 1984, 82, 188. 2 - Kottner, J., Lichterfeld, A. and Blume-Peytavi, U. Br J Dermatol. 2013, 169, 528. 3 - Wan, D.C., Wong, V.W., Longaker, M.T., Yang, G.P. and Wei, F.C. J Clin Aesthet Dermatol. 2014, 7, 25. 4 - Anderson, P.C. and Dinulos, J.G. Curr Opin Pediatr. 2009, 21, 486. 5 - Chularojanamontri, L., Tuchinda, P., Kulthanan, K. and Pongparit, K. J Clin Aesthet Dermatol. 2014, 7, 36. 6 - Varothai, S., Nitayavardhana, S. and Kulthanan, K. Asian Pac J Allergy Immunol. 2013, 31, 91.

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