Note for general public
Study 21E5271

Assessment of the activity of six products using living human skin explants *ex vivo*

According to the study plan n° **D21-0418**

Tested products **Sublio Hyperionic Water Life Device**

A : Thermal water Balaruc F9
Retinol : DERMACEUTIC LABORATOIRE Activ Retinol 1.0 ref. RET1.0030
Calcium Carbonate: $CaCO_3$ ref. 398101

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STUDY

This study was the subject of a complete and detailed study report under reference 21E5271, submitted to SUBLIO France, sole owner of these results.

Date of the **beginning of the study**	02nd July 2021
Date of the end of the **technical phase of the study**	14th February 2022

CONTENTS

TESTED PRODUCTS

Retinol *Commercial formulation Activ Retinol 1.0, DERMACEUTIC LABORATOIRE*

Thermal water *Thermal water of Balaruc-les-bains*

Retinol-Ca *Commercial formulation Activ Retinol 1.0, DERMACEUTIC LABORATOIRE, with 1% of calcium carbonate*

Hyperionization device *The Sublio Hyperionic Water Life device provided by the company SUBLIO France, was used to hyperionize retinol formulations and thermal water, listed above.*

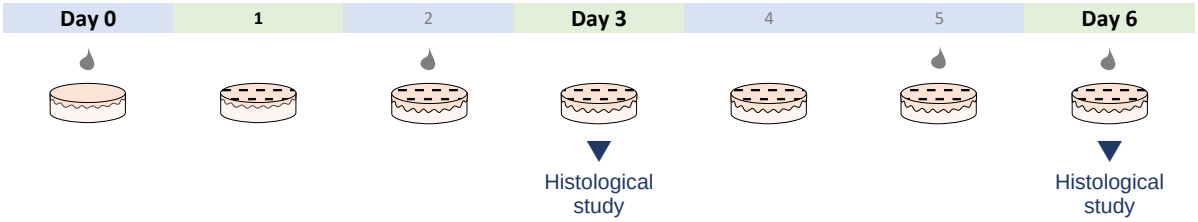
MATERIAL AND METHODS

This study aims to evaluate the biological activity of six treatment conditions on human skin explants in ex vivo survival.

Conditions of treatments :

P1 Retinol
P2 Hyperionized Retinol*
P3 Hyperionized thermal water of Balaruc-les-bains* then Retinol
P4 Hyperionized thermal water of Balaruc-les-bains* then hyperionized Retinol*
P5 Hyperionized Retinol-Ca*
P6 Hyperionized thermal water of Balaruc-les-bains* then Hyperionized Retinol-Ca*

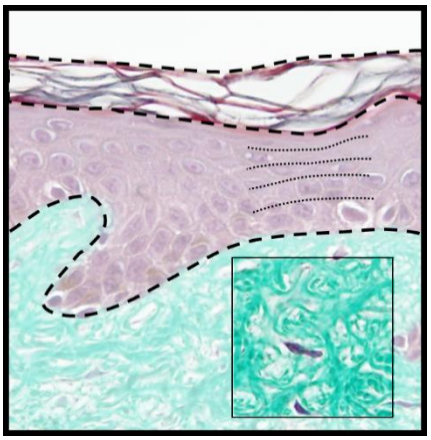
The formulations and water were applied to the surface of the explants at the rate of 1 µL/cm² at ten minutes intervals during double applications (thermal water ± *Hyperionized* then formulation ± *Hyperionized*).



After 3 and 6 days of treatment, a histological study was performed to evaluate the effect of different treatment conditions on the epidermis and dermis.
After staining with Masson's trichrome, cell and tissue morphology was evaluated by microscopic examination. To facilitate the comparison of different treatments, a tissue morphology score is calculated from morphological parameters.

CONCLUSION

Scoring parameters of tissue morphology



Evaluated parameters		*Notions linked*
	Thickness + Laminated aspect	**Barrier function Hydration**
4 at 5	Keratinocytes layers number	**Renewal Stimulation**
	Relief de la JDE	**Decreases with age Elastic network**
	Density of Dermis	**Elastic network Collagen network**

After 3 days of treatment	**Retinol** P1	**Hyperionized Retinol** P2	**Hyperionized water + Retinol** P3	**Hyperionized water + Hyperionized Retinol** P4	**Hyperionized Retinol-Ca** P5	**Hyperionized water + Hyperionized Retinol-Ca** P6
Stratum corneum	↔	↗	↗	↗	↗	↗
Epidermis	↔	↔	↔	↔	↔	↗↗
JDE	↔	↔	↔	↔	↔	↗
Dermis	↔	↔	↗	↗	↔	↗
Tissue morphology score	**67**	**77**	**80**	**80**	**77**	**95**

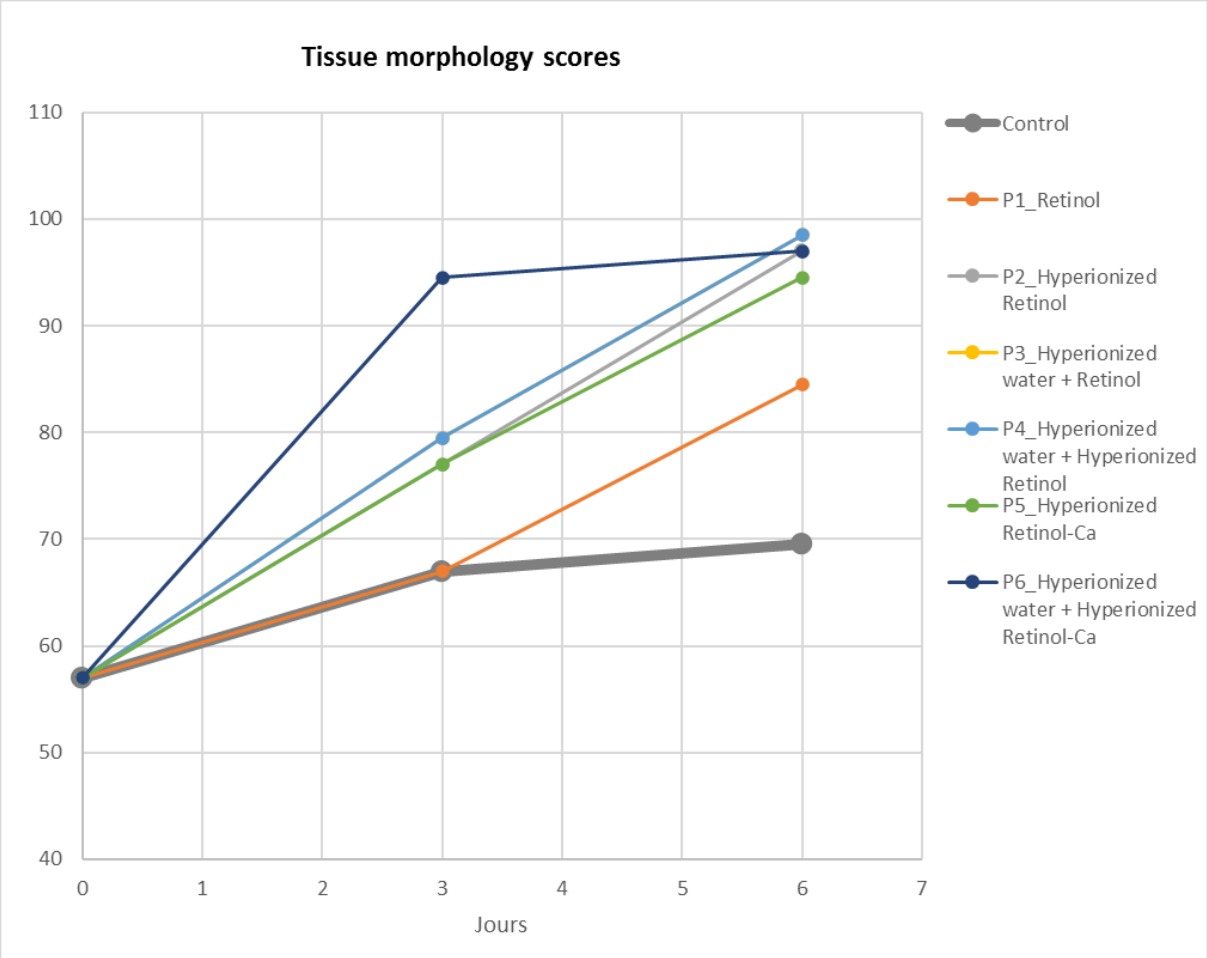
After 6 days of treatment	**Retinol** P1	**Hyperionized Retinol** P2	**Hyperionized water + Retinol** P3	**Hyperionized water + Hyperionized Retinol** P4	**Hyperionized Retinol-Ca** P5	**Hyperionized water + Hyperionized Retinol-Ca** P6
Stratum corneum	↗	↗	↗	↗	↗	↗
Epidermis	↗	↗↗	↗↗	↗↗	↗↗	↗↗
JDE	↗	↗	↗↗	↗↗	↗	↗
Dermis	↔	↗	↔	↔	↔	↗
Tissue morphology score	**85**	**97**	**99**	**99**	**95**	**97**

Decrease		**Increase**	↔	No variation
↘	Slight	↗	SC	*Stratum corneum*
↘↘	Moderate	↗↗	EpiD	Epidermis
↘↘↘	Fairly clear	↗↗↗	JDE	Dermo-epidermal junction
↘↘↘↘	Clear	↗↗↗↗	DP	Papillary dermis

After 3 days of treatment, all combinations of tested products are well tolerated by the skin.
After 6 days of treatment, all combinations of tested products induce epidermal alterations that are typical of a retinoic treatment.

⇨ **Morphology in accordance with retinoic treatments.**

Evolution of the tissue morphology score after 3 and 6 days of treatment.



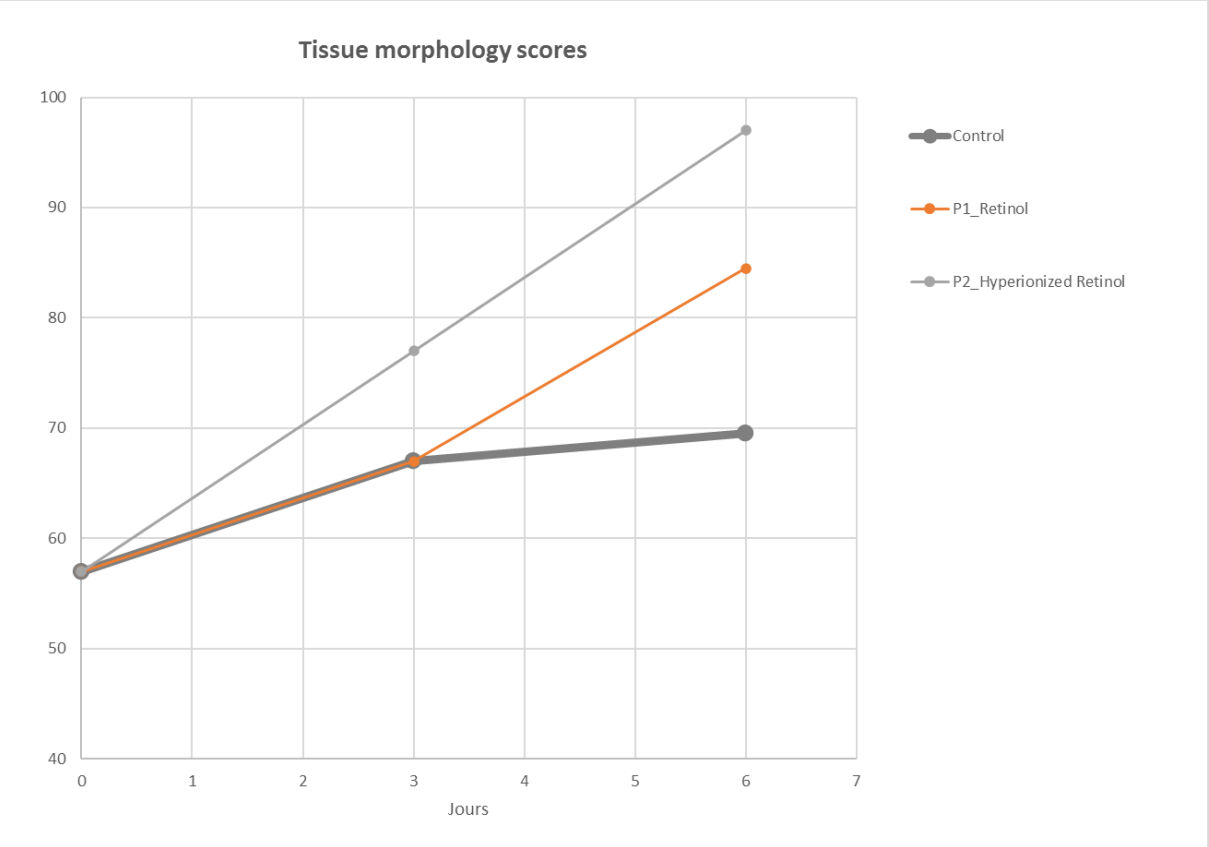
The P4 curve — is the same as P3 — and covers it.

After 3 days of treatment, retinol induces no modification in tissue morphology score. All other treatment combinations are significantly more active, starting on the third day of treatment.

After 6 days of treatment, retinol induces a clear increase in tissue morphology score, reflecting significant anti-aging activity.

All other treatment combinations are significantly more active than retinol alone.

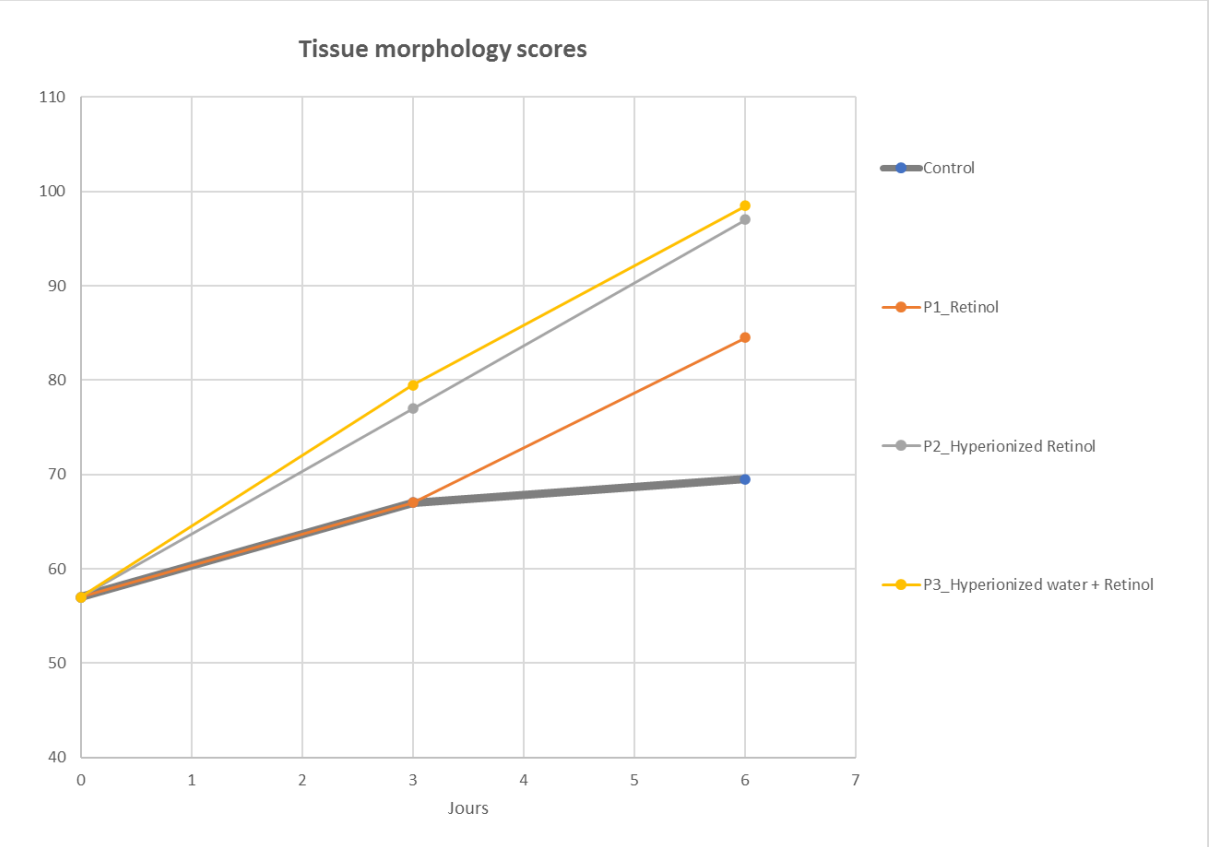
Effect of hyperionization of retinol



Compared to treatment with retinol alone (P1 —), treatment with hyperionized retinol (P2 —) induces an increase in the thickness of the epidermis, as well as a densification of the collagen network in the papillary dermis.
The hyperionization of retinol thanks to the Sublio Hyperionic Water Life device provided by the company SUBLIO France induces a potentiation of the effects of retinol.

⇨ **Hyperionization makes the retinol-based formulation significantly more active and earlier.**

Effect of the application of hyperionized thermal water before retinol



Compared to treatment with retinol alone (P1 —), treatment with hyperionized thermal water Balaruc F9 and then retinol (P3 —) induces a greater increase in the thickness of the epidermis, a densification of the collagen network in the papillary dermis on Day 3, as well as an increase in the relief of the dermal-epidermal junction.

The addition of hyperionized thermal water Balaruc F9 before treatment with retinol induces a potentiation of the effects of retinol.

⇨ **The application of hyperionized thermal water makes the retinol-based formulation significantly more active and earlier.**

Hyperionization of retinol applied after hyperionized water does not bring improvement. The results of P3 and P4, presented on page 4, are superimposed.

Effect of the addition of calcium to retinol

The addition of calcium carbonate to hyperionized retinol (P5 —) does not induce potentiation of the effects of hyperionized retinol (P2 —).

⇨ **The addition of calcium to hyperionized retinol does not improve the effect of hyperionized retinol.**

On the other hand, the addition of hyperionized thermal water before treatment with hyperionized retinol with added calcium (P6 —) makes it possible to potentiate the effects of retinol with an increase in the thickness of the epidermis, a densification of the collagen network in the papillary dermis, as well as an increase in the relief of the dermal-epidermal junction after only 3 days of treatment.

⇨ **The application of hyperionized thermal water before that of retinol with added calcium and hyperionized, makes retinol much more active and earlier.**

MESSAGE TO REMENBER

⇨ **Hyperionization of a cosmetic formulation containing retinol makes it significantly more active and faster.**

⇨ **The application of hyperionized thermal water on the skin before the application of a cosmetic formulation containing retinol makes it significantly more active and more quickly.**

⇨ **The addition of calcium in the hyperionised formulation containing retinol does not alter the effect of retinol.**

⇨ **The application of hyperionized thermal water before the application of a hyperionized cosmetic formulation containing retinol and calcium makes it significantly more active and much more quickly.**

Retinol is a powerful cosmetic active ingredient with also a certain skin intolerance, it is applied at a dose of 1 microliter (µL) per cm² unlike other cosmetic products usually tested at a dose of 2 µL / cm².
Hyperionized thermal water was applied at the same dose of 1 µL/cm².

⇨ **It should be good to notify that these results were obtained with doses of only 1 µL/cm², which makes their activity even more significant.**

For comparison, a drop of eye drops distributed with a dropper bottle represents a volume of 30 to 50 µL.