Outreach note of the study report 21E5272 SUBLIO

According to the study plan **D21-0417**

Exploration of the effect of the hyperionisation of tap water and sea water, by a genomic study on human skin explants *ex vivo*

Tested products

Tap water (Longjumeau) +/- hyperionised with the Sublio Ionic WaterBox *Pro* device

Sea water (Longjumeau) +/- hyperionised with the Sublio Ionic WaterBox *Pro* device

Sponsor

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CONTENTS

STUDY

This study was subject to complete and detailed reports under the reference 21E5272 ; returned to SUBLIO France, only owner of these results.

Date of the beginning of the study	24th June 2021
Date of the beginning of the technical phase of the study	21st September 2021
Subcontracting partners performing the genomic phase	**Laboratoire Genex** 1, Chemin de Saulxier 91160 Longjumeau

TESTED PRODUCTS

P1 Water (Longjumeau city), sterilized by filtration at 0.22µm.

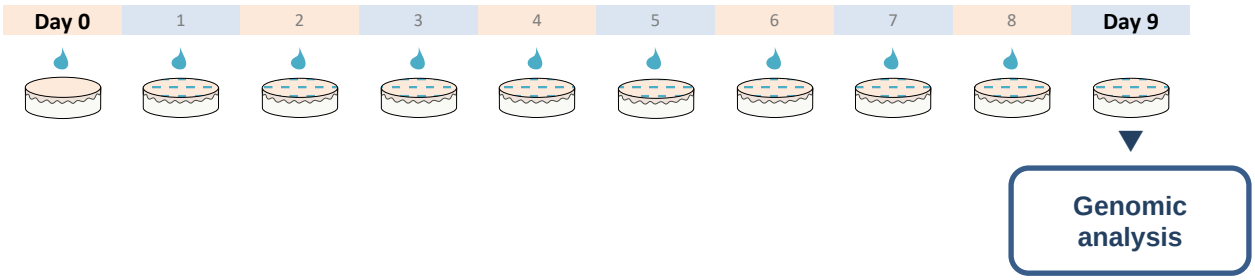
P2 Water (Longjumeau city), sterilized by filtration at 0.22µm and hyper-ionised thanks to the Sublio Ionic WaterBox *Pro* device provided by SUBLIO France company.

P3 Sea water (Saint-Malo city) provided by SUBLIO France company and sterilized by filtration at 0.22µm (sea water is stored at 4°C and must be used within 48 hours after the collect).

P4 Sea water (Saint-Malo city) provided by SUBLIO France company, sterilized by filtration at 0.22µm and hyper-ionised thanks to the Sublio Ionic WaterBox *Pro* device provided by SUBLIO France company (sea water is stored at 4°C and must be used within 48 hours after the collect).

MATERIAL & METHODES

The aim of the study is to apply water (hyperionised or not) daily on human skin explants kept in survival for 9 days. Then, a genomic study was conducted by the Genex laboratory to analysis the expression of 10 genes stimulated or repressed by the treatment.



After 9 days of treatment (2µL/explant), the explants are harvested and fixed in RNAlater to preserve RNA.

After extraction, the quantity and the quality of RNA was controlled.

The extracted RNA were retro-transcribed (iScript, Bio-Rad). They were analyzed and semi-quantified by qPCR (iTaq, Bio-Rad) in order to evaluate the 10 gene of interest.

A histological study has been realized simultaneously in order to check cell and tissue morphology.

RESULTATS

1. Control of the morphology

After 9 days of treatment, the tap water (P1) and the sea water (P3) are well tolerated by the skin.

After 9 days of treatment, the tap water or the sea water hyperionised with the Sublio Ionic WaterBox Pro device (P2 or P4 respectively) are also well tolerated by the skin.

The hyperionisation does not result in any change in the skin tolerance of tap water and sea water.

2. Genomic study

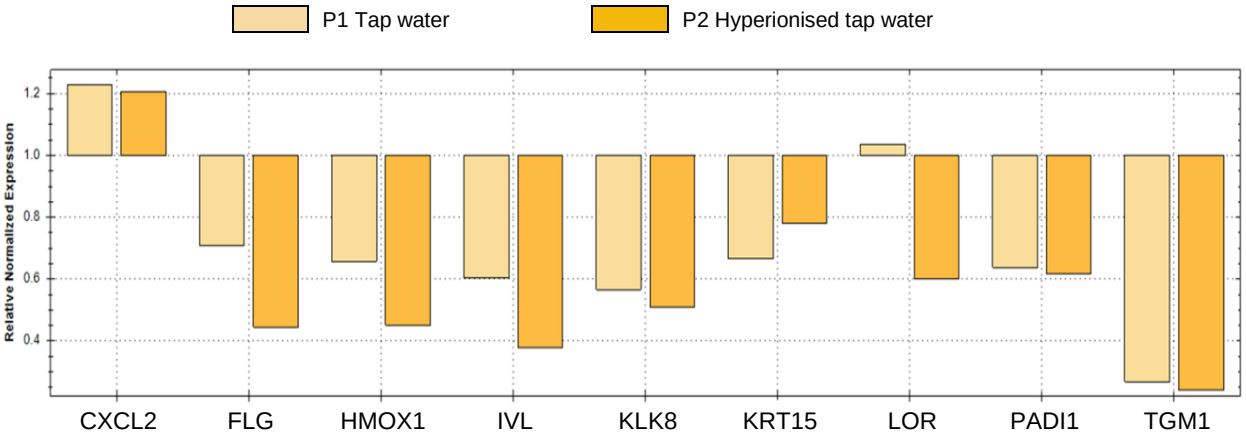
The following table summarizes the symbol and the name of each gene of interest (selected for the study from the results of the previous genomic study 20E5076) with their respective qPCR efficiency and their biological function. Two housekeeping genes (B2M et GAPDH) and a RT (reverse transcriptase) control have also been included in this analysis.

gene	name	qPCR efficiency	Function
CXCL2	chemokine (C-X-C motif) ligand 2, exonic	94%	**Inflammation** Inflammatory mediator
FLG	filaggrin	98%	**Cutaneous barrier** Involved in keratinocyte differentiation and NMF formation
HMOX1	heme oxygenase 1	90%	**Oxidative stress** Detoxifying enzyme
IVL	involucrin	96%	**Cutaneous barrier** Involved in keratinocyte differentiation
KLK8	kallikrein-related peptidase 8, exonic	100%	**Cutaneous barrier** Involved in desquamation
KRT15	keratin 15, exonic	95%	**Epidermal stimulation** cytokeratin specific of the basal layer of keratinocytes
LOR	loricrin (exonic)	102%	**Cutaneous barrier** Involved in keratinocyte differentiation
PADI1	peptidyl arginine deiminase, type I, exonic	98%	**Cutaneous barrier** Involved in NMF formation
SPRR3	small proline-rich protein 3, exonic	99%	**Cutaneous barrier** Involved in keratinocyte differentiation
TGM1	transglutaminase 1, intron-spanning	98%	**Cutaneous barrier** Involved in keratinocyte differentiation
B2M	house keeping	98%	/
GAPDH	house keeping (exonic)	97%	/

The analysis of the housekeeping genes B2M and GAPDH shows a stable amplification with high quality.
An average was calculated from the values of the three explants from the same donor (biological groups) after normalization with the values of the housekeeping genes.
For each gene of interest, a ratio between the different treatment conditions was calculated. Thus, these ratios allow to identify the effect of hyperionisation of tap water and sea water. We have chosen the following modulation threshold: a value greater than 1.15 to define an induced expression and a value lower than 0.8 to define a repressed expression.

Activity of tap water +/- hyperionised

Expression ratios between the two averages of the expression values for tap water (P1) or hyperionised tap water (P2) versus control (T), on day 9.



Comparison P2 vs P1

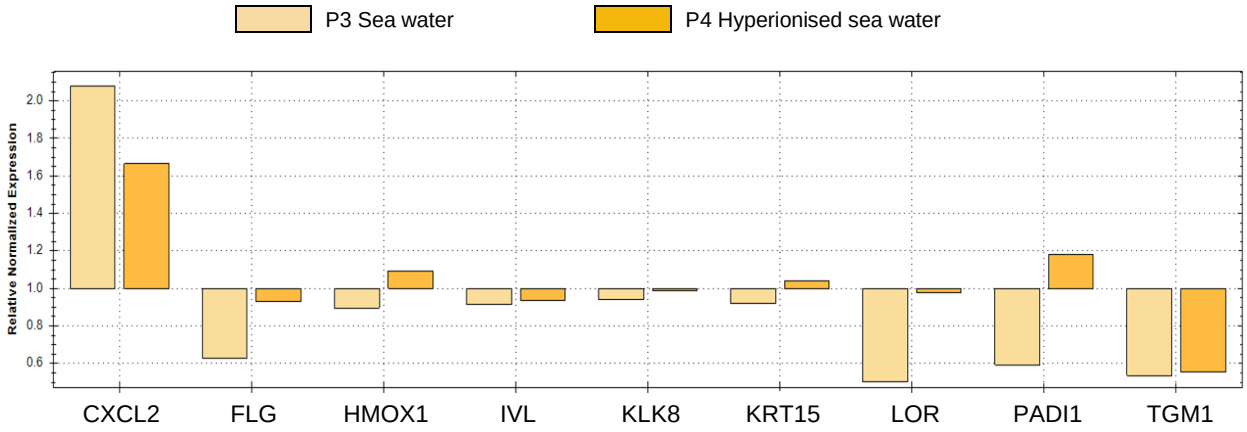
CXCL2	FLG	HMOX1	IVL	KLK8	KRT15	LOR	PADI1	TGM1
↔	↘	↘	↘	↔	↗	↘	↔	↔

For this donor, the tap water (■) induces a significant increase of the inflammation (CXCL2) et a significant decrease of the genes involved in the formation and the regulation of cutaneous barrier (FLG, IVL, KLK8, PADI1 et TGM1).
Furthermore, it decreases epidermal renewal (KRT15) and reduces the basal level of oxidative stress (HMOX1).
All other genes were not modulated.

The hyperionised tap water (■) amplifies the decrease of the expression of gene involved in the formation and the regulation of cutaneous barrier (FLG, IVL et LOR).
This treatment also amplifies the decrease of oxidative stress (HMOX1) and stimulates the epidermal renewal (KRT15).

Activity of sea water +/- hyperionised

Expression ratios between the two averages of the expression values for sea water (P3) or hyperionised sea water (P4) versus control (T), on day 9.



Comparison P4 vs P3

CXCL2	FLG	HMOX1	IVL	KLK8	KRT15	LOR	PADI1	TGM1
↘	↗	↗	↔	↔	↗	↗	↗	↔

The sea water (■) induces an increase of CXCL2, a gene implied in cutaneous inflammation, and a decrease of the genes involved in the formation and the regulation of cutaneous barrier (FLG, LOR, PADI1 et TGM1).

The hyperionised sea water (■) significantly decreases the expression of CXCL2, a gene implied in cutaneous inflammation, and increases the genes involved in the formation and the regulation of cutaneous barrier (FLG, LOR et PADI1). It also increases the expression of HMOX1 which shows antioxidant properties.

CONCLUSION

Tap water (Longjumeau, 3rd donneur)

These results unambiguously show that the treatment of the skin explants with hyperionised tap water amplifies the results obtained with water, with a process that leads to a renewal of cutaneous tissue from D9.

It has been shown that the process begins with a moderate osmotic stress which induces an inflammation. Then, the inflammatory state decreases which allows to the establishment of cell differentiation and consequently, regeneration of cutaneous tissue.
This process requires the formation of factors which inhibit the oxidative stress, decrease the inflammatory state and stimulate the process involved in the stratum corneum formation during terminal epidermal differentiation.

This study concerns the evaluation of hyperionisation of tap water on a third donor. Please refer to the global analysis of the results of the three donors.

Sea water

This first analysis shows that the treatment of the skin explants with sea water induces a slight inflammation associated with a repression of the markers of the cutaneous barrier.

However, these results cover only 10 of the 23 000 human genes. These 10 genes only represent a small part of the many biological effects developed during thalassotherapy.

The repression of filaggrin, loricrin and PADI1 clearly shows nevertheless that the prolonged contact with sea water induces a cutaneous barrier alteration.

The hyperionisation of sea water allows to inhibit these side effects by reducing the skin inflammation, by limiting the decrease of filaggrin and loricrin and by totally preventing the decrease of PADI1.

By reducing skin inflammation and by regulating skin hydration, the hyperionised sea water allows to significantly improve the beneficial effects of thalassotherapy.