Outreach note of the sudy report 20E5076 SUBLIO
According to the study plan D19-732-1

Exploration of the activity of tap water vs the same water hyperionised Sublio, by a transcriptomic study on human skin explants *ex vivo*

Tested products
- **Tap water (Longjumeau)**
- **Tap water (Longjumeau) hyperionised with the Sublio Ionic WaterBox *Pro* device**

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STUDY

This study was subject to a complete and detailed report under the reference 20E5076, returned to SUBLIO France, only owner of these results.

Date of the **beginning of the study**	22nd January 2021
Date of the **end of the technical phase of the study**	26th March 2021
Subcontracting partners performing the genomic phase	**Laboratoire Genex** 1, Chemin de Saulxier 91160 Longjumeau

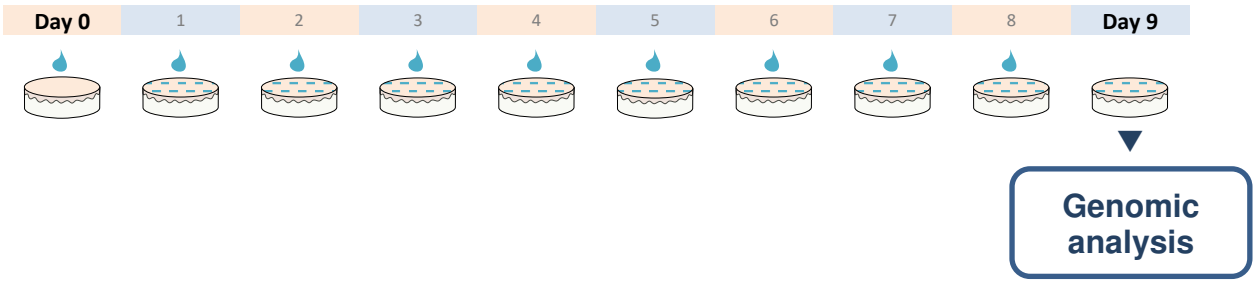
TESTED PRODUCTS

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.

MATERIALS & METHODS

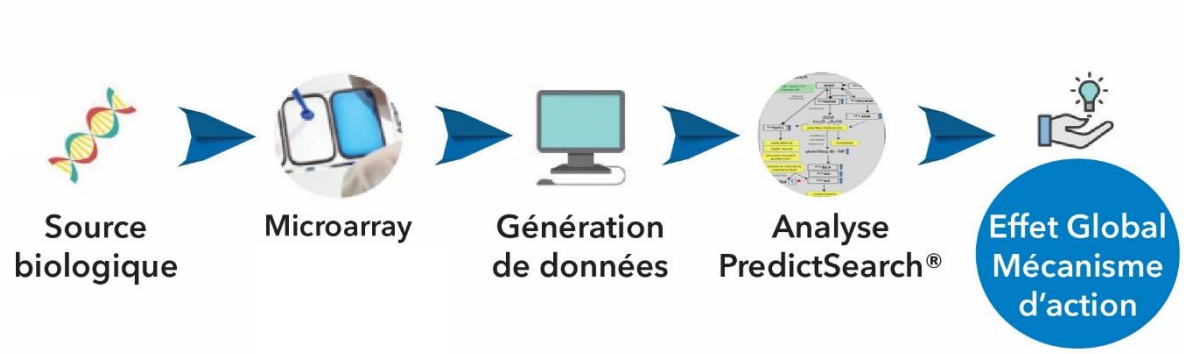
The aim of the study is to apply water (hyperionised or not) daily on human skin explants kept in survival for 9 days. Then, a genomic study was conducted by the Genex laboratory to identify the genes stimulated or repressed by the treatment.



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, amplified, marked with Cyanine-3 and hybridized on chips containing the entire human genome (23 000 genes).

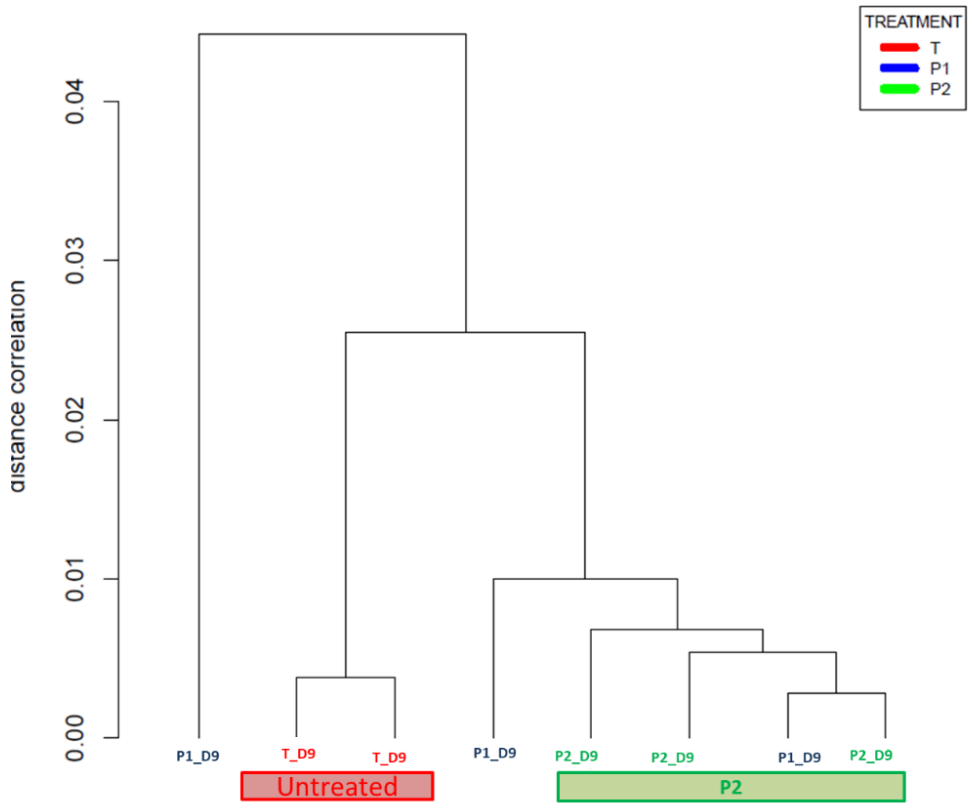


RESULTS

1. Hierarchical classification

The gathering of samples according to the gene expression profiles allows to visualize the separation of non-treated samples (in red) and the gathering of samples in response to P2 (in green). The response P1 is more dispersed (in blue).

Hierarchical classification



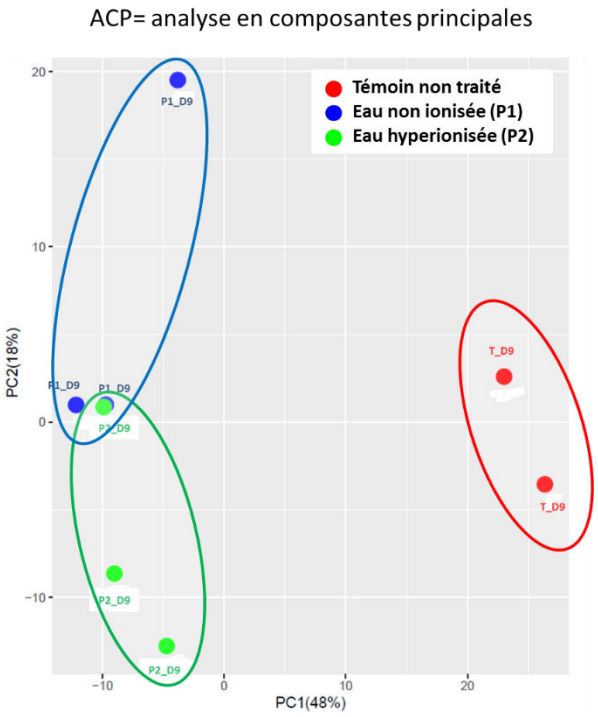
- After P1 treatment, the gene response is dispersed and seems random.
- After P2 treatment (hyperionic water) the gene response is very clustered which shows that it is real, coherent and reproducible.

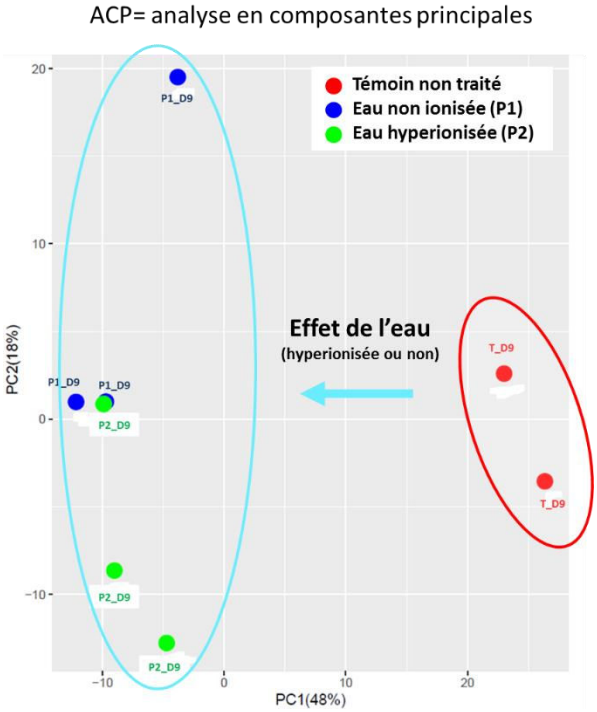
2. Principal component analysis

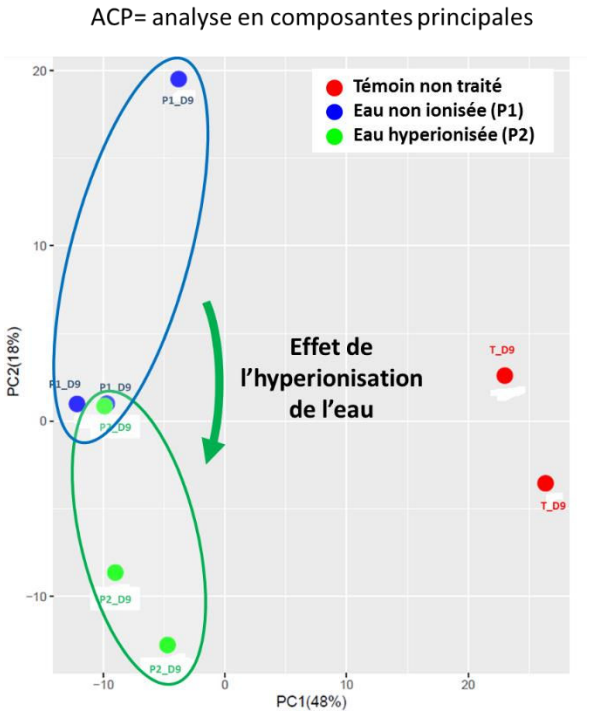
Principal component analysis (PCA) is a powerful tool for information compression and synthesis, which is used to treat and interpret a big number of quantitative data.

For this study, a principal component analysis was performed on gene expression profiles.

The first component shows 48% of variation between non-treated samples on the right and treated samples on the left. The second component (responsible for 18% of variation) is linked to P2 and P1 treatment effects, on the left.



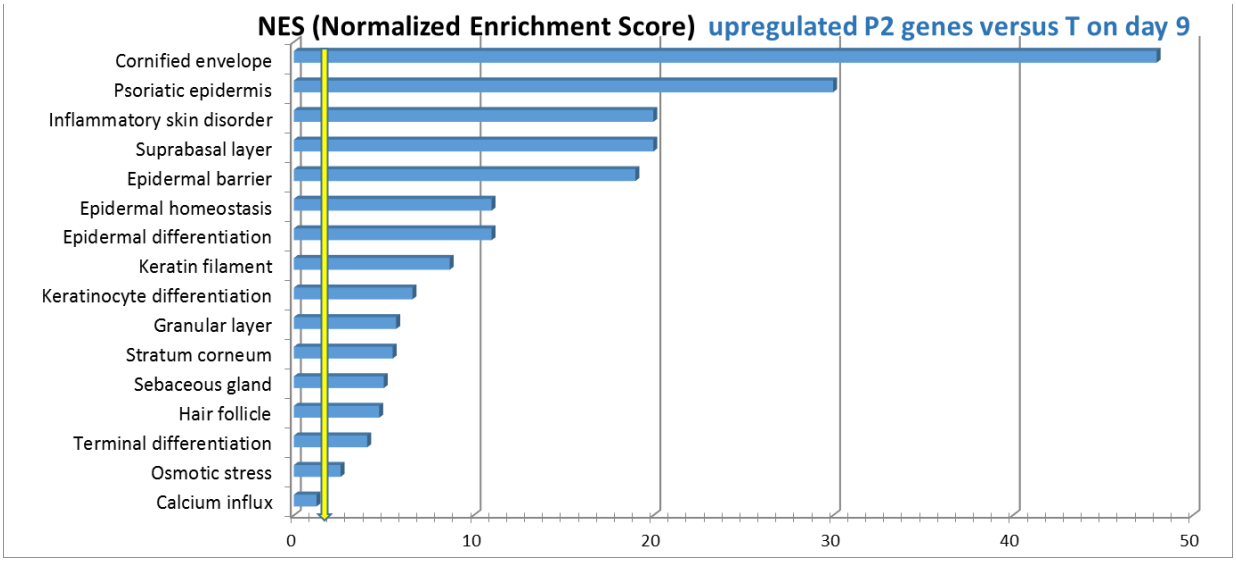




➥ This analysis shows the effect of water on cutaneous gene response and the effect of water hyperionisation.

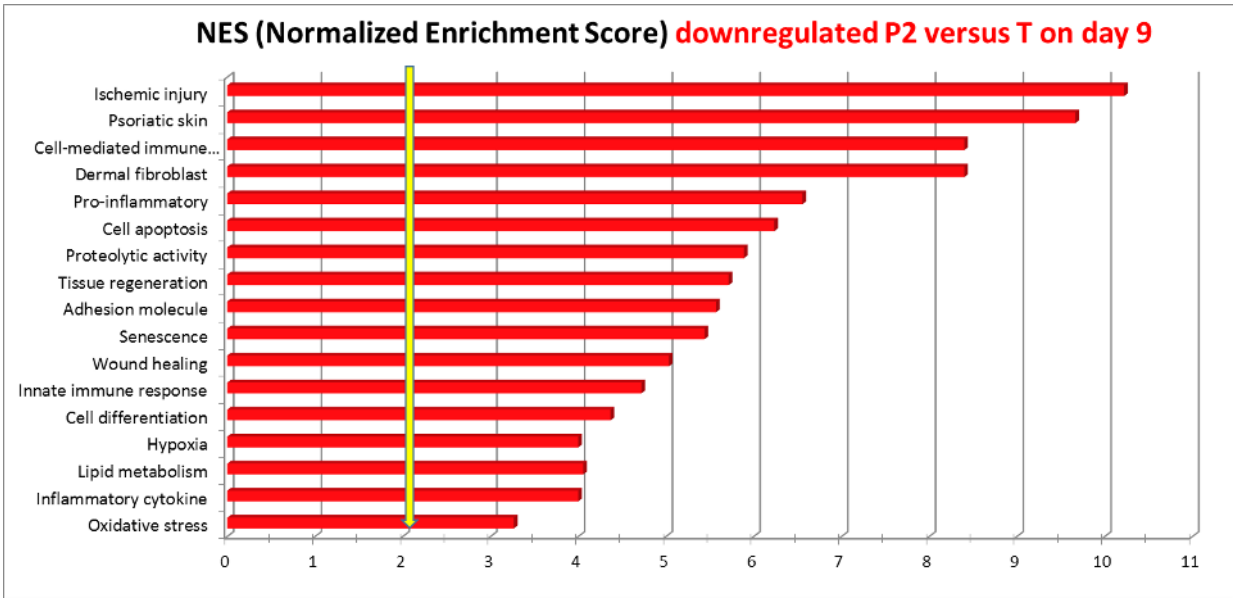
3. Enrichment of genes induced with hyperionised water

The enrichment was deducted from the genes identified with a drastic threshold (≥2,0 for induced genes, and ≤0,5 for repressed genes) because of the big number of modulated genes (188 induced annotated genes and 186 repressed annotated genes whose expression was specifically modulated).



Histogram of the biological terms annotated by PredictSearch® and classified in function of the NES score (Normalized Enrichment Score) for the genes induced in response to hyperionised water on day 9.
The yellow bar indicates the enrichment threshold of 2.

4. Enrichment of genes repressed with hyperionised water



Histogram of the biological terms annotated by PredictSearch® and classified in function of the NES score (Normalized Enrichment Score) for the genes repressed in response to hyperionised water on day 9.
The yellow bar indicates the enrichment threshold of 2.

The first observation on the modulated terms on D9 is that the inflammation is inhibited, and the differentiation is on the contrary induced.

The stimulation of the differentiation is illustrated by the induction of genes of the epidermal differentiation complex. Hence, the most enriched term for the induced genes-associated terms is « cornified envelope ». Among these genes, we can find the genes coding for the proteins of the cornified envelope that are involved in its formation during skin tissue regeneration.

In a consistent manner, a big number of terms identified by the induced genes such as « Epidermal differentiation », « Keratinocyte differentiation » and «Terminal differentiation » confirm without ambiguity the initiation of a differentiation process on D9.
It is important to notice that the genes which appear with the term « inflammatory skin disorder » are not inflammatory genes but are associated with protective activities in response to inflammatory or oxidative stress

On the contrary, the genes associated to inflammation such as « Ischemic injury » and « Proinflammatory » are repressed by hyperionised water on D9. It is the same for the term « Cell differentiation », associated to genes that are known to be linked to an inhibition of the differentiation. For example, LIF (Leukaemia Inhibitory factor) is a pro-inflammatory cytokine which plays a role in keratinocytes proliferation and whose repression precedes their differentiation

CONCLUSION

These results demonstrate without ambiguity that the treatment of explants by hyperionised water amplifies the results obtained with water, by a process that leads to skin tissue regeneration on D9.

The process is initiated with a moderate osmotic stress inducing a medium inflammation which then fades, allowing the establishment of a differentiation and in consequence a tissue regeneration.

This process implies the formation of oxidative stress inhibitory factors that decrease the inflammatory state and activate factors ensuring the development of the cornified cell envelope during a terminal differentiation process.

However, we need to point out that the water hyperionised thanks to the Sublio Ionic WaterBox *Pro* device, in the used conditions, amplifies significantly the modulations induced by the non-ionised water whatever are its biological effects.