FRENCH POLYNESIAN LAGOON WATER FOR A STRONGER SKIN BARRIER

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1 INTRODUCTION

It has been widely demonstrated that minerals have an important role in skin physiology [1]. While high content mineral salts thermal waters, are widely known and used for cosmetic purpose, for sea water only Israeli Dead Sea water has been studied extensively. Thus, it could be interesting to investigate the properties of other sea water such as French Polynesian lagoon water which could have also a specific mineral composition.

It has been widely demonstrated that the quality of the skin barrier function is a major factor for the skin capacity to face exogenous damages and limit trans-epidermal water loss. At cellular level, this barrier function is the result of different biological processes such as keratinocytes differentiation and cornification.

From basal keratinocytes to superficial corneocytes, several proteins are involved such as transglutaminase K and involucrin participating in the formation of the cornified envelope or filaggrin helping in the formation of the corneocyte matrix and involved in the production of Natural Moisturizing Factor (NMF) in the skin. Tight junction proteins such as Claudin-1 also plays a role in the cohesion of the stratum corneum by ensuring the sealing between corneocytes [2].

2 MATERIAL & METHODS

SEA WATER

The sea water tested in this study was reasonably collected in French Polynesia, more precisely in the lagoon of Tahiti around 10 meters depth in no way endangers the maritime ecosystem. The mineral profile of this sea water was analyzed using ion chromatography (IC).

KERATINOCYTES CULTURES TREATMENTS

Cells cultures were performed using Normal Human Epidermal Keratinocytes (NHEK), at the 3rd passage and preliminary inoculated in a supplemented Keratinocyte-serum free medium, in a 96-well plate for 24 hours (TGK, claudin-1 and involucrin assays) or 192 hours with medium renewal after 24 and 96 hours (filaggrin assay).

After first incubation time, the culture medium was replaced with the treatment medium composed of 50% supplemented Keratinocyte-serum free medium and 50% MCDB153 powder medium reconstituted with high mineral salts content water. Controls were also prepared using ultrapure water for medium reconstitution and containing (positive reference) or not (negative control) CaCl2.

All experimental conditions were then incubated for a second time, 72 hours (TGK, Filaggrin, claudin-1) or 144 hours with treatment renewal after 72 hours (involucrin) and, performed in triplicated.

IN-SITU IMMUNOFLUORESCENT LABELLING

At the end of incubation time, the assay medium was discarded, and the cells were rinsed, fixed, permeabilized and then labelled using a specific primary antibody which were then revealed using a fluorescent secondary antibody. In parallel, the cells nuclei were colored using Hoechst solution 33258 (bis-benzimide, Sigma, ref. B1155).

IMAGE ACQUISITION

The image acquisition (5 photos/well) was performed with an INCell AnalyzerTM 2200 (GE Healthcare, x20 objective lens). The labeling was quantified by the measurement of the fluorescence intensity and then normalized to the total number of cells (Integration of numerical data with the Developer Toolbox 1.5, GE Healthcare software).

STATISTICS

Raw data were analyzed using Microsoft Excel® software. The inter-group comparisons were performed by an unpaired Student's t-test. A difference between two groups is considered as statistically significant if the p-value is less than 0.05.

REFERENCES

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[2] Kirschner N and Brandner J M. (2012) Barriers and more: functions of tight junction proteins in the skin. Ann NY Acad Sc. 1257:158-166

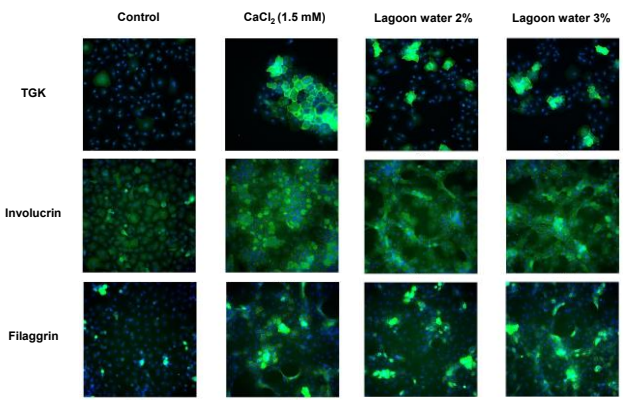
3 RESULTS & DISCUSSIONS

MINERAL PROFILE

Mineral profile analysis using IC of French Polynesian lagoon water shows a high mineral salts content, especially in **sodium** and **chlorine** (consistent with the sea water origin) as well as **calcium** (199 mg/ml), **magnesium** (1236 mg/ml), or **sulphates** (2700 mg/ml) which is a composition representative of batch reference.

EPIDERMIC DIFFERENTIATION PROTEINS EXPRESSION

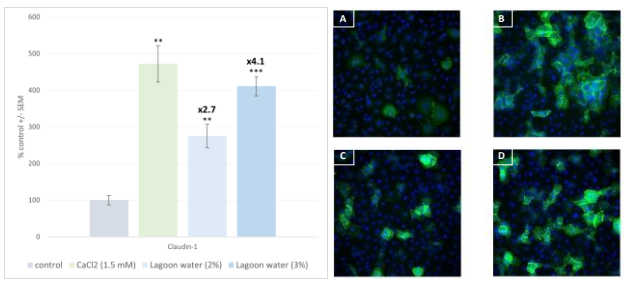
Under the experimental conditions of this study, the high mineral salts content water tested at 2% and 3%, stimulated with a concentration-dependent pattern, the expression of keratinocyte differentiation proteins. A statistically significant stimulation of **TGK** (on average x4.8 and x5.7), **involucrin** (on average x3.2 and x3.8) and **filaggrin** (on average x8.4 and x10.0) expressions was observed.



Representative images of the epidermic differentiation proteins (TGK, involucrin, filaggrin) expression (green) by human keratinocytes, not treated (negative control) or treated with CaCl2 (positive reference) or lagoon water at 2% or 3%.

EPIDERMIC COHESION PROTEINS EXPRESSION

Regarding the tight junction protein expression, the treatment of NHEK with the high mineral salts content water at 2% and 3%, resulted in a statistically significant increase in the cellular expression of **claudin-1** protein with a concentration-dependent pattern (on average x2.7 and x4.1 of the negative control).



*Epidermic cohesion proteins expression by human keratinocytes (Claudin-1), not treated (negative control) or treated with CaCl2 (positive reference) or lagoon water at 2% or 3%. (** p<0.01; ***p<0.001 Student t-test). Representative images of the protein expression: negative control (A), positive reference CaCl2 (B) or lagoon water at 2% (C) or 3% (D).*

4 CONCLUSIONS

In-vitro investigations on high mineral salts content French Polynesian lagoon water show its ability to stimulate, in normal human keratinocytes, the production of major proteins involved in the biological processes of keratinocytes differentiation (TGK, involucrin and filaggrin) and epidermidis cohesion (Claudin-1). These strong stimulations might be the consequences of the specific richness in calcium and magnesium, two minerals described for their key roles in keratinocyte physiology, of the French Polynesian lagoon water tested. These actions highlight our sea water as an interesting active for the reinforcement of the skin barrier function and allow us to go further in the interest of lagoon waters for skincare.

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