FRENCH POLYNESIAN LAGOON WATER FOR A STRONGER SKIN BARRIER

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Abstract

Background: It has been widely demonstrated that minerals have an important role in skin physiology. 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 French Polynesian lagoon water which could have also a specific mineral composition. The objective of this study was to assess if French Polynesian high mineral salts content water might improve keratinocyte differentiation and cohesion, as compared to $CaCl_2$.

Methods: Normal human epidermal keratinocytes were inoculated in culture medium during different times according to the biological marker observed. Cellular expression was then revealed using immunofluorescence labelling.

Results: French Polynesian high mineral salts content water culture medium significantly stimulated the production of keratinocytes differentiation proteins (transglutaminase, involucrin, filaggrin) and cohesion proteins (Claudin-1).

Conclusion: The lagoon water tested stimulates the production of keratinocyte differentiation and cohesion proteins. This action will lead to the reinforcement of the skin barrier function.

Keywords: Polynesian water – skin barrier – keratinocytes differentiation – keratinocytes cohesion.

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].

Materials & 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. Before commercialization the water is filtered and conserved with phenoxyethanol.

The mineral profile of this sea water was analyzed using ion chromatography (IC).

Keratinocyte cultures and treatments:

Cells cultures were performed using Normal Human Epidermal Keratinocytes (NHEK), Bioalternatives reference K341 at the 3rd passage and preliminary inoculated in a Keratinocyte-serum free medium supplemented with 0.25 ng/ml epidermal growth factor, 25µg/ml pituitary extract and 25µg/ml Gentamycin 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 this first incubation time, the culture medium was replaced with the treatment medium composed of 50% Keratinocyte-serum free medium supplemented with 25µg/ml Gentamycin and, 50% MCDB153 powder medium reconstituted with high mineral salts content water previously filtered on 0.22 µm and supplemented with sodium bicarbonate and 25µg/ml Gentamycin.

In parallel, controls were also prepared including NHEK in a treatment medium composed of 50% Keratinocyte-serum free medium supplemented with 25µg/ml Gentamycin and, 50% MCDB153 powder medium reconstituted with ultrapure water containing (positive reference) or not (negative control) $CaCl_2$ supplemented with sodium bicarbonate and 25µg/ml Gentamycin.

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 **(Table 1)**. In parallel, the cells nuclei were colored using Hoechst solution 33258 (bis-benzimide, Sigma, ref. B1155).

Table 1: Primary and secondary antibodies used for *in-situ* immunofluorescent labelling

Protein	**Primary antibody**	**Secondary antibody**
TGK	Anti-TGK Proteintech, ref. 12912-3-AP	GAM-Alexa 488 Invitrogen, ref. A11001
Involucrin	Anti-Involucrin Sigma, ref. I9018	GAM-Alexa 488 Invitrogen, ref. A11001
Filaggrin	Anti-filaggrin Santa Cruz, ref. sc-66192	GAM-Alexa 488 Invitrogen, ref. A11001
Claudin-1	Anti-Claudin-1 Santa Cruz, ref. sc-81796	GAM-Alexa 488 Invitrogen, ref. A11001

Image acquisition:

The image acquisition (5 photos/well) was performed with an INCell Analyzer™ 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.

RESULTS

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, magnesium, or sulphates which is a composition representative of batch reference **(Table 2).**

Table 2: Mineral profile of the French Polynesian lagoon water tested.

Anions / Cations	**[ ] (mg/ml)**
HCO_3^-	493
Cl^-	21604
SO_4^{2-}	2700
Na^+	10809
Mg^{2+}	1236
K^+	334
Ca^{2+}	199

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. Considering negative control at 100% of expression, a statistically significant stimulation of TGK (on average 385% and 478% of the negative control), involucrin (on average 229% and 289% of the negative control) and filaggrin (on average 746% and 906% of the negative control) expressions was observed **(Figure 1 & 2)**.

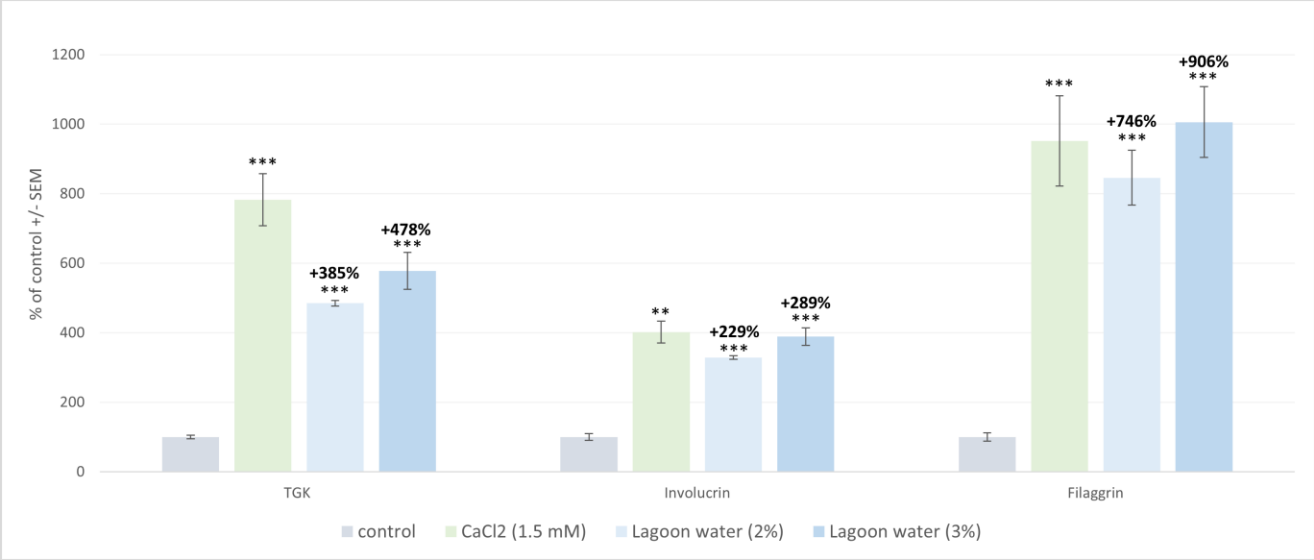

Figure 1: Epidermic differentiation proteins expression by human keratinocytes, not treated (negative control) or treated with $CaCl_2$ (positive reference) or lagoon water at 2% or 3%. (** p<0.01; ***p<0.001 Student t-est)

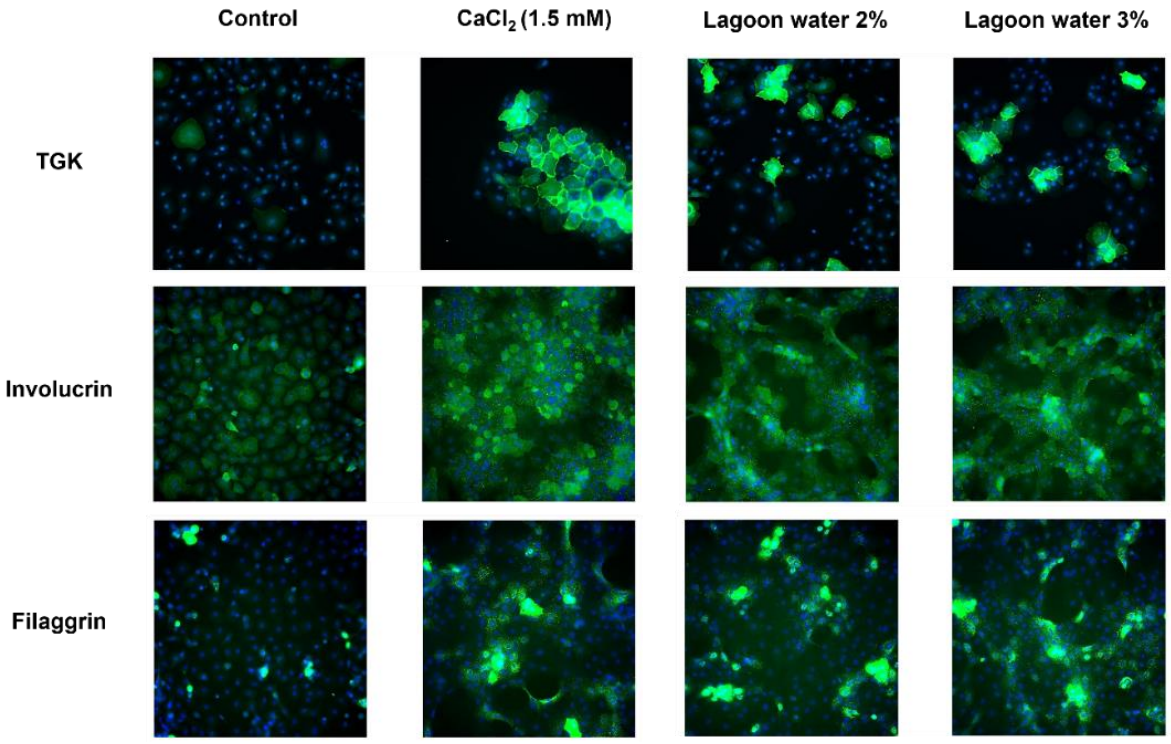

Figure 2: Representative images of the epidermic differentiation proteins (TGK, involucrin, filaggrin) expression (green) by human keratinocytes, not treated (negative control) or treated with $CaCl_2$ (positive reference) or lagoon water at 2% or 3%.

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 175% and 311% of the negative control) ((**Figure 3)**.

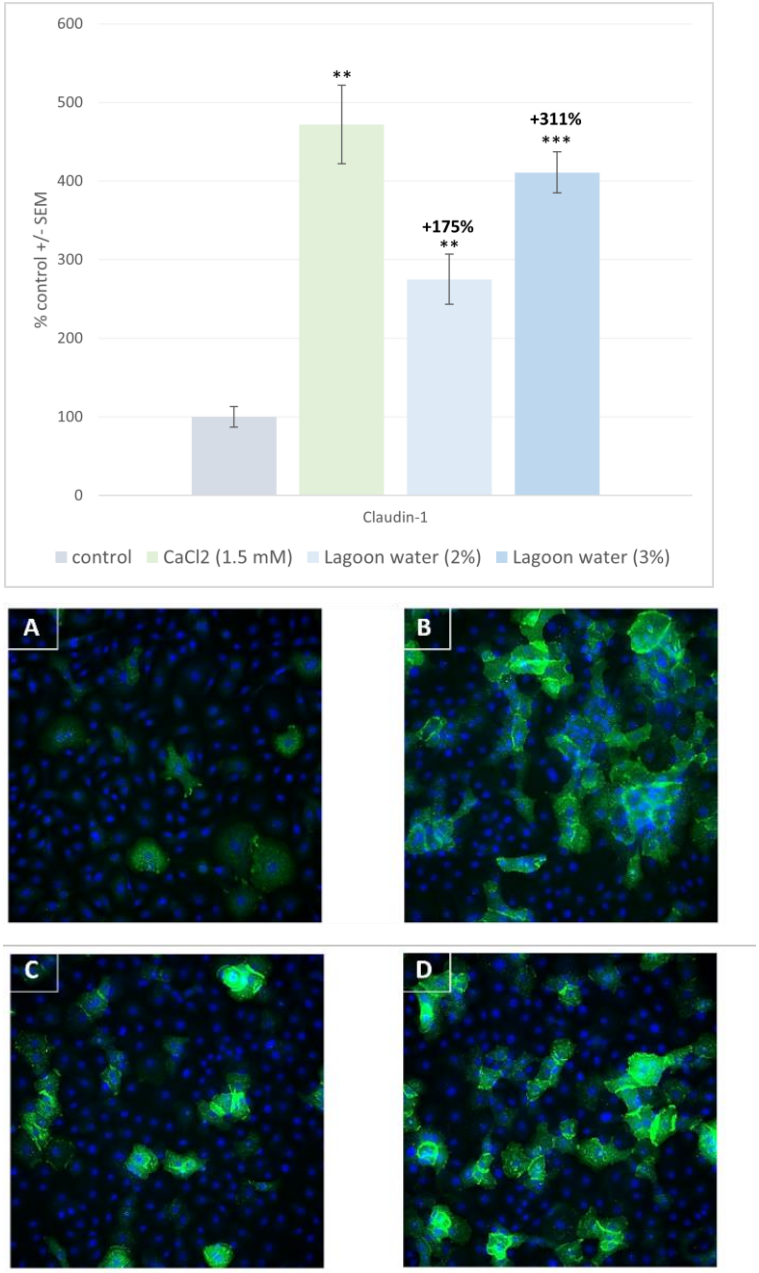

Figure 3: Epidermic cohesion proteins expression by human keratinocytes (Claudin-1), not treated (negative control) or treated with $CaCl_2$ (positive reference) or lagoon water at 2% or 3%. (** $p<0.01$; ***$p<0.001$ Student t-test). Representative images of the protein expression: negative control (A), positive reference $CaCl_2$ (B) or lagoon water at 2% (C) or 3% (D).

Discussion & Conclusion

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.

Conflict of Interest Statement.

NONE, however, all authors are employed by L'OREAL Research & Innovation or CARITA Brand.

Reference

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