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Jonzac Thermal Spring Water Reinforces Skin Barrier Function of Human Skin and Presents a Soothing and Regenerating Effect

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Abstract

The skin is a formidable physical and biological barrier which communicates continuously with the outside of the body. And the *stratum corneum*, the outermost layer of human epidermis, plays a central role in the interaction between the cutaneous tissue and the external environment. The horny layer, and more generally the whole skin layers, avoid the penetration of harmful exogenous agents, produce molecules named anti-microbial peptides which impact the composition of the cutaneous microbiota, regulate the internal corporal temperature, avoid the water loss from the inside of the body and constitute an incredible efficient anti-oxidant network. Nevertheless, nowadays, the skin is more and more solicited by the different elements of the cutaneous exposome, including atmospheric pollution and solar radiations, which can cause a dramatic acceleration of the skin ageing process. As a consequence, due to the multifunctional protective role of the skin, during the recent decade the cosmetic industry invested massively in the development of new raw materials and end-products (dermo-cosmetics) able to preserve an optimal state of the skin regarding the external environment. Based on their physical-chemical properties thermal spring waters, which are extremely rich in inorganics ions, are interesting and powerful candidates to be part, as integral component, of new efficient dermo-cosmetic formulations dedicated to protect the skin from the external stimuli. The aim of the present work was to investigate and characterize the activity of Jonzac thermal spring water on the skin. Using different models, we proved for the first time that Jonzac thermal spring water reinforces the barrier function of the skin by modulating the expression of key markers including filaggrin and human beta defensin 2 on *ex vivo* human skin. The *ex vivo* and *in vivo* hydration activity, by

Raman spectroscopy and corneometry respectively, has been also demonstrated. We have also shown that Jonzac thermal spring water ameliorates significantly the cutaneous microrelief *in vivo*. To conclude, we characterize the soothing effect of Jonzac thermal spring water by the analysis of histamine release in Substance P treated skin explants and by measuring the redness of the skin following UV exposure of the skin *in vivo*. We observed that both parameters decreased following a preventive treatment of the skin with Jonzac thermal spring water. Taken together our results indicate that Jonzac thermal spring water is a promising and powerful dermo-cosmetic which can be used to preserve an optimal state of the cutaneous tissue.

Keywords

Jonzac Thermal Spring Water, Skin Barrier Function, Soothing Effect, Regenerating Effect, Skin Hydration, Isotonicity, Dermo-Cosmetics, *Ex Vivo* Explants

1. Introduction

The aim of the present study was to characterize for the first time the effects of Jonzac thermal spring water (JTSW) on skin physiology. In particular we analyzed the impact of JTSW on skin barrier function, cellular regeneration and hydration on *ex vivo* (human skin explants) and *in vivo* human skin models. The soothing effect of JTSW was also investigated by analyzing mast cell activation and histamine release *ex vivo* and by measuring skin UV-induced skin redness *in vivo*.

The skin is a fascinating organ which plays a pivotal role in various biological processes. From the outermost *stratum corneum* to the deepest adipocyte tissue, each cutaneous layer contributes importantly to skin functions.

In particular, the *stratum corneum* provides a first line of defense against the environmental stimuli and regulates global skin homeostasis.

For long time it has been considered a simple physical barrier, serving as inert wrapping to separate the human body and external environment. At the beginning it was described by the two-compartment model named "brick and mortal" model [1]. The bricks were the corneocytes, held together by a homogeneous extracellular matrix composed by lipids and proteins, which served as mortar.

This was obviously an oversimplification of the architecture of this extraordinary skin compartment, which is actually well-known to present an extremely complex structure [2] and playing not only a role of physical barrier but also representing a biological site responsible for maintaining healthy skin.

It participates in multiple protective functions including the formation of a permeability barrier [3], it provides a first line of defense against pathogenic bacteria trough lipids [4] or anti-microbial peptides secretion [5], it ensures the formation of an important antioxidant network [6] and it represents an efficient

protection against UV-B irradiation by generating trans-urocanic acid [7].

Besides skin disorders that can affect deeply the horny layer integrity and functionality including atopic dermatitis [8], psoriasis [9] and rosacea [10], the skin and in particular the *stratum corneum* are continuously exposed to exogenous elements able to perturbate skin barrier function.

Recently Huang and collaborators [11] show that traffic-derived air pollution could deteriorate skin physical and antioxidant barrier properties. In another work it has been shown that ozone exposure induces chemical modifications of the unsaturated bonds in ceramides and cholesterol [12]. On the other hand, the deleterious impact of solar ultraviolets on mechanical and structural properties of human *stratum corneum* has been also described [13].

Besides changes in skin barrier function, it has been also proved that environmental stresses including UV [14] and air pollutants [15] are able to induce skin inflammation and irritation.

Interesting, very recently, Masutani and collaborators showed that detergents including sodium dodecyl sulphate induce mast cell-independent skin inflammation with H1-antihistamine-refractory itch in mouse skin [16].

With regards to the importance to maintain an optimal status of the barrier function of the skin, the dermo-cosmetic industry made big efforts in developing moisturizer or barrier repair cream able to improve physiological states where *stratum corneum* functionality is affected [17] [18] [19] [20].

During the recent years different studies have investigated the dermo-cosmetic properties of thermal waters [21] [22] [23].

The relative high concentration of minerals which present different biological activities including keratolytic, regenerative, and antioxidant effects [24] [25] [26] is a key feature that makes thermal waters good dermo-cosmetic candidates for maintaining an optimal physiological status of the skin.

JTSW is drawn in Charente-Maritime from its original underground environment at a depth of 1850 meters ensuring consequently a purity from microbiological agents and environmental pollutants. JTSW is a sulfured and sodium chloride water, it is hyper-mineralized (dry residual ≈ 7000 mg per liter) and is composed by a large variety of mineral salts and trace elements.

Due to these relevant features, since its discovery in 1979, JTSW has been deeply analyzed and numerous studies have demonstrated the rheumatological and phlebology properties of JTSW as well as its ability to treat respiratory disorders [27] [28], but the effect of JTSW on the skin were not analyzed so far.

In this context, we decided to analyze for the first time the effects of JTSW on the cutaneous tissue by using *ex vivo* and *in vivo* human skin.

2. Material and Methods

2.1. JTSW Characterization

The water characteristics were determined in an accredited laboratory (IANESCO, Poitiers, France) using standardized procedures (**Table S1**).

The water isotonicity was determined using human dermal fibroblast cultured in supplemented Dulbecco's modified Eagle's medium (DMEM) at 37˚C, 5% CO_2, 95% humidity. After washing with phosphate buffered saline (PBS) (Sigma Aldrich, Saint Quentin Fallavier, France), cells (non-confluent cell layer) were incubated with JTSW, with 0.9% wt/vol sodium chloride solution (isotonic control) or with distilled water (hypotonic control). Phenotype of cells has been assessed by monitoring cell morphology. The morphology monitoring starts from the contact of pure JTSW or control solution using an optical microscope (NIKON Eclipse TS100) with image capture system (NIKON DS Fi1) at ×200 magnification. Shootings have been done automatically every 3 minutes for 39 minutes for JTSW and for the isotonic control. Shooting is performed automatically every minute for 13 minutes for the hypotonic control.

2.2. Preparation of *Ex Vivo* Human Skin Explants

Full-thickness human skin biopsies were obtained from the abdominal area of three healthy female donors (26 - 73 years) who had undergone plastic surgery. Skin samples were collected from the patient and processed as described previously by Percoco and collaborators [29].

Briefly, circular explants of 1 cm in diameter were prepared using a biopsy punch, placed in 2 ml of BIO-EC's Explant Medium (BEM) and cultured under classical conditions (37˚C in 5% CO_2). The different treatments are shown in **Figure S1**. The day of the skin explants preparation has been defined as day 0 for each experiment.

BIO-EC Laboratory possesses an authorization from the Bioethics group of the general director services of the French research and innovation ministry (registered under n_DC-2008_542) to use human skin from surgical waste since 5th May 2010. The study was performed in accordance with the Declaration of Helsinki after the patients had given informed consent to use their skin samples by BIO-EC Laboratory.

2.3. Treatment of Stripped Human Skin Samples

On day 0, the human skin explants coming from the abdoplasty of a 69-year-old female donor and from the abdoplasty of a 26-year-old female donor were tape stripped 5 to 10 times respectively using adhesive scotch tape Magic 3 M.

From day 0 (just after stripping) to day 8, the human skin explants have been treated with 2 mg per cm^2 of unmixed or diluted JTSW in sterile distillated water for 4 or 5 times (**Figure S1**).

For the experiment analyzing the effect of JTSW on filaggrin expression, skin explants were stripped on day 0 and then treated topically with JTSW on day 0 (following the strippings), day 2, day 3 and day 6 (**Figure S1(a)**).

For the experiment analyzing the effect of JTSW on beta-defins-2 expression, skin explants were stripped on day 0 and then treated topically with JTSW on day 0 (following the strippings), day 1, day 2, day 3, day 6 and day 7 (**Figure S1(b)**).

On day 8, for both experiments, the skin explants have been collected and processed for histological analysis.

2.4. Microscopy and Image Analysis

For histological analysis, half of skin explants was frozen at −80˚C and half was fixed for 24 h in buffered formalin solution (VWR International, Fontenay-sous-Bois, France) and successively processed as described by Percoco and collaborators [29].

The cell viability has been assessed on formalin-fixed paraffin-embedded (FFPE) sections stained with Masson-Goldner's trichrome.

Both immunohistochemistry and immunofluorescence stainings were performed on either 5 µm-FFPE skin sections or 7 µm-frozen sections. Each primary antibody was diluted in PBS containing 0.3% of a blocking agent and supplemented with 0.05% Tween® 20 (Sigma Aldrich, Saint Quentin Fallavier, France). They were diluted as following: monoclonal anti-filaggrin antibody at 1:100 (sc-66192, Santa Cruz Biotechnology, Santa Cruz, CA, USA), polyclonal anti-beta-defensin 2 antibody at 1:100 (sc-20798, Santa Cruz Biotechnology, Santa Cruz, CA, USA), monoclonal anti-collagen I antibody at 1:800 (ab138492-1001, Abcam, Cambridge, UK).

Filaggrin and beta-defensin 2 immunostaining was revealed by immunofluorescence using an Alexa Fluor® 488-conjugated secondary antibody diluted in 0.3% BSA-PBS at 1:1000 (A11001, Thermofisher Scientific, Montigny Le Bretonne, France). Nuclei were counterstained with propidium iodide at 0.02% in PBS.

Skin sections were observed with an Olympus BX43 microscope (Olympus, Rungis, France) equipped with a DP72 digital camera (Olympus, France) or with an Olympus BX63 microscope (Olympus, Rungis, France) equipped with a DP74 digital camera (Olympus, France), stored and analyzed using Cell^D software (Olympus, Rungis, France).

The image analyses were performed using Cell^D or cellSens software (Olympus, Rungis, France). The percentage of stained surface of the region of interest of stripped explants was compared to the untreated explants or the stripped explants ($n = 9$).

2.5. Raman Spectroscopic Measurement of Water Content

On day 0, the human skin explants coming from an abdoplasty of a 73-year-old female donor have been treated with 10 mg per cm^2 of a gel containing more than 80% of JTSW. Two hours and 8 hours after treatment, molecular analysis of water content was performed on 10 µm thick frozen skin sections with a confocal Raman microscope (Labram, Horiba, Palaiseau, France), using a frequency-doubled, diode-pumped 660 nm Nd:YAG laser. Point-by-point analysis was performed by Raman spectroscopic measurement at 60 distinct points on the *stratum corneum* and 60 distinct points on the un-

derlying living epidermis of each explant. After correction of the baseline and smoothing of the Raman spectra, the data were normalized using the spectral region from 1300 cm^{-1} to 1800 cm^{-1}. After normalization, water content was measured by the integrated intensity of the spectral band between 3200 cm^{-1} and 3400 cm^{-1}, corresponding to the region of vibration of hydrogen-oxygen bonds within water molecules.

2.6. Evaluation of *Stratum corneum* Hydration *in Vivo*

Stratum corneum hydration was measured directly using a CM 825 corneometer [30] on 12 volunteers presenting forearm dry skin 30 minutes, 8 hours and 24 hours following a unique application of 2 mg per cm^2 of pure JSTW. Three successive measurements were taken at 5 seconds intervals, and the mean value calculated for each skin site (treated and untreated area). Results were in expressed in *stratum corneum* hydration rate (in percentage) compared to baseline value according to the following formula:

$$\Delta = \frac{\left(ZTt_i - ZTt_0\right) - \left(ZNTt_i - ZNTt_0\right)}{ZTt_0 + \left(ZNTt_i - ZNTt_0\right)} \times 100(\%)$$

with ZT: mean value for treated area;

ZNT: mean value for untreated area;

t_0: before treatment;

t_i: at the different time points after treatment.

2.7. Analysis of Skin Microrelief

The cutaneous microrelief was analysed from 10 volunteers presenting normal or dry skin on cyanoacrylate skin surface stripping using DIAGNOSKIN® system [31].

Briefly, a gel containing 98.10% JTSW was applied twice (in the morning and in the evening) on the face of 10 volunteers for 29 days.

The analysis of the microrelief by DIAGNOSKIN® system was realized before, immediately after a unique application of the gel containing JTSW and after a 30 days long treatment.

2.8. Mast Cell Activation and Histamine Release

Human skin samples of 0.8 cm in diameter (Biopredic, France) were placed on culture inserts (filter pore size 0.4 µm; Costar, Poly-Labo Paul Block, France) with the epithelium uppermost at an air/liquid interface. The inserts were set on 24 well plates (Costar) and culture medium was added to the wells so that the surface of the medium reached the filter level. Organ cultures were performed using DMEM.

After 4 hours of stabilization under classical culture conditions (37˚C in 5% CO_2), human skin explants were treated with JTSW at 20% or distilled water (control condition) diluted in PBS. 24 h after treatment, 30 µM of substance P (SP) (Neosystem, France) suspended in PBS were added to the culture medium

and skin samples were maintained in culture for 2 additional hours.

Skin inflammatory status was then assessed by histological evaluation (degranulation) of mast cells and measurement of histamine released in the supernatant by a spectrofluorometric method.

For histological evaluation, human skin explants were then embedded in paraffin. Thick sections of 50 µm were stained with haematoxylin-eosin and coloured with Giemsa stain. The mast cells present in the dermis were revealed with Giemsa stain. Histologically, a more or less intense blue-violet and granular appearance of the mast cells was observed, in combination with the more or less significant presence in their cytoplasm of granulations. The degranulated mast cells were then counted under Axiopht microscope (Zeiss, Germany). The results are expressed in the following manner: for each condition, the percentage of mast cells for each score was calculated relative to the total number of mast cells. Thirty images for each condition have been analyzed for histological analysis. For histamine release analysis, 2 h after SP application, explant culture supernatants were collected and processed using a commercially available ELISA kit (IBL International, Hamburg) and histamine was then quantified by spectrofluorometric method. Histamine concentrations were expressed in ng/ml.

2.9. Erythema Evaluation on UV-Exposed Human Skin *in Vivo*

Following treatment with pure JTSW, the volar forearms of healthy volunteers (n = 22; subjects with sensitive skin; from 25- to 50-year-old skin with a light phototype; 2 delimited areas) were irradiated with 2 MED of UV radiation. One hour, 2 h, 4 h and 24 h after UV radiation, chromameter reading were taken to determine the degree of skin erythema.

For quantitative measurement of skin colour, a chromameter was used (Minolta CR-400, Osaka, Japan). It measures the erythema and skin colour based on the International Commission illumination L*a*b* colour space (CIELAB). In this system the L* coordinate correlates with the intensity of the reflected light (brightness) and the a* and b* coordinates are chromatic, covering the spectrum from red to green and from yellow to blue, respectively. The a* value is well recognized to linearly correlate with skin erythema and it has been used to assess the redness of the skin.

Each volunteer signed a formal consent prior in order to participate to the study with the obligation to give possible side effects.

The study was performed according to the World Medical Association Declaration of Helsinki and the EU Directive 2001/20/EC of 4 April 2021.

2.10. Statistical Analysis

The data from each experiment were analyzed statistically by the Student's t-test. Data are expressed as mean ± SD. $p \leq 0.05$ was considered statistically significant. * for $p < 0.05$ and ** for $p < 0.01$

3. Results

3.1. JTSW Characterization

The first aim of the present project was to determine the chemical composition of JTSW.

Results are shown in Table 1. The chemical characterisation reveals that

Table 1. Chemical composition of JTSW.

Element	Values
pH (17˚C)	8.0
Turbidity	<0.30 NFU
Conductivity (25˚C)	10 800 µS·cm^{-1}
Complete alkalimetric title (CAT)	12.1˚F
Hydrotrimetric title (TH)	180 ˚F
Dry residuals at 180˚C	7660 mg·L^{-1}
Silica	31 mg·L^{-1}
Calcium	620 mg·L^{-1}
Magnesium	120 mg·L^{-1}
Total Sulphur	780 mg·L^{-1}
Sodium	2100 mg·L^{-1}
Potassium	130 mg·L^{-1}
Carbonate	0 mg·L^{-1}
Hydrogen carbonate	148 mg·L^{-1}
Sulphates	2500 mg·L^{-1}
Ammonium	1.7 mg·L^{-1}
Chlorides	2900 mg·L^{-1}
Nitrogen	1.5 mg·L^{-1}
Bore	0.25 mg·L^{-1}
Manganese	190 mg·L^{-1}
Strontium	15 mg·L^{-1}
Dissolved selenium	0.7 µg·L^{-1}
Total cyanides	*<5 µg·L^{-1}
Nitrates	*<0.5 mg·L^{-1}
Nitrites	*<0.01 mg·L^{-1}
m and p-xylene	*<0.1 µg·L^{-1}
o-xylene	*<0.1 µg·L^{-1}
Total phosphorus	*<0.01 mg·L^{-1}

*: below the limit of quantification.

JTSW is a water rich in sulphates (2500 mg/L), chlorides (2900 mg/L), sodium (2100 mg/L) and calcium (620 mg/L).

Important levels of soluble silica (31 mg/L), potassium (130 mg/L), magnesium (120 mg/L), strontium (15 mg/L) and lithium (3.5 mg/L) have been also observed.

Importantly, no quantifiable traces of environmental pollutants including cyanides, xylene derivates, nitrates, nitrites and phosphates have been observed (Table 1).

In parallel, the isotonic effect of JTSW on human dermal fibroblast *in vitro* was also assessed.

The results have shown that during the whole exposure no significant morphological changes of dermal fibroblasts *in vitro* were observed (Figure S2) demonstrating that JTSW is isotonic compared with the intracellular medium.

3.2. Effect of JTSW on Skin Barrier Properties *ex Vivo*

The skin barrier properties of JTSW were investigated using a stripping-based human skin *ex vivo* model.

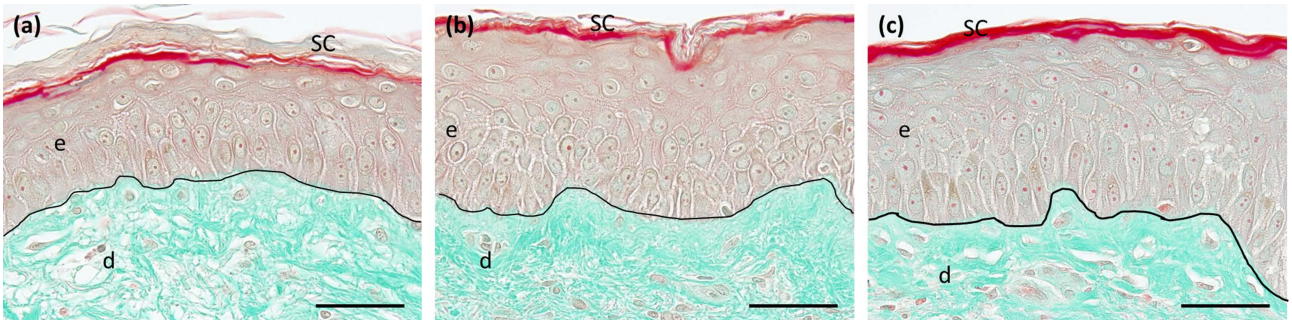
Firstly, the impact of JTSW at 20% on tissular viability of skin explants was investigated.

The results showed that JTSW does not impact the epidermal and dermal morphology of the skin following several successive applications indicating, as a consequence, that JTSW presents no toxicity on skin cells (Figure 1).

After that the impact of JTSW at 20% on skin barrier function on skin explant with altered horny layer was investigated. To do that, the *stratum corneum* integrity of human skin explants *ex vivo* was fragilized by successive strippings which induced a significant decrease (−29%** vs untreated control) of filaggrin immunolocalization (Figure 2).

The preventive application of JTSW at 20% induces a significant increase (+27%*) of filaggrin expression compared to stripped skin (Figure 2) suggesting that JTSW reinforces skin barrier properties of human skin.

In the frame of the same experiment, a slight effect of JTSW at 20% on collagen



Abbreviations: d, dermis; e, epidermis; SC, *stratum corneum.* Scale bar: 50 µm.

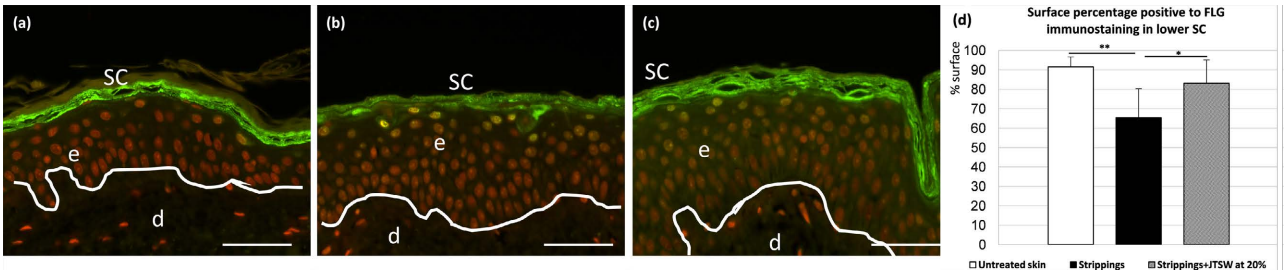
Figure 1. Analysis of cellular and tissular viability. The viability of skin explants was assessed on FFPE skin section following Masson's trichrome staining, Goldener variant. In (a) untreated skin, in (b) skin following strippings and in (c) skin following strippings + JTSW.

I synthesis was observed (+8% vs stripped skin) (Figure S3).

The moisturizing activity of gel containing more than 80% of JTSW was also assessed by Raman spectroscopy by analysing the intensity of water region (3200 - 3400 cm^{-1}) on both *stratum corneum* and viable epidermis.

The results showed JTSW improves hydration (water content) of *stratum corneum* and epidermis by 26%** and 80%** respectively, 8H after a unique application (Figure 3).

Interesting, a strong induction of hBD2 immunolocalization (+63%* vs untreated control) was also observed (Figure 4) on stripped skin explants treated



Abbreviations: d, dermis; e, epidermis; SC, *stratum corneum.* Scale bar: 50 µm.

Figure 2. Immunolocalization of filaggrin. Immunostaining of filaggrin on FFPE skin sections. In (a) untreated skin, in (b) skin following strippings and in (c) skin following strippings + JTSW. In (d) semi-quantification of filaggrin by image analysis.

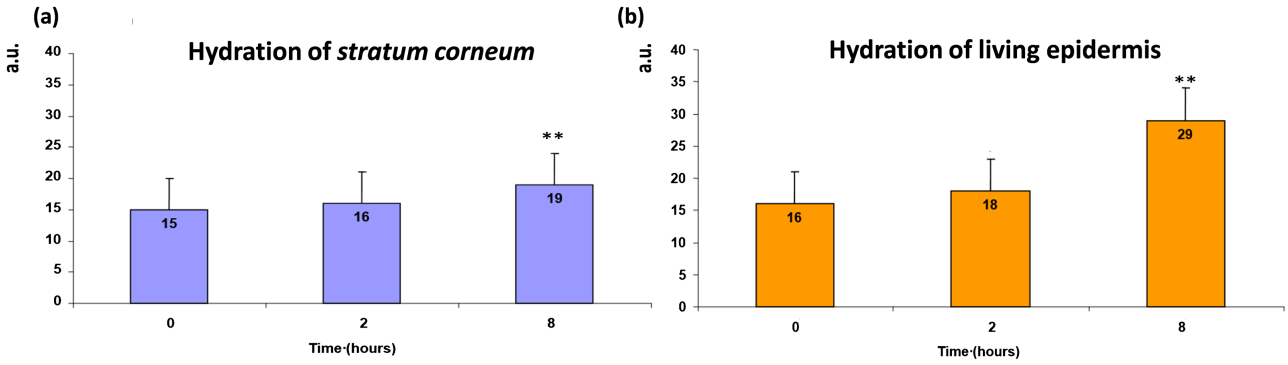
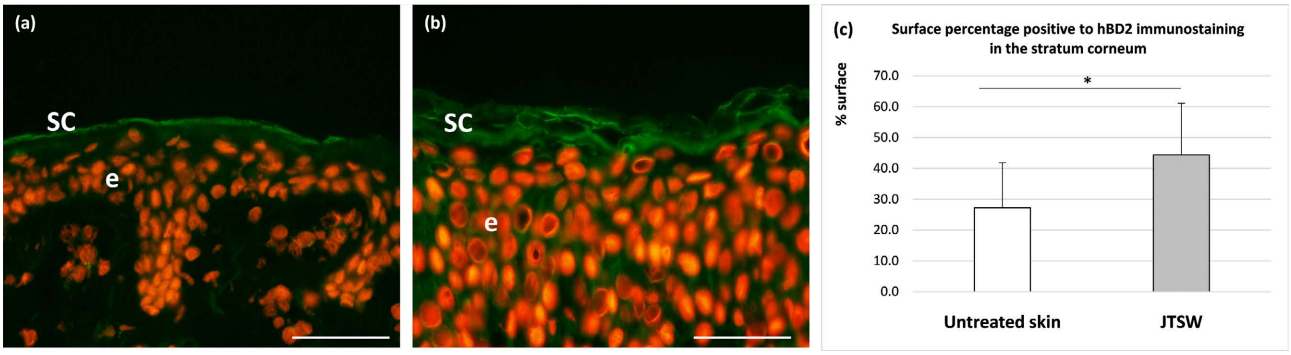


Figure 3. Hydration of *stratum corneum* and viable epidermis measured by Raman spectroscopy following JTSW treatment.



Abbreviations: e, epidermis; SC, *stratum corneum.* Scale bar: 50 µm.

Figure 4. Immunolocalization of hBD2. Immunostaining of hBD2 on frozen skin sections. In (a) untreated skin, in (b) following JTSW treatment. In (c) semi-quantification of hBD2 by image analysis.

with JTSW showing that JTSW reinforces innate immunity defence of the skin.

It is important to note that in the frame of this experiment an increase of epidermal thickness (+20%** vs untreated control) following JTSP treatment for 8 days has been also observed (Figure 5).

The impact of JTSW was assessed also *in vivo* on 12 volunteers presenting forearm dry skin. The results shown that JTSW increased significantly *stratum corneum* hydration rate compared to baseline value 30 minutes (+8.6%*), 8 hours (+10.8%**) and 24 hours (19.4% **) following a unique application (Figure 6).

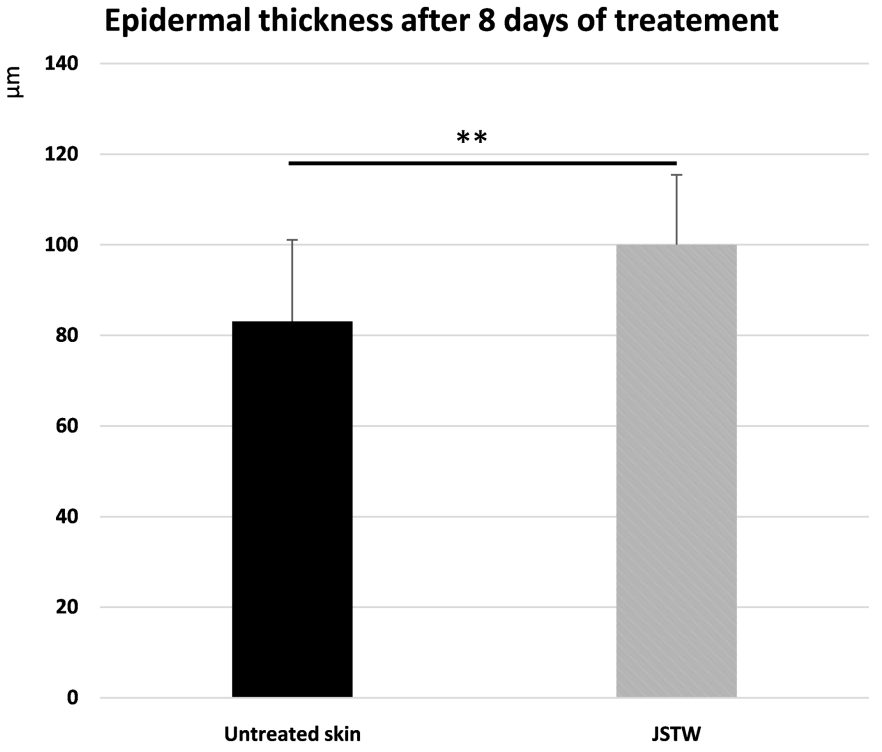


Figure 5. Epidermal thickness. Analysis of the thickness of the epidermis on skin explants treated for 8 days with JTSW.

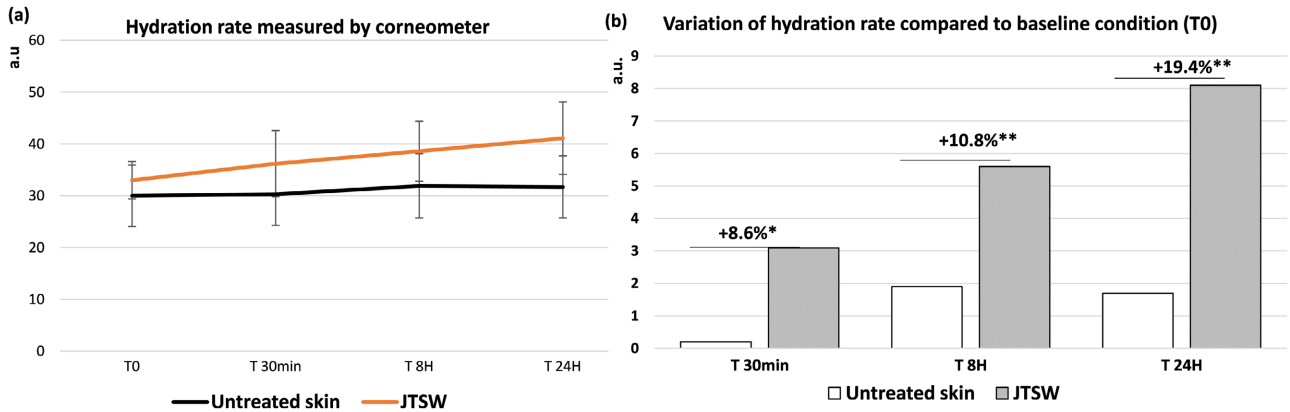


Figure 6. In (a) hydration rate measured *in vivo* on healthy female volunteers and in (b) variation of hydration rate on each sampling time compared to baseline condition.

The effect of JTSW on skin microrelief has been also investigated *in vivo* (Figure 7).

The aspect of the cutaneous microrelief is affected by several factors that can be intrinsic (natural ageing) or extrinsic (pollution, UV exposure, cigarette smoke etc.) [32] [33].

A young skin is characterized by a regular network of primary and secondary lines which in turn defines the polygonal plateaus. On aged skin the cutaneous network is perturbed and polygonal plateaus are destructed.

As a consequence, the structure of the cutaneous microrelief is a reflection of the structural integrity of all skin layers, from the horny layer until the papillary dermis.

Using the Diagnoskin® system a score from 1 to 10 is assigned to the cyanoacrylate skin surface stripping. The more the score is high, the better the organization of the cutaneous microrelief is.

The results showed that no effect of JTSW on microrelief aspect has been observed after a unique application (+8%^{ns} *vs* T_0). On the contrary a significant increase (+27%** vs T_0) of the microrelief score has been observed following 30 days of treatment, reflecting a better organization of the cutaneous microrelief.

3.3. Soothing Effect of JTSW

The skin is continuously exposed to external stimuli which in turn are able to trigger a cutaneous inflammatory reaction. In particular nociceptive stimuli including extreme temperatures, mechanical aggressions and irritants induce local liberation of neurotransmitters such as Substance P which results in neurogenic inflammation involving leukocyte adhesion to venular endothelium and the degranulation of mast cells.

Mast cell degranulation provokes the release of inflammatory mediators such as histamine, cytokines, prostaglandin D2, and tryptase into the skin.

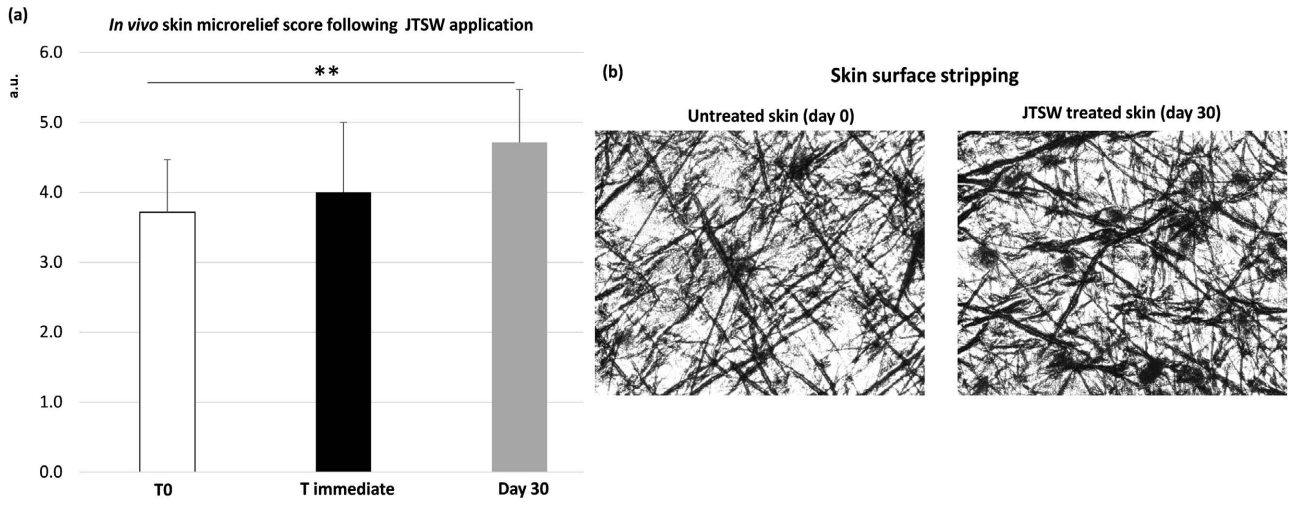


Figure 7. In (a) skin microrelief score assessed by the Diagnoskin® system and in (b) cyanoacrylate surface stripping on untreated skin and on skin treated with JTSW for 30 days.

In the frame of the present project the ability of JTSW to prevent Substance P-induced mast cell degranulation and histamine release was also investigated.

The results showed that JTSW at 20% is able to reduce significantly (−35%*) histamine release in Substance P-stimulated skin explants *ex vivo* (Figure 8). In parallel, we observed that JTSW at 20% reduced the number of granulated mast cells following Substance P stimulation (−79% of mast cells granulation index) (Figure 8).

The soothing effect of JTSW were analysed also *in vivo* in healthy volunteers exposed to 2 MED of UV. The data indicated that JTSW reduces significantly the UV-induced redness of the skin 4 (−10%**) and 24 hours (−11%*) following UV exposure (Figure 9).

4. Discussion

The skin is the outermost layer of human body and it is continuously exposed the several stimuli from the environment [34] that can induce, by different mechanisms, an accelerated skin aging process.

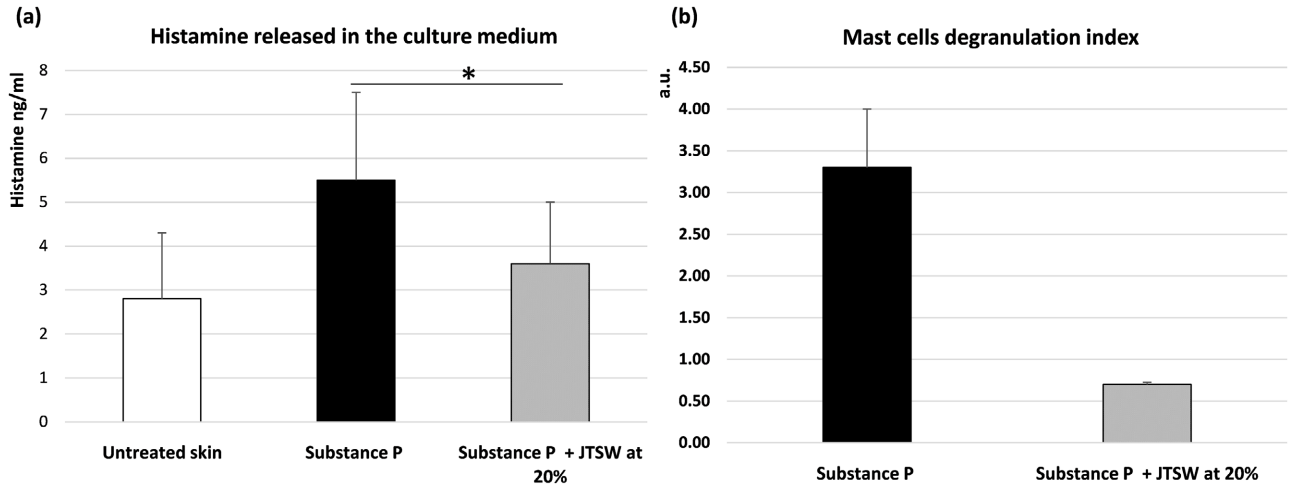


Figure 8. In (a) dosage of histamine release in the culture medium and in (b) mast cells degranulation index.

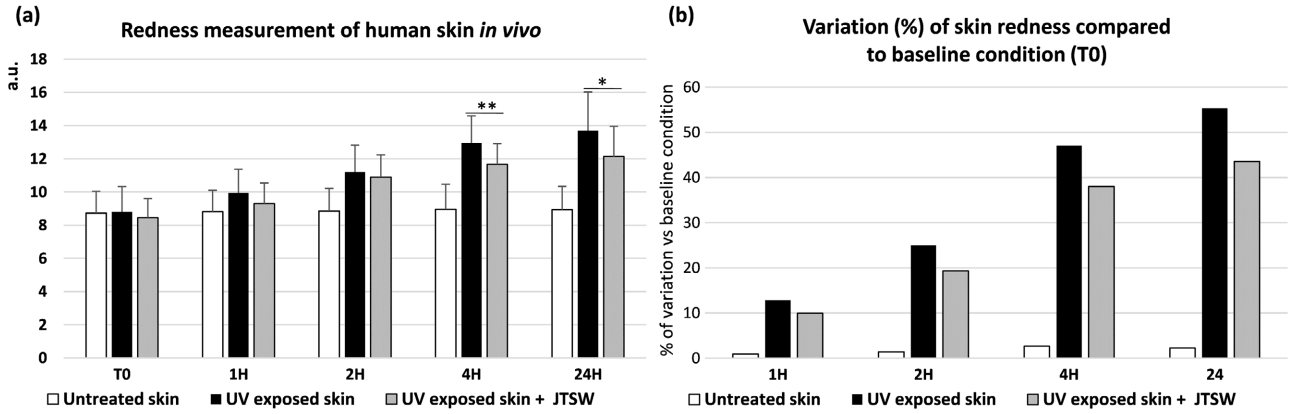


Figure 9. Colorimetric analysis (a* value) of *in vivo* human skin measured by a chromameter. In (a) redness measurement of *in vivo* skin and in (b) variation of skin redness along the time.

The maintain of an optimal integrity of skin barrier function is a key strategy to preserve a healthy state of the whole cutaneous tissue.

During the last years, several studies have demonstrated, in relation to their extremely rich composition, the benefic effects of thermal waters on the cutaneous tissue. In a very recent review, Figueiredo and collaborators [35] have compared and discussed the effects on the skin of six different thermal waters.

Among the several effects described, the anti-inflammatory activity, protection against UV-A and UV-B irradiations, reduction of skin sensitivity, anti-bacterial properties and the reinforcement of skin barrier function were the more often described activities [36]-[48].

In the present work firstly, we characterized chemically the composition of Jonzac thermal spring water and secondly, we assessed the ability of JTSW to reinforce skin barrier function of human skin. Thirdly, the soothing effect of JTSW was also investigated.

The quantitative and qualitative analysis of JTSW reveals remarkable high contents of different chemical elements including sulphates (2500 mg/L), chlorides (2900 mg/L), sodium (2100 mg/L) and calcium (620 mg/L).

Important levels of potassium (130 mg/L), magnesium (120 mg/L) and strontium (15 mg/L) have been also observed. This rich chemical composition explains the isotonicity properties of JTSW.

Based on the chemical composition, the skin barrier protection activity of JTSW on an *ex vivo* human skin model presenting a fragilized *stratum corneum* was investigated.

The results showed that the application of JTSW water was able to restore filaggrin deficiency on stripped human skin explants. This activity can be certainly explained by the high content of calcium observed in JTSW.

Calcium ion is a key regulator of keratinocyte differentiation and proliferation [49] [50] [51] and it is has been demonstrated that calcium contents needed for pro-filaggrin expression are higher than those required for other earlier differentiation markers including keratin 1 and keratin 10 suggesting that calcium is essential for a proper barrier formation process. In addition, it can directly bind to specific sites of pro-filaggrin initiating the cleavage of pro-filaggrin in mature filaggrin [52]. In another work Pernet and collaborators demonstrated that calcium triggers human beta defensin(hBD)-2 and -3 expression in activated keratinocytes [53]. These data were also confirmed in the present work, where we find that topical application of JTSW significantly induces the expression of hBD-2 in explants from healthy donor.

We also demonstrated that JTSW ameliorates skin hydration on both *ex vivo* and *in vivo* conditions.

Optimal skin hydration is essential to maintain a normal activity of enzymes implicated in desquamation. If the hydration levels of the horny layer are insufficient, the whole desquamation process is impacted resulting in accumulation of corneocytes in the *stratum corneum* and macroscopic consequences on skin aspect including dryness and roughness can be observed [54].

Here we also demonstrated the moisturizing activity of JTSW on an *ex vivo* skin model by Raman spectroscopy analysis and *in vivo* by measuring skin hydration by corneometry.

Both approaches showed that topical applications of JTSW improve significantly the hydration of *stratum corneum* and the epidermis proving the moisturizing activity of the thermal water.

The effect of JTSW on *in vivo* skin microrelief organization was also investigated by Diagnoskin® system.

The results showed that a 30-days long treatment with JTSW ameliorates the aspect of the cutaneous microrelief suggesting a cellular regeneration activity of JTSW.

These data are partially supported by collagen I analysis by immunochemistry, showing a slight increase of collagen I synthesis in the papillary dermis following JTSW application. Moreover an increase of the epidermal thickness following JTSW was also observed, indicating that JTSW ameliorates the cellular renewing of the epidermis.

In a third part of the present project, we investigated the soothing effect of JTSW.

We have shown, using human skin explants *ex vivo* stimulated with Substance P, that JTSW is able to reduce the production of histamine, a neurotransmitter involved in physiologic and pathologic processes such as pruritus and inflammation [55] [56].

In addition, the ability of JTSW to decrease mast cells degranulation following Substance P stimulation has been also demonstrated.

As discussed above, the composition of JTSW is extremely rich in ions elements and levels of strontium observed (15 mg/L) are particularly interesting.

In fact, several studies have shown the anti-itch and anti-irritation activity of this inorganic ion [57] [58] [59].

Consequently, we suggest that the soothing activity of JTSW observed in the present work can be related to the high content of strontium.

Finally, an *in vivo* study that we realized on 22 volunteers exposed to 2 MED of UV-A showed that JTSW is able to reduce skin redness, confirming the soothing and anti-irritation activities previously described.

5. Conclusions

In the present work we characterized for the first time the skin barrier reinforcement and soothing activities of Jonzac thermal spring water using *ex vivo* human skin and *in vivo* tests.

This thermal water is extremely rich in chemical elements, including inorganic ions such as calcium and strontium that can be easily associated to the observed activities of JTSW on the skin.

The results obtained here suggest that the Jonzac thermal spring water can be considered as relevant active ingredient to be incorporated in dermo-cosmetics

products which aim to treat skins characterized by a fragilized barrier function including atopic dermatitis.

In addition, due to the ability to decrease the Substance P effect on the skin, including histamine production and mast cells degranulation, Jonzac thermal spring water is a good candidate to treat itchy skins.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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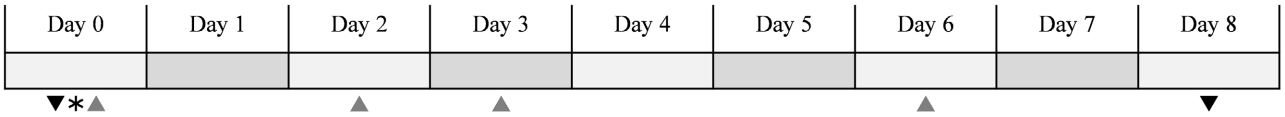
Supplemental Material

Table S1. Methods used for JTSW physical-chemical characterization by an accredited laboratory (IANESCO, Poitiers, France) using standardized procedures

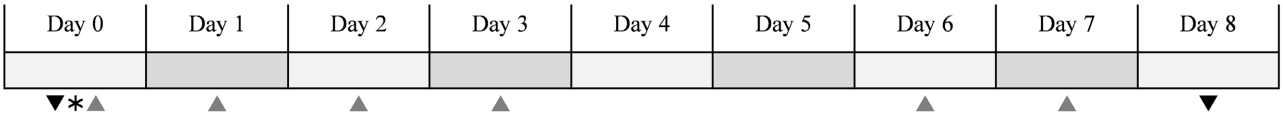
Element	Methods
pH (17˚C)	NF EN ISO 10523
Turbidity	NF EN ISO 7027-1
Conductivity (25˚C)	NF EN 27888
Complete alkalimetric title (CAT)	NF EN ISO 9963-1 (potentiometry)
Hydrotrimetric title (TH)	NF T 90-003
Dry residuals at 180˚C	NF T 90-029
Silica	NF ISO 15923-1
Calcium	NF EN ISO 11885 (ICP-OES)
Magnesium	NF EN ISO 11885 (ICP-OES)
Sulphur	NF EN ISO 11885 (ICP-OES)
Sodium	NF EN ISO 11885 (ICP-OES)
Potassium	NF EN ISO 11885 (ICP-OES)
Carbonate	NF EN ISO 9963-1 (potentiometry)
Hydrogen carbonate	NF EN ISO 9963-1 (potentiometry)
Sulphates	NF ISO 15923-1
Ammonium	NF ISO 15923-1
Chlorides	NF ISO 15923-1
Nitrogen	NF EN 25663
Bore	Internal method MA-EE-212 from NF T 90-041
Manganese	NF EN ISO 17294-2 (ICP-MS)
Strontium	NF EN ISO 11885 (ICP-OES)
Dissolved selenium	NF EN ISO 17294-2 (ICP-MS)
Total cyanides	NF EN ISO 14403-2
Nitrates	NF ISO 15923-1
Nitrites	NF ISO 15923-1
m and p-xylene	Internal method MA-MPO-106 (HS-GCMS) from NF EN 11423-1
o-xylene	Internal method MA-MPO-106 (HS-GCMS)
Total phosphorus	Internal method MA-EE-246 from NF EN ISO 6878 T90-023

(a) Effect of JTSW on filaggrin expression analysis on human stripped skin explant *ex vivo*



▼ Skin explant sampling
∗ Skin explants strippings x10
▲ Product application (JTSW 20%)

(b) Effect of JTSW on β-defensin 2 expression analysis on human stripped skin explant *ex vivo*



▼ Skin explant sampling
∗ Skin explants strippings x5
▲ Product application (Unmixed JTSW)

*Internal method developed by IANESCO laboratory.

Figure S1. Experimental procedure used for the treatment of human skin explants *ex vivo*.

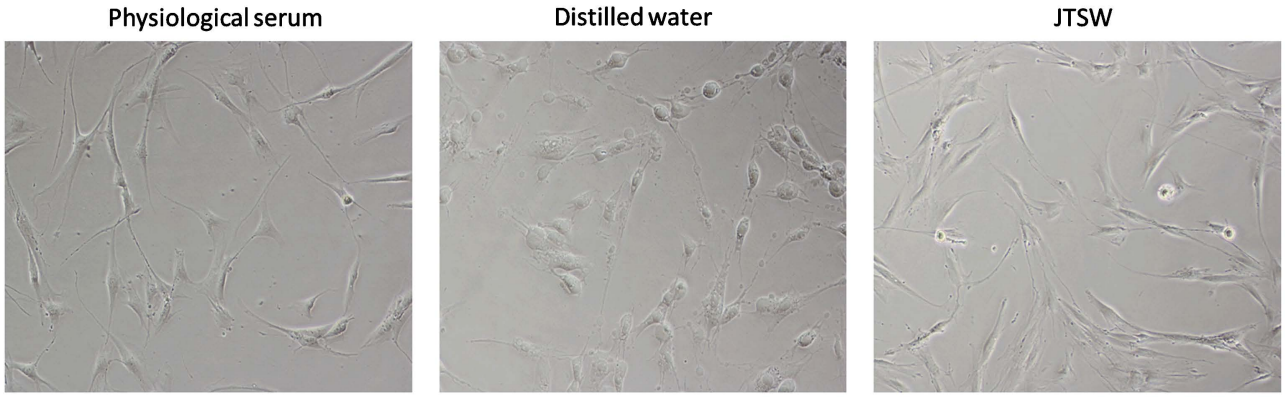
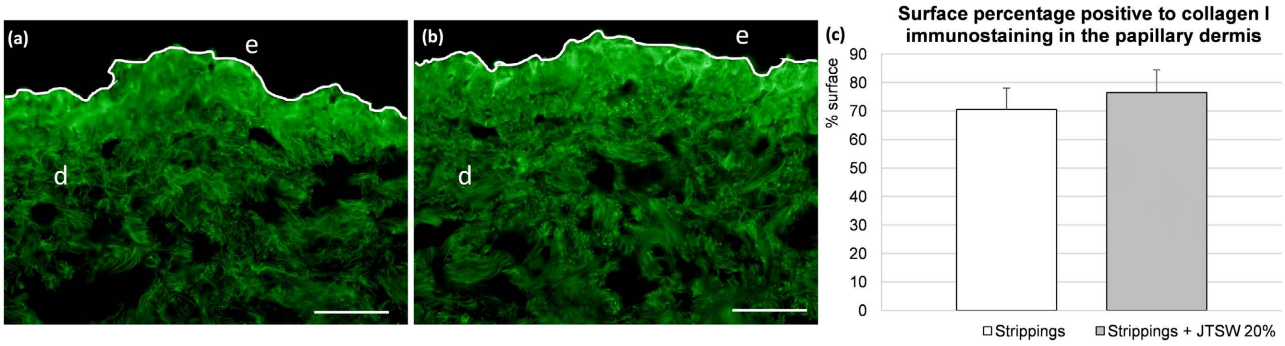


Figure S2. Isotonicity test. Microscopical images of dermal fibroblast *in vitro* culture in physiological serum (isotonic control), distilled water (hypotonic control) or in JTSW. The images shown an intact cellular morphology, similar to the one observed on the isotonic positive control, after JTSW incubation attesting the isotonic properties of JTSW. After the incubation of fibroblasts with distilled water an important cellular swelling has been observed.



Abbreviations: d, dermis; e, epidermis. Scale bar: 50 µm.

Figure S3. Immunolocalization of collagen I. Immunostaining of collagen I on frozen skin sections. In (a) skin following strippings and in (b) skin following strippings + JTSW. In (c) semi-quantification of filaggrin by image analysis.