

# *In Vivo* Improvements in Facial Appearance and *in Vitro* Changes in Gene Expression Using a Topical Formulation Designed to Repair Environmentally Induced DNA Damage

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## Abstract

**Background:** While sunscreen has been accepted as a mainline defence against photodamage from ultraviolet, visible light and near-infrared radiation, there appears to be a lack of research into photorepair. The concept of protecting the skin during the day and repairing cellular damage at night is intuitive, yet specific strategies revolving around combinations of proven reparative active ingredients remain unelucidated. **Purpose:** To investigate the efficacy of a solar repair Formulation following ultraviolet and environmental exposure in order to improve overall skin health and appearance through three hypotheses: The Formulation increases expression of DNA repair mechanisms markers; The Formulation enhances overall skin appearance through reducing signs of inflammation, elevating hydration, reinforcing skin firmness and amplifying radiance; *In-Vivo* efficacy test results are aligned with measured gene expression changes. **Methods:** The Formulation (#6NIC1.V1.1-1) was tested for: *In-vitro* LDH cytotoxicity activity, *In-vitro* qPCR gene expression with and without ultraviolet exposure on a reconstructed 3-dimensional skin model, and *In-Vivo* efficacy study on a panel of 22 participants objectively and subjectively. **Results:** Skin radiance, firmness, hydration, redness, and inflammation are significantly improved after *In-Vivo* skin exposure to the Formulation and environmental challenges such as ultraviolet radiation. These outcomes were confirmed by *in-vitro* genetic testing on a reconstructed human skin model. **Conclusion:** The studies allowed us to identify and group results in four main skin functions that were significantly enhanced following the application of the Formulation: firmness, hydration, radiance and soothing.

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## Keywords

Photoprotection, Photorepair, DNA Repair, Anti-Photoaging, Gene Expression, Antioxidant, Rejuvenation

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## 1. Introduction

As the body's first line defence against environmental damage, the skin is constantly exposed to multiple stressors which negatively impact its health and beauty. It is now widely accepted that solar damage accounts for approximately 80% of facial aging [1], and increasingly urban air pollution is implicated as another major threat to human skin health worldwide.

Distinct from intrinsic aging primarily caused by genetic factors and hormonal status changes over time, extrinsic aging is essentially photoaging caused by the combined effects. The combined effects of sunlight (including ultraviolet (UV), visible light (VL) and near-infrared (NIR) radiation) are universally acknowledged by dermatologists [2] [3], although our understanding of the biochemical mechanisms involved remains incomplete.

Despite this knowledge, however, formulations and regimens designed to protect the skin from solar and environmental damage by day and repair cellular injury at night are not well defined or researched.

The authors previously reported that most globally available sunscreen formulations, whether they are SPF 50+, PA+++ or ++++ are incapable of providing complete skin protection from the entire solar spectrum, which includes not only UV but also VL and NIR radiation [4]-[13]. Most sunscreens only offer protection in the UV range, when in reality skin damage also results from VL and NIR exposure [4]-[13]. Increasingly, dermatologists are recommending comprehensive skin regime that protects from UV, VL and NIR.

We also reported that a comprehensive solar protection and repair regime (including skin immune boosters, antioxidants, zinc oxide sunscreens, barrier lipids, hydroxy acids and retinoids) elicits significant improvements skin texture, appearance, clarity, and firmness as demonstrated in multidimensional 2D and 3D assessments [4]-[13].

To investigate the efficacy of a solar repair Formulation following UV and environmental exposure in order to improve overall skin health and appearance, we assessed *in vitro* gene expression changes and *in-vivo* efficacy in improving overall skin appearance through reducing signs of inflammation, elevating hydration, reinforcing skin firmness and amplifying radiance.

## 2. Materials and Methods

### 2.1. Topical Formulation

The Formulation is a complex composition of functional ingredients forming

the delivery system as well as performant active ingredients such as Vitamin A in two different forms, 15 pure amino acids and one modified amino acid and more (Table 1). This complex blend, #6NIC1.V1.1-1, is the studied Test Material (TM).

### Test Materials

Four TMs were used to complete the studies described below. The Untreated Control consists of tissue samples not treated with any substances. This TM was used to assess cytotoxicity. Triton X-100, a nonionic surfactant with cell lysing properties that disrupt cell walls leading to cellular death was used as a positive control for cytotoxicity assessment. 100% cell death is expected when cells are exposed to Triton X-100. In order to assess the gene expression changes in the 3-dimensional reconstituted skin tissue model as well as to confirm the cytotoxicity rate of the Formulation, a 0.9% Saline solution was used as a negative control. This solution is considered neutral and inert to the skin cells. Therefore it should induce very low to zero cytotoxicity and changes in gene expression. The results obtained with this solution serve as a comparison with the tested Formulation results. Finally, #6NIC1.V1.1-1 also referred to as the Full Formulation is described in Table 1. This is the main material to be assessed in order to determine how much change will be induced after using the Full Formulation in the cytotoxicity, gene expression changes and *In-Vivo* studies. Table 2 expands further on the Test Materials design.

## 2.2. Skin Model and Gene Expression

The gene expression and cytotoxicity studies were performed on a commercially available 3-dimensional *in vitro* skin model (Mattek EFT-400) composed of epidermal keratinocytes and dermal fibroblasts. Tissues were equilibrated prior to inoculation with 15  $\mu$ L of any of the above test materials (TM). For each treatment

**Table 1.** List of ingredients functions composing the formulation.

| Topical Formulation Ingredients Functions |
|---|
| Vitamin A, E                              |
| Complex and Essential Fatty Acids         |
| 16 Pure and Modified Amino Acids          |
| Humectants & Penetration Enhancers        |
| Australian Botanicals Extracts            |
| Emollients and Humectants                 |
| Stabilizers and Preservatives             |
| Thickener & Emulsifiers                   |
| Extracts, Ferments, Minerals & Sugars     |
| Pigments                                  |

**Table 2.** Test groups and result expectations.

| Test Groups                        | Comments                      | Expectations   |
|------------------------------------|-------------------------------|--|
| Untreated Control                  | Cytotoxicity Negative Control | No cytotoxicity should be observed                       |
| Triton X-100                       | Cytotoxicity Positive Control | Maximum cytotoxicity should be observed                  |
| 0.9% Saline, no UV                 | Gene Expression Control       | Very low cytotoxicity should be observed                 |
| 0.9% Saline + UV + 0.9% Saline     | Gene Expression UV Control    | Some cytotoxicity should be observed due to UV radiation |
| #6NIC1.V1.1-1, no UV               | Full Formula                  | Very low cytotoxicity should be observed                 |
| #6NIC1.V1.1-1 + UV + #6NIC1.V1.1-1 | Full Formula + UV Exposure    | Some cytotoxicity should be observed due to UV radiation |

group, four tissues were included. Post distribution of treatment materials, the tissues were placed in an incubator at 37°C with 5% CO<sub>2</sub> and ~95% relative humidity for 24 hours. At the issue of the 24 hour incubation, each tissue's surface was washed to remove TM. Depending on the treatment group, tissues were exposed to UVB at 200 mJ/cm. Following full exposure to UVB, 15 uL of TM was re-applied to the tissues which were then re-incubated at 37°C with 5% CO<sub>2</sub> and ~95% relative humidity for 24 hours. Gene expression was assessed after a total of 48 hours exposure to the TM utilising Genemarkers' qPCR-based Environmental Stress gene Panel (ESP) containing 107 target genes (Table A1). After full incubation, tissue surfaces were washed, and each culture was placed in contact of RNA later solution ready for RNA isolation (Figure 1).

Following a qPCR process, statistical data analysis was performed using relative quantitation (RQ) method and converting any linear RQ values into linear fold-change values.

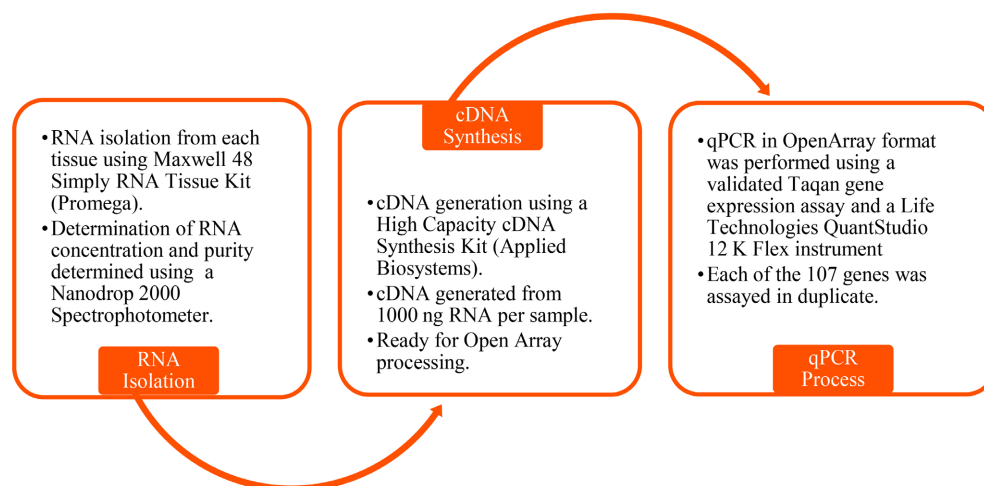
### 2.3. LDH Cytotoxicity Assessment

Controls:

- High Control: 100 µL of 15 Triton X-100 was inoculated on the surface of two tissues as per process above.
- Low Control: Four untreated (UNT) tissues

To assess the LDH activity (cytotoxicity) of the TM, the culture medium of each treated tissue was used and diluted 1:10 with Phosphate Buffered Saline (PBS). Every dilution was then combined with the LDH reaction mix at a ratio 1:1 and followed by a 20 minute incubation at room temperature in the dark. A 1.0 N HCl solution was used to stop the reaction of each dilution and absorbance was measured at 492 nm with a reference filter at 620 nm.

The LDH activity or cytotoxicity was calculated relative to the absorbance of the low control (0% cytotoxicity) and high control (100% cytotoxicity) following the below formula:



**Figure 1.** Gene expression process from inoculated tissues to raw data generation.

$$\% \text{ Cytotoxicity} = \frac{(\text{Test Media Value} - \text{Low Control})}{(\text{High Control} - \text{Low Control})} * 100$$

## 2.4. *In-Vivo* Clinical Evaluation

### Patients

Patients were selected based on inclusion and exclusion criteria defined as **Tables 3-5**. Patients were directed not to use any other skincare products or undergo any form of esthetic procedure during the study. Additionally, they were instructed to continue with their normal diet throughout the study. As this study involved a retrospective review of patients previously treated, the approval of an ethics committee was not required. All patients signed an informed consent document of participation following an explanation of the study design and execution, and agreed to publication of results and images.

### Kinetics

Parameters tested and the time points are described below.

## 3. Results

### LDH Cytotoxicity Assessment

The cytotoxicity and gene expression studies were performed on the below Test Groups incorporating some of the test materials referenced in Material and Methods:

Increased LDH activity is interpreted as an indication of cells being damaged or dead. The positive control, Triton X-100 is the reference for full cytotoxic LDH activity. Levels of cytotoxicity were assessed after 24 and 48 hours and were compared to the untreated, negative control.

After 24 hours, the cytotoxicity levels of the test groups were minimal (**Figure 2**). After 48 hours, the Full Formulation exposed to UV showed slightly higher cytotoxicity although still very low and lower than the tissues inoculated with 9% Saline Solution and exposed to UV radiation.

**Table 3.** Patients inclusion criteria for selection.

| Inclusion Criteria  | Non-Inclusion Criteria   |
|---|--|
| <p style="text-align: center;"><b><u>Specific</u></b></p> <ul style="list-style-type: none"> <li>• Sex: female and male;</li> <li>• Age: between 30 and 65 years old;</li> <li>• Phototype: I to II;</li> <li>• Type: Caucasian;</li> <li>• Subjects with dry, normal and combination skin.</li> </ul> <p style="text-align: center;"><b><u>General</u></b></p> <ul style="list-style-type: none"> <li>• Healthy subject;</li> <li>• Subject having given his/her free informed, written consent;</li> <li>• Subject willing to adhere to the protocol and study procedures.</li> </ul> | <ul style="list-style-type: none"> <li>• Female Specific-pregnant or nursing women or women planning to get pregnant during the study;</li> <li>• Cutaneous pathology on the study zone (eczema, etc.);</li> <li>• Subject with make-up on the day of the visit at the laboratory;</li> <li>• Use of topical or systemic treatment during the previous weeks liable to interfere with the assessment of the cutaneous acceptability/efficacy of the study product:             <ul style="list-style-type: none"> <li>- Change in anti-wrinkle, smoothing, firming and/or brightening topical products within previous week on the studied zones,</li> <li>- Non-invasive procedures within previous month on the studied zones,</li> <li>- Intake of food supplement acting on skin within the three previous months,</li> <li>- Invasive procedures:                 <ul style="list-style-type: none"> <li>○ deep chemical peeling within previous 3 months on the studied zones,</li> <li>○ mesotherapy, dermapen, laser within previous 6 months on the studied zones,</li> <li>○ botox and/or hyaluronic acid injections within previous 12 months on the studied zones;</li> </ul> </li> </ul> </li> <li>• Subject having undergone a surgery under general anesthesia within the previous month;</li> <li>• Excessive exposure to sunlight or UV-rays within the previous month;</li> <li>• Subject enrolled in another clinical trial during the study period (concerns the studied zone).</li> </ul> |

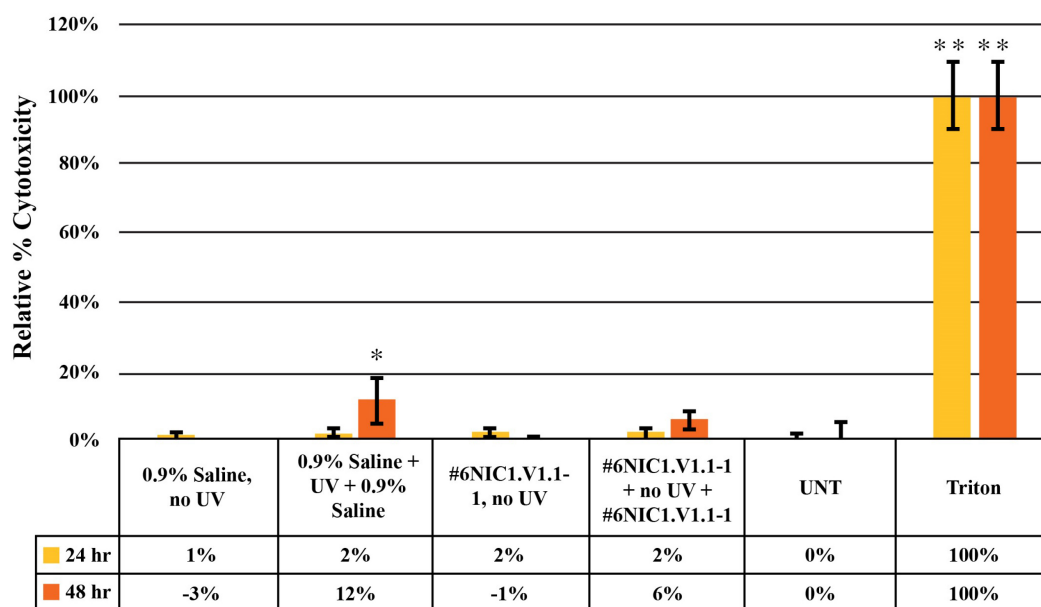
Each patient is his/her own control.

**Table 4.** Formulation application instruction.

| Application Zone | Application Frequency                       | Directions for use   |
|------------------|---|--|
| Face             | At home.<br>Once daily<br>(in the evening). | <p>Use as replacement of the usual evening facial care product.</p> <p>Each morning, apply the usual moisturizer on a dry face and neck (no cleansing of face and neck with the usual cleanser—only water is allowed for face rinsing if needed).</p> <p>Each evening, only cleanse face and neck with usual cleanser and apply the Testing product only as skin care.</p> |

**Table 5.** Kinetics known as *In-Vivo* tests parameters and check points.

| Kinetics  | Evaluation zones | D0 | D28  | D56  |
|---|------------------|----|------|------|
|   |                  |    | (±1) | (±1) |
| Information of the subject about study conditions and collection of his/her informed consent.   |                  | ●  |      |      |
| Verification of inclusion and non-inclusion criteria.   |                  | ●  |      |      |
| Clinical examination by the dermatologist in charge of the study in order to evaluate the cutaneous state of the face.  |                  | ●  | ●    | ●    |
| Clinical score by the dermatologist in charge of the study.   |                  | ●  | ●    | ●    |
| Definition of two zones on the face (one zone on the temple for Cutometer® measurements and one zone on the cheek for Corneometer® measurements and TiVi700® analysis). | Face             | ●  |      |      |
| Distribution/collection of the daily log.   |                  | ●  | ●    | ●    |
| Distribution/collection of the studied product.   |                  | ●  | ●    | ●    |
| Realization of a photograph of the full face at front and each profile using Visia®.  |                  | ●  | ●    | ●    |
| Subjective evaluation questionnaires.   |                  |    | ●    | ●    |
| Measurements using Cutometer®.  | Temple           | ●  | ●    | ●    |
| Measurements using Corneometer®.  |                  | ●  | ●    | ●    |
| Measurements using TiVi700®.  | Cheeks           | ●  | ●    | ●    |

**Figure 2.** Relative % LDH activity (cytotoxicity) as 24 and 48 hours. Note. The \* ( $p \leq 0.05$ ) symbol and. \*\* ( $p \leq 0.001$ ) designates statistical significance after performing unpaired t-test.

### Measured Changes in Gene Expression Following Treatment with Formulation (#6 NC1. V1.1)

Of the 107 genes tested, 60 - 80 presented significant changes in genetic expression with and without UV exposure. Each gene change is reported to influence structural and functional activity within skin cells (anti-inflammation, DNA repair, survival, apoptosis, antioxidation etc). A selection of 5 genetic markers was curated and reported above as their functions and mechanism of action are the most relevant to the study (**Table 6**).

**Table 6.** Gene expression linear fold change before and after UV exposure.

| Gene ID | Gene Name                        | #6NIC1.V1.1-1 No UV |       | v#6NIC1.V1.1-1 + UV + #6NIC1.V1.1-1 |       | Function in the skin                    | Results  |
|---------|----------------------------------|---------------------|-------|-------------------------------------|-------|---|--|
|         |                                  | FC                  | %C    | FC                                  | %C    |   |  |
| DHRS9   | Dehydrogenase/Reductase 9        | 17.98               | 1698% | 18.38                               | 1738% | Oxidative Stress Response               | In keratinocytes, DHRS9 is known to be involved in the process of conversion of retinol into retinoic acid [14] [15]. Its activation is generally considered to be a sign of production of retinoic acid [15]. Its expression might be affected by retinoic acid levels and UVB exposure.  |
| LRAT    | Lecithin Retinol Acyltransferase | 10.65               | 965%  | 6.74                                | 574%  | Oxidative Stress Response               | Responsible for retinoid absorption and storage, its downregulation can lead to depletion of vitamin A in the skin cells which in turn would cause oxidative stress [16]. Acute UVB exposure can be responsible for lower levels of LRAT [17].   |
| ANXA1   | Annexin A1                       | 2.54                | 154%  | 4.98                                | 398%  | Oxidative Stress Response, Inflammation | Marker of collagen production increase, ANXA1 is also known to be a powerful anti-inflammatory within human keratinocytes, inhibiting pro-inflammatory responses [18] [19].  |
| CSF2    | Colony Stimulating Factor 2      | 7.91                | 691%  | 11.64                               | 1064% | Inflammation                            | Involved in multiple biological functions, CSF2 is known for its activity of [20] [21]: <ul style="list-style-type: none"> <li>- Regulating extracellular matrix proteins expression</li> <li>- Inducing keratinocyte proliferation and differentiation in order to facilitate early wound healing</li> <li>- Stimulating the epidermal melanocytes proliferation and differentiation in response to UVB exposure</li> </ul> |
| IL1A    | Interleukin 1 alpha              | 3.31                | 231%  | 3.25                                | 225%  | Inflammation                            | Interleukin-1 alpha is an epidermal resident cytokine acting as a regulator of the skin structure and functions [22] [23]. When upregulated it is known to manage dermal collagen turnover as well as stimulating the production of hyaluronic acid [23]. It potentially can inhibit the tyrosinase activity leading to a reduction of melanogenesis [23].   |

### ***In-Vivo* Clinical Evaluation**

Each participant's skin was assessed at Day 0, 28 and 56 under dermatological supervision, thus representing its own control.

Each subject was provided with the full Formulation to apply every night, a self-assessment questionnaire and a daily log to report any unusual or adverse occurrences.

The population recruited is detailed in **Table 7**.

### **Cutaneous Compatibility**

This assessment was performed by a dermatologist. None of the patients experienced nor reported any clinical, functional nor physical signs of skin irritation during the trial period (**Table 8**). The full results obtained are described in **Table A2**.

### **Cutaneous Microcirculation, Soothing Effect**

Cutaneous microcirculation, assessed via Tivi700 revealed a reduction of redness (**Figure 3**), which may be regarded as a visible symptom of inflammation (**Figure 4**) [24]. A 9% mean reduction in redness across 70% of patients was recorded after 28 days of usage and a further 7.3% across 60% of patients after 56 days (**Table A3**).

### **Cutaneous Hydration**

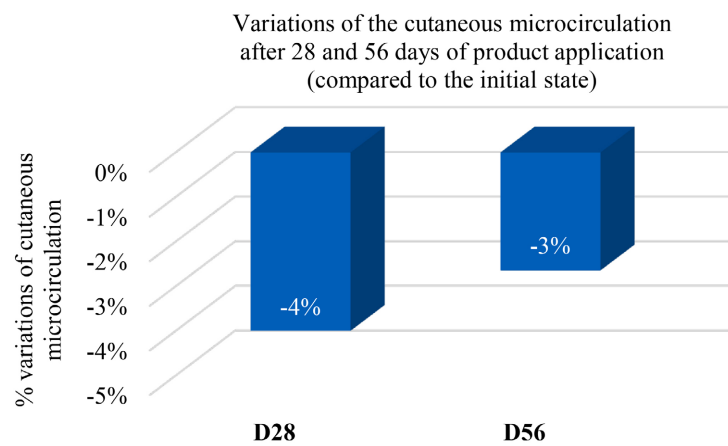
Cutaneous hydration was measured with a Corneometer [25], and results were compared before and after using the Formulation (**Figure 5**). 100% of the patients experienced an increase in hydration after applying the Formulation nightly for 28 days and continuing using it for 56 days. An average 39% elevation in epidermal moisture levels was recorded after 56 days of continual use (**Table A4**).

**Table 7.** *In-Vivo* test demographics (total number of patients n = 22).

| <b>Sex</b>            | <b>N</b> | <b>%</b> |
|-----------------------|----------|----------|
| <b>Female</b>         | 19       | 86%      |
| <b>Male</b>           | 3        | 14%      |
| <b>Phototype</b>      | <b>N</b> | <b>%</b> |
| <b>I</b>              | 5        | 23%      |
| <b>II</b>             | 17       | 77%      |
| <b>Face skin type</b> | <b>N</b> | <b>%</b> |
| <b>Dry</b>            | 7        | 32%      |
| <b>Normal</b>         | 5        | 23%      |
| <b>Combination</b>    | 10       | 45%      |

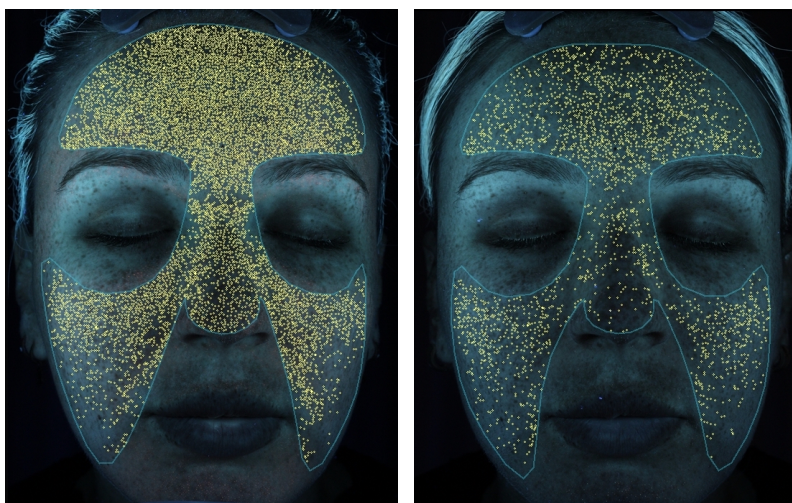
**Table 8.** Clinical and reported signs of non-acceptability.

| Non-Acceptability Signs     |    |      |
|-----------------------------|----|------|
| Assessment at D28           |    |      |
|                             | N  | %    |
| No clinical signs           | 21 | 100% |
| Not relevant clinical signs | 0  | 0%   |
| Relevant clinical signs     | 0  | 0%   |
| No reported signs           | 21 | 100% |
| Not relevant reported signs | 0  | 0%   |
| Relevant reported signs     | 0  | 0%   |
| Assessment at D56           |    |      |
|                             | N  | %    |
| No clinical signs           | 21 | 100% |
| Not relevant clinical signs | 0  | 0%   |
| Relevant clinical signs     | 0  | 0%   |
| No reported signs           | 21 | 100% |
| Not relevant reported signs | 0  | 0%   |
| Relevant reported signs     | 0  | 0%   |



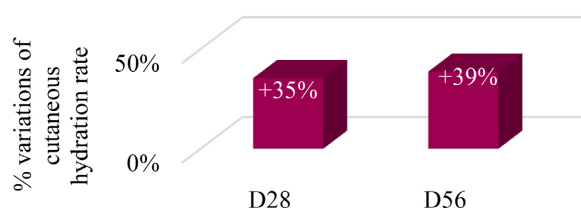
|                           | Parameter                  | Kinetics |            | D Dx-D0 (mean ± SEM) | P value | % of efficacy | % of subjects with soothing effect |
|---------------------------|----------------------------|----------|------------|----------------------|---------|---------------|------------------------------------|
|                           |                            | Delta    | Time Point |                      |         |               |                                    |
| #6NIC1 Batch<br>#6NIC1.28 | Cutaneous microcirculation | Delta    | D28        | -9.0 ± 2.9           | 0.006   | -4%           | 70%                                |
|                           |                            | Delta    | D56        | -7.3 ± 2.4           | 0.006   | -3%           | 60%                                |

**Figure 3.** Variations of cutaneous microcirculation measured with TIVI700 after using the Formulation for 28 and 56 days compared to the initial state. A significant decrease of cutaneous microcirculation characterizes a soothing effect of the Formulation.



**Figure 4.** An amelioration of inflammatory products is supported by a reduction in porphyrin count (yellow dots) after 28 days of using the solar repair Formulation. Further investigation is required to determine the effect of this change on the skin microbiome.

Variations of cutaneous hydration rate after 28 and 56 days of product application (compared to the initial state)



|                              | Parameter                | Kinetics | D Dx-D0<br>(mean ± SEM) | P value | % of efficacy | % of patients presenting an improvement |
|------------------------------|--------------------------|----------|-------------------------|---------|---------------|---|
| #6NIC1<br>Batch<br>#6NIC1.28 | Cutaneous hydration rate | DeltaD28 | 20 ± 2                  | <0.001  | 35%           | 100%                                    |
|                              |                          | DeltaD56 | 21 ± 2                  | <0.001  | 39%           | 100%                                    |

**Figure 5.** Variations of cutaneous hydration rate measured with a corneometer (in arbitrary units) after using the Formulation for up to 56 days compared to the initial rate. A significant increase of hydration rate is a sign of moisturizing effect, no change of the hydration rate shows a non-drying effect.

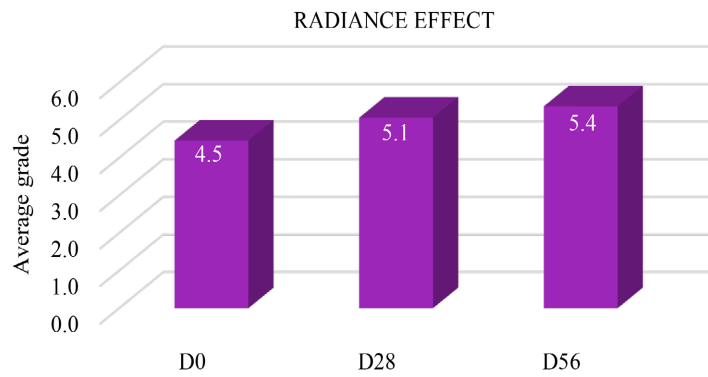
### Clinical Grading of overall radiance through measurements of evenness of skin tone, texture and brightness

Upon Dermatologist assessment, each patient's skin was graded on three different parameters to determine the radiance-enhancing effect of the Formulation (**Figure 6** and **Figure 7**).

- Evenness of the skin tone
- Brightness and complexion radiance
- Skin texture refinement

Overall, all of the patients experienced an average of 20% increase in radiance (**Table A5**).

### Biomechanical properties: firmness, tonicity, and suppleness



|   | ΔDX-D0<br>(mean ± SEM) |     |     |       | Δ% on<br>the mean | P value | Test                      | % of subjects<br>presenting<br>an improvement |
|---|------------------------|-----|-----|-------|-------------------|---------|---------------------------|---|
|   | Δ                      |     |     |       |                   |         |                           |   |
| Evenness skin tone                          | Δ                      | D28 | 0.9 | ± 0.1 | 16%               | <0.0001 | Wilcoxon signed rank test | 81%   |
|   | Δ                      | D56 | 0.8 | ± 0.1 | 16%               | <0.0001 | Wilcoxon signed rank test | 81%   |
| Brightness/complexion<br>radiance           | Δ                      | D28 | 0.7 | ± 0.1 | 15%               | 0.0001  | Wilcoxon signed rank test | 67%   |
|   | Δ                      | D56 | 0.9 | ± 0.1 | 21%               | <0.0001 | Wilcoxon signed rank test | 90%   |
| Skin texture/Fineness<br>of the skintexture | Δ                      | D28 | 0.5 | ± 0.1 | 10%               | 0.0020  | Wilcoxon signed rank test | 48%   |
|   | Δ                      | D56 | 1.1 | ± 0.1 | 24%               | <0.0001 | Wilcoxon signed rank test | 100%  |
| RADIANCE EFFECT                             | Δ                      | D28 | 0.7 | ± 0.1 | 14%               | <0.0001 | paired t-test             | 86%   |
|   | Δ                      | D56 | 0.9 | ± 0.1 | 20%               | <0.0001 | Wilcoxon signed rank test | 100%  |

**Figure 6.** Variations in clinical grading of evenness of skin tone, brightness, skin texture and overall radiance by a dermatologist after using the Formulation for 28 days, continuing on to 56 days compared to initial assessments.



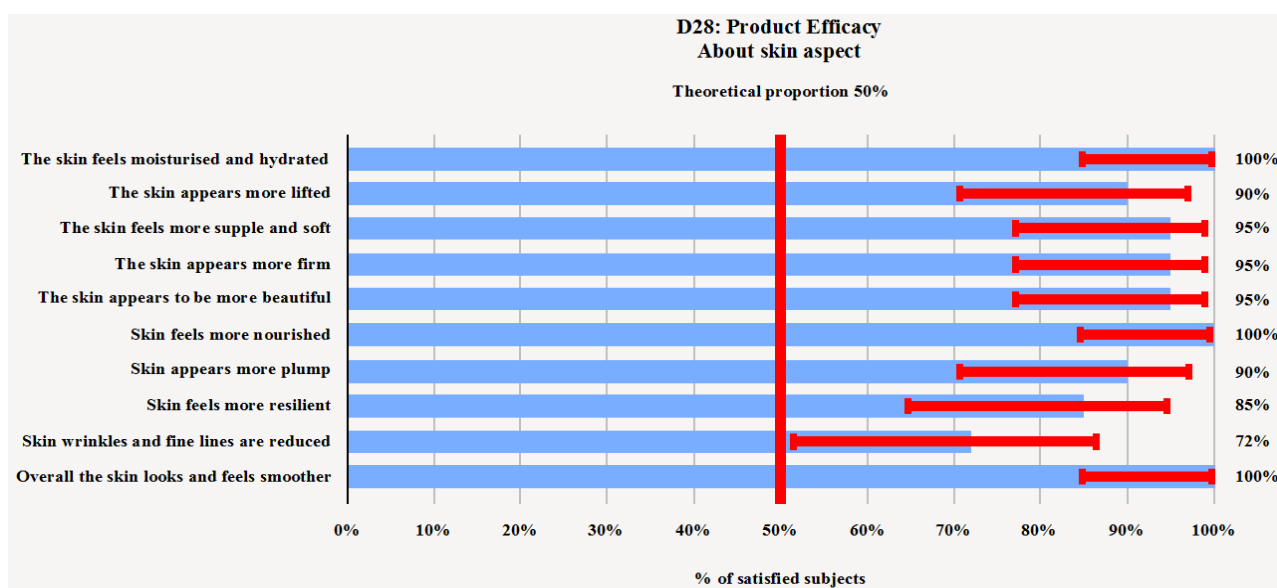
**Figure 7.** Visible improvements in skin tone, texture and radiance after 28 days of using the solar repair Formulation nightly

Using a Cutometer, the firmness of each patient’s skin was measured (see **Table 9**) [26]. After 28 days and after 56 days, 95% of the patients experienced, respectively, an average 13% and 21% improvement in skin firmness. Results for tonicity and suppleness improvement were not significant after 28 or 56 days as this was deemed to be too short a period to affect changes in skin connective tissue integrity (**Table A6**).

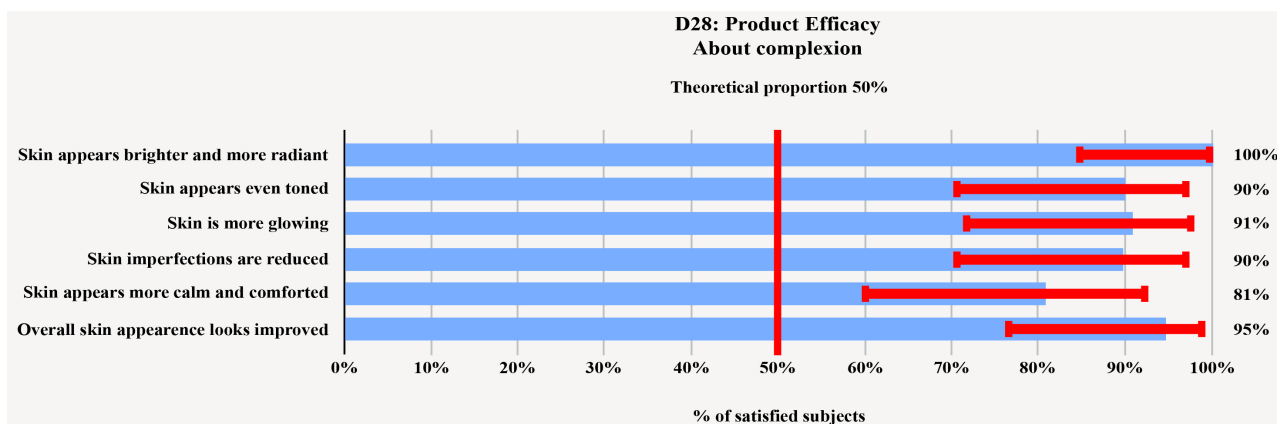
**Table 9.** Variations of the skin biomechanical properties using a cutometer after using the Formulation for 28 days compared to initial assessments. Statistical significance was examined by T-test.

| Variation of the skin biomechanical properties | Kinetics | Variations (mean $\pm$ SEM) | D% on the mean | % of efficacy | p value | % of subjects with the expected effect |
|--|----------|-----------------------------|----------------|---------------|---------|--|
| Firmness (R0 $\rightarrow$ Uf)                 | D D28    | -0.051 $\pm$ 0.008          | -13%           | +13%          | <0.001  | 95%                                    |
|  | D D56    | -0.074 $\pm$ 0.016          | -21%           | +21%          | <0.001  | 95%                                    |
| Suppleness Ue                                  | D D28    | -0.033 $\pm$ 0.009          | -11%           | -11%          | 0.001*  | 16%                                    |
|  | D D56    | -0.051 $\pm$ 0.014          | -19%           | -19%          | 0.002*  | 11%                                    |
| Tonicity Ur                                    | D D28    | 0.004 $\pm$ 0.006           | 2%             | 2%            | 0.977   | 47%                                    |
|  | D D56    | 0.001 $\pm$ 0.007           | -4%            | -4%           | 0.913   | 42%                                    |

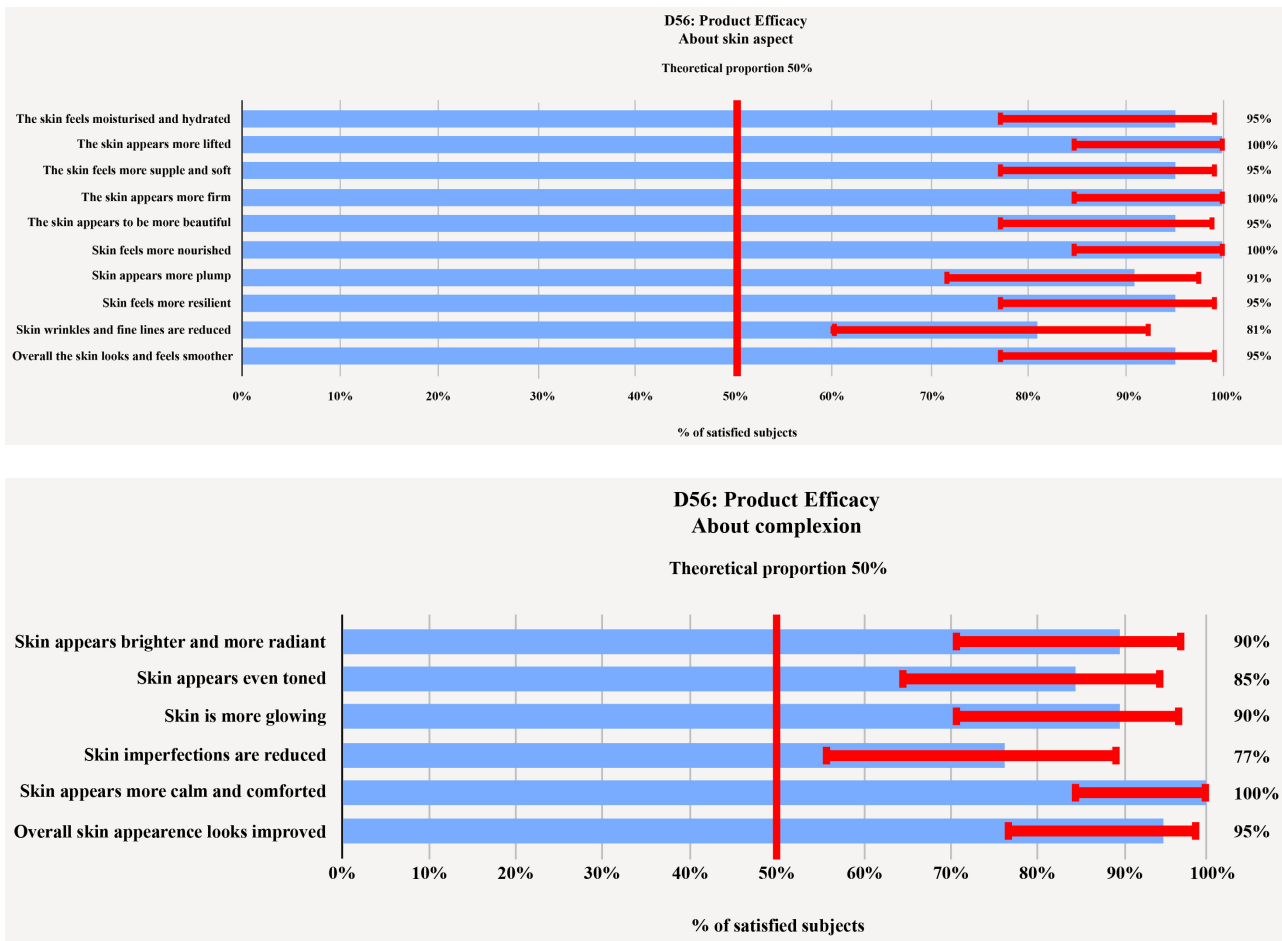
\*Not clinically relevant.



**Figure 8.** Patients self-assessment after using the formulation for 28 days.



**Figure 9.** Patients self-assessment after using the formulation for 28 days.



**Figure 10.** Patients self-assessment after using the formulation for 56 days.

### Self-Assessment Questionnaire

Each participant was requested to fill a questionnaire about perceived efficacy of the Formulation based on various criteria. Affirmations were proposed and patients were asked to rate their degree of agreement with the statement on a four-point scale from agree to disagree (Figure 8, Figure 9 and Figure 10). Where patients agreed or strongly agreed, the results were considered positive. Only 21 patients were considered for this assessment.

Overall, the vast majority of patients observed and reported a global improvement in the appearance and feel of their skin.

## 4. Discussion

Dermatologist assessment protocols on the safety of the Formulation for even the most sensitive skin revealed that all of subjects were able to use the Formulation nightly without skin irritation. On the contrary, in response to TIVI700 microcirculation testing, 70% of patients reported a reduction in redness (mean 9%) and sensitivity. Subjects reported that their skin felt firmer, stronger and more resilient. All of the patients experienced an increase of skin hydration measured with the corneometer and could feel a positive change in skin moistu-

risation and firmness after 28 days of use. Improvements in skin radiance were reported by all of patients, with enhancement of skin tone evenness, brightness and texture also observed in majority of patients after 28 and 56 days. This could potentially be explained by the improved hydration and a complex of active ingredients purposefully curated for this Formulation.

Subjective self-assessment revealed that skin firmness, plumpness, appearance of wrinkles and fine lines were also significantly improved as measured via the cutometer and self-assessment. These results could be attributed to the elevated complex of Vitamin A and peptides incorporated into the Formulation to enhance the skin tone and texture via reparative retinoid and cellular signalling pathways.

With an overwhelmingly positive *in-vivo* response to the Formulation, it can be concluded that the Formulation enhances skin appearance and health based on objective and subjective assessments.

It should be noted that this was an initial clinical study based on 40 patients, 20 of whom were assessed for physiological changes (hydration, skin firmness, radiance etc.) as reported by subjects and confirmed by dermatologist assessment, while the remaining 20 subjects were assessed solely for visual improvements (captured via Visia imaging). These documented outcomes serve as foundational data that could form the basis of further investigation.

*In-vitro* genetic studies on changes in gene expression in 3-D reconstructed human skin models revealed that four highly significant genes responsible for solar skin repair processes following UV assault were significantly upregulated.

Dehydrogenase/reductase SDR family member 9 (DHRS9) is described as a moonlighting protein that functions simultaneously as a metabolite enzyme and as a transcriptional repressor [14]. DHRS9 is a specific, identifiable marker of human regulatory macrophages. In keratinocytes, DHRS9 is involved in the conversion of retinol and its metabolites into retinoic acid, and its activation is evidenced by production of retinoic acid [15]. Its expression is influenced by UVB exposure and existing skin retinoic acid reserves. When inoculated with the solar repair Formulation and exposed to UVB, a 1738% increase in the expression of DHRS9 was recorded.

Lecithin Retinol Acyltransferase (LRAT) responsible for conversion of all-trans-retinol into retinyl esters, LRAT has been characterized at the molecular level. Because of the physiological relevance of LRAT, particularly in the metabolism and storage of vitamin A, its molecular characterization is of vital importance [16]. Acute UVB impairs retinoid storage in the epidermis, and inhibiting retinoic acid synthesis disrupts epidermal differentiation and structure [17]. In this study, we observed a 574% increase in LRAT expression in the order of LRAT following inoculation and UVB exposure.

Annexin A1 (ANXA1) belongs to the annexin family of  $Ca^{2+}$ -dependent phospholipid-binding proteins, preferentially located on the cytosolic face of the plasma membrane, and is a reliable marker of collagen synthesis activation [18] [19]. ANXA1 also acts an anti-inflammatory agent because of the biosynthesis of

potent mediators of inflammation, leukotrienes and prostaglandins [19]. In our study investigating the potential of a solar repair Formulation to reduce inflammation and increase collagen reduction, we documented a 398% increase in the expression of ANXA1 in a 3-D reconstructed human skin model.

Colony Stimulating Factor 2 (CSF2) is a monomeric glycoprotein that functions as a cytokine, and involved in multiple biological activities, including regulating extracellular matrix proteins expression, inducing keratinocyte proliferation and differentiation in order to facilitate early wound healing and modulating epidermal melanocytes proliferation and differentiation in response to UVB exposure [20] [21]. Our research demonstrated an increase in CSF2 expression of 1064%.

Interleukin-1 alpha (IL-1A) is an epidermal resident cytokine acting as a regulator of skin structure and function [22] [23]. IL-1A orchestrates dermal collagen turnover when upregulated, as well as stimulating the production of hyaluronic acid and Natural Moisturising Factors. It possesses the ability to inhibit tyrosinase activity leading to a reduction of melanogenesis.

Each of these results is supported by the *in-vitro* genetic study using a 3-dimensional skin model of epidermal keratinocytes and dermal fibroblasts. 107 genes were assessed but only 5 were selected for this paper, not only for their outstanding significant improvement but also for their link to visual results observed through the *in-vivo* study. Effectively, an improvement in the collagen production will have physical impacts such as a firmer and smoother skin.

Some of these results were, in fact, expected in relation to the active ingredients used in the Formulation. For example, upon incorporating retinoids in the form of retinol and retinaldehyde as well as vitamin A functional boosters, it is expected that the skin will be better equipped to convert, store and utilise retinoid metabolites including retinoic Acid, a known promoter of connective tissue synthesis and repairer of extrinsic photoaging.

## 5. Summary

*In-Vivo* testing has demonstrated that when applied to human skin for 28 and 56 days, significant improvement in skin appearance and skin health were observed:

- Skin radiance improved
- Skin firmness increased
- Skin redness reduced
- Skin hydration elevated.

To confirm these clinical improvements in skin tone and texture, *in vitro* genetic testing validated that not only do skin cells remain healthy upon exposure to the Formulation even when exposed to UV light but also that significant positive changes can be observed in gene expression after use of the Formulation.

- DHRS9, instrumental in modulating the conversion of retinol into retinoic acid was upregulated by up to 1738% after usage of the Formulation.

- LRAT, an enzyme encoding genes responsible for regulating the absorption and storage of retinoids was upregulated up to 574% after usage of the Formulation.
- ANXA1, encoding for collagen synthesis was upregulated by up to 398% after use of the Formulation.
- CFS2, responsible for regulating extracellular matrix proteins expression and inducing keratinocyte proliferation and differentiation was upregulated by 1064% following use of the Formulation

## 6. Conclusion

Each of the gene expression changes recorded in this study corresponds to functional proteins responsible for skin hydration, calmness, firmness (collagen and elastin production) and overall radiance, confirming each of the three proposed hypotheses.

1) DNA repair mechanisms markers are enhanced following daily use of the Formulation.

2) By reducing signs of inflammation, elevating hydration, reinforcing skin firmness and amplifying radiance, the Formulation elicits a visual and tactile improvement in skin appearance and tactility.

3) *In-vitro* changes in gene expression confirm clinical *in-vivo* results.

## Limitations

The absence of a clinical control group and a comparison dosage may limit the significance of these findings.

## Disclosure

The authors disclose that this study was entirely funded by RATIONALE Skincare Pty Ltd., Victoria, Australia. Amaryllis Aganahi and Richard Parker are paid employees of RATIONALE. Yohei Tanaka is a paid consultant plastic surgeon for RATIONALE.

## Conflicts of Interest

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

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## Appendix

**Table A1.** Gene markers environmental stress gene panel list.

| ENVIRONMENTAL STRESS PANEL GENE LIST                    |   |
|---|---|
| DNA REPAIR  | OXIDATIVE STRESS RESPONSE   |
| » ATM: ataxia-telangiectasia mutated                    | » AHR: aryl hydrocarbon receptor  |
| » EGR1: early growth response 1                         | » AIFM2: apoptosis inducing factor mitochondria associated 2              |
| » GADD45A: growth arrest and DNA damage inducible alpha | » ANXA1: annexin a1   |
| » H2AFX: h2A.X Variant histone                          | » CAT: catalase   |
| » MGMT: o-6-methylguanine-DNA methyltransferase         | » CYP1A1: cytochrome p450 family 1 subfamily a member 1                   |
| » PARP1: PARP enzyme (NAD)                              | » CYP1B1: cytochrome p450 family 1 subfamily b member 1                   |
| » PCNA: proliferating cell nuclear antigen              | » DHRS9: dehydrogenase/reductase 9  |
| » POLB: DNA polymerase beta                             | » GPX1: glutathione peroxidase 1  |
| » TDG: thymine DNA glycosylase                          | » GPX3: glutathione peroxidase 3  |
| » XRCC1: x-ray repair cross complementing 1             | » HIF1a: hypoxia inducible factor 1 subunit alpha                         |
| » XRCC2: x-ray repair cross complementing 2             | » HMOX1: heme oxygenase 1   |
| » XRCC4: x-ray repair cross complementing 4             | » HSP90AA1: heat shock protein 90 kDa alpha (cytosolic), class a member 1 |
| EPIGENETICS   | » HSPD1: heat shock 60 kDa protein 1 (chaperonin)                         |
| » DNMT1: DNA methyltransferase 1                        | » KEAP1: kelch like ECH associated protein 1                              |
| » DNMT3A: DNA methyltransferase 3 alpha                 | » LRAT: lecithin retinol acyltransferase                                  |
| » DNMT3B: DNA methyltransferase 3 beta                  | » MAPK14: mitogen-activated protein kinase 14 (p38)                       |
| » FTO: FTO alpha-ketoglutarate dependent dioxygenase    | » MT1G: metallothionein 1G  |
| » HDAC1: histone deacetylase 1                          | » NAMPT: nicotinamide phosphoribosyltransferase                           |
| » HDAC3: histone deacetylase 3                          | » NFE2L2: nuclear factor, erythroid 2 like 2 (NRF2)                       |
| » HDAC8: histone deacetylase 8                          | » NOX4: NADPH oxidase 4   |
| » METTL3: methyltransferase like 3                      | » NQO1: NAD(P)H quinone dehydrogenase 1                                   |
| » TET1: tet methylcytosine dioxygenase 1                | » PDSS1: decaprenyl diphosphate synthase subunit 1                        |
| » TET2: tet methylcytosine dioxygenase 2                | » PRDX2: peroxiredoxin 2  |
| » TIMELESS: timeless circadian regulator                | » PRDX3: peroxiredoxin 3  |
| INFLAMMATION  | » PRDX6: peroxiredoxin 6  |
| » AHR: aryl hydrocarbon receptor                        | » SOD1: superoxide dismutase 1, soluble                                   |
| » ANXA1: annexin a1                                     | » TXNRD1: thioredoxin reductase 1   |
| » CASP1: caspase 1                                      | » UCP1: uncoupling protein 1  |
| » CCL2: C-C motif chemokine ligand 2                    | » UCP2: uncoupling protein 2  |
| » CSF2: colony stimulating factor 2                     | » UCP3: uncoupling protein 3  |

**Continued**

- » CXCL8: C-X-C motif chemokine ligand 8 (IL8)
- » HSPD1: heat shock 60 kDa protein 1 (chaperonin)
- » IFNG: interferon gamma
- » IL18: interleukin 18
- » IL1A: interleukin 1 alpha
- » IL1B: interleukin 1 beta
- » IL24: interleukin 24
- » IL33: interleukin 33
- » IL6: interleukin 6
- » NFKB1: nuclear factor kappa b subunit 1 (p50)
- » NLRP3: NLR family pyrin domain containing 3
- » PTGS2: prostaglandin-endoperoxide synthase 2 (COX-2)
- » PYCARD: PYD and CARD domain containing
- » TLR3: toll like receptor 3
- » TLR5: toll like receptor 5
- » TNF: tumor necrosis factor

**MITOCHONDRIAL FUNCTION & BIOGENESIS**

- » AIFM2: apoptosis inducing factor mitochondria associated 2
- » ATP5F1B: ATP synthase f1 subunit beta
- » COX10: cytochrome c oxidase assembly factor heme a: farnesyltransferase COX10
- » COX4I1: cytochrome c oxidase subunit 4I1
- » COX5B: cytochrome c oxidase subunit 5B
- » COX6B1: cytochrome c oxidase subunit 6B1
- » COX7A1: cytochrome c oxidase subunit 7A1
- » CYC1: cytochrome c1
- » NDUFA11: NADH:ubiquinone oxidoreductase subunit a11
- » POLG1: DNA polymerase gamma, catalytic subunit
- » PPARGC1A: PPARG coactivator 1 alpha
- » SDHB: succinate dehydrogenase complex iron sulfur subunit b
- » SOD2: superoxide dismutase 2, mitochondrial
- » TFAM: transcription factor a, mitochondrial
- » UCP1: uncoupling protein 1
- » UCP2: uncoupling protein 2
- » UCP3: uncoupling protein 3

**SKIN AGING/EXTRACELLULAR MATRIX MODULATION**

- » CASP14: caspase 14
- » CDKN2A: cyclin dependent kinase inhibitor 2A (p16)
- » COL1A1: collagen type i alpha 1 chain
- » ELN: elastin
- » FBN1: fibrillin 1
- » FLG: filaggrin
- » HAS2: hyaluronan synthase 2
- » LOXL1: lysyl oxidase like 1
- » MMP1: matrix metalloproteinase 1
- » MMP9: matrix metalloproteinase 9
- » NAMPT: nicotinamide phosphoribosyltransferase
- » PANK4: pantothenate kinase 4
- » SIRT1: sirtuin 1
- » TIMP1: TIMP metalloproteinase inhibitor 1

**SURVIVAL/APOPTOSIS**

- » BAK1: bCL2-antagonist/killer 1
- » BCL2: bCL2 apoptosis regulator
- » EGR1: early growth response 1
- » FOXO1: forkhead box o1
- » FOXO3: forkhead box o3
- » MAP1LC3A: microtubule associated protein 1 light chain 3 alpha
- » MAPK1: mitogen-activated protein kinase 1
- » MKI67: marker of proliferation ki-67
- » SQSTM1: sequestosome 1 (p62)
- » TP53: tumor protein p53
- » TP73: tumor protein p73

**WOUND HEALING**

- » HIF1a: hypoxia inducible factor 1 subunit alpha
- » HSP90AA1: heat shock protein 90 kDa alpha (cytosolic), class a member 1
- » HSPD1: heat shock 60 kDa protein 1 (chaperonin)

**Table A2.** Cutaneous acceptability, detailed patients assessment results after 28 and 56 days of usage.

| Cutaneous acceptability |                |   |      |           |              |           |          |
|-------------------------|----------------|---|------|-----------|--------------|-----------|----------|
| Subject#                | Face skin type | D28 ( $\pm 1$ )   |      |           |              |           |          |
|                         |                | Clinical signs observed by the dermatologist in charge of the study and Reported signs by the patients (if different or more important than D0) |      |           |              |           |          |
|                         |                | Type of clinical sign   | Area | Intensity | Imputability | Relevance | Comments |
| 1                       | Dry            | None  | -    | -         | -            | -         | -        |
| 2                       | Dry            | None  | -    | -         | -            | -         | -        |
| 3                       | Combination    | None  | -    | -         | -            | -         | -        |
| 4                       | Combination    | None  | -    | -         | -            | -         | -        |
| 5                       | Combination    | None  | -    | -         | -            | -         | -        |
| 6                       | Combination    | None  | -    | -         | -            | -         | -        |
| 7                       | Dry            | None  | -    | -         | -            | -         | -        |
| 8                       | Dry            | None  | -    | -         | -            | -         | -        |
| 9                       | Dry            | None  | -    | -         | -            | -         | -        |
| 10                      | Dry            | None  | -    | -         | -            | -         | -        |
| 11                      | Dry            | None  | -    | -         | -            | -         | -        |
| 12                      | Combination    | None  | -    | -         | -            | -         | -        |
| 13                      | Normal         | None  | -    | -         | -            | -         | -        |
| 14                      | Combination    | None  | -    | -         | -            | -         | -        |
| 15                      | Combination    | None  | -    | -         | -            | -         | -        |
| 16                      | Combination    | None  | -    | -         | -            | -         | -        |
| 17                      | Normal         | None  | -    | -         | -            | -         | -        |
| 18                      | Normal         | None  | -    | -         | -            | -         | -        |
| 19                      | Combination    | None  | -    | -         | -            | -         | -        |
| (20)*                   | (Normal)*      | NC  |      |           |              |           |          |
| 21                      | Normal         | None  | -    | -         | -            | -         | -        |
| 22                      | Combination    | None  | -    | -         | -            | -         | -        |
| D56 ( $\pm 1$ )         |                |   |      |           |              |           |          |
| Subject#                | Face skin type | Clinical signs observed by the dermatologist in charge of the study and Reported signs by the patients (if different or more important than D0) |      |           |              |           |          |
|                         |                | Type of clinical sign   | Area | Intensity | Imputability | Relevance | Comments |
|                         |                | Type of clinical sign   | Area | Intensity | Imputability | Relevance | Comments |
| 1                       | Dry            | None  | -    | -         | -            | -         | -        |
| 2                       | Dry            | None  | -    | -         | -            | -         | -        |
| 3                       | Combination    | None  | -    | -         | -            | -         | -        |
| 4                       | Combination    | None  | -    | -         | -            | -         | -        |
| 5                       | Combination    | None  | -    | -         | -            | -         | -        |

## Continued

|       |                |      |   |   |    |   |   |
|-------|----------------|------|---|---|----|---|---|
| 6     | Combination    | None | - | - | -  | - | - |
| 7     | Dry            | None | - | - | -  | - | - |
| 8     | Dry            | None | - | - | -  | - | - |
| 9     | Dry            | None | - | - | -  | - | - |
| 10    | Dry            | None | - | - | -  | - | - |
| 11    | Dry            | None | - | - | -  | - | - |
| 12    | Combination    | None | - | - | -  | - | - |
| 13    | Normal         | None | - | - | -  | - | - |
| 14    | Combination    | None | - | - | -  | - | - |
| (15)* | (Combination)* |      |   |   | UN |   |   |
| 16    | Combination    | None | - | - | -  | - | - |
| 17    | Normal         | None | - | - | -  | - | - |
| 18    | Normal         | None | - | - | -  | - | - |
| 19    | Combination    | None | - | - | -  | - | - |
| 20    | Normal         | None | - | - | -  | - | - |
| 21    | Normal         | None | - | - | -  | - | - |
| 22    | Combination    | None | - | - | -  | - | - |

Legend: NC: not come, (\*): not included in data analysis, UN: untraceable, ()\*: not included in data analysis.

**Table A3.** Patients TIVI 700 results and variations of cutaneous microcirculation after 28 and 56 days.

**A significant decrease of cutaneous microcirculation characterizes a soothing effect of the product.**

| Subject | Before/after |     |     |       |       |  |
|---------|--------------|-----|-----|-------|-------|--|
|         | D0           | D28 | D56 | Δ D28 | Δ D56 |  |
| 1       | 154          | 147 | 153 | -7    | -1    |  |
| 2       | 165          | 140 | 153 | -25   | -12   |  |
| 3       | 193          | 186 | 183 | -7    | -10   |  |
| 4       | 184          | 175 | 185 | -9    | 1     |  |
| 5       | 158          | 141 | 147 | -17   | -11   |  |
| 6       | 202          | 167 | 177 | -35   | -25   |  |
| 7       | 158          | 149 | 160 | -9    | 2     |  |
| 8       | 221          | 198 | 186 | -23   | -35   |  |
| 9       | 200          | 183 | 183 | -17   | -17   |  |
| 10      | 185          | 155 | 165 | -30   | -20   |  |
| 11      | 137          | 140 | 137 | 3     | 0     |  |
| 12      | 166          | 149 | 155 | -17   | -11   |  |

## Continued

|                |   |              |                                 |                |                |
|----------------|---|--------------|---------------------------------|----------------|----------------|
| 13             | 155   | 151          | 156                             | -4             | 1              |
| 14             | 160   | 161          | 165                             | 1              | 5              |
| 15             | 107   | 114          | UN                              | 7              | UN             |
| 16             | 205   | 196          | 200                             | -9             | -5             |
| 17             | 135   | 144          | 135                             | 9              | 0              |
| 18             | 151   | 149          | 142                             | -2             | -9             |
| 19             | 106   | 109          | 110                             | 3              | 4              |
| 20             | 142   | NC           | 138                             | NC             | -4             |
| 21             | 134   | (157) *AV    | 135                             | (23) *AV       | 1              |
| 22             | 158   | 167          | MV                              | 9              | MV             |
| <b>Mean</b>    | <b>162.5</b>  | <b>156.1</b> | <b>158.3</b>                    | <b>-9.0</b>    | <b>-7.3</b>    |
| Median         | 158.0   | 150.0        | 155.5                           | -8.0           | -4.5           |
| Minimum        | 106.0   | 109.0        | 110.0                           | -35.0          | -35.0          |
| Maximum        | 221.0   | 198.0        | 200.0                           | 9.0            | 5.0            |
| <b>SEM</b>     | <b>6.5</b>  | <b>5.3</b>   | <b>5.0</b>                      | <b>2.9</b>     | <b>2.4</b>     |
| CI 95%         | 13.5  | 11.1         | 10.5                            | 6.0            | 5.0            |
| Nb of subjects | 22  | 20           | 20                              | 20             | 20             |
|                |   |              | <b>Statistical analysis p =</b> | <b>0.006</b>   | <b>0.006</b>   |
|                |   |              | <b>Statistical test</b>         | <b>T. test</b> | <b>T. test</b> |
|                | <b>Δ%</b>   |              |                                 | <b>-4%</b>     | <b>-3%</b>     |
|                | <b>% of subjects with soothing effect (variation &lt; -0.1)</b> |              |                                 | <b>70%</b>     | <b>60%</b>     |

Legend: AV: aberrant value, MV: missing value, NC: not come, UN: untraceable, (\*): not included in data analysis.

**Table A4.** Patients Corneometer results and variations of cutaneous hydration rate after 28 and 56 days.

| Cutaneous hydration rate evaluation in comparison to the initial state: Corneometer® measurement (in arbitrary units) |              |     |     |       |       |
|---|--------------|-----|-----|-------|-------|
| Subject   | Before/after |     |     |       |       |
|   | D0           | D28 | D56 | Δ D28 | Δ D56 |
| 1   | 48           | 79  | 51  | 30    | 3     |
| 2   | 26           | 60  | 64  | 34    | 38    |
| 3   | 49           | 76  | 63  | 27    | 14    |
| 4   | 52           | 77  | 73  | 25    | 21    |
| 5   | 51           | 76  | 78  | 25    | 27    |
| 6   | 58           | 73  | 65  | 15    | 7     |
| 7   | 46           | 64  | 75  | 18    | 28    |
| 8   | 38           | 67  | 67  | 28    | 29    |

Continued

|                |           |  |                                 |                  |                  |
|----------------|-----------|--|---------------------------------|------------------|------------------|
| 9              | 53        | 65   | 65                              | 13               | 13               |
| 10             | 38        | 71   | 80                              | 33               | 42               |
| 11             | 49        | 61   | 77                              | 11               | 28               |
| 12             | 41        | 66   | 67                              | 25               | 25               |
| 13             | 64        | 86   | 86                              | 22               | 22               |
| 14             | 51        | 62   | 65                              | 11               | 13               |
| 15             | 52        | 66   | UN                              | 13               | UN               |
| 16             | 57        | 78   | 87                              | 21               | 30               |
| 17             | 68        | 78   | 79                              | 10               | 11               |
| 18             | 62        | 76   | 78                              | 15               | 16               |
| 19             | 60        | 81   | 81                              | 22               | 21               |
| 20             | 75        | NC   | 82                              | NC               | 7                |
| 21             | 86        | 92   | 93                              | 6                | 7                |
| 22             | 54        | 71   | 84                              | 16               | 30               |
| <b>Mean</b>    | <b>54</b> | <b>73</b>                                      | <b>74</b>                       | <b>20</b>        | <b>21</b>        |
| Median         | 52        | 73   | 77                              | 21               | 21               |
| Minimum        | 26        | 60   | 51                              | 6                | 3                |
| Maximum        | 86        | 92   | 93                              | 34               | 42               |
| <b>SEM</b>     | <b>3</b>  | <b>2</b>                                       | <b>2</b>                        | <b>2</b>         | <b>2</b>         |
| CI 95%         | 6         | 4  | 5                               | 4                | 5                |
| Nb of subjects | 22        | 21   | 21                              | 21               | 21               |
|                |           |  | <b>Statistical analysis p =</b> | <b>&lt;0.001</b> | <b>&lt;0.001</b> |
|                |           |  | <b>Statistical test</b>         | <b>T-test</b>    | <b>T-test</b>    |
|                |           | <b>% of variation of cutaneous hydration</b>   |                                 | <b>35%</b>       | <b>39%</b>       |
|                |           | <b>% of subjects presenting an improvement</b> |                                 | <b>100%</b>      | <b>100%</b>      |

Legend: NC: not come, UN: untraceable subject, (\*): not included in data analysis.

**Table A5.** Patients radiance clinical score assessment by Dermatologist results and variations after 28 and 56 days.

| Evenness skin tone                  |    |     |     |     |     |  |
|-------------------------------------|----|-----|-----|-----|-----|--|
| 0 = not even at all; 10 = very even |    |     |     |     |     |  |
| Variations Δ                        |    |     |     |     |     |  |
| Sub#                                | D0 | D28 | D56 | D28 | D56 |  |
| 1                                   | 4  | 5   | 5   | 1   | 1   |  |
| 2                                   | 4  | 5   | 4   | 1   | 0   |  |
| 3                                   | 4  | 4   | 5   | 0   | 1   |  |
| 4                                   | 5  | 6   | 6   | 1   | 1   |  |
| 5                                   | 4  | 5   | 5   | 1   | 1   |  |

Continued

|   |            |  |            |                                  |                                  |
|---|------------|--|------------|----------------------------------|----------------------------------|
| 6   | 4          | 5  | 4          | 1                                | 0                                |
| 7   | 5          | 6  | 5          | 1                                | 0                                |
| 8   | 3          | 4  | 4          | 1                                | 1                                |
| 9   | 3          | 4  | 4          | 1                                | 1                                |
| 10  | 4          | 5  | 5          | 1                                | 1                                |
| 11  | 6          | 7  | 7          | 1                                | 1                                |
| 12  | 5          | 6  | 6          | 1                                | 1                                |
| 13  | 5          | 6  | 6          | 1                                | 1                                |
| 14  | 4          | 5  | 5          | 1                                | 1                                |
| 15  | 6          | 6  | UN         | 0                                | UN                               |
| 16  | 3          | 4  | 3          | 1                                | 0                                |
| 17  | 5          | 6  | 6          | 1                                | 1                                |
| 18  | 5          | 6  | 6          | 1                                | 1                                |
| 19  | 6          | 6  | 7          | 0                                | 1                                |
| 20  | 7          | NC   | 8          | NC                               | 1                                |
| 21  | 3          | 5  | 4          | 2                                | 1                                |
| 22  | 6          | 6  | 7          | 0                                | 1                                |
| <b>Mean</b>   | <b>4.6</b> | <b>5.3</b>                                     | <b>5.3</b> | <b>0.9</b>                       | <b>0.8</b>                       |
| Med   | 4.5        | 5.0  | 5.0        | 1.0                              | 1.0                              |
| Min   | 3.0        | 4.0  | 3.0        | 0.0                              | 0.0                              |
| Max   | 7.0        | 7.0  | 8.0        | 2.0                              | 1.0                              |
| <b>SEM</b>  | <b>0.2</b> | <b>0.2</b>                                     | <b>0.3</b> | <b>0.1</b>                       | <b>0.1</b>                       |
| 95% CI  | 0.5        | 0.4  | 0.6        | 0.2                              | 0.2                              |
| # of subjects   | 22         | 21   | 21         | 21                               | 21                               |
|   |            | <b>Δ %</b>                                     |            | <b>16%</b>                       | <b>16%</b>                       |
|   |            | <b>Statistical analysis p =</b>                |            | <b>&lt;0.0001</b>                | <b>&lt;0.0001</b>                |
|   |            | <b>Statistical test</b>                        |            | <b>Wilcoxon signed rank test</b> | <b>Wilcoxon signed rank test</b> |
|   |            | <b>% of subjects presenting an improvement</b> |            | <b>81%</b>                       | <b>81%</b>                       |
|   |            | <b>% of subjects without change</b>            |            | <b>19%</b>                       | <b>19%</b>                       |
|   |            | <b>% of subjects presenting worsening</b>      |            | <b>0%</b>                        | <b>0%</b>                        |
| <b>Brightness/complexion radiance</b>                 |            |  |            |                                  |                                  |
| 0 = dull complexion; 10 = radiant/luminous complexion |            |  |            |                                  |                                  |
| <b>Variations Δ</b>                                   |            |  |            |                                  |                                  |
| <b>Sub#</b>   | <b>D0</b>  | <b>D28</b>                                     | <b>D56</b> | <b>D28</b>                       | <b>D56</b>                       |
| 1   | 3          | 4  | 4          | 1                                | 1                                |

Continued

|               |            |  |            |                                  |                                  |
|---------------|------------|--|------------|----------------------------------|----------------------------------|
| 2             | 5          | 6  | 5          | 1                                | 0                                |
| 3             | 3          | 4  | 4          | 1                                | 1                                |
| 4             | 4          | 5  | 5          | 1                                | 1                                |
| 5             | 4          | 5  | 5          | 1                                | 1                                |
| 6             | 3          | 4  | 3          | 1                                | 0                                |
| 7             | 5          | 5  | 6          | 0                                | 1                                |
| 8             | 3          | 3  | 4          | 0                                | 1                                |
| 9             | 3          | 4  | 4          | 1                                | 1                                |
| 10            | 4          | 5  | 5          | 1                                | 1                                |
| 11            | 6          | 7  | 7          | 1                                | 1                                |
| 12            | 5          | 5  | 6          | 0                                | 1                                |
| 13            | 5          | 6  | 6          | 1                                | 1                                |
| 14            | 4          | 5  | 5          | 1                                | 1                                |
| 15            | 4          | 4  | UN         | 0                                | UN                               |
| 16            | 3          | 4  | 4          | 1                                | 1                                |
| 17            | 4          | 5  | 5          | 1                                | 1                                |
| 18            | 5          | 5  | 6          | 0                                | 1                                |
| 19            | 6          | 6  | 7          | 0                                | 1                                |
| 20            | 6          | NC   | 7          | NC                               | 1                                |
| 21            | 4          | 6  | 5          | 2                                | 1                                |
| 22            | 6          | 6  | 7          | 0                                | 1                                |
| <b>Mean</b>   | <b>4.3</b> | <b>5.0</b>                                     | <b>5.2</b> | <b>0.7</b>                       | <b>0.9</b>                       |
| Med           | 4.0        | 5.0  | 5.0        | 1.0                              | 1.0                              |
| Min           | 3.0        | 3.0  | 3.0        | 0.0                              | 0.0                              |
| Max           | 6.0        | 7.0  | 7.0        | 2.0                              | 1.0                              |
| <b>SEM</b>    | <b>0.2</b> | <b>0.2</b>                                     | <b>0.3</b> | <b>0.1</b>                       | <b>0.1</b>                       |
| 95% CI        | 0.5        | 0.4  | 0.5        | 0.3                              | 0.1                              |
| # of subjects | 22         | 21   | 21         | 21                               | 21                               |
|               |            | <b>Δ %</b>                                     |            | <b>15%</b>                       | <b>21%</b>                       |
|               |            | <b>Statistical analysis p =</b>                |            | <b>0.0001</b>                    | <b>&lt;0.0001</b>                |
|               |            | <b>Statistical test</b>                        |            | <b>Wilcoxon signed rank test</b> | <b>Wilcoxon signed rank test</b> |
|               |            | <b>% of subjects presenting an improvement</b> |            | <b>67%</b>                       | <b>90%</b>                       |
|               |            | <b>% of subjects without change</b>            |            | <b>33%</b>                       | <b>10%</b>                       |
|               |            | <b>% of subjects presenting worsening</b>      |            | <b>0%</b>                        | <b>0%</b>                        |

Continued

| Skin texture/Fineness of the skin texture |            |                                 |            |                                  |                                  |
|---|------------|---------------------------------|------------|----------------------------------|----------------------------------|
| 0 = coarse; 10 = fine                     |            |                                 |            |                                  |                                  |
| Variations $\Delta$                       |            |                                 |            |                                  |                                  |
| Sub#                                      | D0         | D28                             | D56        | D28                              | D56                              |
| 1   | 4          | 4                               | 5          | 0                                | 1                                |
| 2   | 4          | 5                               | 5          | 1                                | 1                                |
| 3   | 4          | 4                               | 5          | 0                                | 1                                |
| 4   | 4          | 5                               | 5          | 1                                | 1                                |
| 5   | 4          | 4                               | 5          | 0                                | 1                                |
| 6   | 4          | 4                               | 5          | 0                                | 1                                |
| 7   | 5          | 6                               | 6          | 1                                | 1                                |
| 8   | 3          | 3                               | 4          | 0                                | 1                                |
| 9   | 3          | 4                               | 4          | 1                                | 1                                |
| 10  | 5          | 5                               | 6          | 0                                | 1                                |
| 11  | 6          | 6                               | 7          | 0                                | 1                                |
| 12  | 5          | 5                               | 6          | 0                                | 1                                |
| 13  | 5          | 6                               | 7          | 1                                | 2                                |
| 14  | 3          | 4                               | 4          | 1                                | 1                                |
| 15  | 5          | 5                               | UN         | 0                                | UN                               |
| 16  | 2          | 3                               | 3          | 1                                | 1                                |
| 17  | 5          | 6                               | 6          | 1                                | 1                                |
| 18  | 5          | 6                               | 7          | 1                                | 2                                |
| 19  | 6          | 6                               | 7          | 0                                | 1                                |
| 20  | 6          | NC                              | 7          | NC                               | 1                                |
| 21  | 4          | 6                               | 5          | 2                                | 1                                |
| 22  | 6          | 6                               | 7          | 0                                | 1                                |
| <b>Mean</b>                               | <b>4.5</b> | <b>4.9</b>                      | <b>5.5</b> | <b>0.5</b>                       | <b>1.1</b>                       |
| Med                                       | 4.5        | 5.0                             | 5.0        | 0.0                              | 1.0                              |
| Min                                       | 2.0        | 3.0                             | 3.0        | 0.0                              | 1.0                              |
| Max                                       | 6.0        | 6.0                             | 7.0        | 2.0                              | 2.0                              |
| <b>SEM</b>                                | <b>0.2</b> | <b>0.2</b>                      | <b>0.3</b> | <b>0.1</b>                       | <b>0.1</b>                       |
| 95% CI                                    | 0.5        | 0.5                             | 0.6        | 0.3                              | 0.1                              |
| # of subjects                             | 22         | 21                              | 21         | 21                               | 21                               |
|   |            | <b><math>\Delta</math> %</b>    |            | <b>10%</b>                       | <b>24%</b>                       |
|   |            | <b>Statistical analysis p =</b> |            | <b>0.0020</b>                    | <b>&lt;0.0001</b>                |
|   |            | <b>Statistical test</b>         |            | <b>Wilcoxon signed rank test</b> | <b>Wilcoxon signed rank test</b> |

Continued

|  |            |             |
|--|------------|-------------|
| <b>% of subjects presenting an improvement</b> | <b>48%</b> | <b>100%</b> |
| <b>% of subjects without change</b>            | 52%        | 0%          |
| <b>% of subjects presenting worsening</b>      | 0%         | 0%          |

**RADIANCE EFFECT (average grade)**

*Note: a compilation of the three grades is performed. A significant increase of the average grade allows to conclude a radiance effect (if no worsening is observed on the separately taken scores).*

| Sub#        | Variations $\Delta$ |            |            |            |            |
|-------------|---------------------|------------|------------|------------|------------|
|             | D0                  | D28        | D56        | D28        | D56        |
| 1           | 3.7                 | 4.3        | 4.7        | 0.7        | 1.0        |
| 2           | 4.3                 | 5.3        | 4.7        | 1.0        | 0.3        |
| 3           | 3.7                 | 4.0        | 4.7        | 0.3        | 1.0        |
| 4           | 4.3                 | 5.3        | 5.3        | 1.0        | 1.0        |
| 5           | 4.0                 | 4.7        | 5.0        | 0.7        | 1.0        |
| 6           | 3.7                 | 4.3        | 4.0        | 0.7        | 0.3        |
| 7           | 5.0                 | 5.7        | 5.7        | 0.7        | 0.7        |
| 8           | 3.0                 | 3.3        | 4.0        | 0.3        | 1.0        |
| 9           | 3.0                 | 4.0        | 4.0        | 1.0        | 1.0        |
| 10          | 4.3                 | 5.0        | 5.3        | 0.7        | 1.0        |
| 11          | 6.0                 | 6.7        | 7.0        | 0.7        | 1.0        |
| 12          | 5.0                 | 5.3        | 6.0        | 0.3        | 1.0        |
| 13          | 5.0                 | 6.0        | 6.3        | 1.0        | 1.3        |
| 14          | 3.7                 | 4.7        | 4.7        | 1.0        | 1.0        |
| 15          | 5.0                 | 5.0        | UN         | 0.0        | UN         |
| 16          | 2.7                 | 3.7        | 3.3        | 1.0        | 0.7        |
| 17          | 4.7                 | 5.7        | 5.7        | 1.0        | 1.0        |
| 18          | 5.0                 | 5.7        | 6.3        | 0.7        | 1.3        |
| 19          | 6.0                 | 6.0        | 7.0        | 0.0        | 1.0        |
| 20          | 6.3                 | NC         | 7.3        | NC         | 1.0        |
| 21          | 3.7                 | 5.7        | 4.7        | 2.0        | 1.0        |
| 22          | 6.0                 | 6.0        | 7.0        | 0.0        | 1.0        |
| <b>Mean</b> | <b>4.5</b>          | <b>5.1</b> | <b>5.4</b> | <b>0.7</b> | <b>0.9</b> |
| Med         | 4.3                 | 5.3        | 5.3        | 0.7        | 1.0        |
| Min         | 2.7                 | 3.3        | 3.3        | 0.0        | 0.3        |
| Max         | 6.3                 | 6.7        | 7.3        | 2.0        | 1.3        |
| <b>SEM</b>  | <b>0.2</b>          | <b>0.2</b> | <b>0.3</b> | <b>0.1</b> | <b>0.1</b> |

**Continued**

|  |                                 |     |     |                      |                                  |
|--|---------------------------------|-----|-----|----------------------|----------------------------------|
| 95% CI   | 0.5                             | 0.4 | 0.5 | 0.2                  | 0.1                              |
| # of subjects                                  | 22                              | 21  | 21  | 21                   | 21                               |
|  | <b>D%</b>                       |     |     | <b>14%</b>           | <b>20%</b>                       |
|  | <b>Statistical analysis p =</b> |     |     | <b>&lt;0.0001</b>    | <b>&lt;0.0001</b>                |
|  | <b>Statistical test</b>         |     |     | <b>paired t-test</b> | <b>Wilcoxon signed rank test</b> |
| <b>% of subjects presenting an improvement</b> |                                 |     |     | <b>86%</b>           | <b>100%</b>                      |
| <b>% of subjects without change</b>            |                                 |     |     | 14%                  | 0%                               |
| <b>% of subjects presenting worsening</b>      |                                 |     |     | 0%                   | 0%                               |

Legend: UN: untraceable, NC: not come.

**Table A6.** Patients Cutometer biomechanical properties results and variations after 28 and 56 days.

| <b>CUTOMETER' MEASUREMENTS</b> |              |              |              |                     |               |
|--------------------------------|--------------|--------------|--------------|---------------------|---------------|
| <b>Firmness (R0 → Uf)</b>      |              |              |              |                     |               |
| <b>Sub#</b>                    |              |              |              | <b>Variations Δ</b> |               |
|                                | <b>D0</b>    | <b>D28</b>   | <b>D56</b>   | <b>D28</b>          | <b>D56</b>    |
| 1                              | 0.249        | 0.180        | 0.291        | -0.070              | 0.042         |
| 2                              | 0.382        | 0.290        | 0.252        | -0.092              | -0.130        |
| 3                              | 0.275        | 0.250        | 0.250        | -0.026              | -0.026        |
| 4                              | 0.318        | 0.261        | 0.296        | -0.057              | -0.022        |
| (5)*                           | (0.350) * AV | (0.293) * AV | (0.295) * AV | (-0.058) * AV       | (-0.055) * AV |
| 6                              | 0.465        | 0.308        | 0.178        | -0.157              | -0.287        |
| 7                              | 0.421        | 0.325        | 0.305        | -0.096              | -0.116        |
| 8                              | 0.297        | 0.233        | 0.221        | -0.065              | -0.077        |
| 9                              | 0.301        | 0.247        | 0.254        | -0.054              | -0.047        |
| 10                             | 0.321        | 0.271        | 0.171        | -0.050              | -0.150        |
| 11                             | 0.336        | 0.308        | 0.235        | -0.028              | -0.101        |
| (12)*                          | (0.288) * AV | (0.244) * AV | (0.159) * AV | (-0.044) * AV       | (-0.129) * AV |
| 13                             | 0.467        | 0.450        | 0.390        | -0.017              | -0.077        |
| 14                             | 0.397        | 0.369        | 0.347        | -0.028              | -0.051        |
| 15                             | 0.436        | 0.390        | UN           | -0.047              | UN            |
| 16                             | 0.352        | 0.316        | 0.257        | -0.036              | -0.095        |
| 17                             | 0.342        | 0.339        | 0.323        | -0.003              | -0.019        |
| 18                             | 0.420        | 0.377        | 0.345        | -0.043              | -0.075        |
| 19                             | 0.531        | 0.488        | 0.457        | -0.043              | -0.075        |
| 20                             | 0.295        | NC           | 0.280        | NC                  | -0.015        |

## Continued

|                      |  |               |              |                  |                  |
|----------------------|--|---------------|--------------|------------------|------------------|
| 21                   | 0.344  | 0.324         | 0.319        | -0.021           | -0.025           |
| 22                   | 0.359  | 0.316         | 0.298        | -0.044           | -0.061           |
| <b>Mean</b>          | <b>0.365</b>   | <b>0.318</b>  | <b>0.288</b> | <b>-0.051</b>    | <b>-0.074</b>    |
| <b>Med</b>           | 0.348  | 0.316         | 0.291        | -0.044           | -0.075           |
| <b>Min</b>           | 0.249  | 0.180         | 0.171        | -0.157           | -0.287           |
| <b>Max</b>           | 0.531  | 0.488         | 0.457        | -0.003           | 0.042            |
| <b>SEM</b>           | <b>0.016</b>   | <b>0.017</b>  | <b>0.016</b> | <b>0.008</b>     | <b>0.016</b>     |
| <b>95% CI</b>        | 0.034  | 0.036         | 0.033        | 0.017            | 0.033            |
| <b># of subjects</b> | 20   | 19            | 19           | 19               | 19               |
|                      | <b>Δ %</b>   |               |              | <b>-13%</b>      | <b>-21%</b>      |
|                      | <b>% of cutaneous firmness improvement: firming effect (-R0)</b>                                     |               |              | <b>+13%</b>      | <b>+21%</b>      |
|                      | <b>Statistical analysis p =</b>  |               |              | <b>&lt;0.001</b> | <b>&lt;0.001</b> |
|                      | <b>Statistical test</b>  |               |              | <b>T-test</b>    | <b>T-test</b>    |
|                      | <b>% of subjects with an improvement in the cutaneous firmness<br/>(NB: if variation &lt; -0.01)</b> |               |              | <b>95%</b>       | <b>95%</b>       |
| <b>Suppleness Ue</b> |  |               |              |                  |                  |
| <b>Variations Δ</b>  |  |               |              |                  |                  |
| <b>Sub#</b>          | <b>D0</b>  | <b>D28</b>    | <b>D56</b>   | <b>D28</b>       | <b>D56</b>       |
| 1                    | 0.179  | 0.124         | 0.213        | -0.055           | 0.034            |
| 2                    | 0.280  | 0.232         | 0.186        | -0.048           | -0.094           |
| 3                    | 0.181  | 0.170         | 0.172        | -0.011           | -0.009           |
| 4                    | 0.224  | 0.166         | 0.214        | -0.059           | -0.010           |
| (5)*                 | (0.286) * AV   | (0.219) * AV  | (0.207) * AV | (-0.068) * AV    | (-0.079) * AV    |
| 6                    | 0.350  | 0.213         | 0.118        | -0.138           | -0.232           |
| 7                    | 0.308  | 0.247         | 0.210        | -0.061           | -0.098           |
| 8                    | 0.209  | 0.167         | 0.156        | -0.043           | -0.053           |
| 9                    | 0.227  | 0.175         | 0.185        | -0.052           | -0.042           |
| 10                   | 0.241  | 0.194         | 0.111        | -0.047           | -0.130           |
| 11                   | 0.258  | 0.220         | 0.179        | -0.038           | -0.079           |
| (12)*                | (0.216) * AV   | (-0.164) * AV | (0.123) * AV | (-0.053) * AV    | (-0.093) * AV    |
| 13                   | 0.354  | 0.396         | 0.340        | 0.042            | -0.014           |
| 14                   | 0.306  | 0.320         | 0.298        | 0.014            | -0.008           |
| 15                   | 0.355  | 0.340         | UN           | -0.016           | UN               |
| 16                   | 0.258  | 0.227         | 0.211        | -0.031           | -0.047           |
| 17                   | 0.304  | 0.296         | 0.278        | -0.008           | -0.027           |

## Continued

|                      |              |  |              |               |               |
|----------------------|--------------|--|--------------|---------------|---------------|
| 18                   | 0.341        | 0.322  | 0.293        | -0.019        | -0.048        |
| 19                   | 0.391        | 0.427  | 0.414        | 0.037         | 0.023         |
| 20                   | 0.242        | NC   | 0.229        | NC            | -0.013        |
| 21                   | 0.293        | 0.243  | 0.253        | -0.051        | -0.041        |
| 22                   | 0.310        | 0.266  | 0.234        | -0.045        | -0.076        |
| <b>Mean</b>          | <b>0.280</b> | <b>0.249</b>   | <b>0.226</b> | <b>-0.033</b> | <b>-0.051</b> |
| <b>Med</b>           | 0.286        | 0.232  | 0.213        | -0.043        | -0.042        |
| <b>Min</b>           | 0.179        | 0.124  | 0.111        | -0.138        | -0.232        |
| <b>Max</b>           | 0.391        | 0.427  | 0.414        | 0.042         | 0.034         |
| <b>SEM</b>           | <b>0.014</b> | <b>0.019</b>   | <b>0.017</b> | <b>0.009</b>  | <b>0.014</b>  |
| <b>95% CI</b>        | 0.028        | 0.040  | 0.036        | 0.019         | 0.029         |
| <b># of subjects</b> | 20           | 19   | 19           | 19            | 19            |
|                      |              | <b>Δ %</b>   |              | -11%          | -19%          |
|                      |              | <b>% of cutaneous suppleness improvement (Ue)</b>                    |              | -11%          | -19%          |
|                      |              | <b>Statistical analysis p =</b>                                      |              | 0.001*        | 0.002*        |
|                      |              | <b>Statistical test</b>  |              | T-test        | T-test        |
|                      |              | <b>% of subjects with an improvement in the cutaneous suppleness</b> |              |               |               |
|                      |              | <b>(NB: if variation &gt; 0.01)</b>                                  |              | 16%           | 11%           |
|                      |              | <i>*not clinically relevant</i>                                      |              |               |               |

## Tonicity Ur

| Sub#  | Variations Δ |             |             |              |              |
|-------|--------------|-------------|-------------|--------------|--------------|
|       | D0           | D28         | D56         | D28          | D56          |
| 1     | 0.056        | 0.089       | 0.096       | 0.033        | 0.040        |
| 2     | 0.110        | 0.115       | 0.096       | 0.006        | -0.014       |
| 3     | 0.075        | 0.092       | 0.106       | 0.017        | 0.031        |
| 4     | 0.093        | 0.070       | 0.096       | -0.023       | 0.004        |
| (5)*  | (0.229)* AV  | (0.135)* AV | (0.140)* AV | (-0.095)* AV | (-0.089)* AV |
| 6     | 0.159        | 0.133       | 0.150       | -0.027       | -0.009       |
| 7     | 0.104        | 0.117       | 0.132       | 0.014        | 0.028        |
| 8     | 0.093        | 0.088       | 0.097       | -0.006       | 0.004        |
| 9     | 0.141        | 0.085       | 0.078       | -0.056       | -0.063       |
| 10    | 0.077        | 0.104       | 0.088       | 0.027        | 0.011        |
| 11    | 0.159        | 0.212       | 0.128       | 0.053        | -0.031       |
| (12)* | (0.161)* AV  | (0.089)* AV | (0.053)* AV | (-0.073)* AV | (-0.108)* AV |
| 13    | 0.204        | 0.213       | 0.158       | 0.009        | -0.046       |

## Continued

|                      |              |   |              |              |              |
|----------------------|--------------|---|--------------|--------------|--------------|
| 14                   | 0.149        | 0.160   | 0.192        | 0.011        | 0.044        |
| 15                   | 0.274        | 0.290   | UN           | 0.016        | UN           |
| 16                   | 0.111        | 0.115   | 0.142        | 0.004        | 0.031        |
| 17                   | 0.125        | 0.120   | 0.135        | -0.005       | 0.010        |
| 18                   | 0.217        | 0.244   | 0.191        | 0.028        | -0.026       |
| 19                   | 0.152        | 0.164   | 0.174        | 0.012        | 0.022        |
| 20                   | 0.160        | NC  | 0.162        | NC           | 0.003        |
| 21                   | 0.167        | 0.127   | 0.190        | -0.041       | 0.023        |
| 22                   | 0.198        | 0.204   | 0.152        | 0.006        | -0.046       |
| <b>Mean</b>          | <b>0.141</b> | <b>0.144</b>  | <b>0.135</b> | <b>0.004</b> | <b>0.001</b> |
| <b>Med</b>           | 0.145        | 0.120   | 0.135        | 0.009        | 0.004        |
| <b>Min</b>           | 0.056        | 0.070   | 0.078        | -0.056       | -0.063       |
| <b>Max</b>           | 0.274        | 0.290   | 0.192        | 0.053        | 0.044        |
| <b>SEM</b>           | <b>0.012</b> | <b>0.014</b>  | <b>0.009</b> | <b>0.006</b> | <b>0.007</b> |
| <b>95% CI</b>        | 0.025        | 0.030   | 0.018        | 0.013        | 0.015        |
| <b># of subjects</b> | 20           | 19  | 19           | 19           | 19           |
|                      |              | <b>Δ %</b>  |              | 2%           | -4%          |
|                      |              | <b>% of cutaneous tonicity improvement (Ur)</b>   |              | 2%           | -4%          |
|                      |              | <b>Statistical analysis p =</b>   |              | 0.977        | 0.913        |
|                      |              | <b>Statistical test</b>   |              | T-test       | T-test       |
|                      |              | <b>% of subjects with an improvement in the cutaneous tonicity<br/>(NB: if variation &gt; 0.01)</b> |              | 47%          | 42%          |

Legend: AV: aberrant value, NC: not come, UN: untraceable, (\*): not included in data analysis.