Safety and efficacy of a novel home-use device for light-potentiated (LED) skin treatment

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Abstract
Skin structure and function results from a dynamic interplay between dermal and epidermal cell types. Optimizing skin health through an effective and long-lasting skin care regime therefore requires a global approach, encompassing various mechanisms to stimulate this interplay beyond the action scope of a classical topical solution. This study evaluates the impact of a novel home-use device combining a topical serum, light-emitting diodes and massage on the clinical signs of extrinsic skin aging. The innovative principle relies on potentiating the effect of active ingredients contained in the topical serum with visible and near infra-red photons to prevent extracellular matrix degradation and promote its reconstruction. After in vitro and ex vivo investigations, a clinical study assessed the safety and efficacy of a daily treatment with the home-use device for 28 days. A significant increases in skin density and radiance while reducing the wrinkles was obtained with no side effects.

KEYWORDS
dermatology, home-use device, photobiomodulation, rejuvenation

1 | INTRODUCTION

Latest innovations in esthetic dermatology integrate an increasing understanding of skin physiology to trigger response mechanisms at tissue and molecular level for overall skin rejuvenation. A large consensus is growing toward the combined use of complementary and less invasive treatments to induce gradual and progressive skin remodeling. This global therapeutic approach relies on the diversification of cellular stimulation and in-depth activation of every skin components. A permanent communication between dermal and epidermal cell population is ensured by a close interplay of secreted molecules, also known as “cross-talk”. The cross-talk reveals the...
complexity of cell-cell interaction and skin homeostasis control. Aging results from cumulative physiological dysfunctions affecting globally the skin and particularly unbalancing this healthy cross-talk. An efficient approach toward skin rejuvenation targets multiple chain links in the cross-talk to restore multilayer homeostasis.

To achieve this goal, we propose to extend the approach into a daily routine at home. A repetitive daily and potentiated skin care provides a simple, safe, efficient and easy-to-use solution that ensures a link between medical office and patient self-care. This multimodal strategy is founded on physical and chemical cell stimulation combined in a unique home-use device. Visible and near-infra-red light, mechano-transduction and a topical serum are associated in a single treatment. Light and mechanical stresses have the ability to stimulate every cell type to act globally on the cross-talk and improve topical ingredient efficiency. Considering that each wavelength reaches a different penetration depth and activation of cellular functions [1, 2], both fibroblasts and keratinocytes are targets for photobiomodulation treatment. Similarly, skin cells are sensitive to mechanical activation that stimulates their secretory [3] and ion channels [4] activities.

The present publication will cover the home-use device proof of concept. A large amount of publications have already demonstrated the clinical benefits of photobiomodulation [5, 6]. We choose to explore the combined effect of light and active ingredients by systematic in vitro and ex vivo studies and measure the cellular and molecular changes on skin cell populations [7] before assessing clinical safety and relevance of our results.

First, anti-inflammatory and antiaging properties of 660 nm - niacinamide couple is assessed with in vitro studies on primary human UV-damaged keratinocytes and senescent fibroblasts. Then, an ex vivo study evaluates the photoprotective effect of 440 nm - photolyase in presence of the other topical serum ingredients and two complementary wavelengths. Photoaging damage is induced in human skin explants by artificial UV irradiation. Markers of photodamage are measured in light-, serum- and light and serum-treated explants and untreated controls. Finally, a clinical split-face study investigates the tolerance, wrinkles evolution, skin density and radiance with a daily use of the home-use device for 4 weeks. Four levels of evaluation are provided: (a) instrumental measurements, (b) clinical scoring, (c) experts scoring on photograph and (d) subjects’ perceptions of efficacy and acceptability via questionnaire. Clinical study was performed under the supervision of a dermatologist.

2 | MATERIALS AND METHODS

2.1 | In vitro studies

2.1.1 | Cell culture

Normal human epidermal keratinocytes

Normal human keratinocytes (HPEK) were obtained from the Cellntec (Bern, Switzerland) and grown in CnT-57 PCT Epidermal Keratinocyte Medium supplemented with bovine pituitary extract (25 μg/mL, EP, Cellntec, Bern, Switzerland), epidermal growth factor (0, 25 ng/mL) and gentamicin (25 μg/mL). Cells are incubated at 37° with 5% CO₂.

Cells at passage 3 were seeding on 24-well plates and incubated for 24 hours. Then, each treatment was provided once daily for a total of three treatment. The medium was replaced by minimum quantity of culture medium without EP and EGF to minimize light absorption by the serum. Light treatment was delivered by the Illumination Box before adding new culture medium with or without niacinamide (Niacinamide PC, DSM) at the working concentration (0, 25 mg/mL). 48 hours after seeding and before the second treatment, cells were irradiated by UVB (275 mJ/cm²) and UVA (1, 74 J/cm²). UVs were provided by solar simulation system (SOL500, Dr Hoenle, AG) with a H2 filter. Supernatants were collected 24 hours after the last treatment and placed at −80° before ELISA test.

Normal human dermal fibroblasts

Primary human fibroblasts (NHDF) were grown in DMEM (PAN biotech, Aidenbach, Germany) supplemented with 10% fetal bovine serum (FBS, Life Technology, Grand Island, New York), L-glutamine (2 mM), penicillin (50 U/mL) and streptomycin (50 μg/mL). Cells are incubated at 37° with 5% CO₂.

Passage-17 (P-17) fibroblasts, which display senescent markers, were cultivated and compared to young fibroblasts from passage 8 (P-8) [8]. Cells were seeding on 24-well plates and incubated for 24 hours. Each treatment was provided once daily for three treatments. Culture medium was replaced by minimum quantity of Phosphate-buffered saline solution to minimize light absorption by the serum. Light treatment was delivered by a custom-made light-emitting diodes (LEDs) platform before new culture medium was provided supplemented or not with working concentration of niacinamide (Niacinamide PC, DSM) at the working concentration (0, 4 mg/mL) and reduced FCS concentration (1%). Supernatants were collected 24 hours after the last treatment and placed at −80° before ELISA test.
2.1.2 | Cell viability

Working concentration for niacinamide was determined by a viability assay. After 3 days incubation with the niacinamide, cells were incubated with 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT). The absorbency (A) was measured at a wavelength of 540 nm with a microplate autoreader (VERSAmax, Molecular Device). The working concentration is selected at 80% viability (data not shown). Niacinamide (Niacinamide PC, IES) concentration for NHDF and for NHEK were 0.4 and 1.2 mg/mL, respectively.

2.1.3 | ELISA

Cytokines were quantified by ELISA with the Human DuoSet from R&D systems (Minneapolis, Minnesota), for, IL-6 (DY206), IL-8 (DY208) and procollagen 1α with Procollagen Type I C-Peptide (PIP) Kit (Takara, MK101) according to the manufacturer's protocol.

2.1.4 | Photobiomodulation treatment

Cell cultures were irradiated in vitro with pulsed light dispensed by a custom-made device allowing parallel and homogeneous irradiation in 24-well plates called Illumination Box. The Illumination Box is built with LED providing visible and near infra-red irradiation centered on 440, 660 and 780 nm. The box is controlled by a custom-made software that manages currents in LED cards after a calibration with a Qmini 2 spectrometer (RGB Photonics GmbH, Kelheim, Germany) to provide identical light irradiance in each well. Light homogeneity was evaluated for each well according to the protocol described in [9]. This Illumination Box was used in previous studies to optimize irradiance and pulse parameters.

Light parameters were identical for each wavelength with a period of 10 ms, a duty cycle of 50% and a treatment time of 15 s. Keratinocytes were irradiated with an irradiance of 10.6 mW/cm² while fibroblasts received 5.3 mW/cm² irradiations at 660 nm. In normal skin explant or in vivo skin, fibroblasts located in the dermis are covered by the epidermis responsible for partial light absorption. To keep a coherence between in vitro, ex vivo and the clinical study we chose a reduced irradiance for fibroblasts cultures irradiations by assuming that epidermis absorbs 50% of incident 660 nm light [10].

2.2 | Photoaging study

2.2.1 | Explants

Skin explants were prepared from an abdominal plasty (esthetic surgery) of a 39-years-old female donor with a phototype II by the company BioEC (Longjumeau, France) which performed the study. The subcutaneous fat was removed from the skin and explants of around 12 ± 1 mm in diameter were cut out using a circular scalpel; each conditions were tested in triplicate (n = 3 explants per batch). Skin explants were then put in survival with classical cell culture conditions (37°C, 5% CO₂) in proprietary explant medium developed and owned by the BioEC, half of which was renewed every other day.

2.2.2 | Treatments

Photodamage are induced by UVB irradiation at 300 mJ/cm² with a UV Vilbert Lourmat RMX3 simulator. REPAIR serum made by Lightinderm is used for topical application (2 mg/cm²) containing niacinamide, photolyase and basic ingredients contributing to the serum texture, homogeneity and stability. Light treatment is dispensed in a pulsed mode (DC = 50%) for 15 seconds with a combination of three wavelength: 440 nm (5, 7 mW/cm²), 660 nm (10, 6 mW/cm²) and 780 nm (4 mW/cm²). Light was dispensed with the same device as in clinical investigation. Skin explants were primary exposed to UVB irradiation (D₀). Immediately after, the first treatment was dispensed with the serum alone or in combination with the light treatment. Each explant is treated once daily. Half of the explants cultures is stopped 36 hours after UVB irradiation (two treatments), the other half 60 hours after UVB irradiation (three treatments).

2.2.3 | Explants observation

At the study end, explants are collected and all processed in the same way. After fixation for 24 hours in buffered formalin, samples are dehydrated and impregnated in paraffin using a Leica TP 1020 dehybridation automat (Leica Microsystems, Wetzlar, Germany). Samples are then embedded using a Leica EG 1160 embedding station. About 5-mm-thick, sections are made using a Leica RM 2125 Minot-type microtome, and the sections are then mounted on Superfrost plus silanized glass slides.

The observation of the general morphology is performed after staining of paraffinized sections according to...
Masson’s trichrome, Goldner variant. The immunostainning of thymin dimers is performed fixed sections with an antithymin dimers antibody (Kamiya, ref. MC-062, clone KTM53) diluted at 1/1600 in PBS with BSA (0.3%) and Tween (0.05%) for an hour at room temperature with a biotin-streptavadin enhancement system (Vector, Burlingame, California, Vectastain) and revealed by VIP peroxidase substrate (Vector SK 4600).

General morphology thymin dimers immunostaining are observed using a Leica optical microscope type DMLB at the magnification of 40×. Photos are taken with an Olympus DP72 camera.

2.3 Clinical study

Clinical studies were conducted according to the guideline for Good Clinical Practice and the Declaration of Helsinki (GCP-ICH). Due to being cosmetic product studies, ethics committee approval was not required. All subjects gave written informed consent.

2.3.1 Study objectives

The primary objective of the study is the assessment of the safety and efficacy of the REPAIR program designed by Lightinderm, which combine a topical serum and a home-use device. The efficacy is focused on the reduction aging marks: wrinkles, skin density and skin radiance. Secondary objective evaluates subjects capacity to observe treatment efficiency and subjects willingness to adopt the treatment in their daily routine.

2.3.2 Population

Between October and December 2018, a single-center open randomized study was conducted to assess the efficacy of the REPAIR product on the sign of aging. Inclusion criteria were woman; age (between 35 and 45 years); phototype (I-III); skin type (dry, normal, combination or slightly oily); nonsmoking subjects; healthy subjects without any skin disorders; subject with light to moderate wrinkles on crow’s feet (grade between 2 and 4 according to the BAZIN scale); subjects with dull and tired skin; subject living in an urban environment and who will not have had intense or prolonged sun (or sunbed) exposure within at least 1 month preceding the study; subject shall refrain from using cosmetic products other than the serum on the face for 2 weeks before the test and must report to the testing facility with clean washed skin.

Exclusion criteria were as follows: pregnant or nursing woman or woman planning to get pregnant during the study; subject having changed, initiated or stopped her oral contraceptive or hormone treatment for less than 1, 5 month; cutaneous pathology on the study zone (eczema, etc); 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; subject having undergone a surgery under general anesthesia within the previous month; subject having done facial injections and/or lifting; subject having allergy to certain cosmetic or dermo-pharmaceutical products.

2.3.3 Protocol

Before baseline measurements, a wash-out period was performed. Subjects were instructed to apply the Serum Base for 2 weeks. This period normalizes the cutaneous response to the serum base and texture, and avoids potential adaptation period, which would interfere with study results. During the 28 days of the study, volunteers were asked to apply the Serum Base on the whole face once daily. This was a split face study where subjects received once daily by trained staff of the external study center (Dermscan, Lyon, France), 6 days a week (no application on Sunday).
REPAIR program including light treatment, topical serum and massage protocol with the Lightinderm device as described in Figure 1. Treated side was randomly chosen by the external study center for each subject at the beginning of the study. Subjects were asked to come every day to the study center to receive their treatment. Serum Base was applied on the nontreated side.

2.3.4 | Treatment

Device+Serum treatment is dispensed by a novel patented device designed and developed by Lightinderm Paris, France [11]. The device is constituted of a hand piece and a capsule (Figure 1A). The hand piece contains the battery, electronics, mechanical motor and LED. The capsule made of transparent plastic contains a serum volume for a week of treatment (seven treatments), a piston and a glass ball. Once the capsule is inserted in the hand piece, light is emitted through an optimized optical system (lens and capsule walls) to concentrate light rays in the glass ball and to dispense the light directly on the skin. The light is constituted of three wavelengths: 440, 660 and 780 nm. In parallel, the motor pushing the capsule piston combined with the glass ball massage allow to deliver controlled volume of topical serum on the treated zone. Figure 1B indicates six selected zones massaged on treated half-face. Each zone is treated 2 × 15 seconds for a total treatment time of 3 minutes.

2.3.5 | Tolerance evaluation

Subjects were examined by the dermatologist before (D0) and after the study (D28) to assess: erythema, edema, dryness, desquamation and roughness. On D0, the subjects are also asked about their usual sensations: tightness, stinging, itching, warm/burning sensation.

At the end of the study, the cutaneous acceptability of the product is assessed by taking into account the relevant elements reported by the subject (functional and physical signs) as well as those noted during the examination (clinical signs).

2.3.6 | Instrumental measurements

Apparatus used during clinical trial was chosen by the external partner Dermscan (Lyon, France). The redensifying effects were assessed using the high frequency echograph Dermascan C 2D (Cortex Technology, Denmark). Wrinkles were evaluated with Primos 3D Lite (phaseshift rapid in vivo measurement of skin) along with macrophotographs taken with VISIA (CANFIELD imaging systems). Skin colorimetry was measured with Spectrophotometer CM700 -d (Konica Minolta).

2.3.7 | Clinical scoring

In addition to tolerance evaluation, the study dermatologist is asked to evaluate Crow’s feet wrinkles and skin smoothness at D0 and D28 without knowing the distribution of Device + Serum - and Serum Base treated sides.

2.3.8 | Experts scoring

The study center chose five experts, trained to evaluate treatment efficacy on high-resolution macrophotographs provided by the VISIA apparatus. Experts are asked to evaluate the lifting effect and the radiant complexion at D0, D14 and D28 for both sides without knowing whether the scored side is treated with Device + Serum or Serum Base.

2.3.9 | Self-evaluation questionnaire

Subjects are asked to respond to a self-evaluation questionnaire at D0, D14 and D28 to gather their opinion on the treatment safety and efficacy as well as to investigate their keenness to continue to use the home-use device.

2.3.10 | Data analysis

Results and kinetics evaluations were made with the Excel software. Variation is given as a percentage of D0 measurements: \[ \Delta\% = \frac{M_{D28} - M_{D0}}{M_{D0}} \times 100 \] or a difference between two variation \[ \Delta\Delta\% = \Delta\% \text{ (treatment)} - \Delta\% \text{ (placebo)} \]. Statistical analysis was calculated with the ANOVA model except for clinical scoring where Wilcoxon model was used.

3 | RESULTS

3.1 | In vitro study: 660 nm - niacinamide

3.1.1 | Anti-inflammatory effect

UV irradiation promotes the release of significant amount of IL-6 and IL-8 in human epidermal
keratinocytes cultures while nonirradiated control released less than 5% of UV control levels. Treatment with niacinamide alone reduces IL-6 release of 67% (Figure 2A) while no significant effect was measured on IL-8 release (Figure 2B). Light treatment alone at 660 nm does not affect interleukins secretion, but the combination of 660 nm and niacinamide reduces significantly the level of IL-6 and IL-8 of 50% and 37%, respectively, vs niacinamide only.

### 3.1.2 Collagen and MMP

Human primary fibroblasts at passage 17 are used to mimic artificial aging and evaluate antiaging properties of 660 nm - niacinamide couple. Figure 3A compares procollagen 1α release of P-17 fibroblasts with P-8 fibroblasts when treated with or without niacinamide. The aging model is validated as P-17 fibroblasts release twice less procollagen 1α than P-8 fibroblasts. This reduction in
procollagen 1α production does not suppress P-17 fibroblast capacity of collagen synthesis as the adjunction of TGF-β boosts new procollagen 1α synthesis. The niacinamide has no effect on procollagen 1α release either on young and aged fibroblasts. Figure 3B shows that neither niacinamide nor 660 nm light when used alone stimulates new procollagen 1α synthesis. However, the combination of both increases procollagen 1α release of 21% vs nontreated control.

In parallel, MMP-1 are measured in fibroblasts supernatants. Aging model is validated as P-17 fibroblasts culture release higher amount of MMP-1 than P-8 fibroblasts (Figure 3C). This time, niacinamide reduces the MMP-1 of 75% and 47% for young and aged fibroblasts, respectively. P-17 fibroblasts treatment with light at 660 nm alone reduces MMP-1 release of only 18% while the combination of 660 nm and niacinamide significantly reduces of 55% the level of secreted MMP-1, which is significantly 8% lower than niacinamide only.

3.1.3 | Photodamage ex vivo study

Photolyase activity is assessed here in addition to complementary wavelengths and ingredients. Skin explants are irradiated with UVB, then DNA repair efficacy is evaluated for the REPAIR serum with or without the light treatment combining 440, 660 and 780 nm. After immunostaining, CPD appear as violet spot on skin explants images (Figure 4). No CPD are observed for the explants without UV irradiations, whether they are treated or not by the serum (picture not shown). However, UV irradiations induce darker and greater amount of violet spots meaning that CPD are induced by UV irradiations. When treated with the serum alone, skin explants display less violet spots and even fewer spots when the serum is combined to light treatment (Figure 4B,C). This observation is confirmed by the measurements of CPD area on image analysis 36 and 60 hours after the UV irradiations (Figure 5). After 36 hours (one treatment), the serum efficacy is not impacted by the PBM treatment. However, after 60 hours (two treatments), the combination treatment shows significant better results than the serum alone with a 34% less CPD than the control. In parallel the serum efficacy seems to merge the natural ability of skin to repair CPDs.

3.1.4 | Clinical trial results

A total of 33 women have been successfully enrolled in the study. Baseline characteristics of the population are given in Table 1. One subject has dropped out on D23 of the study for cutaneous reaction, she presented redness and tingling on both sides, the study center concluded a lack of moisturizing and no direct link to the tested products. 32 subjects finished the study.

\[\text{FIGURE 4} \quad \text{Explants immunostaining with monoclonal anti-CPD antibodies 60 hours after UV irradiation. A, Control, no treatment applied after UV irradiation; B, REPAIR serum only (S); C, combination of light (440 + 660 + 780 nm) and REPAIR serum (L + S)}\]

\[\text{FIGURE 5} \quad \text{Surface of thymin dimers, 36 and 60 hours after UV irradiations with either no treatment, REPAIR serum or combined with 440 + 660 + 780 nm. Significance is calculated according to a Student t test (♯P < .1, *P < .05, **P < .01)}\]
Significant results were observed in four main categories: treatment tolerance, skin density, wrinkles reduction and radiance. When available, four levels of evaluation can be presented: instrumental measurements, clinical scoring, experts scoring on high resolution photographs and self-assessment questionnaires.

### 3.1.5 | Tolerance

No tightness, no stinging, no itching and no warm/burning sensation have been observed during the cutaneous acceptability analysis. This allowed the study center to conclude that both products treatments, Device + Serum and Serum Base, are very well-tolerated on the cutaneous level for all the subjects with no reported side effects by the 32 subjects.

### 3.1.6 | Skin density and lift effect

Skin density is evaluated instrumentally by measuring with ultrasounds nonechogenic surfaces at $D_0$ and $D_{28}$ (Table 2). Nonechogenic surfaces are estimated by integrating dark zone on echographic pictures. Figure 6 shows ultrasound pictures before ($D_0$) and after ($D_{28}$) of a 42-years-old subject. Each picture displays two parts:

**TABLE 1** Study population overview

| Subjects | 33 |
|----------|----|
| Sex      | Female |
| Age      | $40 \pm 1$ y.o. |
| Phototype| I (3%) | II (61%) | III (36%) |
| Skin type| Normal (12%) | dry (30%) | combination (58%) |

**TABLE 2** Clinical results with the comparison with $D_0$ ($\Delta$%) and the comparison between Device + Serum and Serum Base ($\Delta\Delta$%)

| Time  | Device + Serum ($\Delta$%)   | Serum Base ($\Delta$%)  | Comparison ($\Delta\Delta$%) |
|-------|------------------------------|-------------------------|-----------------------------|
| **Instrumental measurements** |                              |                          |                             |
| Nonechogenic surface (%)     | $D_{28}$: $-10\% \pm 1\% (-25\%^{***})$ | $-2\% \pm 1\% (-5\%^{ns})$ | $-8\% \pm 1\% (-20\%^{***})$ |
| Average relief ($R_z$) ($\mu m$) | $D_{14}$: $-3.1 \pm 2.3 (-3\%^{ns})$ | $+3 \pm 2.4 (+3\%^{ns})$ | $-5.8 \pm 2.4 (-6\%^*)$ |
|                                | $D_{28}$: $-6.2 \pm 2.5 (-6\%^*)$ | $+4.1 \pm 2.5 (+4\%^{ns})$ | $-10.5 \pm 2.7 (-10\%^{***})$ |
| **Clinical scoring** |                              |                          |                             |
| Crow’s feet wrinkles          | $D_{28}$: $-0.4 \pm 0.1 (-14\%^{***})$ | $-0.1 \pm 0.1 (-5\%^{ns})$ | $-0.2 \pm 0.1 (-9\%^*)$ |
| Skin smoothness               | $D_{28}$: $+0.8 \pm 0.1 (+15\%^{***})$ | $+0.4 \pm 0.1 (+8\%^{***})$ | $+0.4 \pm 0.1 (+7\%^{***})$ |
| **Expert scoring**            |                              |                          |                             |
| Lifting effect                | $D_{14}$: $+0.22 \pm 0.03 (+4\%^{***})$ | $+0.14 \pm 0.02 (+3\%^{***})$ | $+0.08 \pm 0.03 (+2\%^{*})$ |
|                                | $D_{28}$: $+0.71 \pm 0.04 (+14\%^{***})$ | $+0.24 \pm 0.02 (+5\%^{***})$ | $+0.47 \pm 0.05 (+9\%^{***})$ |
| Radiant complexion            | $D_{14}$: $+0.24 \pm 0.03 (+5\%^{***})$ | $+0.20 \pm 0.03 (+4\%^{***})$ | $+0.05 \pm 0.04 (+1\%^{ns})$ |
|                                | $D_{28}$: $+0.69 \pm 0.06 (+14\%^{***})$ | $+0.47 \pm 0.03 (+10\%^{***})$ | $+0.21 \pm 0.06 (+4\%^{***})$ |

* $P < .05$.
** $P < .01$.
*** $P < .001$. 

**FIGURE 6** Example of nonechogenic area measurement of a 42-years-old subject, before and after 4 weeks treatments with Device + Serum (top) or Serum Base (bottom).
1. A thin and bright layer on the left side corresponding to skin epidermis.
2. A thicker and mixed part representing the dermis.

At D28, brighter colors corresponds to higher ultrasound intensity and a lower nonechogenic surface compared to D0. However, pictures of the control side show almost no changes between D0 and D28. At the population level, the nonechogenic surfaces for the Device + Serum side have significantly decreased of −25% \((P < .001)\) vs D0 while control side has no significant change. The difference between the two sides is also statistically significant \((P < .001)\). This suggests that investigational treatment presents a redensifying effect of the dermis. This effect was observed on 97% of the subjects.

Ultrasound observations are confirmed by clinical scoring with a statistically significant improvement of the skin density \((+6\%, P < .0001)\) and firmness \((+7\%, P = .0004)\) on the Device + Serum treated side. Surprisingly, statistically significant improvements are also observed on the control side, but the difference between the two sides shows a significantly greater skin density on the treated side \((+2\%, P < .05)\). No significant difference is obtained on skin firmness.

As early as D14, statistically significant lifting effects are observed on both sides \((+4\%, P < .0001)\) and \(+3\%, P < .0001\) for treated and control side, respectively) with a greater effect on the Device + Serum treated side (Table 2). The difference is confirmed at D28 with a much larger gap between treated and control side \((+9\%, P < .0001)\). Interestingly, this effect was observed on 100% of the subjects, which supports a good respond to the global treatment observed by the ultrasound measurements. Figure 7 illustrates the lifting effect assessed by experts. Lifting is mainly observed on the cheek upper part raising as well as on the reduction of the nasolabial fold at D28 vs D0.

### 3.1.7 Wrinkles and smoothness

The effect of the investigational treatment on wrinkles volume and depth is evaluated with projected light fringes. It produces 3D images of the skin surface as shown on Figure 8, which compares the effect of the treatment for a 41-years-old subject before and after. Wrinkles depth and count are reduced on the treated side while no changes are observed on Serum Base side. This observation is confirmed at the population level (Table 2) with a nonsignificant increase in wrinkles relief for the Serum Base \((+4\%, P > .05)\) while treated side displays a significant decrease in \(R_z\) \((-6\%, P < .05)\) after 4 weeks treatment. Concerning the kinetics of the effect, at D14 the impact of both Serum Base and Device + Serum are not significant vs D0 but Device + Serum treatment is already significantly better than Serum Base. This difference has strengthened after 4 weeks with a stronger difference \((-10\%, P < .001)\).

Reduction in wrinkles depth and count are confirmed by a clinical scoring of the Crow’s feet wrinkles. No significant evolution could be noticed on the Serum Base side while a decrease of −14% \((P < .0001)\) in the Crow’s feet wrinkles scoring on the treated side. The two facial sides are statistically different. Figure 9 shows before and after Crow’s feet pictures of a 41-years-old subject for both treated and control side. In addition, subjects wrinkles reduction results in skin smoothness improvement for the clinical scoring. The treated side score \((+15\%, P < .0001)\) is significantly greater than the Serum Base side \((+8\%, P < .0001)\). The

**FIGURE 7** Lifting effect observed on a 39-years-old subject. White arrows indicate locations of lifting effects: cheek uplifting, nasolabial fold reduction

**FIGURE 8** 3D pictures of the skin surface made with Primos 3D Lite used to evaluate the effect of the treatment on wrinkles for a 41-years-old subject at D0 and D28.
improvement in skin smoothness is observed for 91% of the treated side and only 78% for the Serum Base side. This means that the Serum Base contributes to skin smoothness improvement. Experts also reported a significant improvement of the fineness of the skin texture.

3.1.8 Radiance

Experts scoring reported a treatment effect on skin radiance as they noticed improved radiance complexion on all the subjects at D_{28} with a mean improvement of +14% (Table 2). The effects are observed as soon as D_{14} for both side, which means that the Serum Base contributes also to the brightening effect. The difference between Device + Serum and Serum Base is not significant at D_{0} but becomes statistically significant at D_{28}.

3.1.9 Subject's perception of efficacy

The investigational product was liked by all of the 32 women involved as 100% of them found the device more efficient than the Serum Base. Self-assessment questionnaire results gathered in Figure 10 show that most of the parameters evaluated in the questionnaire have improved from D_{0} up to D_{28} (dark arrows). A total of 87% participants felt that their skin was smoother with less fine lines. As of 14 days after treatment start, subjects noted a softer and firmer skin (87% at D_{28}), and 81% of participants reported a more luminous and even toned skin at D_{28}.

4 DISCUSSION

The use of home-use device is a complement to rejuvenation strategy. It has gained increasing adoption over the past few years due to their ability to act more globally on skin cross-talk. The increasing number of rejuvenating devices on the market can be classified into three categories [12]: (a) low energy devices combining LED, microcurrents and radiofrequency devices; (b) mechanical stimulation devices such as dermaroller or massaging device; and (c) nonablative lasers for skin rejuvenation. Even though these devices are sometimes completed by topical treatments, unfortunately there is a lack of demonstrated benefits for combining physical and chemical skin cell stimulation.

This study investigated the safety and the efficiency of a novel home-use device combining three cellular stimulation modes. The uniqueness of this novel device consists of the combination of photobiomodulation, mechanical stimulation and a topical serum in one single treatment. Photobiomodulation and massage treatments were selected for their ability to stimulate a deep cellular level and activate dermal matrix remodeling for long term efficiency. The topical serum brings short term effects by acting directly on the epidermis and is potentiated by the action of light. By stimulating simultaneously upper and deeper skin layers, cross-talk mechanisms are activated to increase the global efficiency of the entire treatment.

Due to the multiplicity of parameters involved in the investigational device, the prospective clinical trial was
preceded by in vitro and ex vivo studies to optimize light parameters as well as wavelength—ingredient pairing for the optimization of dermal matrix degradation prevention and its remodeling.

Interestingly, the combination of light with an active ingredient displays improved results vs the light or active alone. The most characteristic example is the measurements of IL-8 (Figure 2B). Both niacinamide and red light have no effect on the release of IL-8 while the combined treatment induces a 37% reduction. Previous work described gene downregulation associated to IL-6 in HaCaT cell culture after UVB irradiations when treated with niacinamide, however no effect was observed on IL-6 gene [13]. As a complement of its anti-inflammatory effect, the same combination of red light and niacinamide was tested on primary human fibroblasts to evaluate their capacity to rebuild extracellular matrix (ECM). Previous work demonstrated the effect of red light in vitro on fibroblasts with its antiaging property in vivo [14, 15] by describing a stimulation of procollagen 1 and a reduction of MMP-1 of 632 nm. These results were confirmed here in the case of the combination of niacinamide and 660 nm light (Figure 3).

A second light and serum combination was investigated in the photodamage study: 440 nm - Photolyase. The DNA-repair dynamics of photolyase activated by 440 nm light is well known [16] and was validated here when the active ingredient is associated with niacinamide and complementary wavelength (660, 780 nm). The serum is efficient when combined with the light treatment by accelerating the thymin dimers repair (Figure 5), therefore the combination alters the continuous and progressive evolution associated with inflammaging [17].

This study goes beyond the combination of single wavelength-ingredient couple. The entire treatment associates three different emission ranges able to activate cellular functions and to penetrate skin depths specific to each wavelength [10, 18–20]. Three different molecular pathways are activated by the three wavelengths. They can cooperate directly in intracellular communication or indirectly through dermal-epidermal cross-talk. As an example, red light contributes both to epidermal barrier function recovery [21] and to dermal matrix remodeling, especially when combined with near-infra red light [15, 22, 23]. However, previous work suggests that light treatment is more efficient when combining individual wavelengths than broadening spectral ranges [24].

The results obtained from optimizing light-ingredient combinations in the vitro and ex vivo models translated into clinical observations with significant impact on skin density, wrinkles and skin radiance. The most significant result in our study was the ultrasound intensity measured after 4 weeks treatment (Figure 6). High-frequency ultrasound is a modern, noninvasive method that allows the assessment of the efficacy of antiaging therapies [25]. New echogenic pixels reveals the activation of dermal protein synthesis (collagen, elastin), and suggests long term and progressive effects. Both photobiomodulation and mechano-transduction contribute to redensifying and remodeling effects by acting in-depth directly on fibroblasts. They stimulate their natural ability to produce and put in tension new ECM fibers. A potential explanation previously described is the release of latent TGF-β, a key cross-talk cytokine stored in ECM released either by mechanical stimulation [26] or by low level ROS produced by photobiomodulation [27]. When exposed to TGF-β, fibroblasts differentiate into their active myofibroblast form [28]. With a well-developed cytoskeleton, myofibroblasts are very sensitive to mechanical strain and also, they contract to restore tension in the ECM fibers networks [29].

ECM reconstruction does not only contribute to skin mechanical functions but also to the skin’s optical properties. Aged skin is often associated with dullness and lack of skin radiance. This is mainly due to accumulation of glycated fibers and ECM degradation byproducts, which favors light scattering and reduces light reflection on deep skin fibers. The remodeling effect produces new and well organized collagen fibers and therefore, improves skin translucency [30]. In this study, improved skin radiance was observed for 100% of the subject (Table 2).

Contrary to traditional photobiomodulation treatments made in dermatological offices where patients receive light treatment once in a month or a week, home-use device make daily treatment possible. Repetitive use of the device sustains progressive and built-up improvements as observed in this study between D14 and D28 with enhanced clinical scores on skin density, radiance, and reduced wrinkles and fine lines (Table 2). Similarly, self-evaluations showed improved scores on the second part of the study for most evaluated parameters (Figure 10). Knowing that aging is a continuous process resulting from repetitive exposure to extrinsic and intrinsic aggressions, a daily care intervenes rapidly at the root cause of the aging process by limiting the period of proinflammatory conditions, which results in less ECM degradation [31]. The self-evaluation questionnaires also revealed that the device was perceived comfortable and measurable which suggest a treatment compliance by future patients.

Even though a daily use seems to benefit the patients, very few studies have investigated the impact of photobiomodulation treatments frequency. R. Lanzafame investigated in vivo the impact of a daily application of red light at
5 mJ/cm² on an ulcer wound. The same fluence was applied either in one or two daily treatments. They found that at low irradiance, a single treatment daily was more efficient than treatments twice a day with the same light quantities [32]. Over stimulation of cellular mechanisms can exhaust the cell capacity to deal with PBM byproducts such as ROS and can cause opposite effects described by the later phase of the Arndt-Schulz curve [33].

Treatment frequency is also related to the characteristic time in tissue remodeling. Brief light irradiations activate mechanisms involving signaling and metabolic enzymes with short kinetics. Those signaling pathways lead to downstream cellular functions up to effects at the tissular level with an increasing characteristic time-dependency [34]. Consequently, a flash of few milliseconds with visible light will result in the production and self-assembly of new collagen fibers within a week [35].

The home-use device format brings constraints on the choice of light pulsing parameters. We selected 5 ms pulse duration to answer ergonomic limitation as well as avoiding flickering effects and protect the users of potential visual and neurological effects [36]. This choice contrasts with two previous opposite pulse parameters optimizations [37, 38].

We provide here a very safe, pleasant and easy-to-use device directly at home, which allows long term and repetitive treatments. Aging is a continuous process, which requires daily care for both reduction of physiological function degradation and the activation of long-term repair mechanisms. Further studies should investigate the device efficacy over a longer period of use.

5 | CONCLUSION

The investigational device brings a new way of thinking cosmetic by combining physical and chemical stimulation for the global approach of skin care. Stimulating the skin’s dynamic interplay simultaneously through LED light-enhanced ingredients and massage was found to be a safe and efficient way to rejuvenate on the skin aging. The exploration of further combinations could be source of discovery to treat skin at home. Daily use of such technology creates a link between patients and dermatologists with mutual benefits through enhanced expected clinical results. Consequently, dermatologists can broaden easily their therapeutic efficiency plan with associated home-use device prescription.

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CONFLICT OF INTEREST

Cyprien Guermonprez, Lieve Declercq and Géraldine Decaux are employees of Lightinderm, who sponsored these studies. Jean-Alexis Grimaud is the scientific director of Lightinderm. The authors report no other conflicts of interest in this work.

DATA AVAILABILITY STATEMENT

DAS N/A - paper submitted before Expects Data policy came into force.

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