Healing the downs of psycho-dermatology

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1. ABSTRACT

Telomeres are DNA-based caps located at the end of chromosome tips of eukaryotic cells and are important for cell replication during lifespan. Telomeres shorten with mitosis, and telomere length is a marker of cellular aging. Psychological Stress (PS) may influence telomere detriment triggering aging mechanisms. The crosstalk between brain and skin, under PS, stimulates the release of hormones and neuropeptides that have a direct effect on the skin. In this study, we focus on the effect of a Micrococcus ferment lysate (BML_72-4) on the brain skin connection.

Telomere protection was evaluated through TRF1 (shelterin complex) and 53BP1 proteins and telomere length. The effect of the ferment on PS response was analyzed by gene expression and cortisol and keratin10 protein levels, nuclear translocation of NR3C1 and wound healing on an epidermal stressed model. Effects on the Stratum Corneum (SC) were evaluated on skin explants. Clinical study was performed on 20 volunteers (wrinkles, hydration and elasticity).

Ferment increased TRF1 levels (+19%), recovered telomere length and decreased DNA damage (-71%*); modulated genes related to PS; increased keratin 10 levels (+25%*), reduced cortisol (-18%*), NR3C1 translocation (-20%*) and increased wound healing (+37*). On explants it recovered SC properties. On volunteers, it improved body's hydration (+39%*) and elasticity (+8%*) and reduced wrinkles depth (-13%*), in 28 days.

PS can be linked with skin psycho-physiological aging and according to efficacy studies, BML_72-4 acts as a novel ingredient in the field of psycho-cosmetics recovering skin allostasis lost with stress.

Keywords: Telomere length, psychological stress, cortisol, brain-skin communication, aging.

2. INTRODUCTION

Telomeres are non-coding repetitive DNA sequences associated with specific proteins situated at the ends of chromosomes protecting terminal regions of chromosomes from degradation in eukaryotic cells [1-2]. These proteins (TRF1, TRF2, POT1 and others) form a large complex termed the “shelterin complex” which is important for protecting and regulating telomeric structure and function [3-5]. Telomeres shorten with mitosis, and telomere length is a marker of cellular aging since this complex cell aging system regulates the longevity of cells as well as senescence and apoptosis. Several studies indicate that chronic psychological stress (PS) have been associated with shorter telomeres [6]. In fact, diverse studies showed the existence of a powerful brain-skin communication [7-8], since skin has a functional peripheral equivalent of the hypothalamic-pituitary-adrenal axis (HPA) [9]. Continuous exposure to PS increases endogenous glucocorticoids by activating HPA axis. Actually, under stress the brain responds realising corticotropin-releasing hormone (CRH) which stimulates the release of other hormones and neuropeptides that will regulate the stress response such as cortisol and substance P (SP), respectively, among others [7] (Fig. 1). Cortisol is the primary stress hormone in humans and it is necessary for the adaptation to acute stress, but it can be pathogenic when the organism is persistently exposed during a long period of time [10,11]. A few studies have revealed that larger cortisol responses to mental stress are connected with shorter telomeres in adults and children [12]. Cortisol works by binding to the glucocorticoids receptors (GR), creating a complex, which finally translocates into the nucleus, where it can interact directly with specific DNA sequences and other transcription factors that will actively participate in the stress response [13]. Furthermore, cortisol is regulating a wide range of stress responses in skin, including proliferative effects on, inhibition of epidermal lipid synthesis, wound healing delay, decrease expression of antimicrobial peptides, epidermal barrier disfunction and alteration of extracellular matrix (ECM) [14-18].

Substance P is another stress mediator in skin with multiple bioactivities other than neurotransmission, such as capillary vasodilatation, fibroblast, keratinocyte and macrophage proliferation, mast cell degranulation and secretion of cytokines such as interleukin IL-1, IL-6. It is considered a major mediator of neurogenic inflammation and itch [19,20]. In addition, it has been proved that SP can induce cortisol release [21], promoting the damaging effects of cortisol on the skin.

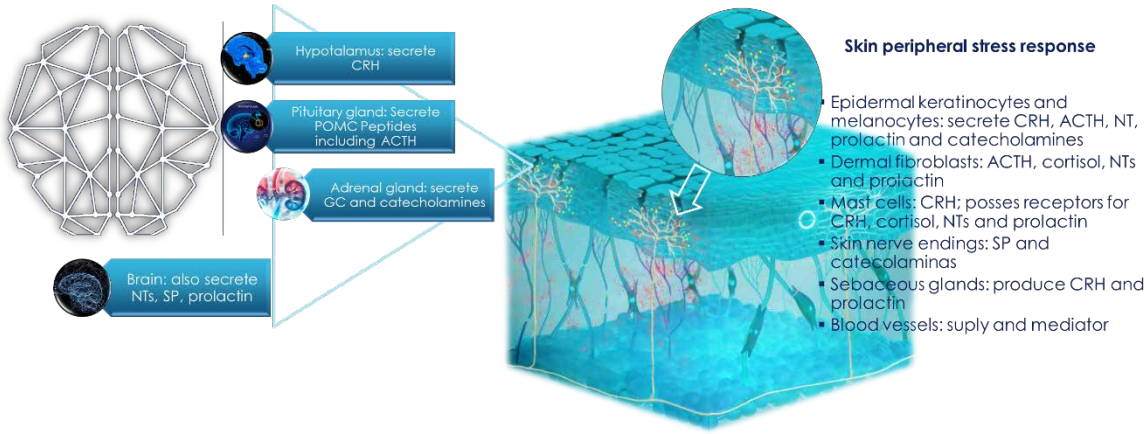

Fig 1. Central stress response and skin peripheral stress response (Adapted from Chen & Lyga, 2014).

Clinical observations indicate that psychological stress leads to onset or aggravation of multiple skin concerns such as delayed healing, acne lesions, dull or thick skin, a dehydrated skin surface and loss of elasticity and density [22]. Considering that PS is a common human condition and everyone experiences it at some point in their lives, the development of novel active ingredients in the field of psycho-cosmetics is needed. Due to this, the aim of this study was evaluating the effect of a Micrococcus ferment lysate (BML_72-4), obtained from a sea breeze and selected after an extensive screening done by the Spanish National Cancer Research Center (CNIO), on the triad among emotional stress-telomere length-skin aging.

3. MATERIALS AND METHODS

3.1 Sample collection and identification

Micrococcus sp. (internal code BML_72-4) was sampled from ocean aerosol layers at international waters near Tonga. It was identified by genome sequence.

3.2 Microorganism fermentation

BML_72-4 is obtained by fermentation of a Micrococcus sp., in a bioreactor, at optimal conditions where parameters such as agitation, temperature, oxygen level and pH are monitored. Finally, an aliquot of the filtered ferment is concentrated by lyophilization for analysis. The ferment is characterized by SDS-PAGE, HPLC and metabolomics.

3.3 Screening of a library of compounds for a potential effect on telomere protection through TRF1 levels

This study was performed by the CNIO. To carry out this study CHA9.3 cells were used to screen several compounds from different origins. CHA9.3 cells were seeded in a 96-well microplate and treated with the different compounds at a set concentration. After a 24 h incubation, cells were fixed and stained for TRF1 (Telomere Repeat Binding Factor 1), and nuclei. Image acquisition and quantitative measurement of TRF1 levels was performed using Opera High Content Screening (HCS) system. The selected candidates were further validated in a dose-response manner using the same methodology. Each condition was performed at least n=2 and N=3. Statistical data analysis was performed using Student t-test by comparison of % viability from test substance vs non-treated cells, *p<0.05, **p<0.01, ***p<0.001.

3.4 Effect of bacterial ferment on telomere length protection through TRF1 and on the formation of double-strand DNA breaks in HEKa cells

This study was performed by the CNIO. The study of the effect of the *Micrococcus* ferment lysate on the telomere length and DNA damage protection was performed by Telomere Repeat Binding Factor 1 (TRF1) and p53-binding protein 1 (53BP1) immunostaining. For TRF1, HEKa cells were treated with BML_74-2 at 0.1 mg/mL for 48 h. Regarding 53BP1 staining, cells were treated with 100 µM H_2O_2 in presence or absence of 0.1 mg/mL or 0.2 mg/mL of BML_72-4 for 24 h. Then, cells were fixed and TRF1 and 53BP1 were stained for immunofluorescence. Fluorescent signals on slides were visualized in a confocal ultra-spectral microscope SP5-MP (Leica). Signal was quantified using the Definiens Developer XD.2 Software. In both cases, one experiment was performed in triplicate and mean values and SEM of the experiment were calculated. Statistical data analysis was performed using Student t-test by comparison of the number of TRF1 or 53BP1/nucleus in non-treated or BML_72-4 treated samples vs the number of TRF1 or 53BP1/nucleus in H_2O_2-treated samples, *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

3.5 Analysis of the effects of BML_72-4 on the expression of extracellular matrix-related genes in HDFa stressed with cortisol

To evaluate the effect of BML_72-4 on extracellular matrix (ECM) related genes on HDFa previously stressed with cortisol, a qPCR was carried out. Briefly, HDFa cells were incubated in the presence of 10 µM cortisol for 24 h. Next, cells were washed and treated for 24 h more with complete medium alone or in the presence of 0.05 mg/mL BML_72-4. Then, RNA extraction and retrotranscription to cDNA were performed. Modulation of expression of a selection of extracellular matrix related-genes were evaluated by qPCR. The obtained data were analysed using the ΔΔCt method for treated versus untreated cells.

3.6 Study of the effect of BML_72-4 on telomere length of HDFa cells

To perform this study, HDFa, in different stages of the aging process, were seeded in 12-well plates at a concentration of 150.000 cells/well and maintained at standard culture conditions for 24 hours. Next, fibroblasts from a 73 y/o donor ($HDFa_{73}$) were treated with 0.1 mg/mL BML_72-4 for 48 h in the incubator. Then, DNA was extracted using Qiagen DNeasy Mini kit following manufacturer's instructions. Finally, telomere DNA vs nuclear DNA ratio was stablished using Absolute Human Telomere Length Quantification qPCR Assay Kit, according to manufacturer instructions. Each condition was performed at least n=3 and N=3. Statistical data analysis was performed using Student t-test by comparison of telomere length of $HDFa_{73}$ untreated (Basal) vs the rest of samples, *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

3.7 Analysis of the effect of bacterial extract on cortisol release in HEKa cells

For this study, HEKa cells were seeded in a 96-well plate at a density of 20.000 cells/well and maintained at standard culture conditions for 24 h. Then, keratinocytes were treated for 48 h with BML_72-4 (0.01, 0.05 and 0.1 mg/mL) in the presence of 50 µg/mL SP in complete DCBM medium. After treatment, supernatants were collected by centrifugation. Cortisol was quantified with a Cortisol Parameter Assay Kit according to manufacturer instructions. Viability was determined by MTT. Finally, OD at 570 nm was determined with spectrophotometer Multiskan. Each condition was performed at least n=3 and N=3. Statistical data analysis was performed using Student t-test by comparison of % cortisol protein release from treated cells vs. non-treated cells (basal), *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$.

3.8 Evaluation of the effect of BML_72-4 on subcellular localization of the glucocorticoids receptor (GR) in HEKa cells

To perform this study, HEKa cells were treated with 10 µM cortisol alone or in the presence of 0.05 mg/mL BML_72-4 for 24 h. Then, cells were fixed and GR was stained for immunofluorescence. Images were acquired using a fluorescence microscope and Image J software was used to determine nuclear GR/total GR ratio. Statistical data analysis was performed using Student t-test by comparison of % of nuclear fluorescence from non-treated or BML_72-4-treated samples vs cortisol-treated samples, *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

3.9 Evaluation of the efficacy of a *Micrococcus* ferment on HEKa cells to stimulate the liberation of KRT10 in presence of cortisol

To carry out this test HEKa cells were cultivated in presence and absence of Cortisol and in presence of the bacterial ferment, at different concentrations. The levels of KRT10 were evaluated by ELISA Kit assay according to supplier protocol for 24 h. The results are expressed as mean value ± standard deviations of KRT10 content and as percentage variation compared to C-. Each condition was performed at least n=3 and N=2. Obtained data in experimental groups treated with samples under study were subjected to statistical analysis and compared to positive controls according to t-test. *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

3.10 Analysis of the effects of BML_72-4 bacterial ferment on the migration of human keratinocytes subjected to cortisol-mediated stress

HaCaT cells were seeded on 24-well culture plates and allowed to reach confluence. Then, the cell monolayer was scraped in a straight line with a pipette tip to create a “scratch”. Cells were then serum-deprived and treated with 10 µM cortisol, alone or in the presence of different concentrations of BML_72-4. Medium supplemented with 10% FBS was used as positive control. A phase-contrast microscope was used to monitor cell migration immediately after scratch and 24 h later. Finally, the acquired images were quantitatively analysed with Image J software. Each condition was performed at least n=4 and N=3. Statistical data analysis was performed using Student t-test by comparison vs cortisol-treated samples *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$.

3.11 Evaluation of the protective effect of BML_72-4 on a stressed stratum corneum of human skin explants

To carry out this study skin explants were obtained from abdominal surgery of a 42 years old female Caucasian donor. The explants were distributed in 3 experimental groups. After stabilization of the explants, the layers of stratum corneum of the group's "stress" and "stress + active" were removed with a stripping procedure on skin explant surface. Then, a solution containing 0.001% of cortisol was topically applied on each explant of stressed groups. BML_72-4 was also topically applied at 0.05 mg/mL for both groups each 24 h for 5 days as stressed group. The group "Control" did not receive any treatment except the culture medium renewal. Cryofixed sections were stained with 1% Safranin-O red solution, Hematoxylin and Eosin and Lucifer Yellow to evaluate the number of corneocyte layers, Stratum Corneum thickness and skin barrier integrity, respectively. DAPI (4',6-diamidino-2-phenylindole) was used for nuclear detection. Light and fluorescence microscopies were used for image acquisition. Three images per explant (9 per condition) were used to quantify the above-mentioned parameters. Finally, the inverse of the obtained results (1/ value parameter x) was normalized over the stress group (%). Data treatment and statistical analysis were achieved by t-test binary comparisons versus Stress group. *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$.

3.12 *In vivo* efficacy study

To evaluate the *in vivo* efficacy of the active ingredient on face and body, 20 healthy Caucasian female subjects aged between 35 and 50-years old with clinical sign of skin aging, such as slight to moderate face wrinkles and dull skin complexion were selected. In particular, subjects exposed to emotional familiar or work stress which could induce high level of cortisol and itching sensation were enrolled. They applied a cream containing 2% of the active ingredient on half face/body, twice daily for 28 days. A placebo was applied on the other half of the face/body. Product evaluation was at different timepoints (1 h, T7 and T28) by means of non-invasive bioengineering techniques able to measure skin profilometry (wrinkle depth – Primos 3D), skin moisturization (Corneometer®), elasticity and firmness (Cutometer®). Illustrative images were taken with VISIA®. The instrumental analysis is integrated with the self-assessment questionnaires filled in by the enrolled subjects.

Data are submitted to two-way test t of Student for paired data. Variations are considered statistically significant when p value is ≤ 0.05. #, $p<0.1$, *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$.

4. RESULTS

4.1 Screening of a library of compounds for a potential effect on telomere protection through TRF1 levels

The effect of the different candidates on telomere length was evaluated by TRF1 staining. The overall results show that most of the candidates cause an increase in TRF1 levels between 10 and 20% with respect to the basal condition. BML_72-4 significant increases the level of TRF1 by 26% suggesting a potential role in counteracting telomere length attrition (results not shown).

4.2 Effect of BML_72-4 on telomere length protection through TRF1 and on the formation of double- strand DNA breaks in HEKa cells

To perform this study, Human Epidermal Keratinocytes from adult (HEKa) were fixed and TRF1, a key component of the shelterin complex which is essential for telomere protection and 53BP1, a nuclear protein that colocalizes with double-strand DNA breaks, were stained for immunofluorescence.

The bacterial ferment used for this experiment was selected from the previous screening, performed by the CNIO, on TRF1 on a cell line model, where BML_72-4 was able to increase, significantly, the levels of this protein by 26%. However, when HEKa cells exposed to BML_72-4, the increase of TRF1 levels was of 19%, when compared to basal conditions (results not shown).

Concerning the effect of BML_72-4 on the formation of DSBs under oxidative stress conditions it was seen that H_2O_2 was able to induce the apparition of 53BP1 foci when compared to untreated cells. However, BML_72-4 reduced significantly the accumulation of DNA damage foci by 68% and 71% at 0.1 mg/mL and 0.2 mg/mL of the ferment, in HEKa cells under oxidative stress (Fig. 2).

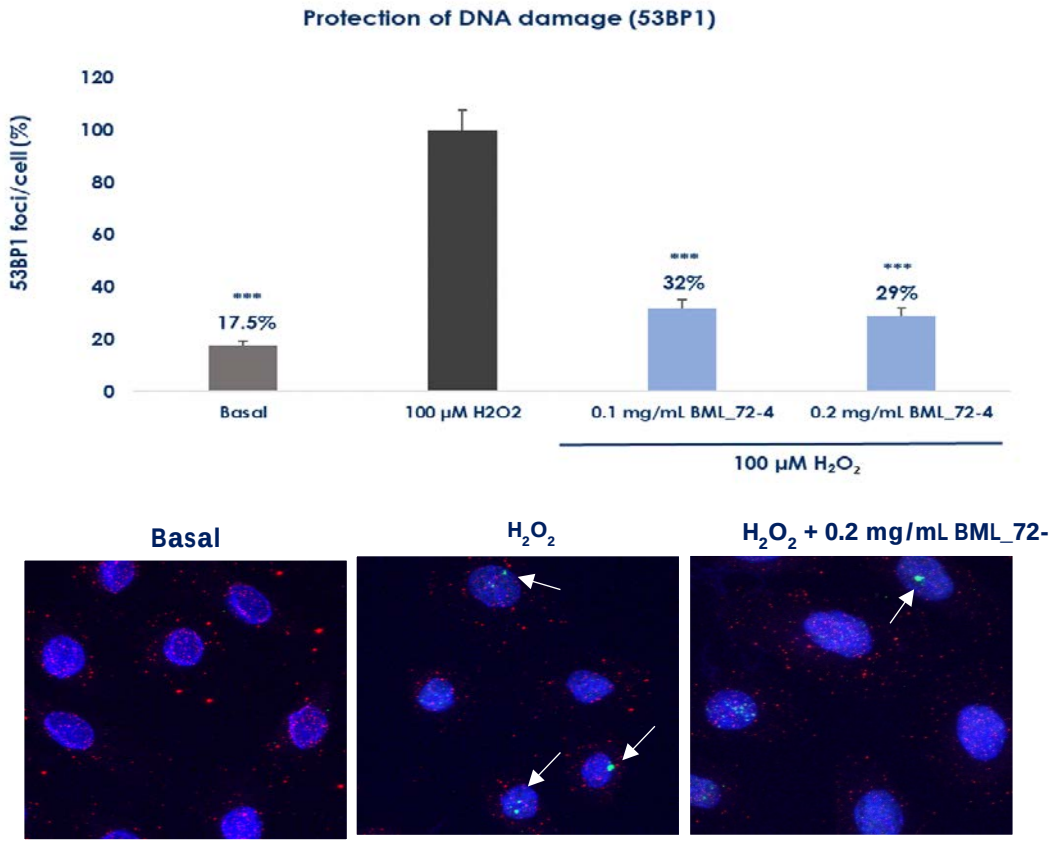

Fig. 2 Bar graph represents the levels of 53BP1 foci/cells after 24 h treatment of HEKa with H_2O_2 alone or in the presence of BML_72-4. Graphic shows the mean ± standard error of the mean (SEM). Images acquired with a fluorescence microscope (green: 53BP1 foci; red: TRF1; blue: nuclei). N=1 and n=3. Student t-test by comparison of the number of TRF1 or 53BP1/nucleus in non-treated or BML_72-4 treated samples vs the number of TRF1 or 53BP1/nucleus in H_2O_2-treated samples, ***, $p < 0.001$.

4.3 Analysis of the effects of BML_72-4 on the expression of extracellular matrix-related genes in HDFa cells stressed with cortisol

To determine the potential of BML_72-4 to revert the effects of cortisol-mediated stress on the expression of ECM-related genes in Human Dermal Fibroblasts (HDFa) a qPCR was performed. Results show that 24 h treatment of HDFa with 10 µM cortisol induces a downregulation of genes COL1A1, COL3A1 and COL5A1, and upregulation of gene MMP2 (data not shown). However, 24 h treatment of cortisol-stressed cells with 0.05 mg/mL BML_72-4 recovers the expression of these genes to normal values, upregulating collagen-related genes such as COL3A1 (+1.15), COL1A1 (+1.20), COL5A1 (+1.40) and downregulating MMP2 (-1.58) (data not shown).

4.4 Study of the effect of BML_72-4 on telomere length of HDFa cells

$HDFa_{73}$ presented telomeres shorter than $HDFa_{25}$ (fold change = 0.8). However, $HDFa_{73}$ incubated with 0.1 mg/mL BML_72-4 for 48 h showed telomeres 1.3x longer than untreated $HDFa_{73}$ (Fig. 3).

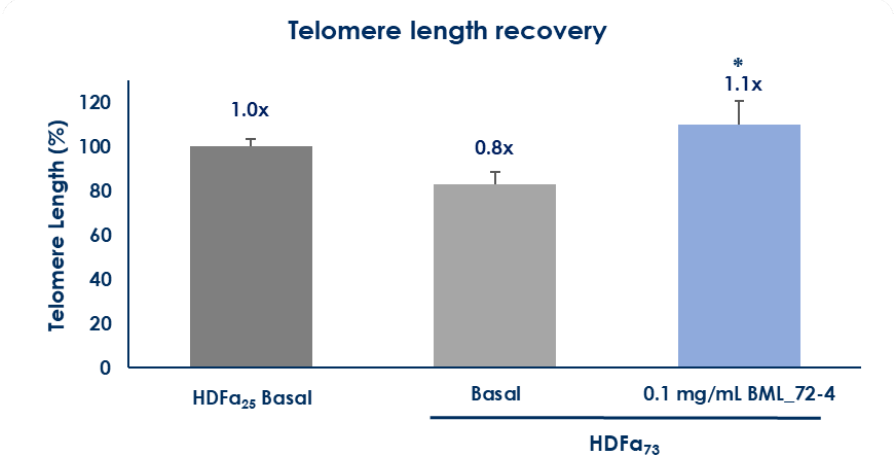

Fig 3 Relative telomere length of HDFa cells treated with different BML_72-4 concentrations. Graphic shows the mean ± standard error of the mean (SEM). Telomere length of $HDFa_{25}$ Basal and treated $HDFa_{73}$ were compared and no significant differences were founded between both conditions. N=3 and n=3. *, $p < 0.05$.

4.5 Analysis of the effect of bacterial extract BML_72-4 on cortisol release in HEKa

Cortisol release in HEKa treated with bacterial ferment BML_72-4 was quantified in cell culture supernatant by Enzyme-linked immunosorbent assay (ELISA). Results show that after 48 h of treatment with 0.01, 0.05 or 0.1 mg/mL BML_72-4, together with SP at 50 µg/mL, cortisol protein release is significantly decreased up to 18%, at the highest concentration tested, when compared to SP alone-treated cells, thus returning cortisol levels to the basal state (Fig. 4).

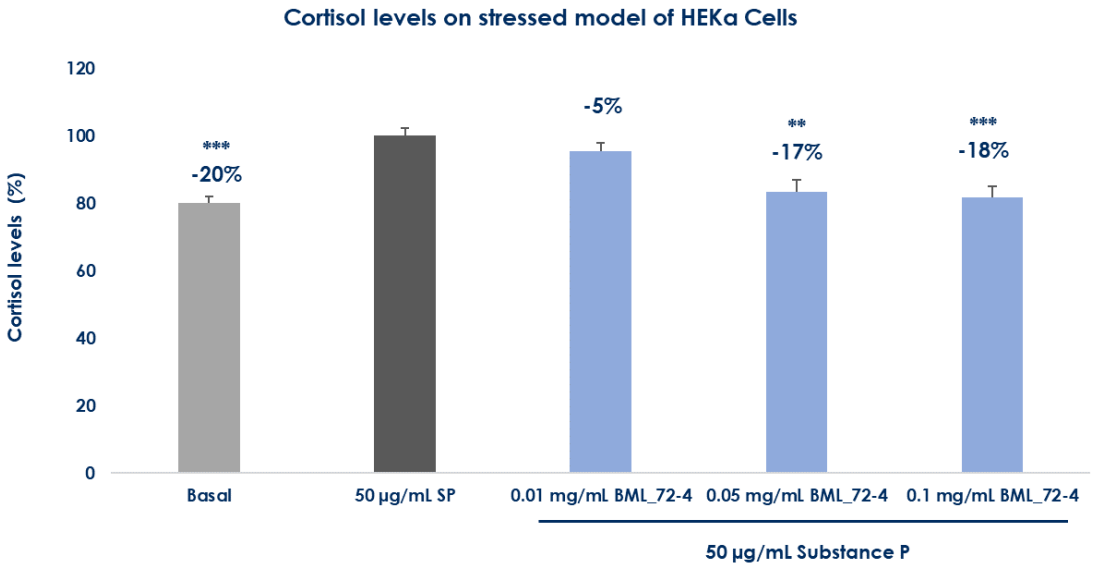

Fig. 4 Cortisol release on HEKa, after 48 h treatment with BML_72-4 and SP. Graphic shows the mean ± standard error of the mean (SEM). Two-tailed Student t-test has been performed comparing treated cells with untreated ones (Basal). N=3 and n=3. **, $p<0.01$ and ***, $p<0.001$.

4.6 Evaluation of the effect of BML_72-4 on subcellular localization of the glucocorticoids receptor (GR) in HEKa cells

The effect of BML_72-4 on GR subcellular localization was evaluated by immunofluorescence. Results show that after 24 h treatment with 10 µM cortisol, GR translocate from cytoplasm to nuclei, switching the staining colour of this organelle from blue to green. However, the presence of 0.05 mg/mL BML_72-4 decreases the quantity of GR relocated to the nuclei, recovering the DAPI blue signal (Fig. 5).

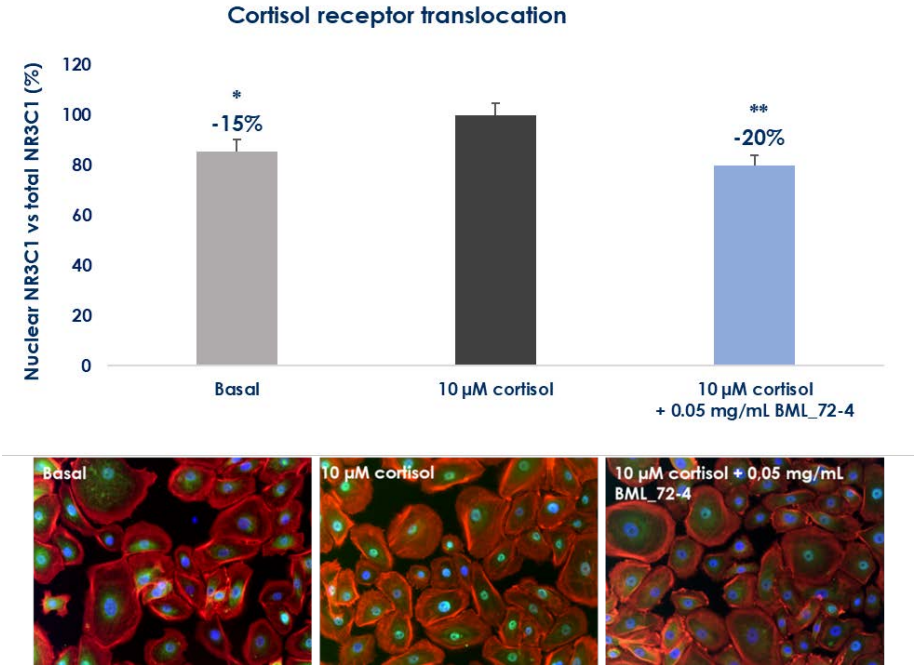

Fig. 5 Nuclear GR fluorescence on Human Epidermal Keratinocytes after 24 h treatment with 10 µM cortisol alone or in the presence of 0.05 mg/mL BML_72-4 h. A) Images acquired with a fluorescence microscope (green: GR, red: phalloidin, blue: nuclei). B) Nuclear fluorescence quantification of the images. Graphic shows the mean ± standard error of the mean (SEM). Two-tailed student t-test has been performed comparing cortisol-treated samples with untreated o BML_72-4 treated samples, *, $p < 0.05$, **, $p < 0.01$.

4.7 Evaluation of the efficacy of a *Micrococcus* ferment on HEKa cells to stimulate the liberation of KRT10 in presence of cortisol

To eluate the effect of BML_74-2 on the levels of KRT10 on an epidermal stressed model, HEKa cells were exposed to 0.01, 0.05 and 0.1 mg/mL of the bacterial ferment in presence of 10 µM cortisol during 24 h. Results show that in presence of BML_74-2 the KRT10 levels is significantly recovered by 25% in HEKa stressed model with cortisol (Results not shown).

4.8 Analysis of the effects of BML_72-4 on the migration of human keratinocytes subjected to cortisol-mediated stress

HaCaT cells treated with cortisol present a significant decrease of cell migration when compared with untreated cells (-25%). However, the presence of BML_72-4 during cortisol treatments restore cell migration ability in a concentration-dependent manner, reaching a complete

restoration with the highest concentration tested (+37% vs cortisol treated cells) **(Fig. 6).**

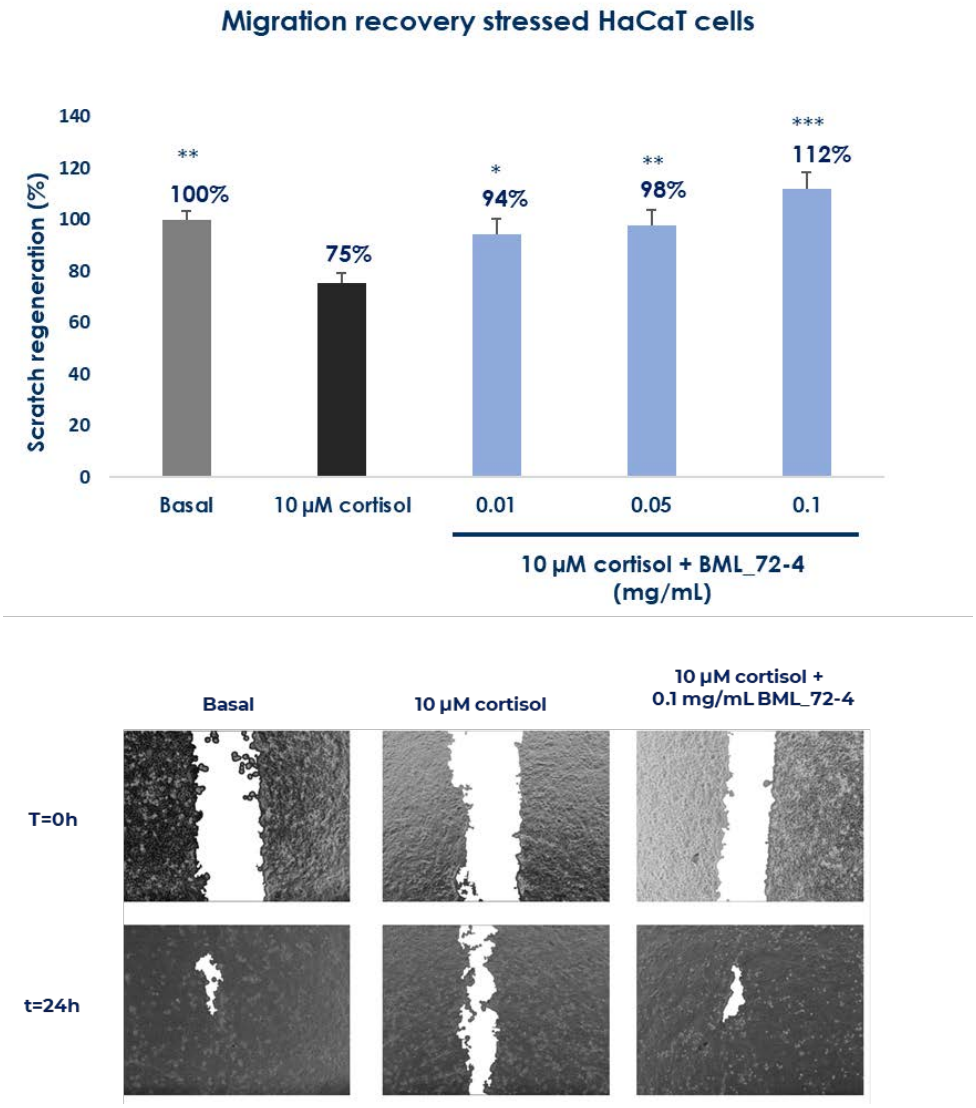

Fig. 6 Bar Graph represents the percentage of increase in HaCaT cell migration observed after 24 hours of treatment with cortisol alone or in the presence of different concentrations of BML_72. Graphic shows the mean ± standard error of the mean (SEM). Bellow, 10X phase-contrast images of migrating HaCaT cells at t=0h (top) and t=24 hours (bottom) after "scratch". Student t-test with two tails has been done comparing each condition with cells treated with 10 µM cortisol alone. N=3 and n=4. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$

4.9 Evaluation of the protective effect of BML_72-4 on a stressed stratum corneum of human skin explants

To evaluate the protective effect of the bacterial ferment on the skin explant integrity, skin explants from a 42-years old Caucasian female were stripped (except untreated condition) to remove the Stratum Corneum (SC) and then the they were stressed with cortisol or stressed and treated with the bacterial ferment each 24 h daily. Untreated skin explant was used as control. The following parameters were measured: SC thickness, corneocyte layers and skin barrier integrity.

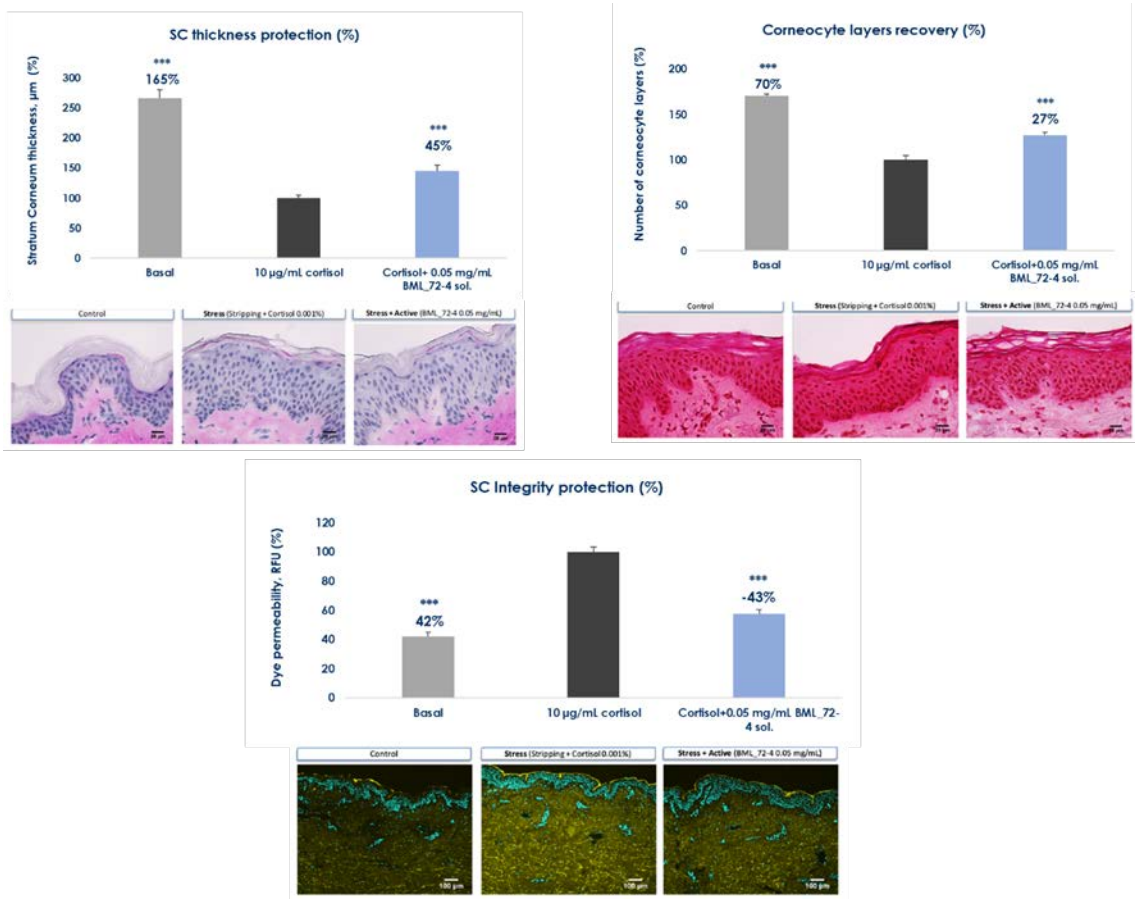

Fig. 7 Bar graphs represent the percentage of Stratum Corneum (SC) thickness, corneocyte layers and barrier integrity in skin explants where the SC was previously removed for "stress" and "stress + BML_72-4" conditions through a stripping. After removal of SC, the ferment and cortisol were applied each 24 hours during 5 days. Cryofixed sections were stained with Haematoxylin and Eosin, 1% Safranin-O red solution and Lucifer yellow, respectively.
The images were obtained with light and fluorescence microscopes. Three (3) images per explant (9 per condition). Data treatment and statistical analysis were achieved by t-test binary comparisons versus stress group. ***, $p<0.001$.

The exposure to the stress conditions showed a significant decrease of the number of corneocyte layers and in the SC thickness. In addition, an increased penetration of a fluorescent dye which gives us an idea of skin barrier integrity impairment are observed after exposure to stress. When compared to the stress conditions, the presence of the active ingredient BML_72-4 significantly preserved the skin from the decrease in number of corneocyte layers (45%), from the decrease in the stratum corneum thickness (27%) and from the impairment of skin barrier integrity (43%) (Fig. 7).

4.10 *In vivo* efficacy study

To evaluate the clinical efficacy of BML_72-4 active ingredient on face and body, 20 healthy Caucasian female subjects aged between 35 and 50-years with clinical sign of skin aging and exposed to emotional stress applied a cream containing 2% of the active ingredient on half face/body, twice daily during 28 days. Placebo was used as control. Parameters as wrinkle depth, skin moisturization, elasticity and firmness at different timepoints were evaluated.

The results show that the active ingredient improves, significantly, skin hydration, elasticity and firmness by 39%, 8% and 9%, respectively on arms. The active also increases all these parameters on legs by 33%, 8% and 9%, respectively (results not shown). Furthermore, the active lessens wrinkles depth in the crow's feet area by 14%, and reduces the perceived age by 6-years old when compared to placebo after 28 days of treatment (Fig. 8-9).

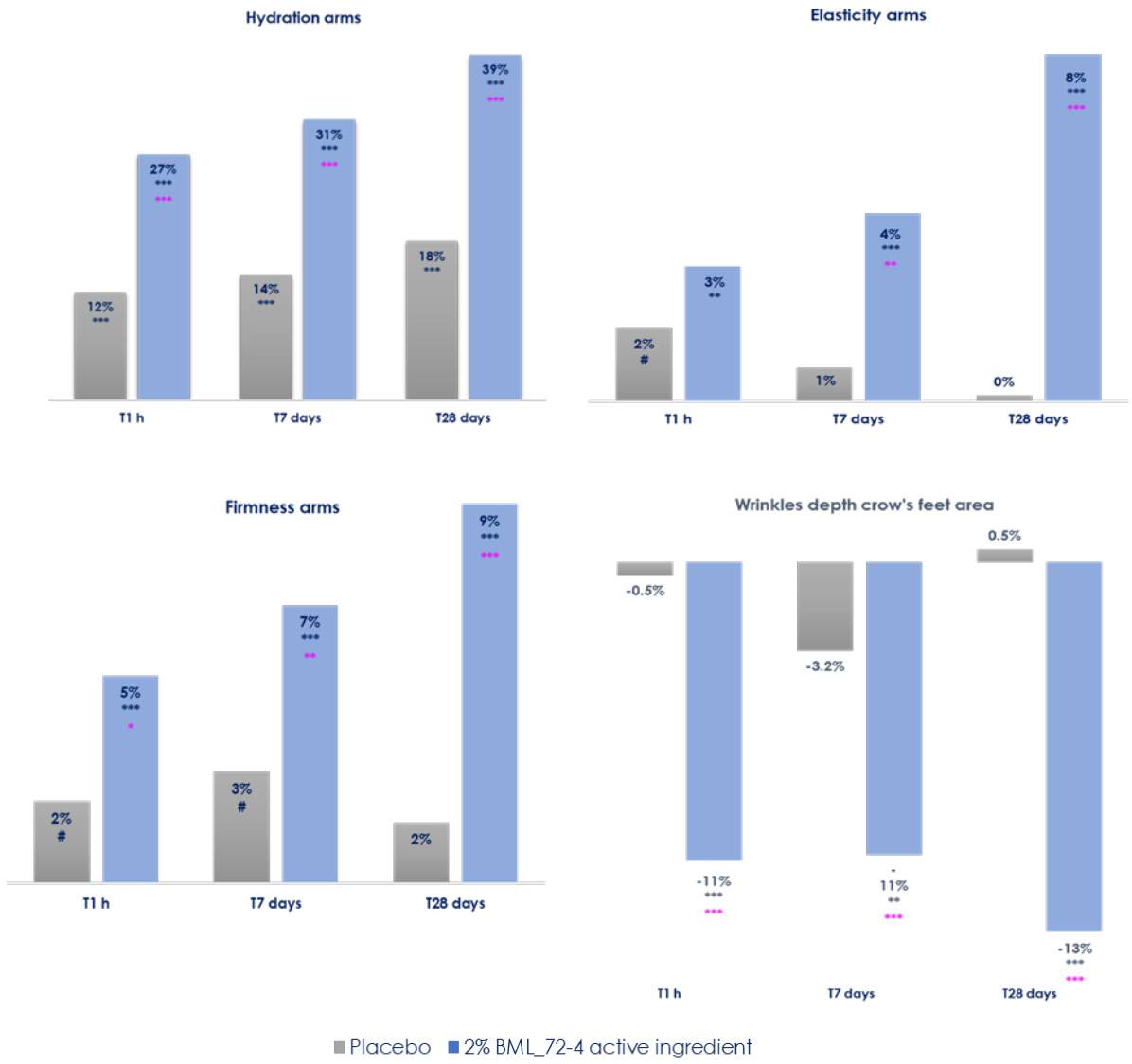

Fig. 8 Bar Graph represents the percentage of improvement of skin hydration (Corneometer®), elasticity and firmness (Cutometer®) on arms and wrinkles depth on the crow's feet area (Primos 3D) at different timepoints. Student t-test with two tails was performed comparing each condition with initial time or versus placebo. ***, $p<0.001$, **, $p<0.01$ and #, $p<0.1$. *Blue vs T0 and pink vs placebo.

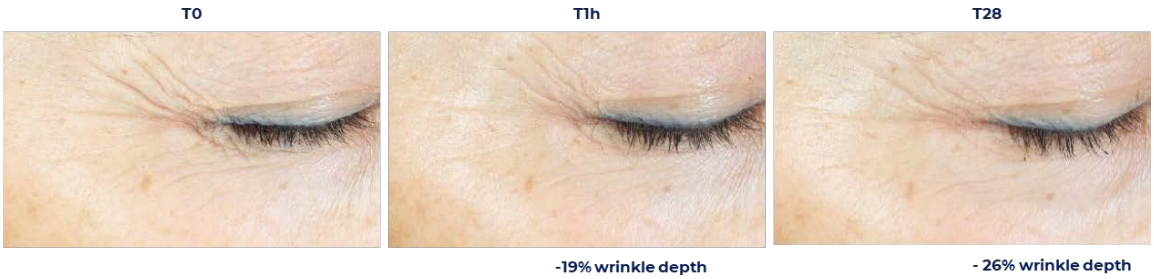

Fig. 9 VISIA® Illustrative images of the wrinkles in the crow's feet area during different timepoints of the study.

5. DISCUSSION

In this study, the contribution of a Micrococcus ferment lysate (BML_72-4) was evaluated in reducing the negative effects of PS on the skin. This bacterial ferment was chosen after a wide-ranging screening study done by the CNIO, where it was evaluated the effect of several compounds on TRF1 (protein of the Sheltering Complex) that, together with other proteins, protects telomere structure at chromosome ends [23]. The result was confirmed evaluating the effect of BML_72-4 on TRF1 levels and 53BP1, a nuclear protein that colocalizes with double-strand DNA breaks [24]. In this sense, the ferment increases the levels of TRF1 and decreases 53BP1 avoiding the telomere detriment seen in the study of the effect of the ferment on the telomere length of young and mature fibroblasts.

Multiples studies demonstrate reliable relationships between short telomeres and chronic PS due the continuously release of hormones and neuropeptides that regulate the stress response as cortisol and substance P, showing that skin acts as an endocrine organ [7, 25]. Furthermore, PS can play important roles in skin's inflammation responses, barrier function, and wound healing. Long-term chronic stress can also lead to premature skin aging [7]. On the effect of BML_72-4 on stressed cell models, the results show that the ferment reduces the levels of cortisol and the nuclear translocation of GR (cortisol receptor), increases the levels of proteins which expression or synthesis are highly affected by the increase of cortisol or substance P such as collagen, elastin, MMPs and KRTs [26-27]. In addition, the ferment restores the ability of human keratinocytes to regenerate under stress conditions and it also recovers SC thickness, the corneocytes layers and decreases skin barrier permeability of stressed skin explants. These results suggest a potential role of BML_72-4 to modulate cortisol-substance P-stress response.

As the skin is an organ of perception, the internal disturbance promoted by PS generates multiple skin problems [22] that the active ingredient could counteract, increasing the skin hydration, firmness and elasticity and reducing the depth of wrinkles and perceived age on stressed volunteers

probably due to the set of metabolites that comprises this ferment. In fact, metabolomic analyses show that BML_72-4 fermentation is increasing metabolites such as tryptophan, that has anti-inflammatory properties involved in dermal reconstruction and wound healing [28-29], Jasmonic acid, with skin healing and antiaging properties [30], Phloionolic acid, an antioxidant [31], and synthetizing new compounds belonging to the flavonoid family such as Isoorientin 6, widely described for its antioxidant properties [32]. Regarding the phytochemical compounds, the most outstanding was Luteolin due to its photobiological effects on the skin, as well as antioxidant and anti-inflammatory activities [33].

According to all the results, the bacterial ferment is capable of reducing the undesirable effects of communication between the brain and the skin in stressful conditions.

6. CONCLUSIONS

Psychological stress can be linked with skin aging since it negatively affects telomere length and epidermal/dermal functions, thereby accelerating the natural senescence process. According to efficacy studies, this bacterial ferment is capable of restoring “skin allostasis” by reducing the effect of stress neuroendocrine response promoting the healing the downs of psycho-dermatology.

7. CONFLICT OF INTEREST STATEMENT

None.

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