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Original Article

Bioactive molecules from the Blue Lagoon: *in vitro* and *in vivo* assessment of silica mud and microalgae extracts for their effects on skin barrier function and prevention of skin ageing

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Abstract: Bathing in the Blue Lagoon, a specific geothermal biotope in Iceland has been known for many years to be beneficial for human skin in general and for patients with psoriasis and atopic dermatitis in particular. The scientific rationale for this empirical observation, however has remained elusive. We now report that extracts prepared from silica mud and two different microalgae species derived from the Blue Lagoon are capable of inducing involucrin, loricrin, transglutaminase-1 and filaggrin gene expression in primary human epidermal keratinocytes. The same extracts also affects primary human dermal fibroblasts, because extracts from silica mud and one type of algae inhibited UVA radiation-induced upregulation of matrix metalloproteinase-1 expression and both algae, as well as silica mud extracts induced collagen 1A1 and 1A2 gene expression in this cell type. These effects were not restricted to the *in vitro* situation because topical treatment of healthy human skin ($n = 20$) with a galenic formulation containing all three extracts induced identical gene regulatory effects *in vivo*, which were associated with a significant reduction of transepidermal water loss. In aggregate, these results suggest that the bioactives in Blue Lagoon have the capacity to improve skin barrier function and to prevent premature skin ageing. These observations explain at least some of the beneficial effects of bathing in the Blue Lagoon and provide a scientific basis for the use of Blue Lagoon extracts in cosmetic and/or medical products.

Key words: collagen synthesis – keratinocyte differentiation – MMP-1

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Introduction

Within recent years, bioactive molecules have been introduced as ingredients for cosmetic products (1,2). Bioactive molecules or 'actives' are characterized by their capacity to actively modulate biological processes which take place in human skin, e.g. by stimulating beneficial properties or by interfering with signalling pathways which are known to lead to skin damage. Cosmetic products containing such active molecules have been termed cosmeceuticals to indicate the pharmaceutical properties of their active components (3). The increasing use of actives already has and will continue to change our perception of cosmetic products, because the more we know about the biological properties of these molecules the better we can use them for the development of more efficient and specifically targeted products. In view of the ongoing demographical changes in Western societies (4), the need for new actives to protect human skin against environmental threats and in particular against extrinsic skin ageing is constantly growing and thus, studies about the mode of action of these molecules are of great interest.

In general, actives can either be chemically synthesized or they can be directly derived from natural sources. The latter strategy is by far the more popular one because 'nature-born' products are very appealing to the consumer and even more important is 'mother nature' or certain natural biotopes very often prove to be a surprisingly rich source of highly effective actives with a

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multitude of biological activities. A prominent example appears to be the Blue Lagoon in Iceland. This geothermal basin contains water originating from underground reservoirs filled with geothermal seawater. The salinity of the water indicates that it is composed of 65% seawater and 35% freshwater. The lagoon water has an average temperature of about 37°C with local and seasonal variations in the range of about 30–45°C, a pH of about 7.5 and a salt content of 2.5% (wt %). Bathing in the Blue Lagoon was reported to have beneficial effects for patients with psoriasis, atopic dermatitis as well as healthy normal skin (5,6). Despite the increasing popularity of bathing in and using skin care products from the Blue Lagoon, virtually nothing is known about the biological activities present in these Blue Lagoon bioactives. Patients, medical staff, dermatologists and regular visitors of the Blue Lagoon agree in an empirical manner that not only bathing in the Blue Lagoon water, but also topical application of the Blue Lagoon silica mud is beneficial for human skin.

In view of this information, we have combined *in vitro* and *in vivo* assay systems in an attempt to identify biological activities in the Blue Lagoon. By employing molecular biological techniques together with conventional skin physiological measurements, we have been able to show that both the silica mud and two microalgae species growing in the Blue Lagoon induce skin barrier improvement and prevent extrinsic skin ageing.

Materials and methods

In vitro studies

Materials

Silica mud from the Blue Lagoon was prepared at the Blue Lagoon in Iceland and then directly shipped to Düsseldorf for further analysis. Similarly, Blue Lagoon coccoid algae and filamentous algae were harvested from the Blue Lagoon water by centrifugation. Cell pellets were then stored at −20°C, shipped on dry ice to the IUF and further stored at −20°C until extract preparation.

Preparation of silica mud extracts

The mud was washed using distilled water. To remove the coarse particles, the mud was subjected to a strainer with a mesh size of 200 μm (Sigma, Munich, Germany). The severely strained silica was subsequently centrifuged at 4000 rpm using a Kendro Megafuge 1.0 with a 2704 Rotor (Thermo Scientific, Langenselbold, Germany) for 15 min. The supernatant was discarded and the remaining sediment was used for the determination of the wet weight. For testing, 50 mg silica mud (wet weight) was dissolved in 1 ml Aqua dest.

Preparation of microalgae extracts

To preserve any potential biological activities, microalgae extracts were prepared by a mechanical procedure that completely avoids heating or any chemical extraction steps. Specifically, 30 g (wet weight, 1 part of volume) Blue Lagoon coccoid and filamentous algae were added in a mortar together with 2 parts of volume of Alumina A2039, type A-5, particle size of 6–10 μm (Sigma). Phosphate-buffered saline (PBS) without magnesium and calcium at a pH of 7 (Gibco Invitrogen, Karlsruhe, Germany) was added drop by drop to increase the volume three-fold and a mortar and pestle was used meanwhile to grind the mass until a uniform viscous paste had formed. This mass was subsequently grinded using the pestle for another 30 min at 4°C. The resulting solution was centrifuged for 1 h at 11 500 rpm (Sorvall Evolution RC equipped with Rotor SLA 1500 [Thermo Fisher Scientific, Langenselbold, Germany]), after which the supernatant was carefully taken off from the pelleted cell debris and alumina. Prior to testing, the protein content of the supernatant was determined by Bradford (7) using the Biorad Protein assay (Biorad, Munich, Germany). In some cases, several dilution steps were necessary to meet the requirement for linearity of the protein determination assay. The supernatants, i.e. crude whole cell extracts were stored at −20°C until testing.

Cell culture

Primary human epidermal keratinocytes were prepared from neonatal foreskin as described previously (8) and maintained in culture under serum-free conditions using a defined keratinocyte growth medium, Keratinocyte SFM (Invitrogen, Heidelberg, Germany) supplemented with bovine pituitary extract (Invitrogen) and recombinant epidermal growth factor (Invitrogen). Cells were propagated up to a passage two or three at 37°C in 5% CO_2 (9). For induction of differentiation, normal human epidermal keratinocytes were seeded in 6-well plates and grown up to confluence.

Human dermal fibroblasts (HDF) prepared from neonatal foreskin were cultured in Eagle's Minimum Essential Medium (Life Technologies GmbH, Eggenstein, Germany) supplemented with 5% fetal calf serum (Greiner, Frickenhausen, Germany), 0.1% L-glutamine, 2.5% $NaHCO_3$ and 1% streptomycin/amphotericin B in a humidified atmosphere containing 5% CO_2 for 4 days until they reached confluence as described (10). For all studies, only early passage (<12) fibroblasts have been used to avoid changes in their original phenotype during subculture. Cells were kept in 6-well plates for culture and irradiation.

Viability test

Viability was tested using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay – cytotoxicity of actives was evaluated using the MTT colorimetric

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assay according to Mosmann (11) as described earlier (12). Briefly, NHEK or fibroblasts were seeded in 96-well plates at 15 000 cells/200 μl in each well. The next day, the cells were treated with the substances of interest for 24 h. After 21 h, 25 μl of MTT (2 mg/ml PBS) was added and the mixture was incubated for another 3 h. Finally, solutions were removed, formazan crystals were dissolved in 200 μl of Me_2SO, and absorption was measured using a microplate reader (Labsystems; Global Medical Instruments Inc., Albertsville, MI, USA) at 540 nm. Viability was calculated as a percentage of control from three individual experiments (13).

RNA Isolation and PCR

Total RNA was isolated using RNeasy Total RNA Kits (Qiagen, Hilden; Germany). The RNA concentration was determined via photometric measurement at 260/280 (Biophotometer, Eppendorf, Hamburg, Germany). Aliquots of total RNA (100 ng) were applied for cDNA-Synthesis using Superscript™III First-Strand synthesis system for the reverse transcription step with random healers (Invitrogen). For each gene, a specific primer pair was designed by PRIMER EXPRESS™ 2.0 software (Applied Biosystems, Darmstadt, Germany) based on the cDNA sequence published as indicated (Table 1, 14–23). Three independent experiments were performed with two determinations each and the mean value of these was calculated. The PCR reactions were carried out on an Opticon 1 (MJ Research, Waltham, MA, USA) using SYBR Green® PCR Master Mix (Applied Biosystems). Each sample was analyzed in double employing the universal protocol over 46 cycles. In detail, 10 min of 94°C activation of hot start taw polymerase, 95°C penetration for 20 s, 55°C annealing for 20 s, 72°C extension for 30 s. For comparison of relative expression in real time PCR in control cells and treated cells, the $2^{-\Delta\Delta C(T)}$ method was used (24).

Table 1. Primer pairs for real time PCR

Gene	Primer pairs	Reference
18S rRNA	5′-GCCGCTAGAGGTGAAATTCTTG-3′ 5′-CATTCTTGGCAAATGCTTTCG′-3′	14
Transglutaminase-1	5′- CCCCCGCAATGAGATCTACA-3′ 5′-ATCCTCATGGTCCACGTACACA-3′	15
Involucrin	5′-CCCATCAGGAGCAAATGAAAC-3′ 5′-GCTCGACAGGCACCTTCTG-3′	16
Filaggrin	5′-AAGGAACTTCTGGAAAAGGAATTTC-3′ 5′-TTGTGGTCTATATCCAAGTGATCCAT-3′	17
Loricrin	5′-TCACATTGCCAGCATCTTCTCT-3′ 5′-GGCTGCTTTTTCTGATAAGACATCT-3′	18
Collagen 1A1	5′-CCTGCGTGTACCCCACTCA-3′ 5′-ACCAGACATGCCTCTTGTCCTT-3′	19
Collagen 1A2	5′-GATTGAGACCCTTCTTACTCCTGAA-3′ 5′-GGGTGGCTGAGTCTCAAGTCA-3′	20
MMP-1	5′-GGGAGATCATCGGGACAACTC-3′ 5′-GGGCCTGGTTGAAAAGCAT-3′	21
Interleukin-1 (IL-1)	5′-TGTATGTGACTGCCCAAGATGAA-3′ 5′-ACTACCTGTGATGGTTTTGGGTATC-3′	22
Interleukin-6 (IL-6)	5′-AGCCGCCCCACACAGA-3′ 5′-CCGTCGAGGATGTACCGAAT-3′	23

In vitro irradiation

Primary HDFs were exposed to a dose of 30 J/cm² UVA radiation, which was previously found to be optimal in inducing gene expression without affecting viability in this cell type (25). In brief, medium was replaced by PBS, lids were removed, and cells were exposed to UVA1 radiation using a SELLAMED 2.000 system (Dr. Sellmeier, Sellas GmbH, Gevelsberg, Germany). The UVA1 output was determined with a UVAMETER type II (Waldmann, Villingen-Schwenningen, Germany) and was found to be approximately 150 mW per cm² UVA1 at a tube to target distance of 30 cm by Grether-Beck et al. (9,26,27). Vitamin E (tocopherol succinate) served as a control for inhibition of UVA-induced upregulation of gene expression (26).

In vivo studies

Volunteers

Approval had been obtained from the Ethics Committee of the Heinrich-Heine University. The study has been conducted according to the ethical rules stated in the Declaration of Helsinki Principles and the ICH GCP guideline was observed so far, as applicable. Twenty healthy human volunteers (eight female and twelve male) were enroled after written informed consent. Their age ranged from 18 to 71 years and all individuals were non-smokers and had no history of any severe skin disease, especially no photosensitivity disorders. Skin types ranged from Fitzpatrick type I to IV. Their buttock skin had not been exposed to natural or artificial UV radiation for a minimum of 1 year. None of the volunteers used dietary supplements during the study.

Topical treatment

For *in vivo* experiments a galenic formulation was prepared. This preparation which for the purpose of this study will be termed Blue Lagoon (BL) actives, contained the active ingredients silica mud (3.25%) and extracts from Blue Lagoon filamentous algae (0.25%) and Blue Lagoon coccoid algae (2.5%) in the given concentrations. All volunteers were treated once daily for 4 weeks at three different skin sites (4 cm × 4 cm large): one area at the volar forearm and two areas at their buttock skin.

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In vivo irradiation
In each volunteer, one of the treated and an untreated skin area (4 × 4 cm) of their buttock skin was exposed to a single dose of UVA1 radiation (100 J/cm^2) from a SELLAMED 2000 (Sellas GmbH) irradiation device. This dose was chosen because it can be easily reached under physiological conditions and in previous studies was shown to consistently induce gene expression *in vivo* in human skin. Twenty-four hours after irradiation, 4-mm punch biopsies were taken from (i) a sham-irradiated control area (no UVA), (ii) a UVA-irradiated skin area (UVA), a BL-actives pretreated, (iii) UVA-irradiated skin area (pretreated, UVA) and (iv) a BL-actives pretreated, unirradiated skin area (pretreatment, no UVA). Biopsies were snap-frozen in liquid nitrogen and stored at −20°C for further analysis.

Assessment of gene expression in skin biopsies
For assessment of gene expression, total DNA was extracted from frozen biopsies and gene expression measured by semi-quantitative Reverse-Transcriptase PCR (RT-PCR) as previously described. In brief, for isolation of RNA from frozen skin biopsies, 600 μl lysis buffer from PeqGold Total RNA Kit (PeqLab, Erlangen, Germany) was added and the samples were disrupted in a MixerMill MM300 (Retsch, Haan, Germany) three times for 3 min with 30 Hz. Total RNA of 50 ng was used for cDNA synthesis. PCR reactions were performed in an Opticon 1 (MJ Research, Waltham, MA, USA) using SYBR QPCR Supermix with Rox (Invitrogen). PCR conditions were as follows: activation of hot start *Taq* polymerase at 94°C for 15 min; denaturation at 95°C for 20 s; annealing at 55°C for 20 s; extension to 72°C for 30 s. Each sample was subjected to PCR in double using the appropriate primer pairs for 45–50 cycles.

Assessment of transepidermal water loss
All skin physiological measurements have been carried out by the same investigator in an air-conditioned room (room temperature 18–22°C, air humidity c. 30–50%). Transepidermal water loss has been measured before and after application of BL actives once daily over a period of 4 weeks at the volar forearm with an evaporimeter (Tewameter TM300, Courage and Khazaka Electronic GmbH, Cologne, Germany) according to the guidelines for measurement of transepidermal water loss by the European Society of Contact Dermatitis (28). The results were expressed as mg/h cm^2.

Statistical analysis
Two-tailed, paired Student's *t*-tests were employed for the statistical analysis and *P*-values of less than 0.05 were considered statistically significant.

Results

Preparation of extracts and cell viability assays

To be able to screen the biomaterial obtained from the Blue Lagoon for potentially beneficial biological effects, we first established standardized procedures that allowed us to prepare extracts from the silica mud and the two microalgae species. Silica mud is more or less water insoluble and we therefore developed a protocol that combined washing and centrifugation steps with the use of a strainer and ultimately gave us a silica solution free of coarse particles (for details see Materials and methods), which could be added to the cell culture medium. The two different algae species from the Blue Lagoon that were used in this study will be termed Blue Lagoon coccoid algae and Blue Lagoon filamentous algae. Both algae species are readily obtainable and grow abundantly in the saline hot water conditions of the Blue Lagoon biotop (29). When preparing extracts from these two microalgae species we were concerned about losing any biological activities prior to testing and therefore completely avoided any chemical extraction or heating steps. Instead, extracts were prepared by a purely mechanical procedure employing a mortar and a pestle (for details see Materials and methods). For all *in vitro* and *in vivo* experiments described in this study, extracts from silica mud or microalgae have exclusively been prepared by strictly following these two standardized protocols.

To assess whether addition of these extracts to skin cells would affect their viability, primary human epidermal keratinocytes and primary HDFs were cultured for 24 h in the presence of silica mud and algae extracts which were added over a broad concentration range (Fig. 1) and cell viability was determined by means of the colorimetric MTT assay. In general, keratinocytes tolerated all three treatments better than fibroblasts and in both cell types, the silica mud extract was less toxic than the two microalgae extracts. In fact, the streamed silica mud did not reduce the viability of both cell types over a concentration ranging from 50 to 5000 μg/ml, whereas the algae extracts were toxic at higher concentrations. Accordingly, for epidermal keratinocytes, extracts from type 1 algae did not reduce cell viability in the range of 31–334 μg/ml, whereas extracts from algae type 2 were more toxic and were only tolerated by this cell type when added at a concentration below 49 μg/ml. For dermal fibroblasts, even lower concentrations had to be used, i.e. for both algae extracts, non-toxic concentrations were between 1 and 10 μg/l.

Effects on keratinocyte differentiation markers

Both skin diseases which are known to benefit from bathing in the Blue Lagoon, i.e. psoriasis and atopic

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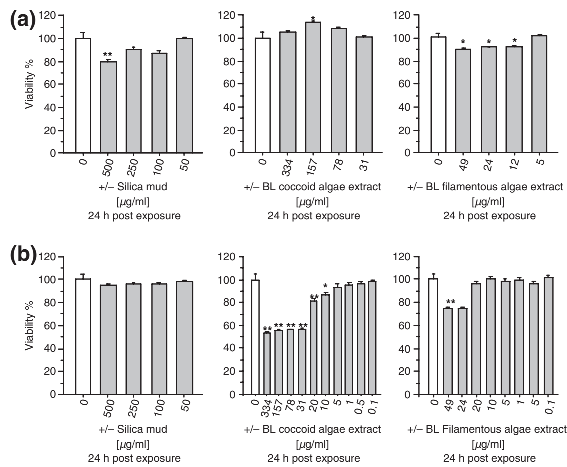

Figure 1. Determination of cell viability in (a) primary human keratinocytes and (b) human dermal fibroblasts 24 h post-treatment using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells were cultured with various concentrations of silica mud, or extracts from Blue Lagoon coccoid algae or Blue Lagoon filamentous algae as indicated. Values (grey bars) represent the mean ± SD from six samples. *$P<0.05$, **$P<0.01$ when compared to untreated controls (white bars; paired student's *t*-test).

dermatitis are characterized by a perturbed skin barrier function (30). We therefore speculated that silica mud and microalgae extracts may contain biological activities that might improve skin barrier function. Among many other factors, keratinocyte differentiation is a key component in determining the quality of the skin barrier. Keratinocyte differentiation is a highly complex process which involves the increased expression of a number of genes which are transcriptionally regulated (31). In the present study, we have measured the transcriptional expression of these genes, which include involucrin, filaggrin and transglutaminase-1 as surrogate markers for keratinocyte differentiation (32). As is shown in Fig. 2a, stimulation of keratinocytes with silica mud extracts increased mRNA steady-state levels for involucrin, filaggrin and transglutaminase-1 in a time- and dose-dependent manner. Similarly, expression of keratinocyte differentiation markers was also increased upon stimulation of cells with extracts from algae type 1, although to a lesser extent from algae type 2 (Fig. 2b). In comparison to silica mud, upregulation of keratinocyte differentiation markers by algae extracts was weaker and less well-balanced. The magnitude of the responses obtained by all three stimuli was similar to that observed after incubating keratinocytes in the presence of 10 μM Skin-Mimics® (Centerchem, Inc., Norwalk, CT, USA) containing mainly skin-identical lipid concentrate (Ceteareth-25; glycerine; cetyl alcohol; behenic acid; cholesterol; ceramide EOP; ceramide EOS; ceramide NP; ceramide NS; ceramide AP; caprooyl-phytosphingosine; caprooyl-sphingosine) (Fig. 2c), previously shown to induce expression of these genes *in vitro* and *in vivo* was thus used as a positive control in this study.

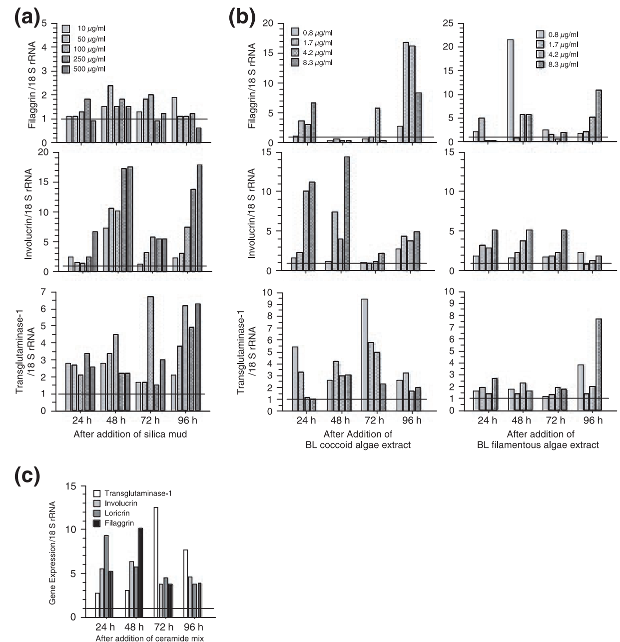

Figure 2. Gene expression of keratinocyte differentiation markers was studied using confluent primary human keratinocytes which were stimulated with various concentrations as indicated of (a) silica mud, (b) extract from Blue Lagoon coccoid or filamentous algae or (c) ceramide mix (10 μM) over a period of 4 days. Gene expression was analyzed using real time PCR and is expressed as induction compared to time-matched untreated controls (vertical line). Data represent one of the two essential identical sets of experiments.

Effects on UV-induced gene expression in dermal fibroblasts

We next searched for bioactivities which might be used to protect human skin against extrinsic ageing. By far, the most important environmental factor responsible for extrinsic skin ageing is UV radiation. A hallmark of photoaged skin is a loss and rarefaction of type I and type III collagen fibres in the dermal compartment, which is thought to be a major cause for wrinkle formation as well as loss of skin firmness and elasticity. There is now compelling evidence that UV radiation causes these changes by at least two different mechanisms: increased degradation and reduced *de novo* synthesis of collagen fibres (33). Specifically, UV radiation induces collagen degradation through the upregulation of matrix metalloproteinase-1 (MMP-1) expression in dermal fibroblasts. This proteolytic enzyme degrades collagen type I, III and VII and is induced by UV radiation directly as well as through an autocrine loop involving the UV-inducible cytokines, interleukin (IL)-1 and IL-6 (34). We therefore next assessed whether a

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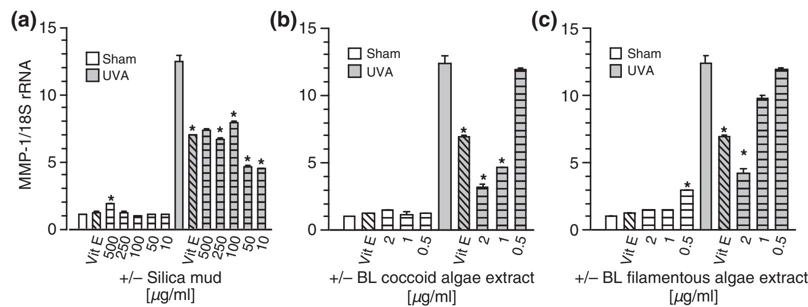

Figure 3. Dose-dependent inhibition of UVA-induced upregulation of matrix metalloproteinase 1 (MMP-1) by (a) silica mud and (b) algae extract from Blue Lagoon coccoid or filamentous algae as indicated. Human dermal fibroblasts were preincubated with these extracts for 24 h prior to irradiation with 30 J/cm^2 UVA. As a control, vitamin E was used at a concentration of 25 μM. MMP-1 expression based on 18S rRNA was determined by real time PCR and is shown as fold induction as compared with untreated controls. Data represent mean values, error bars indicate ± SD of three identical samples. *P<0.05, **P<0.01 when compared with UVA irradiated controls (grey bars; paired student's t-test). These results are one experiment of two.

24-h pretreatment of cultured HDFs with extracts prepared from silica mud or Blue Lagoon microalgae would affect UV radiation-induced MMP-1 expression. As is shown in Fig. 3, extracts from silica mud (Fig. 3a) and Blue Lagoon coccoid and filamentous algae (Fig. 3b) significantly inhibited UV radiation-induced MMP-1 expression. This inhibitory effect was time- and dose-dependent and associated with a concomitant inhibition of UV-induced IL-1 and IL-6 expression.

Effects on constitutive collagen gene expression

The mechanisms leading to a reduction in collagen *de novo* synthesis are less well understood, but UV radiation seems to be capable of downregulating the expression of Collagen 1A1 (COL1A1) and COL1A2 in dermal fibroblasts, i.e. two genes, which are critically involved in collagen synthesis (35). We therefore next asked whether the Blue Lagoon extracts might be able to affect collagen gene expression in HDFs. As is shown in Fig. 4, mRNA steady-state levels of both COL1A1 and COL1A2 were significantly upregulated in cells treated with extracts from Blue Lagoon coccoid and filamentous algae and to a lesser extent in cells treated with silica mud.

Studies addressing the *in vivo* relevance

Our *in vitro* studies suggest that extracts prepared from Blue Lagoon silica mud and microalgae have the capacity to affect the expression of differentiation markers in epidermal keratinocytes and photoageing-associated genes in dermal fibroblasts. To assess the *in vivo* relevance of these findings, we next analyzed the identical parameters in healthy human skin of 20 volunteers that had been treated with a galenic formulation containing all three extracts studied above. Once daily, topical application of this preparation for total of 4 weeks significantly increased mRNA expression for involucrin, filaggrin and transglutaminase-1 (Fig. 5a). Upregulation of these keratinocyte differentiation markers was associated with a significant reduction in transepidermal water loss of treated skin areas (from 23.25 to 9.57 mg/h cm^2, P < 0.000001; Fig. 5b). In addition, topical application of the Blue Lagoon extracts also induced COL1A1 and COL1A2

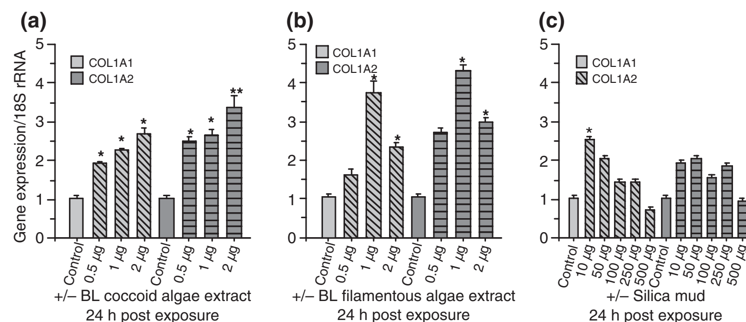

Figure 4. Dose-dependent upregulation of collagen genes COL1A1 and COL1A2 in human dermal fibroblasts 24 h postincubation with extracts from (a) Blue Lagoon coccoid algae, (b) Blue Lagoon filamentous algae or (c) silica mud was determined using real time PCR. Gene expression is shown as increase by folds when compared with untreated time matched controls. Data represent mean values, error bars indicate ± SD of three identical samples. *P<0.05, **P<0.01 when compared with untreated controls (grey bars; paired student's t-test). These results are one experiment of two.

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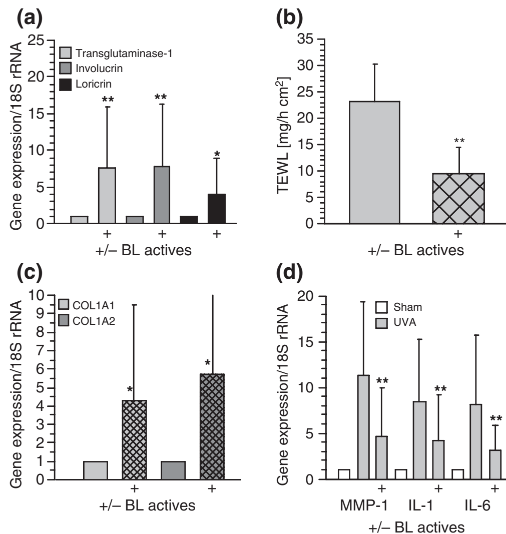

Figure 5. Biological effects *in vivo* in the skin of healthy human volunteeers once daily in a 4-week treatment with 2 mg/cm^2 BL actives. (a) Expression of transglutaminase-1, involucrin and filaggrin mRNA expression in treated versus untreated skin areas. (b) Transepidermal water loss prior and post-treatment of volunteers. (c) COL1A1 and COL1A2 mRNA expression in treated versus untreated skin areas. (d) Inhibition of UVA-induced upregulation of matrix metalloproteinase 1, interleukin (IL)-1 and IL-6 upon treatment with BL actives. *$P<0.05$, **$P<0.01$ when compared with untreated skin areas (grey bars in a, b, c) and to UVA-irradiated areas (grey bars in d; paired student's *t*-test). mRNA expression of the indicated genes was assessed as described in Materials and methods and is shown as fold induction as compared to untreated skin samples of the same volunteer. Data represent mean values of 20 volunteers, error bars indicate ± SD.

mRNA expression in unirradiated skin after 4 weeks of treatment (Fig. 5c). If at the end of the 4-week treatment period buttock skin was exposed to a single dose of UVA radiation, significant upregulation of MMP-1, IL-1 and IL-6 mRNA expression was observed in untreated skin areas (Fig. 5d). In marked contrast, UV-induced gene expression was significantly reduced in the contralateral skin sites which had been treated with the Blue Lagoon extracts prior to UV exposure.

Discussion

In the present study, we provide evidence that silica mud and microalgae from the Blue Lagoon improve skin barrier function and prevent premature skin ageing in humans. These conclusions which are based on *in vitro* and *in vivo* experiments provide a scientific rationale for the empirical observation that the skin of patients with psoriasis and atopic dermatitis and of normal healthy individuals benefits from bathing in the Blue Lagoon.

The capacity to stimulate keratinocyte functions is not unique to silica derived from the Blue Lagoon. Accordingly, stimulation of epidermal keratinocytes with silica has been reported to induce cytokine, in particular IL-1, production already 25 years ago (36). We now corroborate and extend these seminal observations and show that silica particles induce the transcriptional expression of genes which are required for keratinocyte differentiation and thus skin barrier formation. In addition, stimulation of keratinocytes with sodium metasilicate nonahydrate from a commercial supplier gave essentially identical results (data not shown). The underlying signalling pathways are currently unknown, but silica have been reported to affect gene expression in non-skin cells as well (37,38).

Increased keratinocyte differentiation marker expression was also observed upon stimulation of cells with extracts prepared from two different microalgae species, coccoid and filamentous algae derived from the Blue Lagoon. Both silica mud and algae extracts also affected the function of primary HDFs. Accordingly, silica mud as well as extracts from Blue Lagoon coccoid algae protected the skin against UVA radiation-induced MMP-1 expression, whereas extracts from Blue Lagoon filamentous algae were less effective. Both Blue Lagoon algae extract and to a lesser extent the silica mud induced COL1A1 and COL1A2 expression in unirradiated cells. This is in line with studies carried out in primary rat osteoblasts in which collagen synthesis was induced upon stimulation with BG60 silica (39). Beneficial effects of vitamin C for COL1A1 expression *in vitro* (40) or of green tea polyphenols (41) and dietary constituents, such as pantothenate, choline, nicotinamide, histidine, proline, pyridoxine and inositol (42) for skin barrier function *in vivo* have been described. Our observations indicate that the three extracts that were assessed in this study have specific biological properties, which are most likely due to distinct active constituents. Our observation is in line with the concept that extracts derived from the Blue Lagoon are a source for a variety of biologically active ingredients. It should be noted that in the present study we have employed only a limited number of biological assays which were selected on the basis of our interest in skin barrier function and skin ageing. It is thus likely that biological activities other than those described here are present in these three extracts. Further studies using additional biological read-out systems will be required to carefully address this issue.

The major limitation of our study is the use of extracts rather than of highly purified, biochemically well-characterized bioactive material. However, Our study was intended as a first step towards understanding the molecular and biological basis for the beneficial effects that are associated with bathing in the Blue Lagoon. In this regard, we provide for the first time a mechanistic explanation for the empirical observation that bathing in geothermal seawater from the Blue Lagoon has beneficial effects for patients with pso-

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riasis and atopic dermatitis as well as for healthy individuals. The present observation that silica mud and two algae species from the Blue Lagoon contain biologically active material which can be used for skin barrier improvement and protection against extrinsic skin ageing warrants further studies to clarify the precise nature of the responsible molecules. It also forms the scientific basis for the use of these extracts in cosmetic and/or dermatological preparations. It will be interesting to see if the unique environmental conditions, as a whole, define that Blue Lagoon is a source for many more bioactive molecules with beneficial properties for human skin, which extend beyond those described in this study.

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