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Biotechnologically Produced Moss Active Improves Skin Resilience

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abstract

Mosses substantially helped changing primitive earth into a green planet before vascular plants even existed. They encode genes and proteins for extraordinary resilience long abandoned by higher plants. Biotechnology offers a sterile production of the moss *Physcomitrella patens* in a reproducible and sustainable way. MossCellTec™ No. 1 is the first biotechnological moss-based product for cosmetic application.

In our research, we have investigated the importance of *cell nucleus health* as a novel target for anti-aging cosmetics. Interestingly, the moss extract was able to improve the expression of *cell nucleus health* markers in aged skin cells. Additionally, the active ingredient could improve the hot-humid/cold-dry adaptation of reconstructed skin and improved skin homogeneity, barrier and hydration despite stressful weather conditions in a placebo-controlled clinical study. The moss active may therefore enhance the general resilience of the skin against environmental changes.

Introduction

Moss Produced by Biotechnology

Mosses belong to the non-vascular plants and substantially greened our planet. For about 3.3 billion years life was restricted to the sea. To conquer the land one needed to develop strategies to survive on rocky ground. Mosses succeeded by adapting unique strategies [1], and by doing so, subsequently changed the atmosphere and the climate and thereby enabled higher plants to develop.

When mosses conquered the land, they were exposed to harsh conditions: lack of nutrients, strong UV-radiation, cold and heat, dry periods, wind and permanent attacks by bacteria and fungi. They developed a huge set of genes allowing them to adapt promptly to numerous changing environmental conditions.

The flexibility and robustness of moss offers unique opportunities. During evolution, higher specialized vascular plants lost a lot of the genes bearing these resilience information. As a result of the changing environment they were simply not needed anymore.

To this date, mosses however still harbor this valuable DNA and are surviving in niches all over the planet. Mosses still have outstanding resilience properties: they can regrow after being completely dried or after being frozen for more than 1,500 years [2].

To survive on a rocky planet about 470 million years ago, mosses developed the ability to filter the air and rain to accumulate necessary nutrients but also heavy metals and generally toxic compounds which presents no problem for the resilient mosses with their huge arsenal of detoxifying metabolites. But it certainly would be a risky approach using mosses directly from nature with potential contaminants for cosmetic applications. Beside of that, a great number of the approx. 100,000 moss species are endangered as a result of the continuing reduction of natural habitats by humans.

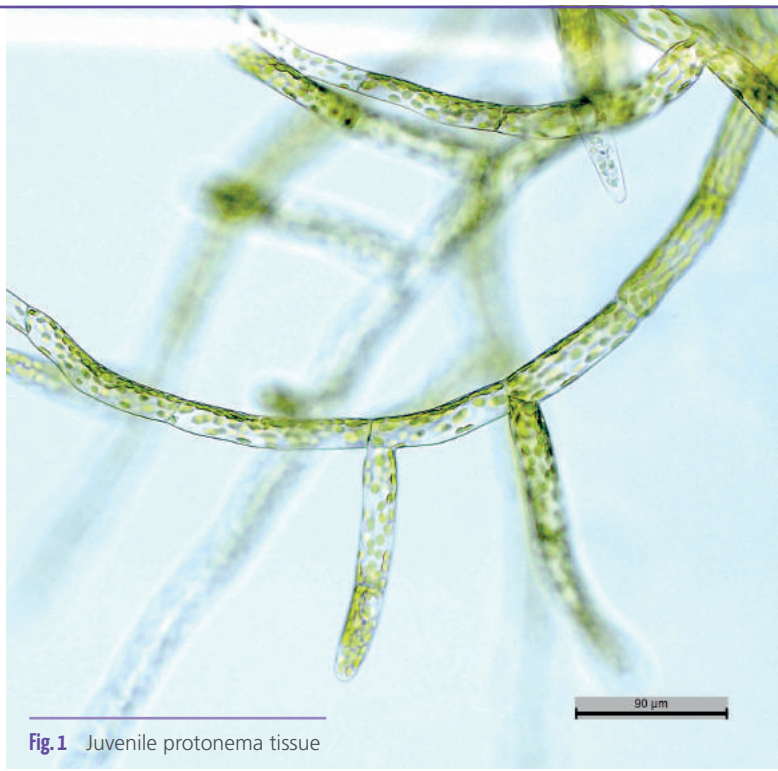

Fig. 1 Juvenile protonema tissue

Therefore, a sustainable and reproducible production of moss under clean, even sterile conditions is highly beneficial. Mibelle Biochemistry developed in close cooperation with Prof. Dr. Reski from the University of Freiburg the production of wild-type *Physcomitrella patens* protonema in large scale. *P. patens* is being researched extensively to study evolution in general [3], but also to discover their resilience mechanisms. Protonema is the juvenile tissue with a root-like structure (**Fig. 1**).

By budding out of the protonema the leafy gametophytes arise. Leafy gametophytes build the part of the plant that is generally recognized as moss. The biotechnological approach utilizes the ability of *P. patens* to regrow protonema out of leafy gametophytes when they are disrupted. By repetitive disruption of the tissue, a homogenous protonema culture can be established and grown in pharma-grade single-use wave bag technology using low energy LED's as a light source.
A novel applied cold press technology allows extracting all the water-soluble valuable metabolites by gentle means. This extraction method avoids the co-extraction of the green chlorophyll, leading to an almost colorless extract. To provide a preservative free cosmetic ingredient, the active is spray-granulated on isomalt: MossCellTec™ No. 1 (INCI: Phytol (and) Isomalt (and) Aqua/Water, from here on termed moss active) is the first ever biotechnology-based moss ingredient for innovative and powerful cosmetics.

Cell nucleus health, a Novel Anti-Aging Concept

In eukaryotic cells, the cell nucleus contains the DNA, the blueprint of the cell and is therefore considered the control center of the cell. It is surrounded by a membrane called the nuclear envelope, which contains holes, the nuclear pores, through which traffic into and out of the nucleus takes place. Only small molecules can freely diffuse through the nuclear pore complexes, larger molecules such as proteins and messenger RNA complexes need to be actively transported to reach their destination. This transport process is highly complex: In a single human cell, there can be up to 5000 nuclear pore complexes and each can transport 1000 molecules per second [4, 5]. This means that in one cell, up to 5 million molecules are transported into and out of the nucleus every second.
A timely transport of signaling molecules is crucial for the adaptation of cells to fast changes such as in temperature and humidity. This is especially true for skin cells which are in close contact with the environment. As we age, the transport becomes less efficient and less selective [6] which can lead to less resilient skin. Furthermore, recent research on premature aging diseases has shown that the correct composition of the nuclear envelope is essential for the maintenance of nuclear shape, DNA stability, and regulated gene expression.

Maintaining the proper stability and shape of the nucleus as well as ensuring an efficient nucleocytoplasmic transport can be summarized as the topic of *cell nucleus health*.
Here we show an improvement of *cell nucleus health* markers in aged keratinocytes with an extract from biotechnologically produced moss. The moss active also improved skin adaptation to climatic stresses *in vitro* as well as *in vivo*.

Materials and Methods

Gene Expression in Old vs. Young Keratinocytes

Normal human epidermal keratinocytes (NHEK) isolated from a younger donor (20 years old female) and an older donor (55 years old female) were cultured for 24 hours. The keratinocytes from the older donor were then incubated or not (control) with different concentrations of *P. patens* extract for 24 hours. All experimental conditions were performed in n=3. Cells were harvested and total RNA was extracted from each sample using TriPure Isolation Reagent (Roche) according to the supplier's instructions. RT-qPCR for the target genes was performed in n=2 using the LightCycler® system (Roche).

Temperature and Humidity Adaptation of 3D Skin Model

19 days old 3D human reconstituted skin (Episkin) was treated with 1 % moss active or just medium (control). After three hours, climatic stress was induced on the 3D skin either mimicking hot/humid summer stress (40 °C, 80 % relative humidity, 30 min) or cold/dry winter stress (10 °C, 40 % relative humidity, 15 min). These climatic stresses were repeated 3 times over 36 hours. Non-climatic stressed 3D skins were included in the experiment as a control. Afterwards, the 3D skins were incubated during 10 hours before processing. After the incubation period, each 3D skin was cryopreserved for histological studies. A Hematoxylin-Eosin staining was performed on one part of the 3D skin. On the other part, an immunostaining with an antibody against LCE1A (Thermo Fischer) was performed using a secondary antibody (Alexa 633 anti-Rabbit), together with DAPI staining to visualize cell nuclei. The images were acquired with a Leica confocal microscope. LCE1A levels were quantified with image analysis using the Leica LasX software.

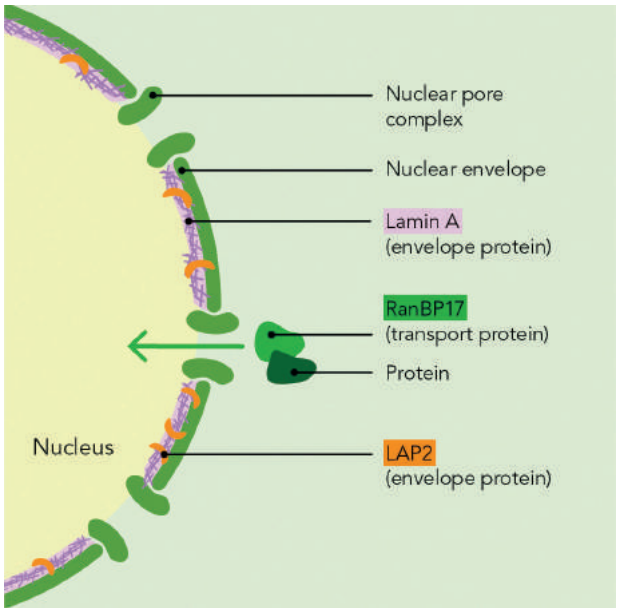

Fig. 2 Schematic illustration of the three investigated *cell nucleus health* markers. Lamin A and LAP2 are localized at the inner nuclear envelope. RanBP17 is a transport protein responsible for importing protein cargo into the nucleus.

Skin Adaptation in a Clinical Study

A double-blind placebo-controlled clinical study on 23 Asian women (39–53 years old) having 2–5 hours outdoor activity daily was performed during the summer in Seoul, Korea. The women applied 2 % moss active and the corresponding placebo cream on each half of their face twice daily for 14 days. Skin hydration was measured using a Corneometer CM 825® (Courage+Khazaka, Germany) and TEWL using a Vapometer® (Delfin, Finland). Additionally, facial photographs were taken using the VISIA® CR (Canfield, USA) and the acquired images were analyzed for the standard deviation value of skin tone (which corresponds to inhomogeneity) on the cheek region by Image-pro®plus (MediaCybernetics, USA). A smaller standard deviation signifies a more even skin tone.

Results and Discussion

P. patens Extract Improves *Cell nucleus health* in Aged Skin Cells

To assess the ability of moss extract to maintain *cell nucleus health*, the gene expression of three nucleus health markers was determined in keratinocytes from an old donor which were treated or not (control) with *P. patens* extract and compared to keratinocytes from a young donor. It has previously been shown that the two nuclear envelope-associated proteins Lamin A and LAP2B are downregulated in old skin and are therefore suitable markers for skin aging and nucleus health (**Fig. 2**) [7]. Notably, RanBP17, a protein responsible for the transport of protein cargo through the nuclear pore complex was shown to be downregulated in several aged cell types, including fibroblasts and neurons, and is therefore considered a universal aging marker [8].

When comparing old and young keratinocytes, the reported downregulation of the three marker genes could be nicely reproduced in old keratinocytes. Treatment of old keratinocytes with *P. patens* extract resulted in a concentration-dependent expression increase of the three marker genes LAP2B, Lamin A and RanBP17 compared to untreated old keratinocytes, closer to the expression of young keratinocytes (**Fig. 3**). Therefore, treatment with the *P. patens* extract has a rejuvenating effect on keratinocytes regarding cell nucleus genes.

Improved Skin Adaptation to Environmental Changes

The timely and efficient adaptation of our skin to different environmental factors is important for a healthy and resilient skin. To test the influence of moss active on the ability of skin to adapt to climatic changes, 3D skin was incubated under different climatic stresses: hot/humid and cold/dry to mimic the exposure of skin in different seasons and switching from heated/air conditioned buildings to a different outside climate. Under these stress conditions, the dermal fiber structure was disorganized and collapsed and fiber density was

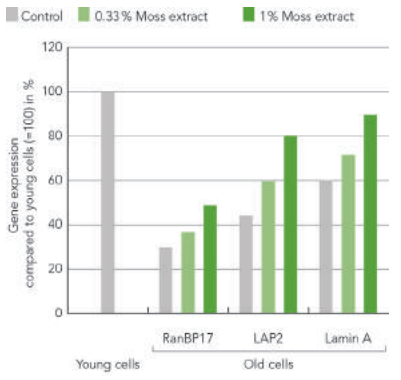

Fig. 3 Gene expression of *cell nucleus health* markers in keratinocytes from a young donor and an old donor treated with *P. patens* extract.

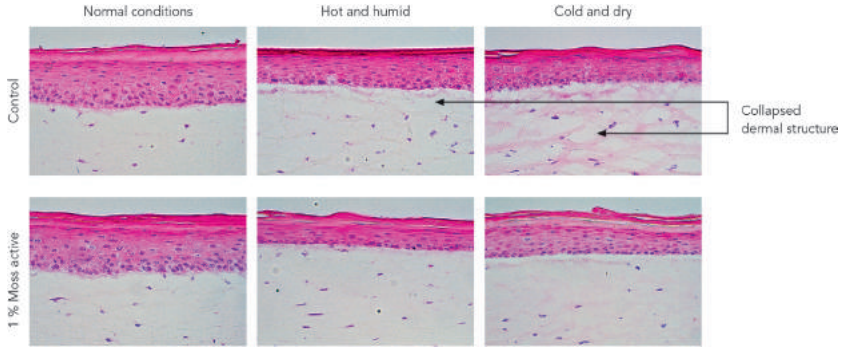

Fig. 4 Adaptation of 3D skin models treated with the moss active to climatic changes.

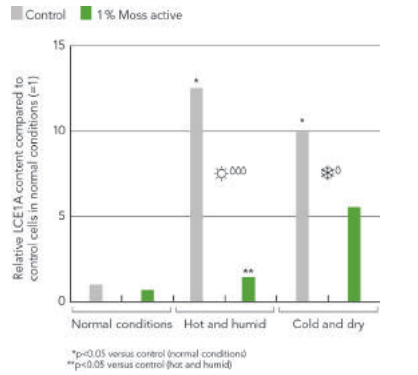

Fig. 5 Expression of the stress marker LCE1A in 3D skin models determined with immunofluorescence and image analysis.

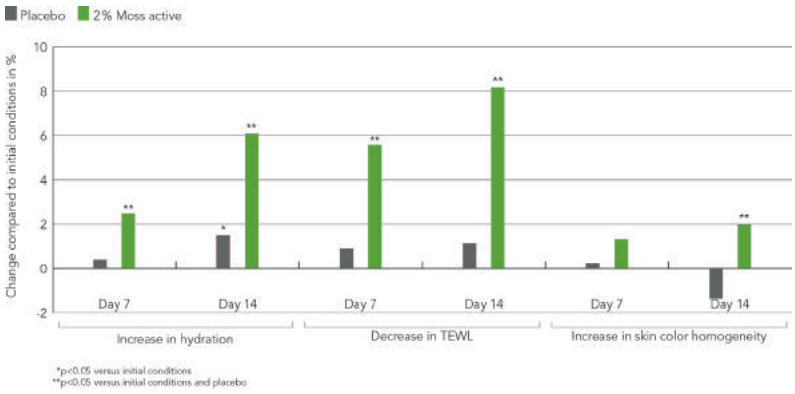

Fig. 6 Improvement of skin parameters with moss active treatment despite stressful climatic conditions.

reduced (**Fig. 4**). Furthermore, expression of the stress marker LCE1A was increased demonstrated via immunofluorescence (**Fig. 5**). Skin that was treated with 1 % moss active did not display these drastic changes in dermal structure and gene expression of stress markers (**Figs. 4, 5**) and could therefore adapt more efficiently to these climatic stresses.

The efficacy of the moss active was also tested in a placebo-controlled clinical study on a panel of Korean women who spent more than 2 hours per day outside in the summer in Seoul. After 14 days of treatment with 2 % moss active, a significant improvement of skin hydration, TEWL and skin tone homogeneity compared to placebo was observed (**Fig. 6**). The positive effect on skin tone homogeneity was also visible in photographs taken of the volunteers (**Fig. 7**). Overall, a skin improvement despite stressful weather conditions and improved skin adaptation to daily environmental changes was observed with moss active treatment.

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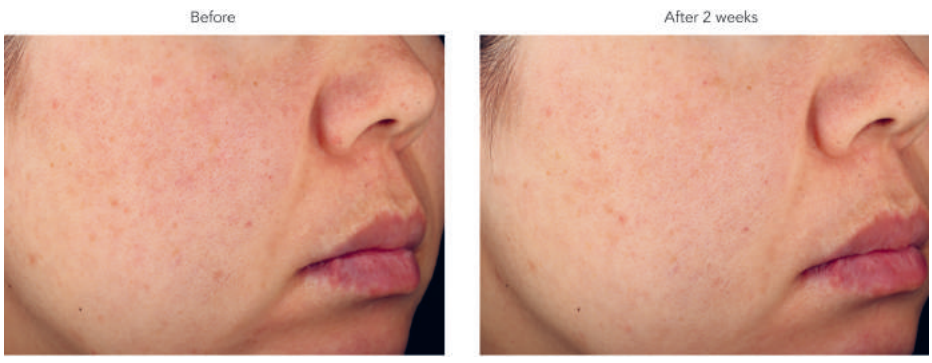

Fig. 7 Visible improvement of skin tone with the moss active.

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