Improved cell nucleus health with moss cell technology

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Abstract

Mosses were among the first plants that conquered the land and they used their extraordinary adaptation abilities to survive from the prehistoric age until the present day. To harvest the resilient properties of moss, an innovative moss cell technology was used to grow moss cells as a culture in the lab. Latest research has shown that the moss active contributes to 'cell nucleus health', a novel anti-ageing concept. The cell nucleus does not only contain the cell's DNA but is also involved in regulating important cellular processes. Efficient transport of molecules into and out of the cell nucleus is crucial for adapting to the ever-changing environment. *In vitro* studies have shown that the moss extract improves expression of cell nucleus health markers in aged cells and helps skin adapt to climatic changes. In a placebo controlled clinical study with women that are exposed to daily temperature changes in the summer, the moss active significantly improved skin hydration, barrier and homogeneity after just two weeks for a more resilient skin.

Higher organisms such as plants and animals are complexes of eukaryotic cells. A hallmark for eukaryotic cells is the presence of a cell nucleus. This organelle 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 (Fig 1). 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.[1,2] 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 signalling molecules is crucial for the adaptation of cells to fast changes such as in temperature and humidity. This is especially true for skin cells who are in close contact with the environment. As we age, the transport becomes less efficient and less selective[3] which can lead to less resilient skin.

For a long time it was believed that the function of the cell nucleus is merely DNA storage and that the nuclear envelope is just a hull that contains the genetic material. This is far from the truth: recent research on premature ageing 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 summarised as the topic of cell nucleus health.

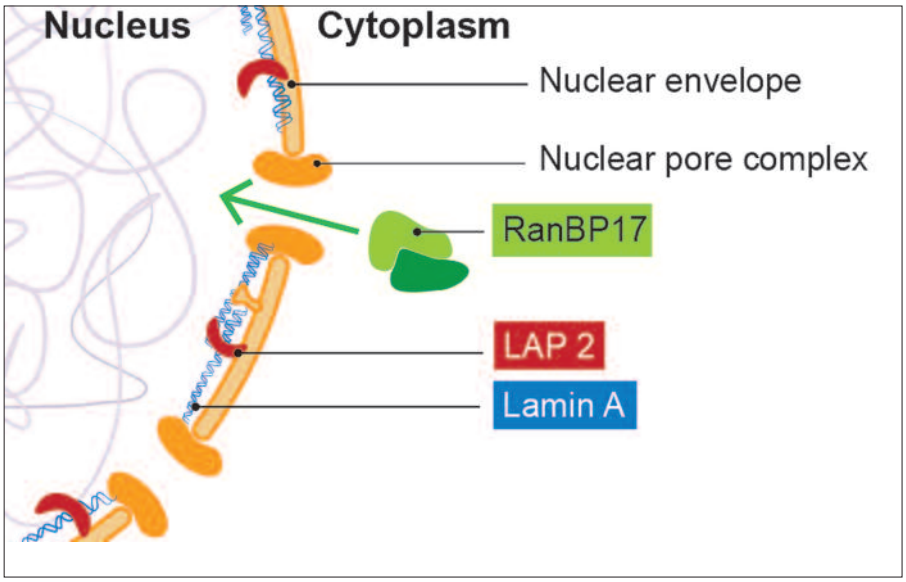

Figure 1: Schematic illustration of the components of the cell nucleus.

Mosses: the first plants on earth

About 470 million years ago mosses were one of the first plants that moved out of the water and conquered the earth.[4] Mosses possess no vertical roots and have a high surface area which makes it difficult to replenish the lost water and nutrients from the soil. Therefore, mosses filter nutrients from the air and rain which makes them susceptible to accumulating pollutants such as heavy metals. In order to cope with the oxidative stress from pollution, mosses developed a particular anti-pollution matrix with a large set of antioxidants.[5] The development of a specialised adaptation strategy was also needed for mosses to inhabit various climatic regions.[6] They are masters in water retention, rehydration, fast recovery and cold resistance. It has recently been shown that mosses are even able to continue to grow after being frozen for 1500 years in permafrost.[7] The molecules that enable such a resilience of the moss are certainly of high interest for the cosmetic industry.

Biotechnology to grow moss in the lab

Although resilient, mosses grow slowly and

are thus often under protection and cannot be harvested in the wild. Additionally, wild mosses filter the air and retain toxins that prevents them from use for cosmetics. To still make the adaptation skills usable for cosmetics, an innovative biotechnology to grow moss cells in a laboratory setting was developed in close collaboration with Greenovation Biotech GmbH and Prof Dr Reski from the University of Freiburg. Sterile cells of the wild-type moss *Physcomitrella patens* in liquid culture were produced. Additionally, a new cold pressing extraction method was established to harvest all water soluble ingredients from the moss cells resulting in a natural end product containing potent molecules. The extract was spray dried on an isomalt matrix and the resulting moss active [MossCellTec™ No.1, INCI: Phytol (and) Isomalt (and) Aqua/Water] was investigated for its ability to influence cell nucleus health and skin resilience.

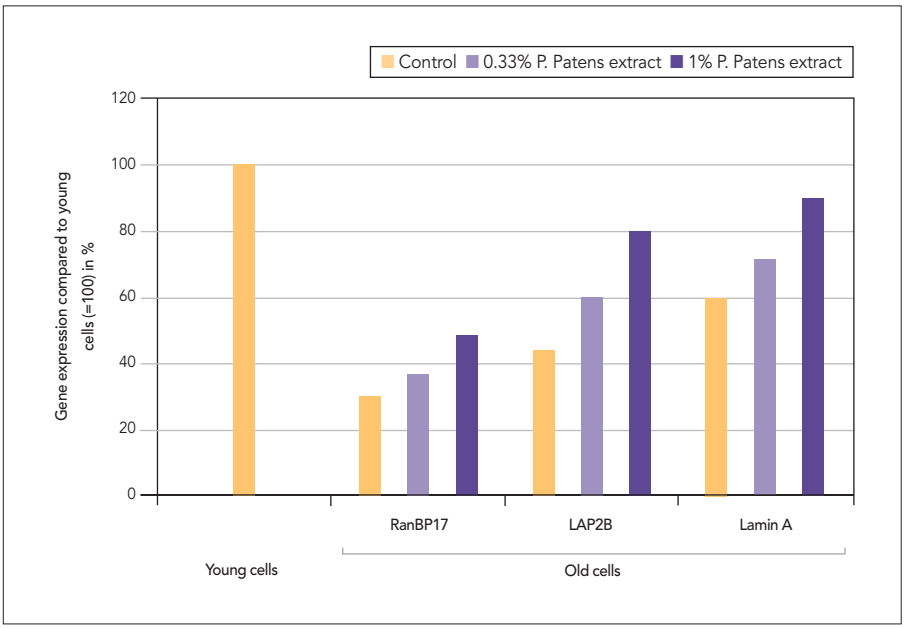

Figure 2: Gene expression of cell nucleus health markers in keratinocytes from a young donor and an old donor treated with *Physcomitrella patens* extract.

Materials and methods

Gene expression in old vs. young keratinocytes

Normal human epidermal keratinocytes (NHEK) isolated from a young donor (20 years old female) and an older donor (55 year old female) were cultured for 24 hours. The keratinocytes from the old donor were then incubated or not (control) with different concentrations of *Physcomitrella 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 day 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 visualise cell nuclei. The images were acquired with a Leica confocal microscope. LCE1A levels were quantified with image analysis using the Leica LasX software.

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

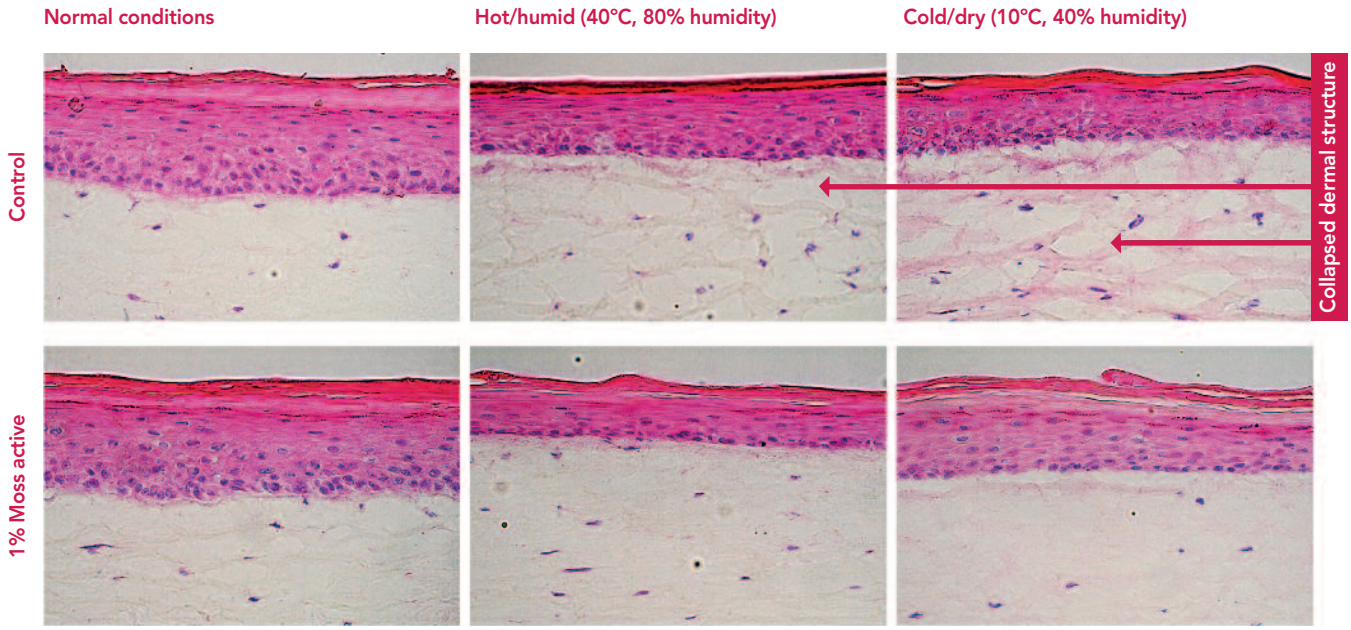

Figure 3: Adaptation of 3D skin models treated with the moss active to climatic changes.

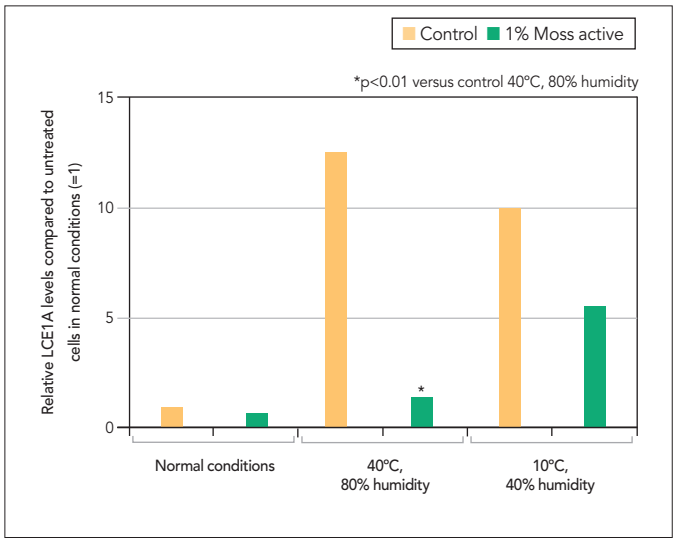

Figure 4: Expression of the stress marker LCE1A in 3D skin models determined with immunofluorescence and image analysis.

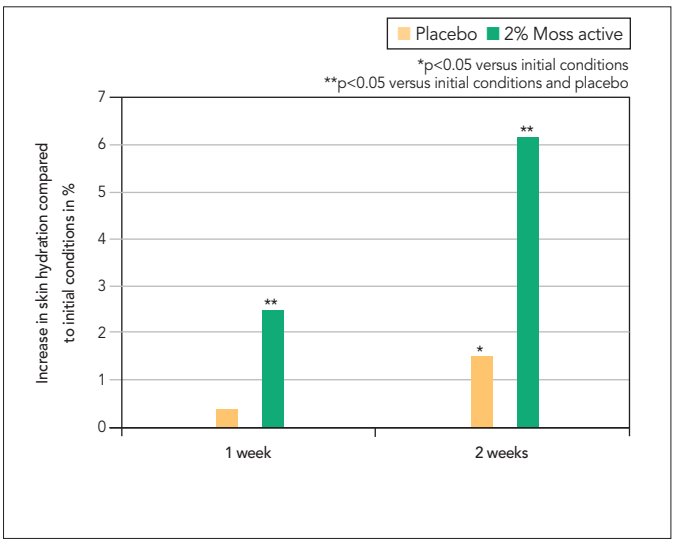

Figure 5: Improvement of skin hydration with the moss active.

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, US) and the acquired images were analysed for the standard deviation value of skin tone (which corresponds to inhomogeneity) on the cheek region by Image-pro®plus (MediaCybernetics, US). A smaller standard deviation signifies a more even skin tone.

Results and discussion

Moss extract improves cell nucleus health in aged skin cells

To assess the ability of *Physcomitrella patens* 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 *Physcomitrella 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 ageing and nucleus health (Fig 1).[8] Notably, RanBP17, a protein responsible for transport of protein

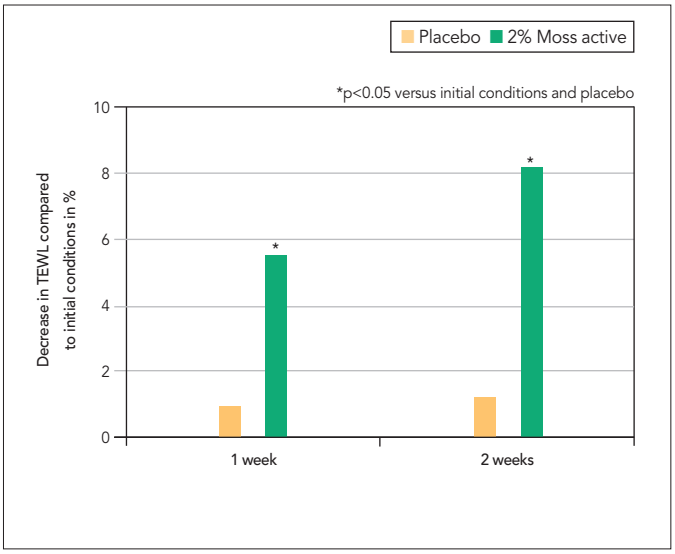

Figure 6: Improvement of TEWL with the moss active.

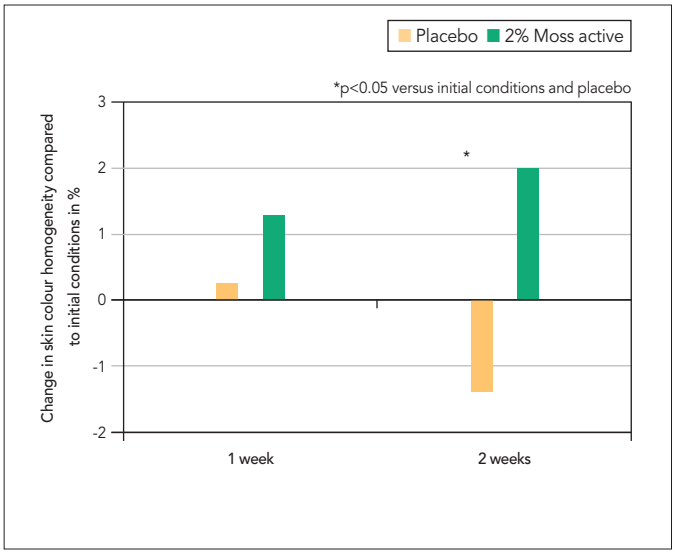

Figure 7: Improvement of skin tone homogeneity with the moss active.

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 ageing marker.[9]

When comparing old and young keratinocytes, we could nicely reproduce the reported downregulation of the three marker genes in old keratinocytes. Treatment of old keratinocytes with *Physcomitrella 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 2). Therefore, treatment with the *Physcomitrella 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 fibre structure was disorganised and collapsed and fibre density was reduced (Fig 3). Furthermore, expression of the stress marker LCE1A was increased demonstrated via immunofluorescence (Fig 4). Skin that was treated with 1% moss active did not display these drastic changes in dermal structure and gene expression of stress markers (Figs 3, 4) 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 (Figs 5-7). The positive effect on skin tone homogeneity was also visible in photographs taken of the volunteers (Fig 8). Overall, a skin improvement despite stressful weather conditions and improved skin adaptation to daily environmental changes was observed with moss active treatment.

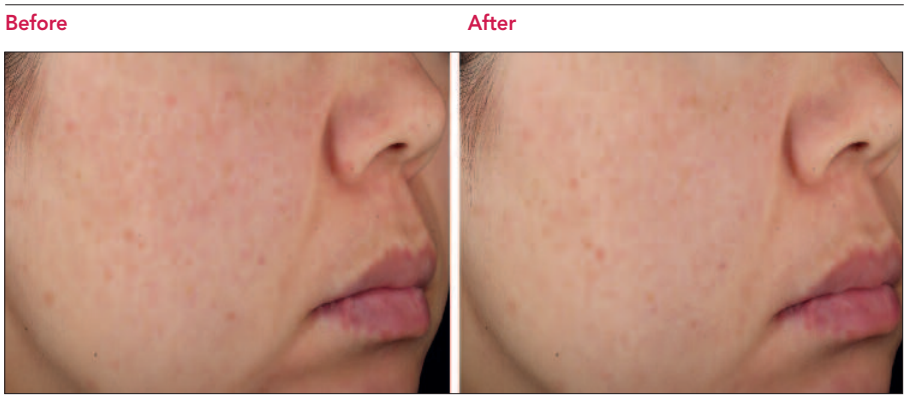

Figure 8: Visible improvement of skin tone with the moss active.

Conclusion

A novel active ingredient was developed using the innovative moss cell technology. The moss active demonstrated a positive influence on cell nucleus health genes and supported skin adaptation to climatic changes *in vitro* and *in vivo*. The result is a more refined skin tone and a more resilient skin after just two weeks. PC

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