Journal of Food, Nutrition and Diet Science
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Short communication

Nutritional supplementation of an apple callus extract to target epidermal aging

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Abstract: The global increase in life expectancy has led to a growing elderly population, which places a significant burden on healthcare systems. As a result, there is a growing interest to improve healthspan and promote healthy aging with accessible means, such as nutrition and lifestyle interventions. The skin, the body's largest organ and first line of defense against environmental stressors, is particularly vulnerable to aging due to both intrinsic and extrinsic factors. Various molecular and biochemical changes can drive skin aging, including the depletion of stem cells, which can lead to impaired skin properties. Nutrition plays a crucial role in skin aging, and dietary supplements could be a viable intervention to support a healthy skin. In this study, a plant callus extract from a specific apple variety (Malus Domestica Borkh), the Uttwiler Spätlauber, was evaluated as a dietary supplement to combat epidermal aging. The extract was found to maintain the function of keratinocyte progenitor cells during aging in vitro. Furthermore, an open-label pilot clinical study involving 31 female volunteers showed that the intake of apple callus extract led to improvements in skin elasticity, density, and firmness after 56 days of supplementation. This study highlights the potential of plant callus extracts as natural dietary supplements to support healthy skin aging. The use of plant callus cultures provides a sustainable and reproducible source of bioactive molecules, which is gaining acceptance in the nutraceutical market. These findings suggest that dietary interventions can play a role in promoting healthy skin aging and contribute to a holistic approach to improving healthspan during the aging process.

Keywords: Epidermal aging, Keratinocyte progenitors, Plant cell culture, Food supplements

1. Introduction

In the last century, the global average life expectancy has greatly increased and is now over 70 years [1]. Consequently, we are confronted with a demographic change in our society, which leads to a marked increase in the elderly population. Currently, the number of people aged 60 years and older is 12 %, a number that is estimated to almost double to 22 % by the year 2050 [2]. As such, the population aging has a large impact on almost all sectors of society, most importantly on the health care sector. The burden of disease increases with age, which results in reduced healthspan (the duration of life spent in good health) and quality of life, despite longer life expectancy. Therefore, improving the healthspan during aging is a strategy set by many professional healthcare organizations. In particularly, non-pharmaceutical and more accessible approaches such as nutrition and lifestyle interventions are of gaining interest [3-5].

The skin is the largest organ of the human body and importantly also the first protective physical barrier to the external environment. As such, it is constantly exposed to

Received: Dec.20, 2023; Revised: Jan.29, 2024; Accepted: Feb.19, 2024; Published: Feb.28, 2024

DOI: https://doi.org/10.55976//fnds.22024123718-28

environmental stressors such as UV radiation, pollutants, or temperature, which can impair the skin and lead to skin aging [6-9]. In addition, intrinsic factors such as age, hormone levels, genotypes and metabolism can contribute to the progressive damage [10-12]. Importantly, extrinsic factors such as personal diet can also modulate individual skin aging [13, 14], which provides a targetable field for anti-aging interventions. Many vitamins have been investigated for their effects on skin aging, including vitamin A, vitamin C and vitamin D [15]. Furthermore, trace elements such as copper and selenium have been proven to be essential for skin homeostasis by driving processes such as protein synthesis, keratinocyte function and antioxidant systems [16, 17]. These beneficial nutrients are often sought to be boosted by the means of dietary supplements.

Aging is driven by various alterations in molecular pathways and biochemical processes, the so-called "hallmarks of aging". Their induction and interaction with each other can lead to cellular damage, resulting in declined physiological function of tissues and organs [18, 19]. One of the hallmarks is stem cell exhaustion, which describes the decline in the number of stem cells and their self-renewal capacity [20]. Progenitor and stem cells are also subject to the hallmarks of aging, which leads to accumulated cellular damage, impaired cell differentiation and eventually to stem cell exhaustion [21]. In the skin, various niches of progenitor and stem cells can be found, including keratinocyte progenitor cells, melanocyte stem cells, hair follicle stem cells, interfollicular epidermal stem cells and dermal stem cells [22]. Age-related dysfunction or depletion of stem cells can lead to impaired skin properties, such as reduced wound healing capacity, epidermal thinning, a decrease in collagen synthesis or dermal atrophy [23-25]. In hair follicles, the depletion of stem cells leads to a reduced regenerative capacity and eventually to hair loss [26]. Therefore, supporting the skin stem cell niche can be a way to support healthy skin aging.

One possibility to maintain a thriving stem cell niche for healthy skin aging would be to support the skin intrinsically with dietary supplements. The global market for dietary supplements and nutraceuticals is continuously increasing, especially the interest in natural and sustainably sourced products [27]. Such sustainable production of plant-based material can be realized with plant cell culture systems, one of which includes the generation of plant callus material. Therein, the material is obtained from plant tissue explants, which are grown in sterile conditions and with the correct media composition, proliferate to a callus of dedifferentiated totipotent plant cells that can be further expanded in liquid culture [28]. These plant "stem cell" cultures often produce similar quantities of beneficial metabolites as found in the full plant or can be subject to elicitation strategies to optimize the yield [29], making plant callus extracts a valuable source of natural dietary supplements.

In this study, the efficacy of a plant callus extract from a special Swiss apple variety, the Uttwiler Spätlauber as a dietary supplement to combat epidermal aging was evaluated. This apple variety is well-known for its very good storability. The fruits can be stored for at least 9 months after harvest without any significant shrivelling effects [30]. A proteomics analysis was performed to identify differences between plant callus extracts and ripe apple tissue. In vitro effects demonstrated that the extract maintains keratinocyte progenitor cell function during aging. Additionally, in a pilot study investigating the clinical effects of the extract as a dietary supplement, anti-aging benefits for the skin were observed.

2. Material and methods

2.1 Apple callus extract preparation

Apple callus cultures were obtained from the Uttwiler Spätlauber variety according to an established in-house protocol that follows the principle of wound induced callus formation [31]. In short, plant tissue materials were wounded with sterile blades and under sterile conditions and the explants were maintained on solid nutrient agar plates until a callus formed. The callus was then submitted to liquid culture in 500 mL flasks containing a liquid medium containing sucrose, vitamins, minerals, trace elements and benzylaminopurine (BAP) (< 0.1 mg/kg) and 2,4-dichlorophenoxyacetic acid (2,4-d) (< 0.25 mg/kg), which are synthetic analogues of the plant hormones auxin and cytokinin, respectively. The cells are grown in the flasks at room temperature with gentle shaking before being transferred to 20 L sterile bioreactor bags (Wave-Biotech AG, Switzerland) and cultured in a flexible bioreactor-system (Wave-Biotech AG, Switzerland) at 25° C, in the dark, set to 9° and 20 rpm/min, with an air flow of 5-10 L/h. Upon complete metabolism of the sugars in the medium (3-4 weeks), the cell suspensions were mixed with 15% ethanol and homogenised under high pressure (1,000 bar) to disrupt the cells, followed by an ultra-high temperature treatment at 113 °C with a flow rate of 300 kg/h.

Unless otherwise specified, the in vitro studies were performed using the described liquid apple callus extract (PCT Md). The clinical study was performed with a powder version (PCT Md Nu) prepared by spray-granulation of the liquid extract on an isomalt carrier (PhytoCellTec™ Md Nu, provided by Mibelle Group Biochemistry, Buchs, Switzerland). For miRNA analysis, apple callus cells were harvested on day 1 and day 14 of liquid culture and immediately frozen at -80 °C without further processing. For proteomic analysis, the apple callus cells were harvested after 24 days of liquid culture and immediately frozen at -80 °C without further processing.

2.2 miRNA analysis

Plant miRNA was isolated using a miRNA purification kit (Norgen Biotek Corp., Canada) according to the manufacturer's instructions. In short, approximately 50 mg of ripe apple tissue and 5 × 106 apple callus cells (cultured and harvested as described above) were homogenized in liquid nitrogen with pestle and mortar. After evaporation of the liquid nitrogen, the assay buffer was added, and the tissue was further homogenized before being centrifuged. The supernatant was supplemented with EtOH (100 %) and vortexed. Small RNA (< 200 nucleotides) was purified by a series of column centrifugation steps. miRNA quantification was performed by UV spectroscopy, measuring the absorbance of each sample at 260 and 280nm (UV-1800 UV-Vis Spectrophotometer, Shimadzu Scientific Instruments Inc., USA). The resulting concentrations were normalized to the dry weight of each sample. All experimental samples were processed in triplicates, from 2 independent experiments (n=6).

2.3 Proteomics analysis

2.3.1 Sample preparation

For a preliminary qualitative proteomic comparison of apple fruit and apple callus cells, 50 mg of ripe apple tissue from Malus domestica Uttwiler Spätlauber and 5 × 106 apple callus cells were homogenized in liquid nitrogen with pestle and mortar, transferred to a tube and extraction buffer (1 M sucrose, 0.5 M Tris-HCl 8.0, 1 % NP-40, 1 % sodium deoxycholate, 50 mM ascorbic acid, 0.1 M KCl, 50 mM EDTA, 10 mM DTT, 1 % protease inhibitor cocktail) was added. After centrifugation (4700 x g for 10 min at 4 °C), the supernatants containing the solubilized proteins were precipitated with cold 0.1 M ammonium acetate/methanol at -20 °C overnight. The following day, after centrifugation (4700 x g for 10 min at 4 °C), the pellets containing the precipitated proteins were washed 3 times with acetone and dissolved in 4 % SDS, 50 mM Tris-HCl 8.0. Protein quantification was performed with Biorad Protein Assay (KüB, Cat#500-0006). Protein samples were precipitated with a final concentration of 5 % trichloroacetic acid. Protein pellets were washed twice with cold acetone and dried. Afterwards the protein pellets were dissolved in 10 mM Tris-HCl 8.2, 2 mM $CaCl_2$. The proteins were reduced and alkylated with TCEP (tris (2-carboxyethyl) phosphine) and chloroacetamide was incubated for 30 min at 37 °C in the dark. Then the proteins were cleaved into peptides by tryptic digestion overnight at 37 °C. To quench the reaction, the samples were acidified to a final concentration of 1 % TFA (trifluoroacetic acid). Afterwards the peptides were desalted by C18 stage tip clean up and dried.

2.3.2 LC-MS/MS and data analysis

Dried peptides were dissolved in 3% acetonitrile, 0.1% formic acid and then transferred into autosampler vials for the analysis of liquid chromatography-mass spectrometry (LC-MS). The samples were injected into an M-class UPLC coupled to a Q-Exactive™ mass spectrometer (Thermo Scientific). The acquired MS data were processed for identification using the search engine MSFragger (Fragpipe v17). The spectra were searched against the UniProt database entry of Malus domestica (Proteome ID: UP000290289). The variable modifications were set to Acetyl (Protein N-term), Oxidation (M), Deamidation (NQ) and the fixed modifications were set to Carbamidomethyl (C). Gene ontology analysis was performed using STRING database [32], specifically setting the origin to "Malus domestica" and filtering for a cut-off False Discovery Rate (FDR) of ≤ 0.01.

2.4 Keratinocyte progenitor cell culture

Keratinocyte progenitor cells were obtained from cultures of primary human keratinocytes isolated from human tissue biopsies according to an established proprietary protocol (CELLnTEC Advanced Cell Systems, Switzerland). The isolated primary keratinocytes were cultured in the keratinocyte growth medium CnT-57 (CELLnTEC) to enrich keratinocyte progenitor cells, in a humidified incubator at 37 °C and 5 % CO_2. CnT-57 is a serum-free keratinocyte progenitor cell culture medium with a low calcium content and a low concentration of bovine pituitary extract [33].

2.5 Colony forming efficiency (CFE)

To determine CFE, 200 keratinocyte progenitor cells were seeded to T-25 flasks, before being respectively treated with 0.01 % (0.1 mg/mL) or 0.04 % (0.4 mg/mL) of apple callus extract (PCT Md) for 7 days, in a humidified incubator at 37 °C with 5 % CO_2. After treatment, cells were stained with crystal violet and colonies were counted manually via brightfield microscopy. The CFE was calculated by dividing the number of colonies counted by the cells seeded: CFE (%) = (colonies counted/cells inoculated) × 100. All conditions were performed in duplicate.

2.6 3D epidermis formation

For the 3D epidermis formation, early passage human progenitor cells (p5), late passage keratinocytes (p14) and late passage keratinocytes (p14) that were previously cultured and maintained in 0.01 % (0.1 mg/mL) PCT Md for 10 passages were used. Cell suspensions (5 × 105 cells) were seeded to polycarbonate inserts (0.4 µm pore size) and cultured in maintenance medium (CnT-07, CELLnTEC), in the presence or absence of 0.01% (0.1mg/mL) PCT Md for 2 days, in a humidified incubator at 37°C with 5 % CO_2. Then the media were changed to differentiation media (CnT-02-3D, CELLnTEC) supplemented with or without 0.01 % PCT Md, the

cultures were airlifted and grown for a further 26 days (total 28 days). Media were replaced every alternate day. On day 14, day 21 and day 28 the 3D models were harvested and fixed over night at 4 °C in 4 % PFA-PBS before being processed for Hematoxylin-Eosin (H&E) staining. All experimental conditions were performed in duplicate.

2.7 H&E staining

Membranes containing the 3D models were carefully removed from the inserts, set between 4 % PFA-soaked biopsy foam pads, and were placed into embedding cassettes. After paraffin embedding, 5 µm sections were cut and mounted to slides (Menzel Gläser, Germany). H&E staining was then performed according to a routine protocol (CELLnTEC). Images were acquired with a brightfield microscope at 20x magnification.

2.8 Clinical study

2.8.1 Participants

A total of 31 female volunteers, Caucasian, aged 42 - 65 years (54.6 ± 6.5), with phototype Fitzpatrick I - III and photo-aged skin were included in an open-label pilot study to test the clinical efficacy of the apple callus extract (PCT Md Nu) by oral supplementation. Exclusion criteria were an ethnicity other than Caucasian, a phototype > III, a severe systemic or dermatologic disease, history, or evidence of drug and/or alcohol abuse, use of any topical (drug-containing) or cosmetic anti-aging product on the test area within the previous four weeks, intake of any kind of dietary supplements within the previous eight weeks, or participation in any other clinical study within the previous eight weeks. The study was conducted at the Skin Test Institute (Neuchâtel, Switzerland) in accordance with the Declaration of Helsinki prior to participants providing written and informed consent.

2.8.2 Study design and product intake

The pilot study was open and monocentric. Participants attended an environmentally controlled facility for the evaluation of skin parameters. During a preconditioning phase of 14 days, participants did not apply any anti-aging products on the measurement area (inner forearm). Following baseline determination (t0) of the skin parameters defined in the section below, participants dissolved 10 mg of PCT Md Nu (in the form of 100mg powder preparation, supplemented with maltodextrin) in 200 mL of a cold liquid with the choice such as water or fruit juice, once daily, in the mornings, for 56 days. After 56 days (t1) the test parameters were measured, and participants completed a self-assessment questionnaire concerning the performance of the treatment.

2.8.3 Skin measurements

The skin parameters were measured on the right inner forearms of the participants. Elasticity measurements were performed with a Cutometer® (Courage & Khazaka, Germany), with 3 replicas taken at each timepoint and the parameter R5 (net elasticity Ur/Ue) selected for analysis. Skin firmness was assessed with a Reviscometer® (RVM 600, Courage & Khazaka), at 7 angles (0°, 30°, 60°, 90°, 120°, 150°, 180°) to the skin with 3 replicas taken at each angle and timepoint. Epidermis and dermis density measurements were performed with a DermaScan® C at 20 MHz, medium focus, B-mode (Cortex Technology, Denmark) and 3 replicas per timepoint. Ultrasound images were recorded and processed by the software provided.

2.9 Statistical analysis

Preclinical data was tested by one-way ANOVA with Tukey post hoc test using GraphPad Prism 8 software (GraphPad, USA), results are depicted as mean ± SD. For the clinical study, paired data were tested by nonparametric permutation analysis using StatXact software (version 5.0.1, Cytel Ldt., USA), results are depicted as mean ± SEM. P values < 0.05 were considered significant.

3. Results

3.1 Apple callus cells: comparison with ripe apple tissue

Apple callus cultures were obtained by wounding of a plant tissue, yielding cells of a less differentiated pluripotent state that were subsequently selected on growth media and propagated in culture (Figure 1). As callus cell cultures contain the full genetic information of whole plants, they also possess the totipotency for the biosynthesis of secondary metabolites [28]. Interestingly, studies have indicated that callus cells may contain higher amounts of secondary metabolites than the fresh fruit of the mother plant [34, 35]. To investigate and compare the proteins present in ripe apple tissue and apple callus cells, a quantitative miRNA analysis was firstly performed. Compared to fresh apple tissue, the callus cells contained significantly higher amounts of small RNA (< 200 nucleotides) after 1 day of liquid culture (Figure 2A). The levels of miRNA were further increased after 14 days of culture, with an almost four-fold increase compared to the fresh apple tissue.

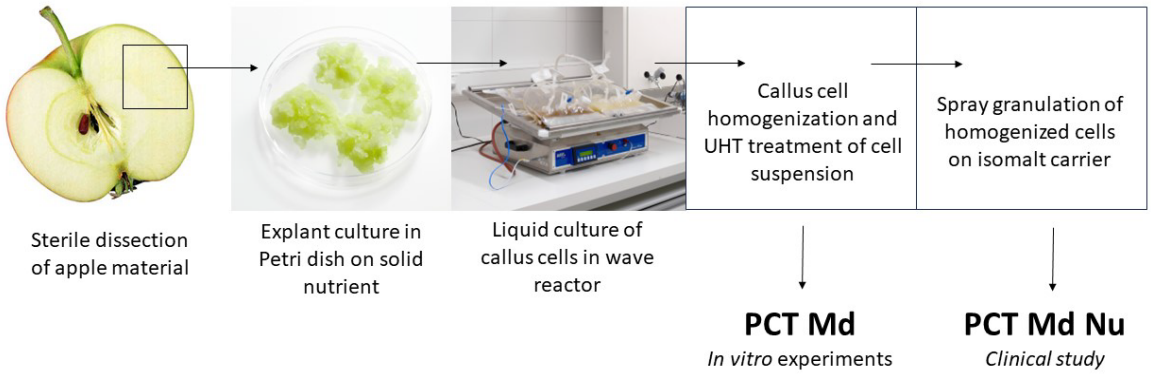

Figure 1. Overview of plant callus production

Explant obtained from sterile dissection of apple tissue is cultured on solid nutrients in a petri dish. After callus formation, cells are transferred to liquid culture in wave reactor bags. Callus cells were then homogenized, and the cell suspensions used for in vitro experiments (PCT Md) or spray granulated on isomalt carrier were used for the clinical study (PCT Md Nu).

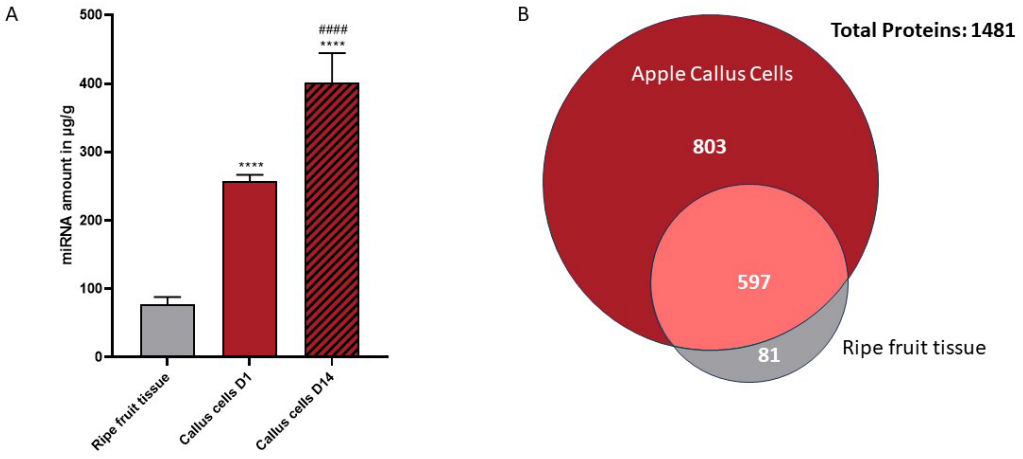

Figure 2. Comparison of apple callus cells and ripe fruit tissue

A) miRNA levels detected µg/g in ripe fruit tissue (grey) versus apple callus cells (magenta) after one day (D1) and 14 days (D14). N=6, mean ± SD, one-way ANOVA, ****p <0.0001 vs ripe fruit tissue, ####p <0.0001 vs callus cells D1. B) Number of genes detected via proteomic analysis in apple callus cells (magenta) and ripe apple tissue (grey). Overlay indicate common genes detected.

To follow up these observations, an investigative proteomic analysis using LC-MS/MS was performed on ripe plant tissue and apple callus cells, respectively. Within the global proteomic investigation, a total of 1481 proteins were identified in apple fruit and apple callus cells (Figure 2B). Therein 678 proteins were identified in the ripe fruit tissue, whereas 1400 proteins were identified in the callus cells. This difference may in part be explained by the characteristics of the samples, as apple fruit has a firmer pulp, and the sample processing may have been affected. Alternatively, the increased number in the callus cells may also be indicative of a higher abundance of proteins and subsequent metabolites, as was assumed from the preliminary miRNA analysis. In total, 597 from the 678 proteins (88.1 %) found in the apple fruit were also identified in the callus culture, which implies that these proteins may also play a functional role in the callus cells. Consequently, 81 proteins were unique to the ripe apple tissue, whereas 803 proteins were specific for the callus culture cells. To further investigate the proteomic profile of the callus culture cells, a gene ontology analysis was performed with the 803 proteins unique to the callus culture. Overall, 94 of the 803 proteins could be assigned to a certain function, therein 22 were specific for the metabolism profile of dedifferentiated cells. Furthermore, the most significant associations were made for proteins

assigned to biosynthesis (n = 12) and metabolic (n = 30) processes. Interestingly, the proteomic analyses revealed that over 33 % of the identified proteins are still considered as uncharacterized with little available information. To sum up, further research on apple fruit protein is needed to explore the complex biological processes and molecular mechanisms for a comprehensive understanding.

3.2 Callus extract stimulates progenitor cell CFE and improves epidermis formation in vitro

The treatment of keratinocyte progenitor cells with PCT Md for 7 days led to a concentration-dependent increase of CFE in human keratinocyte progenitor cells. Compared to control, treatment with 0.01 % (0.1 mg/mL), 0.04 % (0.4 mg/mL) of PCD Md induced CFE by 50 %, 95 %, respectively (Figure 3A). To further elucidate the effects of the callus extract on the function of keratinocyte progenitor cells, the capacity to form a 3D epidermis structure was evaluated in early and late passage cells. Primary human keratinocytes lose their progenitor cell characteristics after prolonged culture, as exemplified by altered cell behaviour, marker expression and the ability to complete the complex process of differentiation [36-38]. Therefore, monitoring 3D epidermal formation allows further insights into the status of progenitor cells. After 28 days of culture, early passage keratinocyte progenitor cells (p5) established a normal 3D model, with all epidermal layers present (stratum basale, spinosum, granulosum, corneum) (Figure 3B).

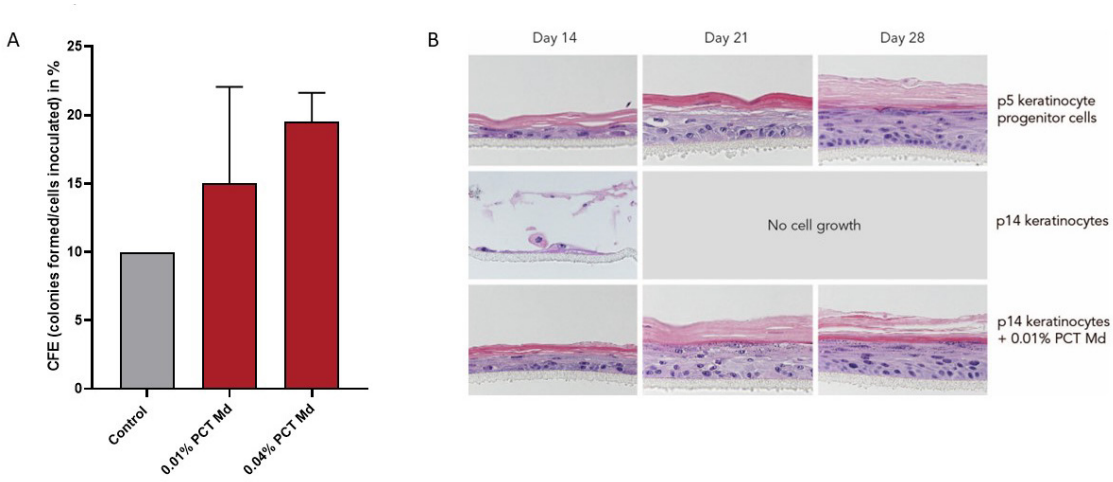

Figure 3. Effects of callus extract (PCT Md) on epidermal stem cells

A) Colony forming efficacy (CFE) of keratinocyte progenitor cells after treatment with 0.01 % (0.1 mg/mL) and 0.04 % (0.4 mg/mL) of the callus cell extract (magenta), compared to untreated controls (grey). N=2, mean ± SD. B) Representative images of 3D epidermis formation with progenitor cells of low ("young") passage p5, and high ("old") passage p14, treated with or without 0.01 % (0.1 mg/mL) PCT Md. N = 2, mean ± SD.

Compared to p5 keratinocyte progenitor cells, late passage cells (p14) were unable to form a 3D epidermal structure. On day 14 only a sparse and poorly structured monolayer was established, which did not develop further. At later timepoints (day 21, day 28), no epidermal structure was visible, which indicated further degradation occurring. In contrast, prolonged culture (10 passages) in media supplemented with 0.01 % (0.1 mg/mL) PCT Md and further treatment during the differentiation process strongly induced the ability of p14 keratinocyte cells to establish a stratified epidermis. These epidermal structures were indistinguishable from the p5 progenitor cell structures. These results show that the callus extract significantly increases the ability of late passage keratinocytes to establish 3D models. It is particularly striking given that untreated p14 cells were completely unable to form a 3D model, whereas extract-treated p14 cells formed a model, which was structurally normal, and seemingly indistinguishable from the control (P5) cells. The results highlight that the culture of keratinocyte progenitor cells with the callus extract can postpone the loss of primary characteristics and enable the cells to complete complex biological processes such as stratification and differentiation, even after serial passaging (aging) of the cells.

3.3 Nutritional supplementation of PCT Md Nu improves skin elasticity, density and firmness

To consolidate the preclinical findings that apple

callus extract has positive effects on skin cells, a pilot trial on healthy female volunteers was performed. 31 participants were instructed to consume 10 mg of PCT Md Nu daily, as a food supplement, for 2 months (56 days). The food supplement was well tolerated by the participants, no adverse effects or intolerance reactions were reported during the study. Moreover, the nutritional supplementation of the PCT Md Nu had beneficial anti-aging effects on the skin. Compared to initial conditions, skin elasticity was increased by 4.7 % (Figure 4A). In addition, skin firmness and density were significantly increased by 5.9 % and 10.5 %, respectively. The increase in skin density was also visible in ultrasonographic images of epidermis and dermis, where a reduction in low echogenic pixels was observed (Figure 4B). These ultrasound images result from increases in extracellular matrix components (collagen, elastin), and a reduction of interstitial water content, which consequently represents an increase in dermal and epidermal density. The positive results were subjectively confirmed by the volunteers. In total, 84 % of the volunteers reported that they felt better about their skin. 81 % noticed that their skin felt smoother and would have continued the treatment with the callus extract.

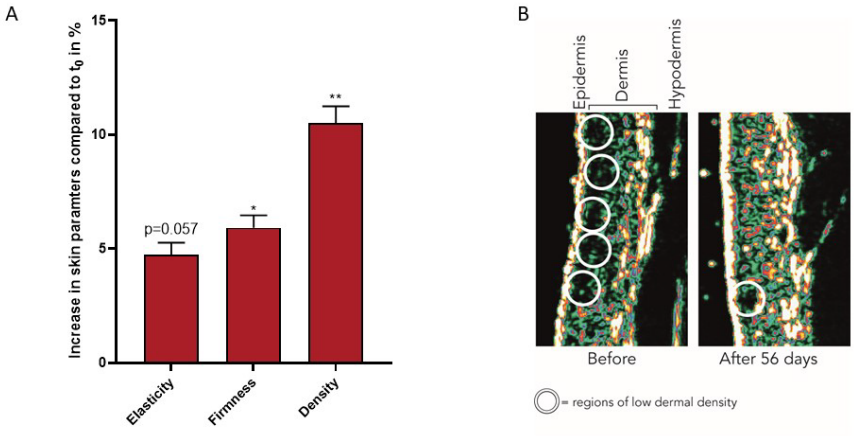

Figure 4. Clinical effects of nutritional supplementation of callus extract

A) Increase in skin elasticity, firmness, and density in the volunteers after daily supplementation of 10 mg PCT Md Nu for 56 days. N = 31, mean ± SEM, *p<0.05, **p<0.01 versus baseline (T0). B) Representative ultrasound images of dermis and epidermis density, before and after daily intake of 10 mg PCT Md Nu for 56 days. High density tissue is depicted in light colors, circles highlight dark areas that represent regions of low dermal density.

4. Discussion

Skin aging is multifactorial and can be influenced by both intrinsic and extrinsic parameters [39]. Aside from topical measures, combating skin aging by means of dietary supplements is an increasingly popular approach [40]. In this study, a plant callus extract was proven to be effective in vitro in preserving epidermal stem cell function during aging, which led to positive clinical effects when ingested as a dietary supplement. Plant material is one of the oldest sources of natural bioactive compounds, but environmental and regional restrictions may often hinder the commercial production of plant extracts. Furthermore, and importantly, the increasing risk of extinction poses a tangible threat to plants and fungi, and therefore requires for alternative means to obtain natural sources [41]. Plant callus cultures are promising sources for the sustainable production of bioactive molecules, and a favourable approach as the culture conditions are more easily controlled compared to whole plant systems [42]. The use of callus cultures not only limits the amount of fresh plant material needed but may also yield higher secondary metabolites compared to the whole plant culture. Indeed, studies have shown that callus cells can contain higher amounts of secondary metabolites than the fresh fruit extract [34, 35]. Our own comparison of callus and fresh fruit extract provided preliminary evidence that the callus may yield higher amounts of beneficial molecules. Further in depth proteomic and metabolomic analyses will provide further insights, and possibly elucidate the molecules of interest that exert the beneficial effects.

The consumption of fruit as a supplement to a healthy and balanced diet is well established. Due to the high content of vitamins, antioxidants and fibre, the nutritional value of fruit is evident, and its consumption is therefore also encouraged with recommended daily amounts in

dietary guidelines [43]. Numerous health-promoting effects of fruit in the diet have been reported, including decreased cardiovascular risk, neurological disease, diabetes and age-related decline [44]. When evaluating the skin, diets rich in fruit have reportedly led to a reduction in signs of aging. A Dutch study demonstrated that fruit-dominant diets were associated with less facial wrinkles [14]. With regard to apple dietary supplements, a clinical study performed in Italy found that daily supplementation of an apple polyphenolic extract (whole fruits, Annurca apple, Malus Pumila Miller cv. Annurca) for 8 weeks improved hair growth, density and keratin content in participants suffering from male and female pattern baldness [45]. Shoji et al further found that administration of an apple polyphenol supplement for 12 weeks in healthy women could reduce UV-induced skin hyperpigmentation, compared to placebo [46]. In both studies with apple extract, the researchers attributed the beneficial effects to the procyanidins content of the extracts, which may be also the case for the apple callus extract of this study.

Dietary supplements to target skin aging are a growing market worldwide and natural and sustainable substrates are of particular interest. Plant callus culture provides a viable approach to produce natural actives, at a reproducible and high yield. In the case of the apple callus extract described in this study, the novel food application was recently granted by the European Food Safety Authority (EFSA) [47], and the extract was classified as safe for use in food supplements. This is a further milestone in the development of novel natural actives, which reflects the growing acceptance of alternative and importantly sustainable sources for the food supplement industry. This benefits not only the nutraceutical market, but importantly also the field of skin aging. The novel food apple callus extract can now be used for dietary interventions that promote healthy skin aging.

Conclusions

Overall, protecting the skin internally with nutritional supplements is a promising approach to protect from exogenous damage and therefore promote a healthy skin. Such non-pharmaceutical lifestyle interventions can therefore contribute to the holistic approach suggested by health professionals and improve healthspan during aging with accessible means.

In conclusion, dietary supplements containing apple callus extract may help to improve skin conditions. Placebo-controlled interventions with a larger population size and longer study duration are desirable to confirm its effectiveness.

Authors' contributions

TG, FW, KN, and JB conceptualized the study and designed the experiments. TG, FW, KN and JB conducted experimental analysis and data interpretation. TG and JB wrote and/or edited the manuscript. All authors read and approved the final version.

Acknowledgments

We acknowledge our colleagues Florian Hofer, Cornelia Schürch Fred Zülli and Carlos Munoz for scientific support and sample preparation. We would also like to thank Alain Béguin and the team of the Skin Test Institute for the conduction of the pilot study.

Funding

The research was funded by Mibelle AG Biochemistry.

Institutional review board statement

This research follows the guidelines of the Declaration of Helsinki. This study was exempt from ethical review and approval due to the following reasons. The clinical study described in this manuscript was an open label pilot study and it was performed in an established Swiss Cosmetic test institute. To our current knowledge, this clinical study for a nutri-cosmetic active did not require an ethical review for human clinical trials due to several factors. Firstly, the study exclusively involved the use of an established cosmetic ingredient, which was recognized as safe for topical application. The extensive pre-existing safety data and a history of use in cosmetic formulations contributed to the exemption from ethical review according to EU/CH legislation.

Additionally, the study employed non-invasive and non-pharmacological interventions, without introducing any new or experimental elements that could pose risks to participants. The interventions adhered to established safety guidelines and were consistent with standard usage of nutritional supplements.

Moreover, the research employed solely non-invasive measurements that focused on cosmetic outcomes and emphasized improvements in skin texture, appearance, or similar aesthetic factors, rather than delved into medical or therapeutic claims. This cosmetic-centric approach further justified the exemption from ethical review.

The study design prioritized participant safety, privacy and informed consent, aligning with the despite the exemption. It was performed according to the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. It exemplifies a balanced approach, recognizing the nature of nutri-cosmetic interventions and ensuring ethical considerations

in line with the specific context and objectives of the research.

Informed consent statement

All participants of the study provided written and informed consent.

Conflict of interest

All authors are employees of Mibelle Biochemistry, Mibelle AG.

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