15TH INTERNATIONAL CONFERENCE

Skin Ageing & Challenges

Abstracts Book

November 5-6, 2024 - Malta

International Society of Microbiota

15th International Conference on

Skin Ageing & Challenges

November 5 - 6, 2024 – Malta

Jean Krutmann

President of Skin Ageing & Challenges

Marvin Edeas

Chairman of the Scientific Committee

Abstract Book DOI

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Welcome to the 15th Skin Ageing & Challenges Meeting

Dear Colleagues,

The scientific committee is pleased to welcome you to the 15th International Conference on Skin Ageing and Challenges 2024, held on November 5-6, 2024 at Corinthia Palace Malta and Online.

Skin Challenges 2024 is organized under the patronage of the International Society of Microbiota (ISM) and the World Mitochondria Society (WMS).

The conference will bring together leading scientists and researchers to explore the latest advancements in skin health, anti-aging, and cellular rejuvenation. Central themes include cellular senescence, mitochondrial function, microbiota's influence, olfactory receptors and iron regulation, each with significant implications for delaying or reversing skin aging. Another key highlight is the exploration of exosomes, tiny extracellular vesicles emerging as potential carriers of regenerative compounds, which offer promising avenues for skin rejuvenation.

We would like to thank all members of scientific committee, speakers and chairpersons of the Skin Ageing & Challenges Community for their contribution.

We hope that you will enjoy the Skin Ageing & Challenges 2024 Conference and that your interactions with colleagues from many countries will stimulate a creative exchange of ideas and challenges

All our regards,

Jean Krutmann
President of Skin Ageing & Challenges
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DOI Information

We are pleased to announce that the Skin Ageing & Challenges 2024 Abstracts Book will be assigned a **DOI (Digital Object Identifier)**, ensuring a permanent and easily accessible online presence for the entire collection of work.

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How to cite your paper in the Abstracts Book?

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Citation example:

Smith, J. (2024, November 5-6). **Innovative therapies in skin aging and skin rejuvenation**. *p. 45*, Skin Ageing & Challenges 2024, Malta. DOI

Practical Information

We would like to take the opportunity to give you some additional information about the meeting arrangements.

The Abstract book contains:
• Speakers' abstracts (the abstracts of the oral presentations follow the order of the program)
• The abstracts of posters on display

Badges

Upon registration you have received your own personal badge. Please wear this badge during the entire meeting including the coffee breaks and lunch.
The conference secretariat will be located in the area in front of the conference hall.

Instructions for participants

Chairpersons: The Chairpersons will be seated at the president's table.

Speakers: Speakers are invited to give their Power Point presentations for downloading on the computer to the technical team. As the schedule is rather tight and to allow sufficient time for discussions, we would be very much obliged if the timing requirements were respected.

Poster Contributors: Please ensure that your poster is displayed at the appropriate location, please respect your poster number. Please remember to remove your posters at the end of the conference. The Poster contributors are invited to stand by their poster during the poster sessions.

Speakers Dinner
A dinner is organized on November 5 at Corinthia Palace Hotel.
If you registered for this dinner, please join the group at 20h00 at the restaurant.

Mobile Phones
As a courtesy to the speakers and other delegates, please turn off your mobile phones or to silent whilst in the conference room. Please do not take pictures of the slides without the consent of the presenting author.

Questions
Please state your name and institution or company before asking your question.

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Personal Belongings
Please keep your valuables and working materials with you at all times. We would advise you to keep your name on the conference notes, as we may not be able to replace these if lost. Skin Challenges Team and Corinthia Palace can't be held responsible for any loss or damage to your property.

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Malta & Online

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Abstracts of Day 1

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REVERSING SKIN AGING: THE POWER OF SENOMORPHICS AND SENOLYTICS

SCHARFFETTER-KOCHANEK, Karin [1,2], SINGH[1,2], Karmveer, AGHAPOUR[1,2], Mahyar, CHENG[1,2], Jinnan, WLASCHEK[1,2], Meinhard, MAITY[1,2], Pallab

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Skin aging is significantly enforced by the accumulation of senescent dermal fibroblasts. Various stressors among them reactive oxygen species damage macromolecules inside and outside fibroblasts are responsible. In addition, natural killer cells fail to adequately remove senescent fibroblasts from skin and other organs. Senescent fibroblasts are characterized by irreversibly arrested growth, failure to undergo apoptosis and an enhanced release of the proinflammatory senescent-associated secretory phenotype (SASP) - which together profoundly impact on endogenous stem cell niches. The SASP spreads senescence to neighboring cells, and, in consequence, promotes skin aging.

In this lecture, we will further discuss redox distress-depending pathways resulting in the irreversible installment of fibroblast senescence and the reduced release of growth factors among them IGF-1. Also, the plasticity of distinct dermal fibroblast phenotypes and the depletion of fibroblast subsets on skin homeostasis and aging will be addressed. Finally, the recently introduced senolytic and senomorphic approaches will be discussed in respect what has been achieved for skin aging.

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SUBSTANCES PROMISING ACTIVE SKIN REJUVENATION EFFECTIVELY SUPPORT FAST SKIN WOUND HEALING

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Several substances with promising anti-aging and rejuvenation activity were extracted from natural sources [1]. It is demonstrated that they synchronously intensify cell division and apoptosis. It was hypothesized that same substances could also support better wound healing. Aging is often accompanied by decreased wound healing efficiency, and the effect of such substances on tissue regeneration was studied. Substantial acceleration of deep wound healing with injections of hyaluronate gel-based compositions containing such substances was experimentally confirmed in laboratory animals. Trials with middle aged and elderly human volunteers confirm high potential of such interventions, including faster healing of infected skin wounds of different etiology resulting in full skin regeneration almost without scar tissue formation, and in recovery of full skin functionality (including the restoration of hair follicles). According to the developed conceptual model of cell number homeostasis, support in effective tissue regeneration and wound healing by mentioned substances could result from a simultaneous effect of accelerated cell proliferation and apoptosis accompanied by a pronounced antimicrobial activity [2]. Extensive cell culture experiments with *ex-vivo* human dermal fibroblasts were carried out to assess the action of the substances. Addition of the substances intensifies the production of collagen and elastin in the fibroblast cell culture without influencing cell vitality. Addition of the substances also accelerates fibroblast functionality recovery after stress-conditioned cultivation (increased pressure, increased O_2 and decreased CO_2 content).

Corresponding presentation will cover results of the cell culture studies and preliminary results on the acceleration of deep skin wound healing, and of the preclinical and limited clinical trials on the accelerated tissue regeneration using hyaluronate gel-based compositions containing small concentrations of the substances that accordingly to the developed cell-level model also exhibit rejuvenation and anti-aging activity.

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A GROUNDBREAKING INTERVENTION EFFECTIVELY REDUCES SENESCENCE IN OLD PRIMARY CELLS BY TARGETING A NEW HALLMARK OF AGEING

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Introduction: The combinatorial action of splicing factors regulates expression of alternative mRNA isoforms. Splicing factor expression declines during ageing and is a major provocation for cellular senescence [1]. We have generated a portfolio of small molecule modulators of splicing factor expression able to ameliorate multiple features of senescence in human primary dermal fibroblasts [2]. Therapies to rejuvenate senescent cells will improve skin appearance and health.

Materials & Methods: Following an in-silico screen of over 200,000 small molecules, we tested various compounds for effects on senescence in senescent primary human dermal fibroblasts. Results were analysed using a proprietary algorithm to identify those molecules that successfully reversed senescence in the cells.

Results: We have identified multiple small molecule compounds that reduce senescent cell load and DNA damage without affecting cell proliferation. Several of these molecules have shown effects comparable to dasatinib and quercetin (DQ), which have already demonstrated benefits in clinical trials.

Conclusion: Cellular senescence plays an important role in age-related skin dysfunction. Dysregulation of mRNA splicing represents a new and druggable driver of cellular ageing, which can be targeted to treat the causes rather than the consequences of cellular ageing. We can create an effect similar to DQ using small molecules suitable for cosmeceuticals.

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SELCOPINTIDE AS A NOVEL THERAPEUTIC AGENT TO COMBAT OXIDATIVE STRESS AND PROMOTE SKIN HEALTH

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Introduction: The skin is continuously exposed to external stimuli, such as ultraviolet (UV) radiation and fine particulate matter, which generate reactive oxygen species (ROS) that lead to skin damage and aging. ROS inhibit collagen synthesis and reduce skin elasticity. In Copine-7 (CPNE7) knockout (KO) mice, the collagen fibers in the dermis layer were found to be disorganized, and ROS levels were significantly elevated. This suggests that CPNE7 plays a key role in regulating oxidative stress and maintaining collagen in the skin. This study aims to investigate whether Selcopintide, a CPNE7-derived peptide, can reduce ROS levels and promote collagen synthesis in the skin.

Materials & Methods: Various concentrations of Selcopintide were applied to skin cells to evaluate its effects on ROS suppression and collagen synthesis promotion. Artificial skin tissues and in vivo models were also used to analyze tissue regeneration, skin thickness, collagen content, and elasticity changes.

Results & Conclusion: Selcopintide effectively suppressed ROS and promoted collagen synthesis in dermal fibroblasts, alleviating skin damage. This suggests that Selcopintide has potential as a therapeutic agent for improving skin health.

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NEW LIFE FOR VITIS VINIFERA L. LEAVES EXTRACTS THROUGH PROBIOTIC-MEDIATED FERMENTATION: INVESTIGATING THEIR ANTIMICROBIAL POTENTIAL TOWARDS METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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Staphylococcus aureus is a skin pathobiont that in dysbiotic conditions may contribute to infections, barrier integrity loss, skin aging, and inflammation[1]. Due to the spread of multidrug resistant strains like methicillin-resistant *S. aureus* (MRSA) ones, new alternative preventive and therapeutic approaches are needed. In this scenario, plant waste like *Vitis vinifera* L. leaves extracts (VVLEs) and derived fermented extracts (fVVLEs) could exert antioxidant, anti-inflammatory, and antipathogen potential[2,3].

Starting from these findings, we compared VVLEs and fVVLEs in terms of: i) analytic profile (i.e., total phenol content and lactic acid content quantification); ii) antioxidant activity (DPPH assay); iii) *in vitro* antipathogen potential towards MRSA ATCC 43300 viability (e.g., alamarBlue assay).

Both the products are rich in polyphenols and have good antioxidant activity. Moreover, fVVLEs show high lactic acid levels. Comparing VVLEs and fVVLEs effects on MRSA, data show their different degree of antimicrobial potential.

Overall, exploitation of grape biomasses satisfies the standards required by the green circular economy model, while providing new alternatives to manage MRSA global burden. Further investigations are needed for their future possible use in the management of dysbiosis-associated skin issues.

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THE EPITRANSCRIPTOME AND SMALL NUCLEOLAR RNAs IN CELLULAR SENESCENCE AND SKIN AGING

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Introduction: The accumulation of senescent cells promotes the aging of several tissues, including the skin. The lack of specific senescence biomarkers complicates the development of pharmacologic and cosmetic interventions. Around 200 2'-O-methylations and pseudouridines decorate ribosomal RNA, but their role in skin aging remains unexplored.

Materials & Methods: We induced senescence by stress exposure in primary human keratinocytes, fibroblasts, and melanocytes to study rRNA modification changes and thereby identify novel biomarkers and intervention targets. Next-generation sequencing was used to determine changes at known rRNA modification sites between senescent and proliferating cells.

Results: We identified several consistently altered sites in the different senescence models. To study the functional consequences of these changes, we depleted snoRNA guiding modifications at the identified sites by antisense oligonucleotides and CRISPR/Cas9. We also visualized these snoRNAs in tissue sections of young and aged human skin by BASEscope in situ hybridization.

Conclusion: Our data show the variability of rRNA modifications and corresponding snoRNAs in different senescent human skin cell types. Understanding the mechanisms underlying the regulation of snoRNA expression and RNA modifications and their physiological function might pave the way for developing novel strategies to target senescent cells and slow skin aging.

IRON METABOLISM OF THE SKIN: RECYCLING VERSUS RELEASE

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Introduction: The epidermis of the skin protects the body against exogenous stressors and undergoes continuous regeneration. The maintenance of the epidermis during aging depends on the sufficient supply with nutrients and trace elements. Insights from recent studies suggest that the metabolic balance of the epidermis is also critically controlled by active and passive transport of chemical substances within the epidermis. Iron is involved in important epidermal processes, such as cellular respiration and detoxification of xenobiotics, and contributes to the control of the skin microbiome.

Materials & Methods: The iron metabolism of the epidermis was investigated by gene expression studies and targeted gene ablations in model systems.

Results: Iron-related genes are differentially expressed in the inner and outer layers of the epidermis, establishing a system that supports the recycling of iron and counteracts release of iron from the skin surface. Specifically, heme oxygenase-1, ferroportin and hephaestin-like 1 are implicated in the recycling of iron in keratinocytes [1].

Conclusion: The epidermal iron metabolism is subjected to a tight control in homeostasis, stress responses and aging, suggesting that molecular regulators of iron are candidate targets of preventive and therapeutic interventions.

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MITOCHONDRIA-PERMEABLE NATURAL ANTIOXIDANTS WITH POTENT IRON CHELATING ACTIVITY FOR SKIN PHOTOPROTECTON

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Introduction: Mitochondrial labile iron (LI) and reactive oxygen species (ROS) make skin cells highly vulnerable to UVA-induced oxidative damage. Synthetic mitochondria-targeted iron chelators offer unprecedented protection against UVA-induced oxidative damage and necrotic cell death.1 This study aimed to identify natural products that can access the mitochondrial compartment, sequester mitochondrial LI and ROS, and provide potent antioxidant and iron chelation properties in the cytosol. Phenolic compounds caffeic acid, and catechin were selected for this purpose.

Materials and Methods: Cytosolic and mitochondrial LI were evaluated with Cytosense LITM and Mitosense LITM sensors before and after UVA irradiation of human skin fibroblasts treated with the compounds.2,3 DCFDA and MitoSOX were used to evaluate intracellular and mitochondrial ROS. Photoprotection was assessed with MTT and Annexin V-propidium iodide assays.

Results: Pretreatment with both compounds significantly reduced both cytosolic and mitochondrial LI pools, correlating with the extent of photoprotection observed after UVA radiation. Iron saturation of compounds significantly reduced their photoprotective activity against UVA. Moreover, the compound pre-treatments caused a significant reduction in intracellular and mitochondrial ROS levels after UVA irradiation.

Conclusion: This is the first study identifying mitochondria-permeable phenolic compounds with both iron chelating and antioxidant activities against UVA damage in skin cells.

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INDUCTION OF FERROPTOSIS IN NORMAL HUMAN DERMAL FIBROBLASTS: IMPLICATIONS FOR SKIN DISEASE PATHOLOGY AND MOLECULAR MECHANISMS

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Introduction: Ferroptosis, a form of regulated cell death, is increasingly recognized for its role in skin disease pathology. Characterized by iron accumulation and lipid peroxidation, ferroptosis has been implicated in the progression of various skin conditions, including cancer, psoriasis and vitiligo [1-3].

Materials & Methods: In this study, ferroptosis was induced in Normal Human Dermal Fibroblasts (NHDF) using Erastin at 5 and 10 μM. After 24 hours, an MTT assay was conducted, and the expression of genes related to the MAPK signalling pathway, iron metabolism pathway and NRF2/HO-1 axis was assessed using RT-qPCR.

Results: The MTT assay showed a significant decrease in cell viability after the addition of the ferroptosis inducer. FTMT expression increased significantly with higher Erastin doses, while NRF2, p53, and TFRC showed slight, dose-dependent increases. p38 expression increased at 5 μM and decreased at 10 mM of Erastin.

Conclusion: Our findings show that Erastin induces ferroptosis in NHDF cells, as indicated by decreased viability and dose-dependent gene expression changes. FTMT upregulation highlights its role in iron metabolism, while increases in NRF2, p53, and TFRC suggest activation of protective pathways. p38's varying expression suggests its involvement in the MAPK pathway, offering insight into ferroptosis mechanisms in skin fibroblasts.

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MITOCHONDRIA: THE REVERSIBLE REGULATORS OF AGING SKIN

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Human cell mitochondria contain thousands of copies of mitochondrial mtDNA (mtDNA) per cell. Mitochondria are generally only known for their role as 'the powerhouse of the cell', but their functions include intra-, inter-, and extracellular information transfer, shaping the innate and adaptive immune response, and providing a site for hormone synthesis. Our experiments with mice genetically modified to allow for conditional mitochondrial dysfunction resulted in skin aging and hair loss in young mice.

We have created an inducible (mtDNA-depleter mouse expressing, in the polymerase domain of POLG1, a dominant-negative mutation to induce depletion of mtDNA in different tissues. These mice showed reduced mtDNA content, changes in mitochondrial protein expression, and reduced stability of mitochondrial oxidative phosphorylation complexes. We demonstrate that the ubiquitous depletion of mtDNA in mice has profound and predominant effects on the skin resulting in wrinkles and hair loss. The development of skin wrinkles was associated with the hyperproliferation of the epidermis, increased expression of MMPs, and decreased expression of TIMP1. We also found increased inflammation that may be an underlying contributing factor in phenotypic changes in the skin. Histopathologic analyses revealed dysfunctional hair follicles. The mice also showed changes in the expression of aging-associated markers, including IGF1R, KLOTHO, VEGF, and MRPS5. The rescue experiment revealed that by turning off the mutant POLG1 transgene expression and restoring the mtDNA content to the wild-type level in the whole animal (mtDNA-repleter) the skin and hair phenotypes revert to normal. These studies present *in vivo* evidence that skin wrinkles and hair loss can be reversed by restoring mitochondrial function.

This mouse model would provide an impetus for developing preventative and therapeutic strategies to augment the mitochondrial functions for treating aging in skin, hair loss and aging-related skin diseases.

EXPLORING MITOCHONDRIAL DYNAMICS AND TRANSFER: IMPLICATIONS FOR SKIN AGING AND NOVEL THERAPEUTIC APPROACHES

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Introduction: The skin's cellular composition involves interactions between melanocytes, keratinocytes, and fibroblasts, crucial for protecting against ultraviolet radiation (UVR) and wound healing (WH)[1]. Horizontal mitochondrial transfer (HMT) supports cell repair by transferring mitochondria between cells[2]. Despite its therapeutic potential, including mitochondrial transplantation (MT), HMT remains underexplored in skin[3].

Materials and Methods: We investigated mitochondrial dynamics among melanocytes, keratinocytes, and fibroblasts under UVR exposure (5.4 mJ/cm² UVA/UVB). Mitochondrial transfer rates were evaluated using 2D co-cultures and transwell assays. Artificial mitochondrial transfer (AMT) from mesenchymal stem cells (MSCs) aimed to boost fibroblast proliferation and reduce oxidative stress. In vivo validation was performed using a mouse model of surgical wounds.

Results: HMT between melanocytes and keratinocytes increased to 39% in co-culture compared to 9% in transwell assays. Minimal transfer occurred between other cell combinations, and fibroblasts exhibited reduced mitochondrial uptake. AMT from MSCs enhanced fibroblast proliferation and decreased reactive oxygen species (ROS) levels. MSC-derived mitochondrial transplantation significantly improved wound healing in mice.

Conclusion: Mitochondrial transfer is essential for maintaining skin integrity and promoting repair. This study highlights the therapeutic potential of AMT and mitochondrial transplantation to enhance regeneration, establishing mitochondria as "living drugs" for novel skin therapies.

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MITOPHAGY AND EXTRACELLULAR VESICLES: MECHANISMS OF MITOCHONDRIAL QUALITY CONTROL IN SKIN AGING

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Introduction: Extrinsic skin aging primarily results from environmental factors like sunlight, pollution, and cigarette smoke. Mitochondrial dysfunction, marked by impaired morphology and dynamics, is a common feature of senescent cells in aged skin. NIX (BNIP3L), a mitochondrial protein, plays a key role in selective mitophagy, enabling the clearance of damaged mitochondria to maintain cellular homeostasis. This study investigated NIX-dependent mitophagy as a mechanism for mitochondrial quality control in UVB-irradiated fibroblasts.

Materials & Methods: Human dermal fibroblasts (HDFs) and NIX-deficient fibroblasts were exposed to UVB to induce senescence and investigate NIX's specific role under these conditions. Techniques were employed to evaluate mitophagy and mitochondrial function.

Results: UVB irradiation in HDFs caused mitochondrial damage, typically managed through NIX-dependent mitophagy, essential for cellular health and survival under UVB stress. NIX-depleted cells showed reduced mitophagy and accumulated damaged mitochondria, which they compensated for by releasing mitochondria-enriched extracellular vesicles (EVs), indicating an alternative pathway for mitochondrial clearance.

Conclusion: Our findings indicate that NIX plays a dual role in mitochondrial quality control, mediating mitophagy and, when deficient, triggering EV-mediated mitochondrial clearance. Exploring this interplay may reveal therapeutic targets to support mitochondrial health and mitigate skin aging, promoting cellular homeostasis.

Reference

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RAMAN FINGERPRINTS & NON-INVASIVE METHODS TO EVALUATE SKIN AGING

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Introduction: Raman microspectroscopy is a promising non-invasive technology for studying skin aging[1,2], based on the inelastic scattering of laser light, usually at 532 nm or 785 nm. Spectra of biological specimens are typically complex and composed of multiple bands representing diverse macromolecules, thus challenging to interpret.

Materials & Methods: We acquired Raman spectra at 532 nm of cell culture supernatants and skin tissue sections and compared samples using multimodal statistical approaches.

Results: We could distinguish full-thickness human skin equivalents containing senescent fibroblasts from those without senescent cells based on Raman spectra of their supernatants[3]. Calcium-fluoride slides were more suitable than conventional glass or plastic substrates for acquiring Raman maps of tissue sections.

Conclusion: Raman microspectroscopy is an exciting tool for non-invasively evaluating the hydration status of the skin or for penetration studies. However, the remaining challenges, such as low sensitivity and reproducibility and sophisticated data analysis, should be solved to facilitate broader usage.

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REAL-TIME QCM-D MONITORING OF ENZYMATIC DEGRADATION OF KEY EXTRACELLULAR MATRIX PROTEINS

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Introduction: collagen and elastin are two crucial proteins for the structural integrity of the extracellular matrix (ECM). Their biomechanical properties complement each other, providing tissues with an optimal balance of flexibility and mechanical resistance [1, 2]. Inflammation-induced elevated activity of proteases, such as collagenase and elastase, leads to excessive ECM degradation. In the skin, this degradation contributes to the loss of elasticity and wrinkle formation, as observed in intrinsic aging and photoaging [3].

Materials & Methods: in this study, we explore the potential of using quartz crystal microbalance with dissipation (QCM-D) monitoring, in combination with localized surface plasmon resonance (LSPR), to investigate the process of enzymatic proteolysis of mammalian collagen and elastin nanofilms in situ.

Results: The complementary QCM-D and LSPR signals allow for real-time tracking of mass changes, as well as alterations in the viscoelastic properties of the collagen and elastin layers during proteolysis. The methodology is tested by conducting experiments at different enzymes concentrations to explore qualitative aspects of collagenase and elastase kinetics. Additionally, prior inhibition of the proteases helps preserve the collagen and elastin adlayers.

Conclusions: Our work presents a novel approach for the characterization of collagen and elastin films, probing interactions between proteases and ECM proteins, and testing inhibitors of collagenase and elastase with potential applications in anti-aging therapies.

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OLFACTORY RECEPTORS: OUTSIDER OR MISUNDERSTOOD KEY STRATEGIC PLAYER?

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Olfactory receptors (ORs) are G protein-coupled receptors that detect odorants and initiate the perception of smell. Although primarily located in the nasal olfactory epithelium, recent studies show that ORs are also present in non-olfactory tissues, indicating broader physiological functions. For example, ORs like MOR23 and hOR17-4 are involved in muscle development and sperm chemotaxis, while Olfr1393 in kidney tubules regulates glucose handling. In skin cells, OR2AT4, activated by the synthetic sandalwood odorant Sandalore, has been shown to promote wound healing by enhancing keratinocyte proliferation and migration through a cAMP-dependent pathway.

However, we will discuss why our study did not confirm these findings.

OLFACTORY RECEPTORS, SKIN AND KERATINIZATION: WHAT DO WE KNOW?

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The skin is first contact point with environment and epidermal keratinocytes contain sensory systems that can detect a variety of environmental factors. For example, several functions of olfactory receptors (ORs) in keratinocytes were reported. However, most studies have focused on individual ORs. Here, we conducted a comprehensive meta-transcriptome analysis of OR gene expression in not only human skin but also several tissues by using publicly available genotype-tissue expression (GTEx) data set. We identified distinct sets of ORs that were highly expressed in each tissue and revealed that OR10A6 in skin is related to epidermal differentiation.

While beneficial aspects of odorants were reported, the skin's protective mechanism against harmful volatiles and small hydrophobic molecules is unclear. We found that odorant binding protein 2A (OBP2A) is expressed in keratinocytes and protects the cells against cytotoxic small hydrophobic molecules. OBP2A is also involved in lipid metabolism and regulates epidermal differentiation process. Interleukin-13 suppressed the expression of OBP2A in keratinocytes and expression of OBP2A was markedly decreased in the epidermis of atopic dermatitis lesional skin.

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MONO-GLUCOSYLATION OF EPIGALLOCATECHIN GALLATE ENHANCES SKIN PENETRATION AND TRIGGERS SKIN AND MICROBIOTA WHITENING-RELATED PATHWAYS

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Introduction: Epigallocatechin Gallate (EGCG) faces challenges in cosmetics due to its poor solubility[1]. As carbohydrates are a major bacteria source of energy, we glycosylated EGCG (EGCGG) through a green, patent-pending cell-free process, increasing its penetration tenfold via a GLUT-based delivery. We assessed its impact on the microbiota and skin.

Materials & Methods: We recorded EGCGG consumption by *Lactobacillus* sp. using HPLC-UV-MS and analyzed the secretome with NMR. Its molecular docking on GLUT1 was assessed *in sillico*. The melanin content and expression level of melanogenesis pathway targets (RTqPCR) were quantified on skin explants. Penetration was assessed using Raman spectroscopy. The whitening effect was evaluated in clinical trials on different skin types (over 206 volunteers).

Results: EGCGG was consumed while no ECGG derivatives increased. It demonstrated significant penetration outperforming non-glucosylated EGCG. Then, it proved a significant whitening effect, decreasing the melanin content by 39%, supported by the reduced expression of melanogenesis-related genes, and a decrease in skin tone in clinical trials.

Conclusion: EGCGG acts as a prebiotic, boosting *Lactobacillus* sp. growth and increasing trigonelline release, a tyrosinase inhibitor[2]. Its effective bacterial consumption and penetration enhance its ability to reduce melanin synthesis across Asian, African, Indian, and Caucasian volunteers.

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GOING BEYOND: RETINOL-LIKE EFFICACY OF SPHINGOLIPID SALICYLOYL PHYTOSPHINGOSINE

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Introduction: The skincare market demands solutions for premature aging caused by exposomal stress [1]. Beyond traditional anti-aging ingredients like retinoids innovative compounds are designed for effective solutions. Sphingolipids are key cosmetic active ingredients and a specifically designed sphingolipid, Salicyloyl Phytosphingosine, is used as anti-aging active [2]. Here, we describe new benefits compared to the well-known anti-aging ingredient retinol.

Material & Methods: We conducted an in vitro study on photo-aged reconstructed skin models for proteomic analysis. In addition, an ex vivo study with photoaged skin was performed to analyze histological and gene expression change focusing on both epidermal and dermal markers.

Results: Proteomic analysis revealed that Salicyloyl Phytosphingosine modulates pathways associated with homeostasis and cellular repair processes. In the ex vivo study on photo-aged skin efficacy comparable to retinol could be shown. In contrast to retinol, Salicyloyl Phytosphingposine does not show deleterious effects. The product improves structures of aged skin by inducing elastin and hyaluronic acid binding proteins in both epidermis and dermis.

Conclusion: Innovation in skincare requires understanding both novel and established ingredients. Our findings show that the sphingolipid Salicyloyl Phytosphingosine exhibits retinol-like efficacy, improving aged skin without retinol's side effects, and reveals new mechanisms for cellular repair and homeostasis.

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SEX AND RACE VARIATIONS IN SKIN STRUCTURE AND BARRIER FUNCTION: RELATIONSHIP TO AGEING AND SENSORIAL PERCEPTION

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The structure of human skin including the components of the epidermis, the basement membrane and the dermis are dependent not only on anatomic site and age, but also on sex and race differences. Furthermore, the lipid and corneocyte components of the outer stratum corneum layer and production of topical sebum are also strongly influenced by racial makeup and sex related hormonal differences. Both are important in skin barrier function, appearance and ageing. Such variations are particularly important in the formulation and composition of topical moisturizing formulations and anti-ageing cosmetic treatments.

We review the principal differences and consider the effects of race and sex on skin structure, barrier function, ageing and sensorial perception. We demonstrate significant differences in the water diffusion kinetics in male and female stratum corneum. The differences lead to marked sex differences in the biomechanical properties of human skin and make have an important role on formulation design and sensorial perception.

Our work includes the development of an extensive human skin computational model providing the highest fidelity 3D biomechanical skin models currently available obtained from in-vivo OCT characterization. Applying the model, we consider sensorial perception of human skin following application of topical formulations and cleansers. We quantitatively elucidate the neural mechanism whereby cutaneous mechanoreceptors and corresponding sensory neurons are activated giving rise to these perceptions and provide a “Master Perception Curve” for future formulation and cleanser development.

MEASURING AGING WITH AI

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Aging is a systems level process and needs systems level models to quantify. We have recently focused our attention on phenotypic images and single cell clocks using a combination of experimental and computational approaches, most recently artificial intelligence (AI). Our deep learning AI models trained on either chronological age or perceived age of the 3D facial images can precisely estimate individuals' aging status, and infer the molecular regulators mediating the impact of lifestyles (Xia et al., 2020).

We further developed thermal facial image based aging clocks using AI and found that compared to 3D facial aging clocks, thermal facial aging clocks are more strongly associated with metabolic states, and the aging rates measured by them are accelerated by metabolic diseases and decelerated by adequate sleep and exercise (Yu et al. 2024).

I will also briefly discuss our single cell based human blood aging clocks and Senescence Identification (SenCID) program (Tao et al. 2024).

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NON-INVASIVE SKIN AGING BIOMARKERS: HAUT.AI'S AI-POWERED SYSTEM FOR ACCURATE AND INCLUSIVE AGE PREDICTION

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Artificial intelligence (AI)-powered systems have found wide applications in dermatology: from the detection of malignant conditions to the non-invasive evaluation of skin ageing. At Haut.AI, we have successfully developed a system for recognizing non-invasive biomarkers of aging in skin photographs using more than 12000 of images with broad demographic representation and expert-labelled data. Advanced neural network architectures like CNNs, GANs, Transformers, and ResNet are used by Haut.AI to detect and simulate ageing biomarkers not only in facial photographs (FaceAge), but also, for the protection of sensitive data, in photographs of the skin around the eyes (PhotoAgeClock) [1] and hands (HandAge) [2]. The accuracy of such predictions on photographs of hands is comparable to that of the system for faces and is on par with the accuracy of current state-of-the-art solutions.

One of the important problems of modern research in dermatology and artificial intelligence is the limited knowledge and data on ethnic skin and its aging. To address this issue, we use generative AI models that allow us to enrich datasets with phenotypes that are underrepresented in current datasets. This allows us to create suitable across wide skin types fair AI solutions that are free from bias.

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AGE, FRAILTY AND INFECTION RISK RESERVOIRS: THE SKIN MICROBIOME OF OLDER ADULTS

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Older adults represent a vulnerable population with elevated risk for numerous morbidities. To explore the association of the microbiome with aging and age-related susceptibilities, including frailty and infectious disease risk, we conducted a longitudinal study of the skin, oral, and gut microbiota in 47 community- or skilled nursing facility-dwelling older adults versus younger adults.

We found that microbiome changes were not associated with chronological age so much as frailty; we identified prominent changes in microbiome features associated with susceptibility to pathogen colonization and disease risk, including diversity, stability, heterogeneity and biogeographic determinism, which were moreover associated with a loss of *Cutibacterium acnes* in the skin microbiome. Strikingly, the skin microbiota were also the primary reservoir for antimicrobial resistance, clinically important pathobionts and nosocomial strains, suggesting a potential role particularly for the skin microbiome in disease risk and dissemination of multidrug resistant pathogens.

WILLAERTIA LYSATE: A HYDROBIOME BIOSOURCED INGREDIENT WITH MULTI-SITE ANTIOXIDATIVE AND ANTIAGING PROPERTIES

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Introduction: Aging is synonymous of skin becoming increasingly thin and fragile associated with a decrease of epidermal cell layers. Beyond this intrinsic aging process, the skin is continually exposed to environmental stressors such as UV radiations that accelerate aging [1 – 2].

Materials & Methods: A comprehensive program has been implemented to evaluate the efficacy of an innovative ingredient, *Willaertia* lysate, through a multi-scale approach encompassing cellular, advanced 3D skin models and human testing.

Results: *Willaertia* lysate, initially sourced from French Alp thermal spring waters, is able to (i) promote cell migration, (ii) improve the quality and the abundance of extra cellular matrix in aged skins and in young skins exposed to UV radiation to similar level as in unexposed young skins, (iii) decrease tyrosinase activity and melanin content, (iv) be efficient on oxidative stress under UV by decreasing exposome markers such as the protein carbonylation and lipid peroxidation expression, (v) improve skin density and dermal texture, and (vi) reduce underneath and crow's feet wrinkles.

Conclusion: This complete set of coherent results demonstrates the global protective and reparative efficacy of *Willaertia* lysate against the effects of (photo)aging. For the first time, the use of a protist lysate is reported in the domain of cosmetics and dermocosmetics as a natural and biosourced postbiotic active ingredient.

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INDOLE-3-ACETIC ACID, A PRODUCT OF THE SKIN MICROBIOME, AND ITS EFFECT ON THE SKIN

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Indole-3-acetic acid (IAA) is widely acknowledged as a plant hormone, yet it is also produced by the skin and intestinal microbiome. Recognized as an aryl hydrocarbon receptor (AhR) agonist, IAA plays a pivotal role in supporting intestinal barrier function. However, limited evidence exists concerning its impact on the skin.

HaCaT keratinocyte viability was assessed using the MTT assay. The influence of IAA on the gene expression of FLG2, OCLN, TJP1, CYP1A1, IL6, and IL8 in both keratinocytes and porcine skin explants (SE) was examined through qRT-PCR. For IL6/8, cells and SE were irradiated with UVA/B. Fluorescent immunohistochemistry (IHF) was employed to detect occludin in SE. Endoplasmic reticulum stress (ERS) was determined by fluorescent detection of unfolded protein aggregates in H_2O_2-treated normal human dermal fibroblasts.

IAA exhibited no detrimental effects on keratinocyte viability up to 0.1 mg/mL. It upregulated the gene expression of FLG2, OCLN, and TJP1 important for skin barrier function, and CYP1A1, a marker of AhR activation. The increased content of occludin was subsequently confirmed on the protein level in SE via IHF. Furthermore, IAA demonstrated anti-inflammatory properties by mitigating UV-induced pro-inflammatory interleukins IL6 and IL8, as well as ERS.

In summary, IAA enhances skin barrier function, possesses anti-inflammatory properties, and diminishes ERS in the skin. AhR activation likely contributes, at least partly, to these beneficial effects.

REGULATION OF FERROPTOSIS IN TUMOR AND NORMAL CELLS: IMPLICATIONS FOR THERAPEUTIC STRATEGIES IN MELANOMA TREATMENT

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Introduction: Cells exhibit variable sensitivity to different death types; cell lines are responsive, while others are resistant to death. This relationship could be targeted in cancers. Cellular response to death induction opens new therapeutic possibilities.

Materials and Methods: We investigated the effects of erastin, a ferroptosis inducer, on various cell lines, including cancerous 1205Lu and normal HaCaT cells. An MTT assay was performed for cell viability, along with tests for ROS levels and apoptosis. Expression for ferroptotic marker genes (ACSL4, TFRC) was analyzed, and co-cultured bystander experiment was conducted to evaluate signal transduction between cells.

Results: TFRC gene expression correlated with iron accumulation in cancer cells, while normal cells had low expression. ACSL4 expression also varied, with sensitive cells (1205Lu) showing increased expression, while resistant cells had silenced expression. Ferroptosis was associated with mitochondrial damage, evidenced by reduced mitochondrial potential and membrane integrity in cancer cells. Co-incubation of HaCaT cells with melanoma led to increased TFRC and ACSL4 expression, suggesting heightened sensitivity to ferroptosis. In contrast, 1205Lu cells showed minimal changes, indicating greater susceptibility to ferroptosis at higher erastin doses.

Conclusion: We confirmed signal transduction between cells, suggesting that cell sensitivity within tumors can be modulated through intercellular communication.

PLANTAGO LANCEOLATA-DERIVED EXTRACELLULAR VESICLES: A POWERFUL STRATEGY FOR SKIN REGENERATION

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Introduction: Plant-derived extracellular vesicles (EVs) have gained attention for their role in transferring bioactive molecules, which influence various biological processes including defense mechanisms, stress responses, and plant development[1]. This study aimed to investigate the effects of *Plantago lanceolata* cell-derived EVs on human skin cells.

Materials & Methods: EVs were isolated from *Plantago lanceolata* cell culture medium through a series of centrifugations and tangential flow filtrations. A hydrosoluble extract, containing 40% plantamajoside, was also obtained from the cells. The effects of a blend composed of EVs and cell extract were assessed in human dermal fibroblasts and keratinocytes by scratch assay, gene expression analysis by digital PCR and protein synthesis determination by ELISA.

Results: The Plantago blend enhanced wound healing in both fibroblasts and keratinocytes, stimulated the growth factors GDF11 and IGF1, and upregulated TFAM and SIRT6, genes associated with cellular metabolism and longevity[2]. Additionally, the EVs reduced melanin content and inflammation by decreasing IL-6 release and increases AQP3 synthesis.

Conclusions: These findings suggest that Plantago lanceolata cell-derived EVs have significant potential in promoting skin regeneration and reducing inflammation. The observed rejuvenation on skin cells could be attributed to their positive effect on growth factors, like GDF11, profoundly involved in skin rejuvenation[3].

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AA PROMOTES YOUTHFUL SKIN THROUGH COLLAGEN NETWORK REACTIVATION

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Introduction: Skin aging is associated with a skin youth structure degradation leading to the appearance of wrinkles, loss of firmness and elasticity [1]. One of the keystones to manage the skin aging process is collagen [2]. Having an extract, AA, reaped from the first plant to be reborn after a fire, we investigated its ability to defend, reinforce and rebuild collagen network.

Material & Methods: Young and aged human fibroblasts along with in-house reconstructed human skin were treated with AA. Then, MMP1, Collagen-I and ROS quantification were carried followed by imaging analysis through atomic force and confocal microscopy.

Results: Following AA treatment, collagen expression was stimulated by 23%, while MMP1 expression was decreased by 36%. Moreover, AA led to decreased ROS production upon oxidative stress exposure translating its protective activity. Image analysis showed a 15-fold functionality reactivation on fibroblasts along with a 43% collagen quality improvement with better defined fibers. Finally, AA showed beneficial effects on extracellular matrix synthesis in a full skin reconstructed model by increasing the expression of 4 different collagen subtypes essential to maintain skin youth structure.

Conclusion: Taken together, these results showed that AA displays interesting anti-ageing effects by reactivating the collagen network.

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COMPARATIVE EFFICACY OF SKINCARE PRODUCTS AND LASER PROCEDURES FOR ROSACEA AND HYPERPIGMENTATION USING ADVANCED IMAGING TECHNIQUES, EXPLORING POTENTIAL AI USE CASE

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Introduction: Skin redness and hyperpigmentation are common dermatological concerns often treated with laser procedures. However, non-invasive skincare products offer a promising alternative, especially for individuals who cannot undergo thermal treatments. This study evaluates the efficacy of AV Laboratories Face Serum, formulated with ferulic acid, niacinamide, caffeine, and beta-glucan, in reducing skin redness and hyperpigmentation.

Materials & Methods: An open-label clinical trial was conducted with participants aged 30-50 years. Participants applied AV Laboratories Face Serum twice daily for 28 days. Skin redness and hyperpigmentation were measured at baseline (Day 0) and after 28 days using the VISIA® Skin Analysis System.

Results: After 28 days, participants exhibited significant reductions in both skin redness and hyperpigmentation for product and laser treatments. However, some results could not be accurately calculated due to inconsistencies in areas of the analysis mask. These discrepancies suggest the potential for AI-driven technologies to enhance accuracy in future assessments by improving consistency between measurements.

Conclusion: AV Laboratories Face Serum effectively reduces redness and hyperpigmentation, offering a non-invasive alternative to laser treatments. This is particularly beneficial for individuals who may be unsuitable for thermal-based treatments. VISIA® analysis confirms the serum's efficacy with consistent use, though future AI applications could further refine measurement accuracy.

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NOVEL APPLICATION OF A MODIFIED STEM CELL CULTURE MEDIUM RESULTS IN IMPROVED WOUND-HEALING OF DERMAL FIBROBLASTS AND KERATINOCYTES WITHOUT INCREASING METABOLISM.

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Introduction: Aging affects skin physiology through reduced elasticity, increased fragility, and diminished wound healing, largely due to compromised stem cell activity[1]. Contributing factors include reduced blood flow, oxidative stress, genetic mutations, abnormal metabolism, and inflammation, which together disrupt skin homeostasis.

Materials & Methods: We explored the application of a modified stem cell medium, AVL SOM3®, based on the clinical medium KSOM. Designed to support skin stem cell function, AVL SOM3® provides essential nutrients and ions. The medium's effects were tested using MeWo fibroblasts, primary dermal fibroblasts, and keratinocytes in an in vitro scratch-wound assay. The AlamarBlue Cell Viability Assay evaluated metabolic activity under varying serum concentrations.

Results: AVL SOM3® improved wound healing compared to other media. Cells cultivated in AVL SOM3® exhibited reduced metabolism, depending on serum levels[2]. Metformin is an anti-diabetic drug, recently shown to slow the pace of aging in a variety of primate tissues[3]. The addition of metformin reduced fibroblast metabolism without affecting AVL SOM3®'s wound healing benefits. Fibroblasts in AVL SOM3® and metformin also showed increased protein production despite lower metabolic output.

Conclusion: These findings suggest AVL SOM3® enhances skin repair and function with minimal metabolic strain, supporting its potential in dermatological applications, including topical formulations, post-treatment therapies, and injectables for enhanced skin regeneration.

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KERATIN-ACETATE DRESSING ACCELERATES WOUND HEALING IN DIABETIC RATS AND STIMULATES M2 MACROPHAGES POLARIZATION

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Introduction: Chronic wounds remain a significant medical challenge. Keratin-based biomaterials are promising in wound healing, particularly those enriched with sodium acetate, of which acid is a product of intestinal microbial fermentation and has anti-inflammatory properties.

Material & Methods: Keratin fibers were obtained from rat fur and soaked in 0.1% sodium acetate. The obtained dressing (FKDP+0.1%Act) was examined *in vitro* and *in vivo* on 30 male Sprague-Dawley rats. Diabetes was induced with a single dose of streptozotocin (65 mg/kg). Next, two full-thickness wounds were created on the dorsum of each rat.

Results: *In vitro* studies on human macrophages revealed increased CD163 expression, a marker for pro-healing M2 macrophages. *In vitro* study also showed reduced expression of pro-inflammatory TNFα. Importantly, immunofluorescence examination of wound biopsies revealed enhanced CD163 marker luminescence in treated wounds, which agrees with the *in vitro* observations. Furthermore, the *in vivo* study showed that FKDP+0.1%Act dressing significantly (p<0.05) accelerated wound healing on days 4, 7 and 14, compared to control wounds.

Conclusion: The FKDP+0.1%Act dressing demonstrated a clear positive effect on wound healing in diabetic rats, with both *in vitro* and *in vivo* studies suggesting enhanced M2 macrophage polarization and accelerated tissue repair.

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15th International Conference on

Skin Ageing & Challenges

Abstracts of Poster Presentations

by Alphabetical Order

CORRELATION BETWEEN TOTAL POLYPHENOLS AND TOTAL FLAVONOIDS WITH ANTIOXIDANT ACTIVITY OF *CHAENOMELES JAPONICA* FRUIT EXTRACT (JAPANESE QUINCE FRUIT) AND ITS POTENTIAL USE FOR COSMETIC PURPOSES

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Introduction: This study investigates the characteristics of an antioxidant cream made from the water-glycerol fruit extract of *Chaenomeles japonica* which is distinguished by their exceptionally strong antioxidant effect and high polyphenol content. The aim of the study was also to determine the antioxidant activity and the sum of polyphenols and flavonoids in different extracts from *Chaenomeles japonica* fruits (water-glycerol, water-methanol, water-ethanol).

Materials & Methods: The antioxidant properties of the tested extracts were determined spectrophotometrically using DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical assay and FRAP (Ferring Reducing Antioxidant Potential) assay. Total polyphenols content (TPC) was determined using Folin-Ciocalteu reagent. The results of total flavonoids content (TFC) were expressed as catechin (CE) equivalent.

Results: Japanese quince fruit extracts have the ability to reduce free radicals by an average of 80% (DPPH assay). The strongest antioxidant properties of water-glycerol extract were correlated with TPC (1125 mg GAE/100 ml) and TFC (4,64 mg CE/100ml).

Conclusion: The presence of antioxidant compounds, as well as the therapeutic effects of *Chaenomeles japonica* fruit extract proven in many studies, provide prospects for the use of the plant in cosmetology. The fruit extract was also a suitable ingredient to produce a cream with good spreadability, homogeneity, consistency, appearance and pH.

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EVALUATION OF BIOACTIVE PROPERTIES AND PHENOLIC COMPOUNDS IN DIFFERENT EXTRACTS PREPARED FROM SALVIA OFFICINALIS L. AND ITS POTENTIAL COSMETIC ACTIVITIES

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Introduction: This study evaluated the antioxidant and antimicrobial activity of different sage (Salvia officinalis) extracts. This plant has been reported to contain a variety of active compounds, including: rosmarinic acid, luteolin-7-glucoside, caffeic acid and 3-caffeoylquinic acid.

Materials & Methods: The antioxidant properties of the tested extracts were determined spectrophotometrically using DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical assay and FRAP (Ferring Reducing Antioxidant Potential) assay. Total polyphenols content (TPC) and total flavonoids content (TFC) were also determined. Their antimicrobial activity was characterized by evaluation of MIC) and MBC/ MFC (in case of C. albicans). The antimicrobial activity of was assessed against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Cutibacterium acnes* and *Candida albicans*.

Results:. The strongest antioxidant properties of methanol extract (56 % in DPPH assay) were correlated with TPC (127 mg GAE/gl) and TFC (876 mg CE/100g). The strongest antibacterial properties of the methanol extract were observed in case of *Cutibacterium acnes* ATCC 11827 (MIC 0.078 mg/mL, MFC 0.312 mg/mL).

Conclusion: This study confirms that sage extracts have potential use as cosmetic skincare ingredients. Thus, *Salvia officinalis* can be considered a promising natural source of readily available, low-cost extracts rich in antioxidant, skincare, and antimicrobial compounds that might be suitable for replacing synthetic compounds in the cosmeceutical industry.

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EXPOSURE OF SKIN FIBROBLASTS TO DECELLULARIZED EXTRACTS FROM HUMAN UMBILICAL CORD TISSUE ENHANCES MIGRATION, PROLIFERATION, AND METABOLIC ACTIVITY

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Introduction: The human umbilical cord (HUC) contains regenerative components such as mesenchymal stem cells (MSCs), growth factors, and cytokines, which possess wound healing and anti-inflammatory properties (1). Decellularized extracts from HUC (HUCDEs) preserve active molecules for use in regenerative therapies (2). Our patented method (SENADI-2022-48957, licensed to Luvigix, Miami, USA) successfully generates HUCDEs with properties that enhance skin fibroblast proliferation and migration (3). This study investigates the effect of HUCDE on fibroblast metabolic activity.

Materials & Methods: Human skin fibroblasts (25,000 cells/well) were seeded into P6 plates. On day two, cells were washed and exposed to different HUCDE concentrations (6 µg/ml) for 72 hours. Metabolic activity was measured using the MTT assay, and data were analyzed with Kruskal-Wallis and Mann-Whitney tests. Five biological replicates and three experimental repeats supported these findings.

Results: HUCDE concentrations between 12.5 and 50 µg/ml increased fibroblast metabolic activity by 20-30% compared to controls (*p<0.05, **p<0.01, **p<0.001).

Conclusion: HUCDE promotes fibroblast proliferation, migration, and metabolic activity, suggesting its potential use in skin regenerative therapies.

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REGULATION OF IRON HOMEOSTASIS AND FERROPTOSIS: ROLE OF IRP2 IN CRISPR-MODIFIED KERATINOCYTES

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Introduction: Cellular iron homeostasis is tightly regulated through proteins (IRP1, IRP2) and iron-responsive elements. Disruption of this balance can lead to ferroptosis, a form of cell death associated with iron accumulation. When iron levels are low, IRPs inhibit the translation of mRNAs encoding proteins involved in iron storage and export, such as FTH1, FTL, and FPN. The study focused on IRP2, a key regulator in eukaryotic cells, which responds to intracellular iron levels.

Materials & Methods: Keratinocyte wild type (WT), HaCaT, and GPX4-knockout (KO) cells, modified using CRISPR/Cas9, were treated with erastin. RT-qPCR gene expression of TFRC, DMT1, and SLC40A1 was measured, while iron was assessed using Prussian blue staining. Lipid oxidation was visualised using confocal microscopy.

Results: Results showed a dose-dependent decrease in TFRC expression in WT cells, while GPX4 KO cells exhibited increased iron accumulation and altered gene expression. IRP2 expression increased in WT cells, suggesting a feedback response to iron depletion, but was silenced in KO cells due to iron overload.

Conclusion: Despite these changes in gene expression, cell viability was unaffected.

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FIBRO-CAPS, A 3D MODEL MIMICKING ALTERATIONS OF A FIBROTIC MICROENVIRONMENT ON THE METABOLISM OF HUMAN MATURE ADIPOCYTES

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Introduction: During ageing, immune cells can accumulate in adipose tissue (AT) contributing to the development of a chronic and low-grade inflammation and to an intense extracellular matrix remodeling, qualified as fibrosis. This network of matricial fibres can exert mechanical forces on adipocytes and induce metabolic alterations.

Materials & Methods: Mature adipocytes (MA) isolated from AT of non-obese women were 3D-cultured in a hydrogel in which extracellular matrix fibers prepared from AT of obese women were incorporated to create 3D adipocyte capsules in fibrotic microenvironment ("Fibro- Caps") before being treated with a combination of Grape Seed and Iris extracts for 6 days. Concentrations of adipocytokines were quantified in secretions. Adipocyte lipolysis, diameter and distribution were evaluated.

Results: MA in fibrotic conditions showed altered stimulated lipolysis and increased IL-6 secretion. Furthermore, adipocyte diameter was increased, associated with more big cells. Grape seed and iris extracts association did not restore lipolysis but decreased adipocyte diameter and IL-6 secretion.

Conclusion: Culture of MA in fibrotic microenvironment induced alterations of their metabolism, with a resistance to lipolysis stimulation and an inflammatory state. Natural ingredients like grape seed and iris extracts are efficient to restore adipocyte metabolism and promote small adipocytes which are more insulin-sensitive and metabolically active.

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EXTRACTS OF *NASTURTIUM OFFICINALE* R. BR. MICROSHOOT CULTURES AS A POTENTIAL COSMETIC MATERIAL WITH ANTI-ELASTASE, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

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Nasturtium officinale R.Br. (Brassicaceae) - watercress, is an aquatic plant, partially protected in some regions of Europe. *N. officinale* herb is a valuable medicinal and cosmetic raw material. Extracts from the herb *N. officinale* are increasingly used in cosmetic preparations, due to anti-inflammatory and anti-aging effects.

The aim of the studies was to evaluate the antioxidant, anti-elastase and antimicrobial activity of extracts from *N. officinale* microshoot cultures grown in bioreactors.

N. officinale microshoots were cultivated in the Plantform™ temporary immersion systems containing 500 mL of the Murashige and Skoog medium with 1 mg/L 6-benzyladenine and 1 mg/L 1-naphthaleneacetic acid. For antioxidant and anti-elastase potential assessment of the two types of extract - water and ethanolic were prepared. For microbiological studies, a dry extract was dissolved in DMSO.

The highest antioxidant potential assessed by ABTS, DPPH and FRAP assays was indicated for ethanol extracts of *N. officinale* microshoot cultures. The water and ethanol extracts had a good anti-elastase activity (79.78% and 75.88%, respectively). Moreover, the antibacterial properties vs. *Cutibacterium acnes* (MIC=0.625mg/mL) and antifungal vs. *Candida albicans* (MIC=1.25mg/mL) strains, were shown.

The study confirmed the high potential of using extracts from *N. officinale* PlantForm™ bioreactor multiplied microshoots in anti-aging and anti-acne cosmetics.

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POSTBIOTIC METABOLITE COMPLEX PROMOTES RELAXATION AND INDUCES SLEEP BY ACCESSING THE SKIN-BRAIN AXIS VIA MICROBIOME MODULATION

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Introduction: The skin-brain axis represents the bidirectional communication between the skin and the central nervous system, mediated through the neuro-immuno-cutaneous system. The skin microbiome plays a pivotal role in this axis, influencing systemic physiological responses including sleep regulation. Melatonin, primarily produced by the pineal gland during sundown, is essential for sleep induction and treatment of insomnia. Optimealth® is a unique metabolite complex produced by AI optimized fermentation technology. These metabolites modulate the skin microbiome towards a state of symbiosis and thereby access the Skin-Brain communication pathways. By promoting pineal serotonin N-acetyltransferase (NAT) activity and enhancing melatonin synthesis, Optimealth®10 W establishes the potential of skin-microbiome modulation as a novel and sustainable approach to improve sleep quality and overall well-being.

Material & Methods: This double-blind, placebo-controlled, randomized clinical trial included 30 participants aged 25 to 55 years. Subjects were randomly assigned to receive either Optimealth® or placebo, with 15 participants in each group. Participants washed their scalp with 5ml of Optimealth® or placebo shampoo at 19:00 hours. Urine samples were collected every 2 hours for 12 hours to measure 6-sulfatoxymelatonin levels. Data were analyzed using appropriate parametric and non-parametric tools.

Results: These results showed that participants have significantly higher levels of melatonin after washing scalp with shampoo with 0.5% Optimealth® compared to placebo group. 95% of test subjects claimed to experience relaxation after application of Optimealth®.

Conclusion: Optimealth® significantly enhances melatonin production and promotes relaxation, demonstrating its potential for sleep induction. By leveraging the skin-brain axis and modulating the skin microbiome, Optimealth® offers a novel approach to managing sleep-related disorders through topical application. These findings support the efficacy of Optimealth® in increasing melatonin levels, providing a promising solution for improving sleep quality and overall well-being.

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SUPPRESSION OF THIOREDOXIN REDUCTASE 1 IN KERATINOCYTE CELL LINE AS A KEY REGULATOR OF THE NRF2 PATHWAY

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Introduction: HaCaT cells, which retain key features of human keratinocytes, are commonly used to study oxidative stress responses in the skin, particularly the NRF2 signaling pathway. As the predominant cell type in the epidermis, keratinocytes are regularly exposed to external stressors like UV radiation and pollutants, which increase reactive oxygen species (ROS). Inhibition of the enzyme TXNRD1 exacerbates oxidative stress, activating the NRF2 pathway to protect cells from damage. This regulation helps prevent skin aging and diseases such as cancer.

Materials & Methods: The study involved three cell lines: wild-type HaCaT (WT), a positive control, and GPX4 knockout (KO) cells created through CRISPR/Cas9 genome editing. These cells were treated with erastin, a ferroptosis inducer, at 5 and 10 μM doses for 24 hours. The expression of NRF2, TRX, and TXNRD1 was measured using RT-qPCR.

Results: Results showed that in GPX4 KO cells, NRF2 and TXNRD1 levels initially rose and then dropped, while TRX levels increased. In HaCaT WT cells, NRF2 and TXNRD1 levels increased, but TRX levels decreased.

Conclusion: These findings suggest that TXNRD1 inhibition disrupts redox balance and triggers NRF2 activation, highlighting TXNRD1's key role in maintaining skin health under stress.

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ANTI-AGING POTENTIAL OF THREE ALGAE EXTRACTS BASED PRODUCTS ACCESSED WITH IN VITRO/EX VIVO TECHNIQUES

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Introduction: The anti-ageing potential of skin care products containing algae extracts (*Laminaria ochroleuca*, *Undaria pinnatifida*, *Cystoseira tamariscifolia, Lithothamnium calcareum*) was investigated.

Materials & Methods: The objective of this study was to examine in vitro effect of day cream (2910), night cream (2911) and eye cream under (2915) on the stimulation of pro-collagen type I and elastin in HDF cells. Furthermore, ex vivo studies were conducted on skin explants derived from a 49-year-old female donor to assess the impact of the products on glycosaminoglycan (GAG), elastin, and collagen formation. In vitro studies were conducted using immunofluorescence labelling of elastin, while the pro-collagen type I was quantified using ELISA assay. Ex vivo tissue staining using alcian blue staining (GAG), Verhoeff–Van Gieson stain (elastin) and Mallory trichome stain (collagen) were performed.

Results: The in vitro studies demonstrated that all products significantly stimulated the synthesis of type I procollagen. Elastin stimulation was observed in the night cream (2911) and the eye cream (2915). The tissue staining indicated that day and night creams stimulated the synthesis of GAGs and collagen. Stimulation of elastin synthesis was not observed. The eye cream stimulated the production of all evaluated structures.

Conclusion: The results indicated that all tested products have anti-ageing properties.

LONG-TERM SKIN TISSUE MAINTENANCE FOR THE BENEFIT OF PRODUCT EFFICIENCY

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Introduction: Skin acts as a protective barrier against external aggressions that our bodies are submitted to on a daily basis. 3D reconstructed skin models are often used in the cosmetic industry as an alternative to in vivo animal models, but although very reproducible, they do not cover the entire complexity of the morphological and physiological characteristics of human skin.

Materials & Methods: Here we present an innovative ex vivo method consisting in a dynamic maintenance of human skin samples in culture, that allows us to study skin response to environmental stresses or cosmetic products over a long period of time. Skin samples were obtained from healthy human donors and processed through various histological techniques.

Results: With this method we managed to maintain healthy skin samples up to 19 days in culture. This allowed us to assess the exfoliating activity of a topically applied cosmetic product through immunostaining of skin barrier function proteins, and to quantify the results using and artificial intelligence application Visiopharm.

Conclusion: In conclusion, ex vivo skin culture is a robust model to assess cosmetic products efficacy and mimic various environmental stresses.

DEVELOPMENT OF SUSTAINABLE COSMETIC INGREDIENTS VIA VITIS VINIFERA LEAFS EXTRACT FERMENTATION

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Fermentation processes can transform natural by-products into high-value cosmetic bioactives, reducing waste, promoting sustainability, and supporting circular economy through upcycling. Fermented Vitis vinifera leafs extracts (FVVLEs) represent a class of innovative ingredients, as they are rich in plant secondary metabolites known for their antioxidant, anti-inflammatory and antimicrobial properties[1] as well as anti-aging activity[2]. In particular, we studied FVVLEs' role in improving skin health and anti-aging properties thanks to its capability to reduce skin blemishes.

FVVLEs were obtained through L. plantarum PBS 067 (DMS 24937) mediated-fermentation: the process was optimized in terms of extract concentrations, pH conditions and temperatures, and later a precision fermentation in a 1L bioreactor was performed. Lastly, antioxidant properties were evaluated through different analysis (DPPH and TPC tests), and efficacy tests on skin health and whitening were performed.

As a result, we identified the optimal conditions for precision fermentation of VVLE using LP; antioxidant assays showed a high antioxidant activity of the fermented product, and efficacy tests confirmed its anti-aging effects and improvement of skin health in general. These results highlight FVVLEs as a potential cosmetic ingredient, especially for the anti-aging application.

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POLYDOPAMINE-LOADED PRUNETIN NANOMATERIALS ACTIVATE DRD2 TO REDUCE UV-INDUCED OXIDATIVE STRESS BY STABILIZING AND PROMOTING NRF2 NUCLEAR TRANSLOCATION

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Introduction: Skin damage caused by UV has been documented clinically. DRD2 possesses various biological functions. However, the mechanism of UV-induced skin inflammation has not been clarified.

Materials & Methods: UV-induced $Drd2^{flox/flox}$ and $Drd2^{flox/flox}$: Cre+ mouse models were established. H@P@M nanomaterials was prepared and characterized. Various molecular biology experiments like qPCR, western blot, immunofluorescence, pull-down, FPLC coupled with mass spectrometry, Duolink, cAMP assay, Co-IP were conducted. A mouse photoaging model was established.

Results: Activated DRD2 can form a complex with Nrf2 via ARRB1 and deubiquitinate Nrf2. H@P@M can activate DRD2 and promote Nrf2 nucleation, which promoted the nuclear translocation of Nrf2 and accomplished anti-inflammatory and antioxidant activities.

Conclusion: The role of DRD2 in UV-induced skin inflammation and oxidative stress was explored, and H@P@M, which act on multiple targets in the anti-inflammatory pathway of DRD2, were developed to maximize the effect of the drug.

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EFFICACY OF GRAPE SEED AND IRIS EXTRACTS ON ADIPOCYTE METABOLISM AND MORPHOLOGY IN FIBROTIC MICROENVIRONMENT

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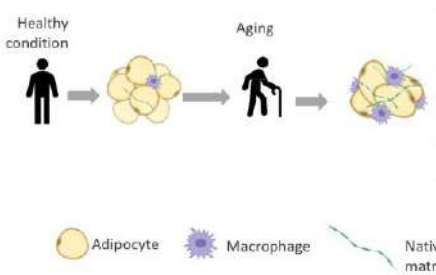
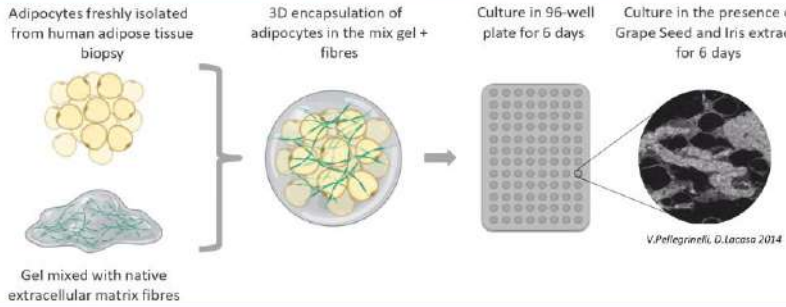
CONTEXT



Adipose tissue, which is the crucial energy reservoir and endocrine organ for the maintenance of systemic glucose, lipid, and energy homeostasis, undergoes significant changes during aging. These changes cause physiological declines and contribute to skin alterations. The **hypodermis**, which is composed of adipose tissue, can be the site of immune cell accumulation contributing to the development and chronicization of local **inflammation**. This inflammatory state can lead to intense matrix remodelling qualified as **fibrosis**. Metabolic alterations of adipocytes can occur in a such fibrotic microenvironment. To assess that, we have developed a model for studying human mature adipocytes in fibrotic conditions and we have evaluated efficacy of **grape seed and iris extracts (GSI)** to reverse subsequent alterations in adipocyte metabolism.

MATERIALS & METHODS

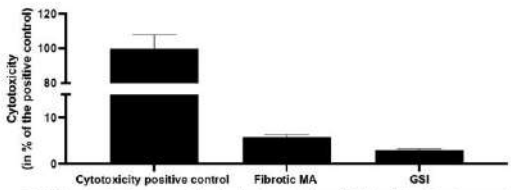


Multiparametric evaluation of compounds:

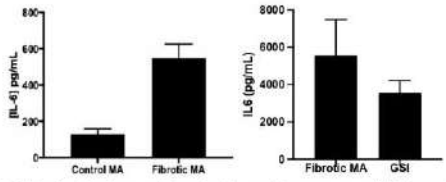
- Cytotoxicity : lactate dehydrogenase activity
- Lipolysis : glycerol release
- Inflammation : interleukin-6 secretion
- Adipocyte size

RESULTS

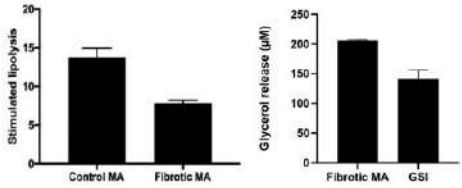
A. GSI extract decreases cytotoxicity in fibrotic microenvironment



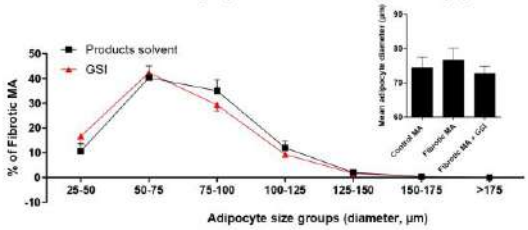
B. GSI extract decreases IL6 secretion induced by fibrosis



C. GSI extract decreases the lipolysis activity of MA in fibrotic microenvironment



D. GSI extract reduces adipocyte size and increases small MA population



CONCLUSION

Fibrosis resulting from chronic inflammation in subcutaneous adipose tissue can contribute to metabolic and morphological alterations of adipocytes and consequently, to skin modifications occuring during aging. Grape seed and iris extracts has proven to be beneficial for adipocytes in this fibrotic microenvironment.

Dr Irena Eris

CENTRE FOR SCIENCE AND RESEARCH

Anti-Aging potential of three algae extract based products accessed with in vitro/ex vivo techniques

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Introduction

The skin is the body's largest organ and is continuously exposed to various environmental factors (ultraviolet rays, smoking, heat, and air pollution). Consequently, skin undergoes extrinsic but also intrinsic aging, the latter of which is also referred to as chronological aging. The process of intrinsic aging can be considered alongside programmed aging and results from continuous chromatic damage by various factors, of which the most representative is oxidative stress caused by reactive oxygen species. The anti-aging potential of skin care products containing algae extracts (*Laminaria ochroleuca, Undaria pinnatifida, Cystoseira tamariscifolia, Lithothamnium calcareum*) was investigated.

Materials & Methods

Anti aging potential of three cosmetic products was evaluated:

- Day cream (2910)
- Night cream (2911)
- Eye cream (2915)

In vitro assays

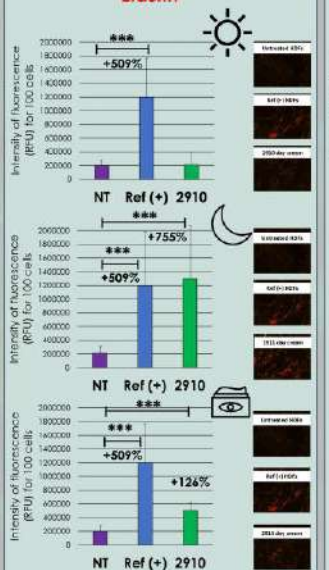
- Studies were performed on human dermal fibroblasts (HDF).
- The presence of **elastin** was assessed by immunofluorescence labelling.
- **Pro-collagen type I** was quantified by ELISA assay.

Ex vivo assays

- Studies were performed on skin explants derived from a 49-year-old female donor
- The presence of **GAGs** was assessed by Alcian blue staining, **elastin** by Verhoeff-Van Gieson staining and **collagen** by Mallory trichrome staining.

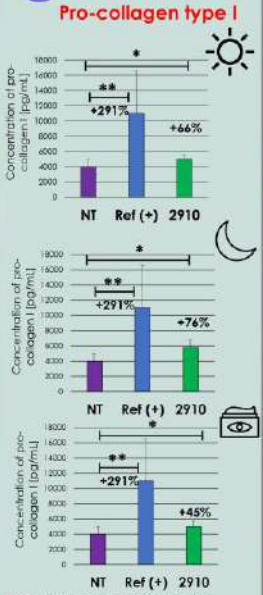
Results

In vitro assay – Elastin



If the p-value is less than 0.01 and 0.001, **p<0.01, ***p<0.001.

In vitro assay – Pro-collagen type I

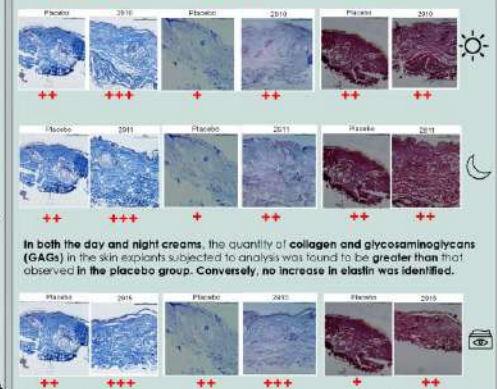


If the p-value is less than 0.01 and 0.001, **p<0.01, ***p<0.001.

Ref: Mix of Vitamin C and TGF-beta

Ex vivo assays

Collagen GAGs Elastin



In both the day and night creams, the quantity of **collagen and glycosaminoglycans (GAGs)** in the skin explants subjected to analysis was found to be **greater than** that observed **in the placebo group. Conversely, no increase in elastin was identified.**

The analysis of the skin explants subjected to testing revealed an **increase in collagen, elastin** and **GAG compared to the placebo.**

The collagen content was determined based on the evaluation of histological preparations, whereby the following scoring system was employed: '+' indicates a low content, '++' denotes a medium content, and '+++' signifies a high content. Placebo: vaseline.

Conclusion

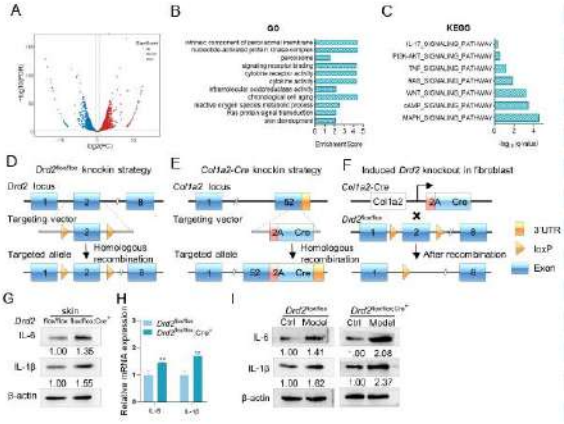
The outcomes demonstrated that all products containing algae extracts as active ingredients exhibited anti-ageing properties.

Polydopamine-loaded prunetin nanomaterials activate DRD2 to reduce UV-induced inflammation by stabilizing and promoting Nrf2 nuclear translocation

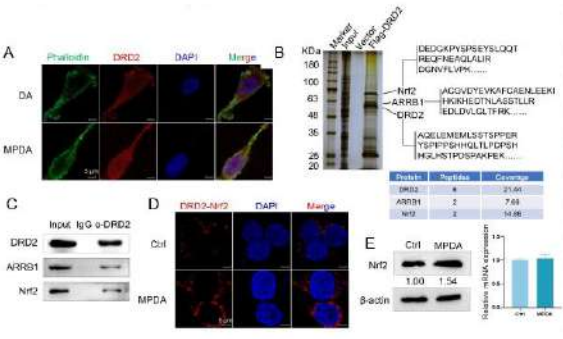
ZHU, Caibin, JIN, Jing, HAN, Jingxia, ZHENG, Shaoting, YAO, Cheng, LIU, Huijuan

Shanghai Cheermore Biotech Co., Ltd. – China

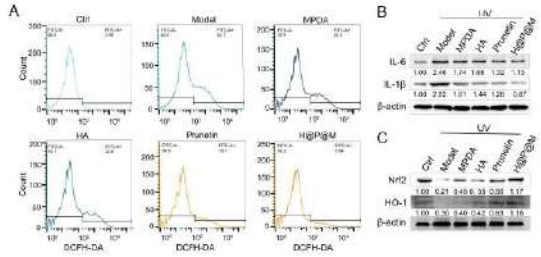
Skin damage caused by UV has been documented clinically and histologically. Dopamine receptor D2 (DRD2) possesses various biological functions. However, the mechanism of UV-induced skin inflammation has not been clarified. DRD2 conditional knockout and UV damage models was established. The results showed that DRD2 played an important role in the treatment of UV-induced skin damage. The internalization of DRD2 after activation can stabilize nuclear factor erythroid 2-related factor 2 (Nrf2). Hyaluronic acid (HA)-coated mesoporous polydopamine (MPDA) nanoparticles (H@P@M) were prepared and characterized. HA facilitated skin epidermal penetration of the nanoparticles to reach the site of inflammation smoothly. MPDA activated DRD2 internalization to stabilize Nrf2. In summary, this study unveiled that in skin inflammation, H@P@M activated and internalized DRD2, which subsequently formed a protein complex with arrestin beta 1–ubiquitin specific protease 8 (USP8)–Nrf2. Deubiquitination was performed to stabilize Nrf2 while promoting the nuclear translocation of Nrf2 to exert anti-inflammatory and antioxidant functions.



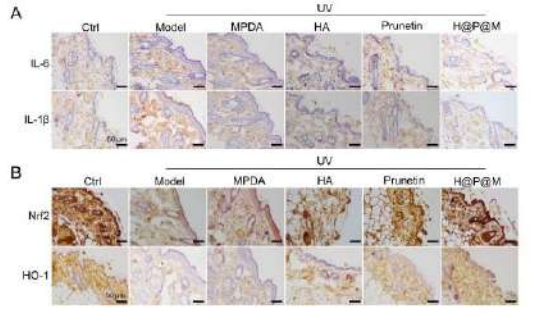
DRD2 deficiency triggered skin inflammation. A. Volcano map of identified genes with changes between groups. B and C. Gene Ontology and KEGG analyses of differentially expressed genes with enriched expressions between groups. D and E. Generation of $Drd2^{flox/+}$ (D) and Col1a2-Cre alleles (E) via homologous recombination. F. Schematic illustrating the experimental strategy. G. Relative protein expression levels of IL-1β and IL-6 in the skin tissues of $Drd2^{flox/flox}$ and $Drd2^{flox/flox}$; Cre^{+} mice. H. mRNA expression levels of IL-1β and IL-6 in skin tissues tested by qPCR.



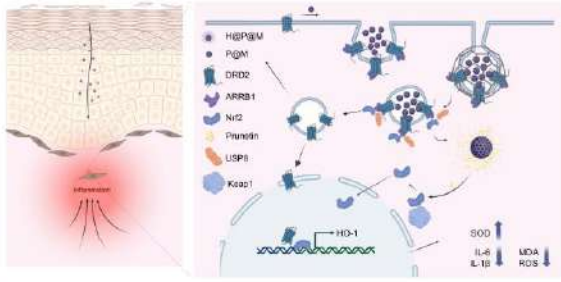
MPDA activated and internalized DRD2 to bind to Nrf2. A. Changes in the cellular location of DRD2 after DA and MPDA treatments. B. Pull-down analysis of DRD2-associated proteins after the addition of MPDA. C. Co-IP analysis of DRD2, ARRB1, and Nrf2 interactions. D. Interaction analysis of DRD2 with Nrf2 using the Duolink proximity assay. E. Relative expression of Nrf2 protein and mRNA in different treatment groups.



Anti-inflammatory and antioxidant effects of H@P@M at the cellular level. A. Relative ROS levels under different treatment groups. B. Relative protein expression levels of IL-1β and IL-6 in different treatment groups. C. Western blot assays of changes in Nrf2 and HO-1 content in different treatment groups.



Anti-inflammatory and antioxidant effects of H@P@M *in vivo*. A and B. Representative IHC images of mouse skin tissue.



The schematic diagram of the molecular mechanism underlying Polydopamine-loaded prunetin nanomaterials's inhibition of UV-induced skin damage.

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CHEERMORE

1 Company Overview

Since 2010, China' s skincare industry has experienced rapid growth, driven particularly by the rise of functional skincare products. As domestic brands have increased their investment in research and development, competition among leading companies has intensified. This surge in R&D has led to a continuous stream of innovations and an increasing number of patents. In 2023 alone, the top 10 cosmetics companies in China applied for approximately 396 patents. Of these, 278 were invention patents, accounting for 70% of the total, while design patents and utility model patents numbered 81 and 38 respectively, making up 20% and 10%.

Founded in 2012, Shanghai Cheermore Group is a comprehensive cosmetics company that integrates research and development, production, sales, and customer service. It is one of the few companies in China with independent R&D capabilities for cosmetic raw materials. Its product range covers beauty, personal care, household cleaning, and maternal and infant care products.

With registered capital exceeding 210 million RMB and a workforce of over 500 employees — including more than 100 researchers — Cheermore Group holds 1,048 registered trademarks and 33 valid invention patents. The company has also secured three international patents in the United States, Japan, Australia, and nine European countries. Additionally, the group has published five high-impact academic papers in top-tier international journals, including Acta Biomaterialia (a leading biomedical journal), British Journal of Pharmacology (a top pharmacology journal), and Cell and Bioscience (a premier medical journal), achieving the highest impact factor in China's cosmetics industry.

SHANGHAI CHEERMORE GROUP

2 Skincare Philosophy

Our approach to skincare is grounded in dermatological science, with a firm commitment to safe and effective formulations. We develop products that provide targeted care for both sensitive and healthy skin, helping restore the skin's natural beauty. By promoting a healthy skincare philosophy and lifestyle, we aim to become a trusted partner for consumers.

Cheermore Group is committed to transforming scientific research into practical, effective solutions for both consumers and businesses. We strive to foster a culture of trust between individuals and the environment. With a rational approach to beauty, we pursue an ideal that helps people rediscover their unique, inherent beauty — this is the ideal of beauty we uphold.

Products

Dermal Defender Series

Specially designed to meet the needs of ultra-sensitive skin, this range soothes the skin's stress responses, providing immediate relief from redness while strengthening the skin's barrier for enhanced resilience.

Soothing Recovery Series

Focused on stabilising and repairing the skin, this series offers comprehensive care for mildly sensitive skin by brightening, firming, and enhancing the skin's defence against oxidative stress.

Total Solution Series

This line provides powerful protection against oxidation and glycation, boosting skin elasticity and reducing the appearance of fine lines and wrinkles, leaving the skin plump and radiant.

Dermal Purefiner Series

Developed for oily and acne-prone skin, this series helps regulate the skin's oil-water balance, minimising pores, combating oxidative damage, and soothing discomfort.

Anti-aging BodyCare Series

More than just body moisturisation, this series applies facial skincare technology and formulations to body care, providing a smooth, refined, and firm experience for the skin.

Rapid Recovery Series

Designed specifically for those undergoing professional medical cosmetic treatments, this series addresses the skin's evolving needs throughout the treatment process. It offers a multi-dimensional skincare solution that enhances recovery and boosts the effects of cosmetic procedures.