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Variability in sourcing and potency of Cannabidiol (CBD) and Hemp-based extracts impact gene expression changes in-vitro, highlighting the need for reliable analytical methods and standards

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Introduction of research

Skincare products claiming benefits from CBD (cannabidiol) and other hemp-based extracts remain a rapidly growing trend in the US and other global markets. However, research in this area is only emerging and scientific validation for the safety and benefits of topical application is not well established. Benefit claims seem to be based on anecdotal testimony, animal model studies, or clinical trials without defined quality standards or analytical characterization of test articles. To properly investigate the potential for skin benefits from the topical application of cannabinoids we understood it is equally important to verify the composition of the test articles being screened. Prior to the Agriculture Improvement Act of 2018, any cannabis material containing Tetrahydrocannabidiol (THC) was considered a schedule-1 drug, so standards and methods were mainly used to detect the presence of certain cannabinoids not for accurate and reliable measures of concentrations. Now the law requires accurate concentration measurements and according to the National Institute of Standards (NIST), determining accurate analytical numbers at levels as low as 0.3% can be a particular challenge for laboratories (1). This challenge may explain and possibly contribute to the infamous findings of the 2017 JAMA study which analyzed 84 products from 31 categories and found that 70 percent of them had labeled the incorrect percentage of CBD (2). So, our research outlined below is composed of two parts; 1. An independent analytical evaluation of several hemp-based cannabinoid extracts, and 2. An investigation of gene expression modulation from these extracts in a human skin cell model. Our hypothesis was that differences in composition and concentration of hemp-based cannabinoid extracts would affect gene expression modulation.

Methodology

In this work, we analyzed six hemp-based extracts including CBD isolates from three separate sources, one broad-spectrum distillate, and one full-spectrum distillate. Our results were then compared to the values declared on the supplier's COA. The instrument used is a Bruker Q-ToF (Quadrupole-Time-of-Flight) mass spectrometer. Data was acquired with LC/MS, where the LC was done with a reverse-phase column using a methanol gradient, in which the mobile phase contained 5 mM ammonium formate and 0.01% formic acid. Cannabinoids found in samples were compared to authentic standards. Three separate Certified Reference Materials (CRMs) of CBD and THC were sourced from Cerilliant/Sigma, Chromadex and Restek.

The potential for skin benefits was investigated in-vitro using a qPCR based model with human keratinocyte and fibroblasts (Genemarkers). Test articles were screened for their influence on 5 endogenous control genes and 163 gene targets known for their important roles in skin biology. We tested CBD isolates from three separate sources (A, B and C) at 0.1% and source (A) at 0.5%. We also tested one broad-spectrum distillate and one full-spectrum distillate, both at 0.1%.

Results

The results from our analysis of the three sources of CBD Isolate are shown in (Fig. 1) with the supplier's COA values to the side for comparison. Measured CBD values are significantly lower than the supplier declared values and samples contain significant diversity in secondary cannabinoid content.

CBD Isolate (A)
Lot: xxxxxx
Sample appearance: White powder

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	55.7	102.16
CBDV	0.10	0.00
CBN	1.11	0.00
CBG	ND	
CBC	ND	
delta-9 THC	ND	
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	<<1	
pesticides/toxic contaminants	ND	

CBD Isolate (B)
Lot: xxxxxx
Sample appearance: White powder

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	56.0	99.8
CBDV	0.23	0.28
CBN	ND	
CBG	ND	
CBC	ND	
delta-9 THC	ND	
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	<<1	
pesticides/toxic contaminants	ND	

CBD Isolate (C)
Lot: xxxxx
Sample appearance: White powder

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	53.5	99.6
CBDV	0.19	0.39
CBN	ND	
CBG	ND	
CBC	ND	
delta-9 THC	ND	
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	<<1	
pesticides/toxic contaminants	ND	

Figure 1. Analytical results for CBD Isolates A, B and C versus Suppliers COA Claims

Results of the characterizations of both the broad-spectrum and full-spectrum distillates are shown in (Fig. 2). Again, the values for CBD are significantly lower than the COA's and additional cannabinoids are present at significant levels. Of note, the full-spectrum distillate in an undiluted state exceeds the legal limit of 0.3% for THC.

Broad-Spectrum Distillate
Lot: xxxxxxx
Sample appearance: Yellow-brown paste

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	44.3	65
CBDV	0.09	Not Tested
CBN	0.01	Not Tested
CBG	ND	
CBC	ND	
delta-9 THC	0.01	0.1
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	0.5	
pesticides/toxic contaminants	ND	

Full-Spectrum Distillate
Lot:xxxxxx
Sample appearance: dark-brown paste

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	47.2	75.5
CBDV	0.23	0.18
CBN	0.31	0.37
CBG	ND	1.09
CBC	ND	2.61
delta-9 THC	1.17	3.46
delta-8 THC	ND	0.26
11-OH-THC	ND	
THC-V	ND	0.00
CBDVA	ND	
CBD/THC isomers and other cannabinoids	6.4	
pesticides/toxic contaminants	ND	

Figure 2. Analytical results and COA values for Broad-Spectrum and Full-Spectrum distillates

An example of several genes differentially affected by varying concentrations, sources and types of extracts is shown in (Fig. 3).

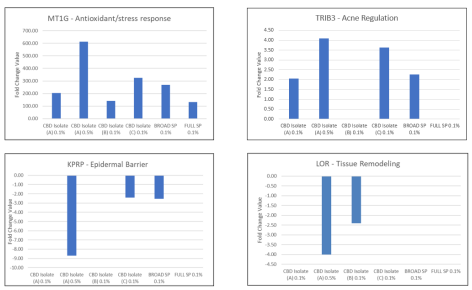

Figure 3. Examples of variability in gene modulation (MT1G, TRIB3, KPRP, LOR) affected by concentration, source and type of extract.

The potential for biphasic responses (3) as a result of differing concentrations is shown in (Fig. 4).

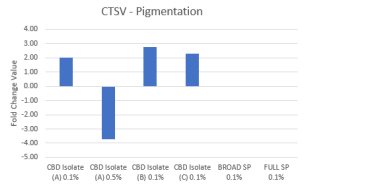

Figure 4. An example of the biphasic potential of gene modulation (CTSV) from different extract concentrations.

And finally, (Fig. 5) demonstrates two examples of genes with modulation suggesting the potential for an 'entourage effect' where biological contributions may be derived from the addition of terpenes (4).

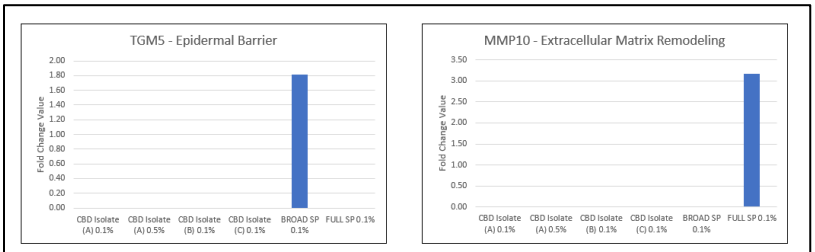

Figure 5. Examples of potential 'Entourage effect' on TGM5 and MMP10.

Conclusion

From the outset, we discovered that the standards and methods for reliable analytical evaluations of CBD and other cannabinoids presented a challenge. Our analytical results showed significant discrepancies between the vendor supplied COA's and our own measurements (Fig. 1-2). In fact, we even found up to 20% variation between the three CRM's sourced as analytical standards (data not shown). Again, this is a well-known challenge and one actively being addressed by the NIST.

The significance of these challenges in analytical accuracy and reproducibility are demonstrated further in out in-vitro gene modulation studies. Dramatic difference in the responses from genes exposed to CBD and other hemp-based extracts arise dependent upon concentration, source and type of extract (Fig. 3-5). The significance of additional known and unknown cannabinoids is a potential cause as well as the biphasic nature of their effects in biological systems. In some cases, these additional cannabinoids are not even tested as a part of the COA. It is an obstacle and a fact that over 100 different cannabinoids have been discovered (5), but most have not been isolated or closely studied to date. It is quite clear, the industry must overcome this challenge of reliable methods and standards for the proper investigation not only of potential benefits, but also risks for humans in the topical and oral use of these cannabinoids.

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About the speaker

Brian Cook received his Bachelor of Science degree in Biology and Master of Science in Biochemistry and Molecular Biology from the University of Georgia. His 20+ years of experience in product development and research has covered polymer science, personal care formulation, and skin & hair research innovations. Brian joined Nu Skin in 2013 first as a senior formulation chemist and currently is a senior associate scientist.

Since joining Nu Skin, Brian has contributed to countless brands, including Epoch, Nutriol, Clear Action, and AP-24. Brian currently serves as the development scientist over ageLOC Galvanic Spa & Body Spa, ageLOC LumiSpa, ageLOC Me, ageLOC Transformations, ageLOC Nutriol, and also supports Nu Skin science through new ingredient research.

Exploration of wrinkle skin microbiome

Allison Garlet PhD; BASF Corporation

Nicolas Del Bene; Neethu Abraham; Lauren Junker PhD and Wendy Chan PhD; Hunter Cameron; Anita Samuga PhD; Nengyi Zhang; BASF Corporation - George Kritikos PhD; Vimal Rawat PhD and Philipp Ternes PhD; BASF Metabolome Solutions GmbH - Sabrina Leoty-Okombi PhD and Valerie Andre PhD; BASF Beauty Care Solutions France SAS.

Introduction

Skin microbiome is extensively studied in dermatological and cosmetic fields to develop solutions for different skin conditions. Although skin microbiome is considered an important component in skin health, its relationship with the skin aspect particularly the aged, wrinkled skin is only partially known.
We sought to understand the differences of skin microbiota composition between young and aged skin, with a focus on wrinkles. We used whole genome sequencing coupled with metabolomic analysis to measure shifts in skin microbiome composition and metabolites.

Methodology

Volunteers were recruited with wrinkle grade (50 with grade 5-6 in old cohort, 50 with grade 0-1 in young cohort). Microbial and metabolite samples were collected by swabbing 3 different zones: in the wrinkle hollow, the crow's feet and under-eye zone, and a control zone (cheek area adjacent to earlobe). One side was dedicated to instrumental measure and metabolite analysis by a combination of GC-MS and LC-MS methods, the other side to microbial DNA study. After DNA extraction (EpiCentre MasterPure kit), human DNA was removed (NEBNext Microbiome DNA Enrichment Kit). Whole metagenome sequencing libraries were prepared using the NEBNext Ultra ll FS DNA Library Prep kit. After quality and quantity check, libraries more than 2nM were sequenced with HiSeq 3000 sequencer. Taxonomic analysis was conducted using MetaPhlan2 to generate a taxonomy abundance profile for each sample.

Results

At first, the metabolomic analysis performed on 49 subjects in the old cohort and 47 subjects in the younger showed a significant decrease of fatty acids and lipids for the old cohort.

Regarding the microbial study performed on 49 subjects in the old cohort and 46 subjects in the younger, alpha diversity was significantly increased in the old *vs* young cohort in all areas. Moreover, *Cutibacterium (*previously *Propionibacterium) acnes* had a lower relative abundance in the older cohort, contrary to *Corynebacterium kroppenstedtii* and *Veillonella. parvula* which had a higher one (Figure 1A).

Surprisingly, the *Lactobacilli* prevalence in young cohort and the decrease of the most abundant in the old wrinkle skin area was unexpected. Indeed, in the 2 cohorts, and among the 12 most prevalent lactic acid bacteria present in the wrinkle hollow (Figure 1B) or the crow's feet area, lot of them were *Lactobacilli.* Moreover, among the 6 most abundant lactic acid bacteria present in the wrinkle hollow, at least 3 *Lactobacilli* were observed in the young cohort and were decreased in the old one, with *Lactobacillus crispatus* being undetectable (Figure1C).

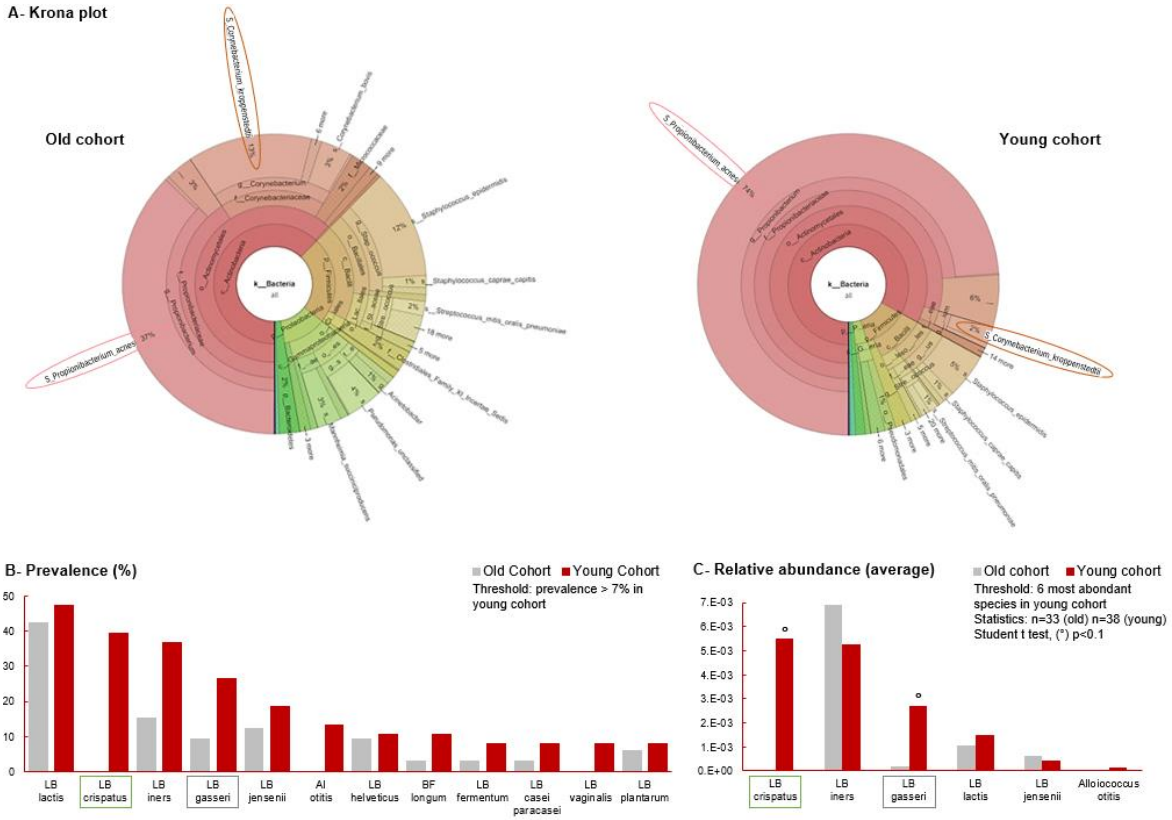

Figure 1: Comparison of bacterial repartition with aging: Krona plots for the crow's feet area (A) and Lactic acid bacteria prevalence (B), or relative abundance (C) in the wrinkle hollow.

Conclusion

We confirmed previous results on diversity shifts relative to age for *Cutibacterium* and *Corynebacterium* [1]. We hypothesized that the significant decrease of both triacylglycerides and free fatty acids in older skin may be responsible for the higher microbial diversity and the shift from *Propionibactericeae* to *Corynebactericeae*. Surprisingly, we discovered that among the lactic bacteria species observed in both cohorts, most of the more prevalent and abundant were *Lactobacilli;* but among them *Lactobacillus crispatus* was particularly decreased in the wrinkle hollow or area. Interestingly, *Lactobacillus crispatus* was previously described to be present in healthy skin but not in atopic dermatitis or psoriatic skin and suggested to have an anti-inflammatory role in both pathologies [2]. Thus, we hypothesized that this commensal *Lactobacillus* strain could also help to improve skin aging signs such as wrinkles.

This study gives deep insight into the microbiota composition into the wrinkle zone. This understanding may contribute to the development of solutions to favor more desirable and young skin phenotype through either direct contact from these beneficial bacteria or use of their metabolites.

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About the speaker

Allison Garlet, MSc - Technical Service Specialist - BASF

Allison is a Technical Service Specialist for BASF's Bioactive ingredients portfolio and is based out of Tarrytown, NY. She has a BSc in Biology from Seton Hall University and a MSc in Microbiology and Molecular Genetics from Rutgers University. She has a background in industrial microbiology research with a focus in microbial physiology, biofilms, and microbiomics.

Skin microbiota modulation made visible:

The prebiotic effects of an *Epilobium fleischeri* extract

Riccardo Sfriso, PhD.; DSM Nutritional Products, Personal Care and Aroma

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Introduction of research

The cutaneous microbiota is being increasingly considered in the cosmetic industry as fundamental to the maintenance of healthy skin. The skin microbiota composition differs highly between body sites [1-3]. Facial skin is a particularly complex environment made of different skin types such as sebaceous (forehead, nose, and chin, also known as T-zone) and dry (cheeks). The composition of the skin microbiota on different facial sites has not been described yet. Therefore, we conducted a clinical study to assess both the bacterial composition on five different facial areas as well as the modulatory effects on the microbiota resulting from the topical application of a plant extract (*Epilobium fleischeri*) known for its sebum regulating properties [4].

Materials and Methods

Clinical study design
Caucasian female volunteers (N=23) in the age range of 20-45 years old were enrolled for the placebo controlled and randomized clinical study. Following a 5 days long conditioning phase, facial application of the leave-on test product, either placebo or active formulation containing 3% of *Epilobium fleischeri* extract, was performed by the study participants at home, twice daily.

Skin microbiome sampling and 16S rRNA gene sequencing
Skin microbiome samples were collected on 5 different facial sites at baseline and after 4 weeks of product application via skin swabbing (Figure 1). DNA was extracted and 16S rRNA sequencing was performed on Illumina's MiSeq platform with paired end 300 bp reads. Pseudoreads were collapsed into 97% OTUs. Their taxonomic classification was performed using the RDP database [5].

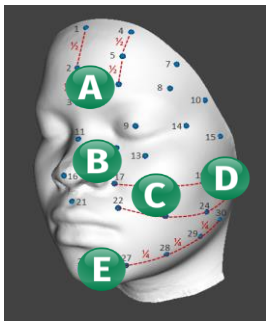

Figure 1. The five microbiome sampling areas on the face. A, forehead; B, nose; C, front cheek; D, lateral cheek; E, chin.

Data filtering and analysis
Co-occurrence filtering methods were applied to establish shifts in the most common microbiota [6]. We built the statistical model testing for differences between the study groups using Songbird [7]. Differentials were calculated to indicate association of the features (OTUs) with the active formulation, using placebo-valued samples as a reference. Songbird allowed to rank microbes based on their association with the active product. Qurro [8] was used to compute log ratios of these ranked features.

Facial colour mapping

Colour maps were generated by combining the mean 3D images and the median values of bacteria pairs' log ratios for each study group. A gradient of blue colour was assigned to indicate higher log-ratio values ($0 < \text{log-ratio} < 2$) whereas a gradient of red colour was assigned to indicate low and negative log-ratio values ($-2 < \text{log-ratio} < 0$). The changes projected onto a 3D face allow for the identification and visualization of the facial sites in which the microbial shift occurred.

Results

All the facial sites considered were highly populated by *Cutibacterium acnes* whose relative abundance ranged from 90% on the forehead, the oiliest site, down to 75% in the lateral cheek, the least oily site. Skin commensals such as *Staphylococcus hominis*, *Staphylococcus epidermidis*, *Micrococcus yunnanensis* were found to be positively associated with the active formulation. In contrast, *Staphylococcus capitis*, *Corynebacterium tuberculostearicum*, *Corynebacterium kroppenstedtii* were found to be negatively associated. Log ratios of bacteria pairs were then calculated and compared over placebo. The results were both plotted in a graph and visualized via an innovative facial colour mapping approach which facilitated the visualization of the microbial modulation effects of the *Epilobium flesicheri* extract (Figure 2-3).

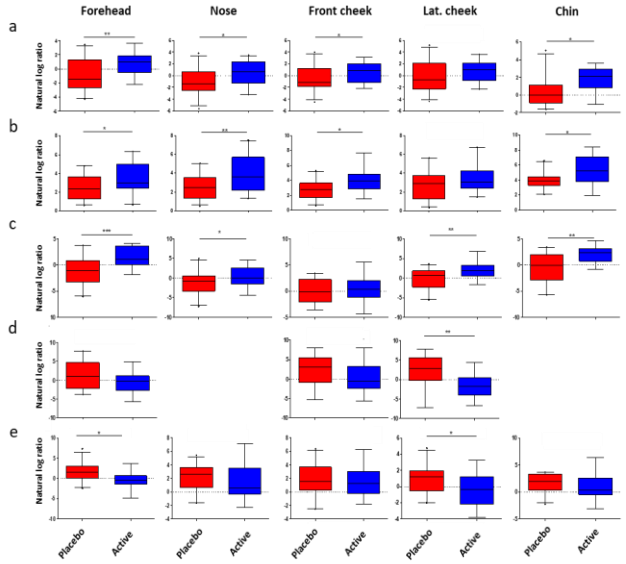

Figure 2. Log ratio of several taxa identified from the differential ranking analysis. Box plots illustrating the natural log-ratio of: a) *S.hominis*/*S.capitis*; b) *S.epidermidis*/*S.capitis*; c) *M.yunannensis*/*S.capitis*; d) *C.kroppenstedtii*/*M.yunannensis*; e) *C.tuberculostearicum*/*M.yunannensis*; across the placebo and active groups. Statistical significance has been calculated via Welch's t-test (*, $p < 0.05$; **, $p < 0.001$; ***, $p<0.0001$).

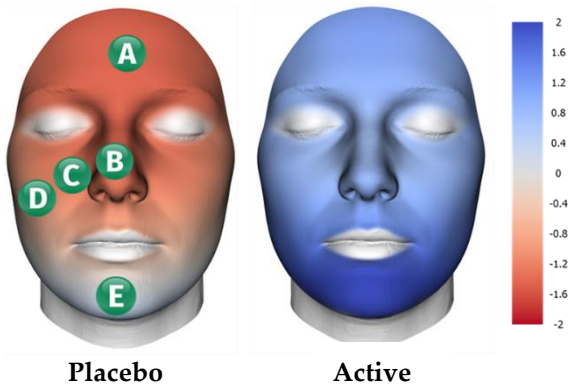

Figure 3. The color maps show log-ratio increase in *S.epidermidis/ S.capitis* after 4 weeks treatment with the placebo (left) and with the product (right). Color code (-2 to 2) is shown on the scale on the right-hand column. (A, B, C, D, E were the facial sampling areas)

The facial microbiota resulted to be significantly enriched in *Micrococcus yunnanensis* ($p=0.0003$, on the forehead), *Staphylococcus epidermidis* ($p=0.03$, on the forehead), and *Staphylococcus hominis* ($p=0.0043$, on the forehead), after 4 weeks. In contrast, a significant lower abundance of *Staphylococcus capitis* ($p=0.0003$, on the forehead), *Corynebacterium tuberculostearicum* ($p=0.01$ on the forehead) and *Corynebacterium kroppenstedtii* ($p=0.004$, on the lateral cheek) was observed.

Conclusion

In summary, our study showed that different facial sites are colonized by different proportions of bacteria, with *C.acnes* being the most abundant, but present in different proportions depending on the biophysical features of the facial skin location i.e. sebaceous area vs dry areas (e.g. forehead vs lateral cheek). Four weeks-long topical application of a natural *Epilobium fleischeri* extract rich in oenothein B resulted in a significant modulation over a series of beneficial facial skin commensals, such as *S.epidermidis*, *S.hominis* and *M.yunnanensis*, providing a beneficial enrichment of these microorganisms in the final microbial composition, while depleting it from opportunistic bacteria such as *S. capitis*, *C. kroppenstedtii* and *C. tuberculostearicum*.

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About the speaker

Riccardo Sfriso, Ph.D.

Lead Scientist Skin Microbiome, Scientific affairs Skin Care

Dr. Riccardo Sfriso joined DSM in April 2019. As a member of the Skin Care Science and Promotion team, he leads all skin microbiome related activities in R&D and is a study director in charge of planning and management of clinical efficacy studies.

Dr. Sfriso holds a PhD in Biomedical Sciences from the University of Bern in Switzerland and a M.Sc. Degree in Chemistry and Pharmaceutical Technology from the University of Padova in Italy.

He published in high-impact scientific journals and has been invited at several congresses and expert meetings as keynote speaker representing DSM's scientific expertise.

Precision Skincare: revealing and addressing true skin needs through a continuous learning model

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Introduction of research

Skin is a dynamic biological tissue, which responds to signals, both internal and external. Today skincare on the market is a trial-and-error approach for many customers. Unaware of their biological needs, consumers must navigate thousands of possible products. If tested, products are tested on a limited set of individuals which may or may not reflect the consumer's skin physiology, ethnicity, and other factors impacting that individual consumer's skin. There is no guarantee that the claims of a given product will provide the same benefits in each individual consumer, fueling the growing interest in safe and science-backed personalized skincare [1-2].

The precision skin care approach consists of an objective system and method for assessing each individual consumer's skin physiology and need and making skincare recommendations and skincare formulations that target those specific underlying skin needs. The approach flips the current model to start with consumers and creates the technology and data tools to educate, inform, and empower consumers that is missing in the industry today. Measuring skin needs and creating a suitable treatment rooted in science allows for a transparent and objective approach to product recommendation and evaluation.

The Precision Skincare Methodology

The precision skincare method is based on measuring a combination of parameters that impact skin health and appearance [3-5]. These parameters are summarized in an overall skin radiance score that describes how light interacts with skin at the surface as well as deeper layers, as a proxy for its underlying biological state. The optical radiance of skin is a composite of the underlying physiology including how much light gets absorbed (by pigment and blood) and where, how much light gets reflected at the skin surface by shine or by skin dryness, how much shadowing is created by lines or under-eye puffiness, and how much light gets bounced back from the collagen to the surface, creating that soft and dewy, diffuse radiance.

The overall radiance score for each individual is decomposed into six high level skin parameters including: skin barrier/hydration, smoothness, skin tone, skin mileu, energy supply and dermal fibers. Those parameters are multiple sub-parameters linking to a series of underlying biological processes that are measured for each individual person. Both visible surface and invisible subsurface skin characteristics can be quantified using known gold standard equipment [6-7]: parallel/cross polarized and UV imaging, spatial frequency domain imaging (SFDI), hyperspectral imaging, and Courage and Khazaka bio-instrumentation measurements. By combining the outcome of these different measurement modalities, we are able to build an individual composite skin signature for each consumer and objectively measure his/her skin needs.

Based on the obtained results, a patented AI data pipeline predicts the most efficient ingredient combination for that specific skin profile. To do so, each individual skin need is evaluated compared to all others, and an overall ranking of individual biological needs is created. Based on the relative weights of needs an optimal combination of ingredients is selected. The optimal combination of active ingredients with known mechanism of action is most likely to improve overall radiance.

Further, via re-measurement an empirical testing feedback loop is created testing predicted efficacy in each individual by tracking their progress in response to their personalized treatment. Thereby continuously optimizing each individual's treatment regimen and improving treatment efficacy for future consumers with similar signatures.

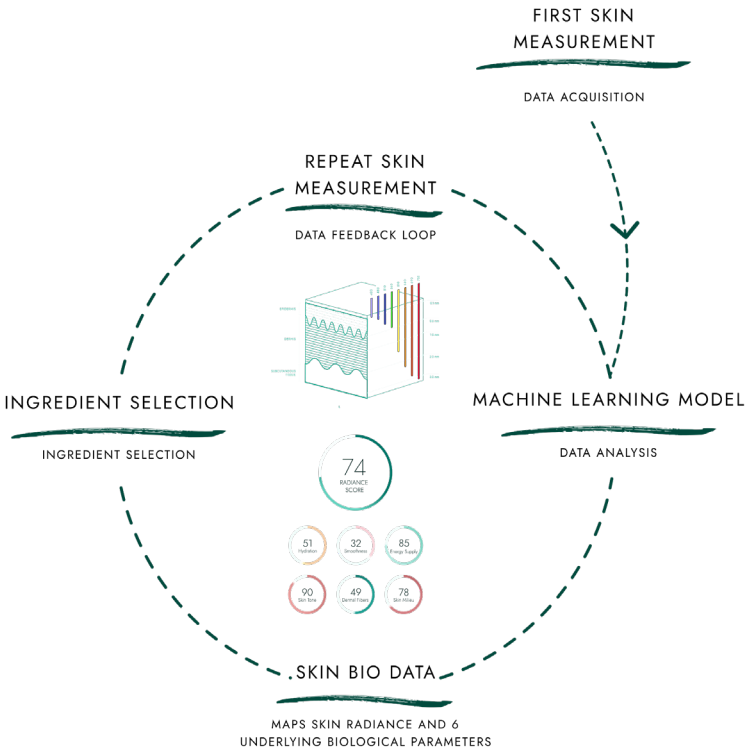

Figure 1: Precision Skincare Learning Loop

Conclusion

The Precision Skincare System redefines consumer education and product selection. It starts with the individual, identifies individual needs, and builds the most optimal product based on those needs. This enables more efficacious treatment, greater consumer satisfaction, and creates a learning model where individual skincare routines continually adapt with the consumer's changing skin needs and the overall efficacy skincare treatment can be improved.

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About the speaker - Liyuan Ji

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