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Skin: Surface to Depth
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Oral Presentations

Wednesday May 2nd

Plenary I: Delivery of Actives

Keynote Lecture - Overcoming the Barrier

No Abstract

Screening Transdermal Patch Compositions to Optimize Delivery

Avadhesh Singh Kushwaha, Judit Bartalis, Edward T Kisak, Elena Alifanova, John M Newsam

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Introduction: Transdermal patches have increasing patient and consumer acceptance, yet the requirements of the drug-in-adhesive format can greatly limit the space of compositions that might viable. Over a number of transdermal patch innovation programs, we have found that high throughput experimentation ("HTE") approaches (which allow larger numbers of patch compositions to be prepared and one or more of the properties of each to be assessed) can be particularly beneficial.

Methodology: We outline these screening methods illustrate their utility in programs directed the research and early development of patched for hormone delivery, analgesia, and other applications. In these programs, we typically apply our HTE technologies and methods to screen some 150-300 compositions in a given patch program.

Results and Conclusions: We will describe how these approaches can provide robust bases for composition of matter patentability.

Mechanisms of Anionic Surfactant Penetration into Human Skin

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Introduction: Anionic surfactants, which are often used to provide the cleansing action in rinse-off products, can induce skin dryness and irritation over time. The objective of this study was to examine mechanisms of surfactant penetration into human skin.

Methodology: Sixteen single and mixed surfactant systems were characterized using tensiometry to determine critical micelle concentration, a zetasizer to determine micelle size and zeta potential, and a dye complexation/filtration method. Surfactant systems were then tested for penetration into human skin *in vitro* using Franz diffusion cells and a radiolabeled tracer.

Results and Conclusions: Neither the monomer penetration theory nor the micelle penetration theory as proposed by Blankschtein's group (JCS 2003) is sufficient to explain anionic surfactant-skin penetration. We propose short term anionic surfactant penetration into the skin is a function of micellar kinetics, while long term skin penetration is a function of charge-induced barrier damage.

Plenary II: Neonatal Skin in honor of Steven B. Hoath

Keynote Lecture - Nature's Multi-Functional Skin Cream: Vernix Caseosa

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Introduction: Have you ever wondered how it is possible for a baby to have such good skin condition despite being immersed in water (amniotic fluid) for nine months? Surely, an understanding of how this happens would likely provide information to use for the development of optimum skin care products. Vernix caseosa, the thick white cream that covers newborn baby skin, is, perhaps the biggest factor in achieving excellent skin condition

Methodology: The literature on vernix caseosa was reviewed to provide an overview of the developments and future potential of this unique material.

Results and Conclusions: Vernix caseosa has multiple physical and biological functions. It contains lysozyme, lactoferrin and other microbials with demonstrated anti-infective properties. Vernix films in vitro impede penetration of the exogenous. enzyme chymotrypsin and, hypothetically, serve to establish epidermal-amniotic fluid gradients of water, nutrients, and electrolytes necessary for normal "inside/outside" architecture and barrier formation. In animals with barrier compromise (via tape stripping), vernix enhances SC formation without increasing epidermal thickness. Vernix functions as a natural moisturizing agent and a skin cleanser, although it is often viewed as a soil itself. Its ow surface energy suggests that it creates a protective hydrophobic layer around the fetus. Vernix contains multiple cytokines such as IL1α, IL1β, TNFα, IL-6, IL-8, and MCP1. The role of these cytokines is just being explored but the association of specific antimicrobials with hydrated granules within vernix supports a mechanism for "quick release" in the presence of chorioamnionitis. Vernix contains cholesterol, ceramides and a number of fatty acids, including oleic, linoleic and long chain species. Fatty acids, particularly linoleic, activate peroxisome proliferator-activated receptor-α (PPARα) which increases the rate of barrier formation. Linoleic acid has anti-inflammatory properties. Overall, vernix facilitates

development of the stratum corneum protective barrier in the normal, full term infant through a variety of protective and adaptive mechanisms.

These functions, coupled with its anti-infective properties, are essential for a premature infant who may not have exposure to vernix in utero. The findings support vernix retention at birth. The World Health Organization recommends that vernix be left in place for at least six hours prior to bathing newborn infants.

Digital Image Analysis Reveals Features of Neonatal Skin Condition after Oil Massage in the Community Setting of Rural Nepal

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Introduction: Nearly all of the annual, global neonatal death toll of 3 million infants occur in developing countries due to premature birth, infections, and complications such as birth asphyxia. Efforts to reduce mortality have shown that specific skin care practices, including topical emollient application and cleansing with chlorhexidine, may reduce neonatal mortality. A compromised or under developed neonatal skin barrier increases the risk of exposure to irritants and infectious agents, particularly because adaptive immunity is not yet operational. As environmental conditions impact stratum corneum development, it is important to understand these effects in combination with skin care practices during the neonatal period. We examined the effects of two emollients for routine massage on skin condition using digital imaging and analysis among a subset of premature and full term infants who participated in a cluster-randomized controlled trial in the high humidity community settings of rural Nepal.

Methodology: Neonates from the main trial were randomized receive to either sunflower seed oil (SSO, intervention) or mustard seed oil (MO, standard community-wide practice) for routine full body massage from July 2012-May 2014. Standardized high resolution digital images were obtained from the chest site of a subset of 209 infants on days 1, 3, 7, 14 and 28. Skin condition (erythema, rash, dryness), skin surface pH, stratum corneum (SC), and transepidermal water loss (TEWL) were also measured. The images were color corrected, converted to CIE Lab color space, separated into individual L*, a* and b* channels and processed to extract objective features using ImageJ (NIH). Image mean, mode, standard deviation, variance, skewness, and kurtosis and skin chroma were analyzed. The effects over time for each oil group using general linear models (GLM). The impacts gestational age and oil treatment were assessed using linear mixed models with repeated measures procedures (SPSS v22). Imaging parameters were compared with the other measures of skin condition.

Results and Conclusions: Infants were stratified by gestational age (GA) into three groups: (1) < 34 weeks GA, (2) 34-<37 weeks GA, and (3) ≥ 37 weeks GA (full term). Mean GAs were 30.6 ± 3.0, 35.8 ± 1.0 and 40.4 ± 2.2 weeks, respectively. For this subset, the skin condition results were generally consistent with those for the total population. Skin pH decreased with differences for days 1, 3 and 7 versus all others ($p < 0.05$). TEWL increased and protein decreased over time Chest erythema first increased then decreased, where day 1 was significantly lower than days 3 and 14. Chest Rash increased until day 14 then decreased. Qualitatively, several features and trends were noted. Miliaria crystallina, i.e., vesicles at the stratum corneum level, appears to be the dominant "rash type". A distinct skin texture developed over time. This is best observed from viewing the case studies. Images from early in the neonatal period tend to have less texture. Miliaria followed the pattern of rash scores with

rapid increase after birth until day 14 and then diminishing by day 28. This may be due to the high humidity and temperature conditions in the region coupled with an immature sweat response in the early postnatal period. The images reveal the development of skin texture, i.e., raised areas, as well as miliaria, that may be related to hydration and eccrine sweat gland occlusion. No significant treatment effects were observed for the imaging parameters. There were significant changes in skin yellow color (increasing), red color (increasing) and lightness (decreasing) over time. Skin chroma was related to skin rash scores with higher values for higher rash scores.

Linear regression modeling was conducted to identify factors that impact skin chroma. Approximately 63% of the variance in chroma was explained by 4 factors: temperature, rash, TEWL and humidity. When humidity was high, chest erythema was low. When rash was high, pH was lower. When TEWL was high, rash was high. From an analysis of the subjects with no visible skin damage, these changes may be maturational and adaptive. The images provide insight regarding the potential mechanisms of changes during the immediate neonatal period.

Objective Assessment of Dry Skin Roughness in a Pediatric Population

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Introduction: Clinical scoring of dry skin includes tactile evaluation of the skin surface to assess roughness. To our knowledge, no instrumental evaluation of roughness has ever been performed to objectively score skin dryness in a pediatric population, nor to document the clinical efficacy of skin care products.

Methodology: A study has been conducted on 80 subjects with normal or dry skin (40 children each), aged from 8 weeks to 4 years. Roughness has been measured on the face using an innovative tribo-acoustic device. Measurements have been performed at T0 and after 21 days of daily face cream product application.

Results and Conclusions: Measurement shows that roughness is significantly higher in dry skin than in normal skin. After 21 days of product application, significant improvement of tribo-acoustic parameter has been observed on normal skin and also on dry skin suggesting global improvement of skin roughness following product application.

This study shows that objective assessment of roughness could be of great interest to characterize dry skin in a pediatric population and to demonstrate clinical efficacy of skin care products

In honor of Steven B. Hoath

Marty Visscher with audience participation

Among its members, the ISBS was privileged to have an imminent scholar, visionary, philosopher, physician, scientist and colleague, the late Steven Bradley Hoath, MD.

Steve' research efforts focused on the ontogeny of premature and full term human skin development. This is important as an understanding of neonatal skin development forms the basis for skin care and development of products for all ages. Understanding the stratum corneum responses to environmental effects enlightens other aspects such as wound healing and skin restoration. All of us who worked with him can attest to the way Steve's energy, enthusiasm and ability to "think outside the box" led him to make very significant contributions to our understanding of skin in general and neonatal skin in particular.

Plenary III A: Barrier Function and Hydration

Keynote Lecture - Atopic Dermatitis and the Barrier

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The epidermis is the interface of the human body to the potentially harmful environment with exogenous stressors like chemicals, UV radiation other physical impact. The epidermal barrier is recognized as a central key pathophysiologic element in inflammatory skin diseases such as atopic dermatitis (AD). Some bases of an impaired barrier have been elucidated on the molecular level e.g. mutation in genes encoding for filaggrin and lipid processing defects. Recently, alterations in the microbiome composition and its relation to altered barrier function were reported.

Multiple non-invasive biophysical measurement instruments are used to assess skin physiology especially in inflammatory skin diseases associated with an altered epidermal barrier e.g. transepidermal water loss, stratum corneum hydration, surface pH, inflammatory signs and surface parameters. Clinical scores for AD disease activity are widely used but rely entirely on subjective criteria in assessing both the severity of lesions and the extent of involvement. Non-invasive biophysical instruments are available and introduced into clinical evaluation of chronic diseases and treatment effects. In AD objective scores including biophysical measurements have been published. Quantifying barrier function, stratum corneum hydration, erythema, scaling, and sub-epidermal edema as well as estimates of involved body surface areas are implemented in assessing the severity of AD. Sensitivity and reliability of these severity scores have been published involving computer assisted software and measurement devices. New models are now developed to use standardized approaches in AD in clinical studies. Recently, non- or minimal-invasive methods have been used in different AD research areas. These methods include multidimensional imaging, in vivo multiphoton spectroscopy, optical coherence tomography, atomic force microscopy, near-infrared spectroscopy (NIR), in vivo Raman micro-spectroscopy and in vivo reflectance Raman spectroscopy. The state of the art of established non-invasive novel methods and their value/limitations in in AD research will be discussed. The combination of established approaches with cutting edge methods will allow to gain a deeper understanding of barrier related inflammatory skin diseases. Eventually biomarkers can be derived from these studies for diagnostic and preventive purposes as well as monitoring of disease activity during specific treatment regiments.

Skin Barrier Impairment Due To Occlusion by Firefighter Clothing

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Introduction: At fire scenes, firefighters are exposed to potentially harmful substances. Post firefighting studies showed increased biomarkers of carcinogenic combustion products in firefighters bodies. Besides inhalation, skin contamination and risk of dermal absorption is getting more attention. In this perspective, skin barrier impairment due to the occlusive effect of firefighter clothes could be a mechanism for enhanced penetration risk of hazardous substances.

Methodology: In a paired comparison involving 16 volunteers, the effect of cellophane and a firefighter coat were studied. TEWL, SSWL and skin permittivity were measured at three time intervals;(1) before (2) immediately after 30 minutes of occlusion and (3) 30 minutes after occlusion. Reflectance confocal microscopy was used to study the skin morphology.

Results and Conclusions: TEWL values immediately after wearing a firefighter coat were significantly increased. This is an indication of an occlusive effect of the firefighter coat. The skin barrier was fully restored after 30 minutes. In conclusion, wearing a firefighter coat contributes to an increased risk of dermal absorption.

In Vivo Confocal Microscopy: From the Optical Biopsy of the *Stratum Corneum* to the Quantification of the Barrier Function

Rouaud-Tinguely Pauline, Boudier David, Breugnot Josselin, Rondeau Delphine, Le Guillou Maud, Bordes Sylvie, Closs Brigitte

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Introduction: To better understand the modifications of the *stratum corneum* (SC) when cutaneous barrier is altered, we needed to develop a new and dedicated method to study the quality of this latter. The *in vivo* confocal microscopy, which is a non-invasive tool allowing optical skin biopsies, is helpful to study the SC with fluorescence modality. However, the images acquired with the microscope are individual images and do not allow a complete view of the organization of the cells of the SC. We developed an innovative approach to get more spatial information in order to obtain images that can be interpreted by an expert or a clinician.

Methodology: Images were acquired from skin surface up to 18µm. Then, dedicated algorithms to reconstruct a single image from the several images acquired were designed and programmed to access to a complete information of the structure of the SC which is not possible by looking at image by image. This complete information obtained thanks to the reconstructed image of the SC help to develop a scoring scale adapted to study the squama and the cellular shape and arrangement. To validate this approach, we studied the impact of an artificial disturbance induced by sodium lauryl sulfate (SLS) on 9 volunteers with normal skin. Images reconstructed with the algorithm were quantified by experts.

Results and Conclusions: Compared to day 0, SLS treatment increased the presence of squama and modified corneocytes shape towards a more rounded shape with arrangement in scales (decrease by an average of 2 stages; $P<0.05$). The study also showed a negative correlation between TEWL and cell organization (-95.9%; $P<0.001$). Altogether, these results demonstrated that this innovative approach enabled a quick analysis of the SC. Supplemented with in vivo confocal Raman microspectroscopy allowing analysis of molecular descriptors of skin barrier function, this approach could support the substantiation of restructuring dermo-cosmetic products in clinical studies.

Plenary III B: Stratum Corneum Hydration

Joint Session of the International Society for Stratum Corneum Research and ISBS

Keynote Lecture - Application of Corneocyte Analyses

Tetsuji Hirao

Chiba Institute of Science

Introduction: Cornified envelope (CE) is an insoluble membrane-like structure surrounding corneocytes, and is essential for barrier function of the stratum corneum (SC). In this presentation, I will introduce non-invasive evaluation of CE maturity using tape-stripped SC and its applications.

Methodology: The outermost SC samples were collected by tape-stripping, and CE was stained with anti-involucrin and fluorescein-labelled secondary antibody, followed by Nile-red staining. This involucrin/Nile red staining can discriminate involucrin-positive immature CE from Nile red-positive mature CE.

Results and Conclusions: The face SC contained much amount of immature CE even in the outermost SC. The SC of inflammatory skin disorders, psoriasis and atopic dermatitis, exhibited increased ratio of immature CE. Immature CE in the face retains a potential to mature by endogenous transglutaminase activity. However, decrease in SC water content resulted in suppression of CE maturation. Application of humectants onto the SC upon incubation could mitigate CE maturation. The evaluation of CE maturity can also be applied to evaluation of efficacy of skincare cosmetics.

Capacitance Imaging of Barrier Disruption

Tim Houser[1], Gary Grove[1,2], Charles Zerweck[1], Lee Wildman[1,2]

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Introduction: Capacitance imaging technology which allows one to produce a detailed image of skin surface microtopography and moisture is starting to become more available. These devices, based on biometric fingerprint sensors, use a capacitive sensor and convert the collected matrix of data to a gray scale image. Measurements of changes in surface texture patterns, moisture and moisture accumulation can be made from these images, providing additional information not available via other methods.

Methodology: Capacitive imaging was used to assess changes in the skin barrier via 3 common forms of disruption: SLS damage, repetitive tape stripping and razor trauma from dry shaving. Images with other polarized imaging systems, TEWL measurements and moisture measurements were collected.

Results and Conclusions: Data from this research suggest that TEWL rates may be calculated from capacitance imaging devices while providing additional information regarding patterns of barrier disruption. Additionally, these devices may be better suited to differentiate between TEWL and EWL as well as elucidate the relationship between dry skin and TEWL.

Skin Barrier Function: Impact of the Skin "Very" Aging on Stratum Corneum Lipids Organization and Water Content *In Vivo*

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Introduction: Clinical manifestations of skin aging like xerosis, wrinkles and slackness are related to underlying complex molecular phenomena in the different layers of the skin. The combinations of classical biometric measurements with more complex and informative techniques like *in vivo* Raman spectroscopy can provide interesting information on the organization of lipids in the *Stratum Corneum (SC)*, their barrier function and on water content and mobility, in order to better characterize the skin aging.

Methodology: Biometric information (TEWL, corneometry, sebumetry, skin pH, mechanical stress) and Raman spectra and in-depth profiles were collected from the forehead of twenty-two young womens (18-24 years old) and eighteen elderly womens (70-75 years old).

Results and Conclusions: Important modifications on biometric skin parameters, structure of the SC and water mobility can be observed for elderly. Our results show a good association between biometric parameters and *in vivo* Raman descriptors. Interestingly, higher compacity of lipids, higher total water content and lower unbound water content are observed for elderly.

Characterizing Skin Hydration Dynamics of Human Skin Using Confocal Raman Microscopy

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Introduction: Confocal Raman microscopy (CRM) can directly quantify water and deuterated water (D_2O) content inside the skin. In this study, we explored the capability of CRM in combination with D_2O as a probe molecule to thoroughly evaluate absorption and desorption of exogenous 'water' (D_2O) to characterize changes in hydration status.

Methodology: A customized confocal Raman spectrometer (modified from Skin Analyzer 3510, RiverD, Netherlands) and a CRM (WITec Alpha-300R Plus, Ulm, Germany) have been exploited for *the in vivo* and *ex vivo* experiments of this study, respectively. The absorption and desorption of D_2O was used to characterize skin hydration changes under different treatment conditions.

Results and Conclusions: Occlusive patching of D_2O alone penetrated into the stratum corneum, with a short desorption period (~15 min) after patch removal. With application of humectants, D_2O penetrated deeper and was retained longer (~30 min) inside the skin. 2D distribution of D_2O inside skin explants was obtained as well. In summary, the developed methodology can be used to characterize hydration dynamics and to evaluate product effects.

Thursday May 3rd

Plenary IV A: Skin Imaging in Honor of Nick Kollias

Keynote Lifetime achievement lecture in Honor of Nik Kolias

G.K. Menon,PhD
California Academy of Sciences, San Francisco, CA..

Nik Kollias; Physicist extraordinaire, continues to inspire us to seek new frontiers in skin biology

As a Biologist, I feel woefully under-qualified to comment on the details of Nik's innumerable experimental work, which he carried out as a Physicist with characteristic precision and attention to specifics of the tools he employed, to quantify the various skin attributes. In our interactions and numerous discussions, he must have been frustrated about explaining to me the technical nuances of his work, but he never showed it. He did appreciate the fact that Biology is an " inexact science" , less measurable and quantifiable than the physical sciences- which only seemed to excite him about his results and motivate him to ask his " stupid questions" to hapless biologists who knew they have more work to do . I mostly tried to get away using " it depends' – as a patent attorney taught me. But Nik would surely follow up later, until at least we had a mutually acceptable theory to put to test.

In this rather limited presentation, I would try to show some of Nik's work that fascinated and motivated me to explore it's significance to skin biology, and possibly to biology in general. A case in point is the seminal work on the Tryptophan fluorescence in human skin .Another is the use of Photoacoustic waves to permeabilize stratum corneum for drug delivery. Some of Nik's close associates have helped me in this task, by providing slides from their work, who are acknowledged in this presentation.

Characterization and Statistical Modeling of Facial Skin Radiance in Senior Women

Hourblin Virginie[1], Nouveau Stéphanie[1], Faugère Julien[1], Gomes Charles[1], Tardy Isabelle[2], Aguilar Luc[1]

[1] L'Oréal Research and Innovation, Aulnay-sous-bois, France
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Introduction: Dull skin is a major concern for senior women but even though some parameters such as optical parameters seem to be involved in the perception of skin radiance, there is a lack of objective assessments, and it remains difficult to assess. A typological study was conducted in order to characterize the drivers of lack of facial skin radiance in senior women using a holistic and cartographic approach. Knowing this, the change level required for each key driver was determined to improve overall skin radiance then confirmed through a validation study.

Methodology: In a first step, a typological study was carried out on 150 French women, phototype II or III, aged over 55 years, and distributed in two groups according to their lack of radiance as scored by a dermatologist (severe versus light to moderate). A large number of parameters including skin type and texture, skin aging signs, wrinkles, pigmentary disorders and dark circles were assessed by a dermatologist and by self-assessments. Instrumental measurements were also performed, skin color using the L*a*b* system (Spectrophotometer® CM-700d), skin shininess (Lightcam®), backscattered light (Translucymeter® TLS850), current level of sebum (Sebumeter® SM815), skin conductance (Corneometer® CM810), and skin density by ultrasounds (DUB®SkinScanner 75). Qualitative and quantitative Bayesian Belief Networks were designed to characterize the lack of radiance and to set a predictive model of radiance improvement for both women and dermatologist. In a second step, a

validation study was carried out on 90 women with a similar profile, and presenting a lack of radiance according to the key features. The predictive model was used to define the expected change range of each feature; this prediction was validated with a combined cosmetic routine.

Results and Conclusions: Bayesian statistical approach was effective for identifying and ranking the key drivers of facial skin radiance. The first striking result was that lack of radiance as assessed by the expert was driven by dark circles, skin shininess, pigmentary disorders, backscattered light and skin density, but usual aging signs such as wrinkles did not contribute to it. Interestingly, these key drivers were also perceived as such by the women enrolled in the study. According to the statistical model, improvement of facial skin radiance in senior women can be reached by decreasing dark circles and skin tone unevenness and by increasing the skin shininess. For each of the key features, we were able to define target values (clinical scores or instrumental measurements) in order to improve the overall radiance. These targets have been validated through the second study, by clinical and self-assessments of radiance after combined skin care and make up applications. These two clinical studies allow us to have now, a tool based on objective clinical targets, in order to get more radiant skin in senior population.

A Novel Method to Measure Skin Mechanical Properties with Three-Dimensional Digital Image Correlation

Zhequan Xu, Jomer Dela Cruz, Christina Fthenakis, Claude Saliou

The Estee Lauder Companies, Inc. Clinical Innovation and Research Division

Introduction: Measuring skin mechanical properties have been of great interest in the skincare industry. We present 3D digital image correlation (3D-DIC) as a high accuracy and non-invasive optical technique to quantify skin movement and deformation under mechanical perturbations.

Methodology: Studies were conducted with subjects aged 25-65 years. After a refined speckle pattern was applied to the skin, a force pulled the skin at a constant velocity. Images were taken and correlated by DIC. DIC's reproducibility across multiple pattern applications, measurement repeatability and sensitivity were investigated. In addition, Cutometer® measurements were taken on the same area.

Results and Conclusions: Several DIC metrics were used to quantify skin mechanical properties. The method is repeatable and reproducible as most random errors are small compared to the mean responses. Some Cutometer® parameters showed good agreement with skin displacement measured by DIC. Other DIC metrics potentially offer new insights into skin mechanical properties not previously revealed by conventional techniques. High resolution, large measurement areas, and ease of direct visualization are substantial advantages of DIC.

Skin Surface Oiliness & Shine Measurement using Polarized Images

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Introduction: We demonstrate use of differential skin reflectance measurement obtained from parallel & cross polarized images for quantitative measurement of skin surface oiliness & shine. This is unfortunately the last project we have conseptualized and worked with Dr. Kollias.

Methodology: Different levels of skin surface oiliness were simulated in 20 subjects within a 2 inch square area of their cheek. Parallel & cross polarized images were captured using Canfield Scientific's VISIA-CR, OLÉ & LAB Imager. Captured images were evaluated by investigators for clinical assessment

of oiliness. Shiny & glossy areas were segemented from the differential reflectance image & their mean intensity values were computed as a measure of oiliness.

Results & Conclusions: Strong correlation is observed between the amount of skin surface oil and the mean intensity measurement from the differential reflectance image. The relationship between oiliness and proposed differential reflectance measurement is independent of the incident and reflection angles. Non-invasive quantitative assessment of skin surface oiliness is possible using the proposed technique. It will also provide spatial distribution and hetrogenety of skin oiliness information.

Plenary IV B: Skin Imaging

Keynote Lecture: From Image Analysis to Molecular Imaging

Chilhwan Oh, M.D., Ph.D.

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Introduction: Quantitative measuring of skin surface character could be done by three dimensional measuring and skin color distribution analysis. Molecular imaging could be categorized into three basic modality : optical, MR and PET molecular imaging

Methodology: Measuring the three dimensional skin surface were taken by stereoimage optical topometer (SOT). We apply the SOT in skin aging, new scoring system of patch test, SCORAD index at atopic dermatitis, quantification of PASI score in psoriasis, skin pore measuring, and acne scar measuring. Skin color analysis were taken by skin color distribution analyzer (SCDA). We had been studied using SCDA such as color analysis in dermoscopic finding, new MASI (melasma area & severity index) score, and various pigmented skin disease. Optical molecular imaging were study in wound healing, apoptosis and stem cell tracking. PET molecular imaging were used in established xenograft melanoma model. MR molecular imaging were study for melanoma model, cerebral infection model.

Conclusion: SOT and SCDA are using useful methodology in skin surface characterization, severity measure the skin disease and new drug efficacy evaluation. Advantage of molecular imaging are disease process monitoring, in vivo stem cell tracking, possible longitudinal study in single animal, and new drug development.

A Method to characterize age-related changes in fiber orientation and density of multiphoton microscopy images

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Introduction: Most methods for analyzing multiphoton microscopy (MPM) images are based on averaging the intensity over the image and therefore disregard the spatial information relating to dermal fibers. The goal of this study was to develop a new algorithm that characterizes the orientation and density of dermal fibers aiming to document differences between young and aged skin.

Methodology: MPM images were acquired from three body sites of two female age groups: 20–30 and 60-80 years old. K-means clustering and local gradient was used to extract information about fiber density and orientation. The preferential alignment of the fibers was characterized using the anisotropy ratio.

Results and Conclusions: Both collagen and elastin fibers showed higher anisotropy ratio for the older group, with the greatest difference in elastin images from upper inner arm. Collagen fiber density decreased with age, while elastin fiber density increased with age. The developed method considers the spatial information inherent to MPM images and provides additional insights on how the dermal fiber structure is affected by the aging process.

Characterization of Fitzpatrick Skin Types I-III for UV Tolerance and Extrinsic Aging Using Classical and Computer Generated Modalities

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Introduction: Popular Fitzpatrick skin type's I-III are used for sunscreen testing and antiaging studies. These skin types are more prone to the adverse effects of extrinsic aging due to their low melanin content and propensity to burn upon exposure to UV light. To better characterize these lighter skin subjects, tolerance to solar simulated light and extrinsic aging parameters were examined.

Methodology: 275 Fitzpatrick skin type's I-III were screened from a data base using MED exposure times. A representative aged matched subject pool from each Fitzpatrick skin type was chosen for wrinkle, ITA determination and smoothness analysis via the 3D imaging analysis.

Results and Conclusions: A correlation between lighter skin individuals and the energy needed to generate an erythemic reaction was established.   These findings substantiate chromameter based ITA values used to define Fitzpatrick skin types. Image data collected shows that wrinkle and texture values are increased in the lighter skin phenotypes further supporting that these individuals are most susceptible to extrinsic aging factors and as a result are preferred candidates for sunscreen and antiaging clinical studies.

The Relationship of Skin Color Parameters and the Perception of Skin Brightness

Rong Kong, Elvin Chun, Jianwei Liu, Xiaojuan Wang, Yinbei Chen

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Introduction: In many cultures skin brightness is an important element of overall beauty. Consequently, skin brightening products have become an essential category in the skin care industry. Claims of skin brightening benefits are typically supported by either color measurements (e.g., L* and ITA) or consumer/expert perception. Our goal is to investigate the correlation between the objective color parameters and the consumer perception of the skin brightness, and build a quantitative model to predict the perception of skin brightness based on color measurements.

Methodology: The study was conducted in two countries, with one panel of 39 women enrolled in the US (Ada, MI) and another panel of 36 women enrolled in China (Shanghai and Guangzhou). The L*, a*, b* values of facial images of Asian and Caucasian women were digitally manipulated by increasing or decreasing 0, 1 or 2 units with all L*, a*, b* combinations using MATLAB (Mathworks, Natick, MA). Each image after manipulation was shown side by side with the original image to the panels and the perceived brighter image between each pair was picked. The results of skin brightness perception and L*, a*, b* changes were analyzed using MATLAB Statistics and Machine Learning Toolbox.

Results and Conclusions: Our results showed that all three color parameters (L*, a* and b*) contributed significantly to the perception of skin brightness. The impact of b*, the indicator of skin yellowness, was comparable to that of L*, which indicates skin lightness and has been previously regarded as the main factor for skin brightness. The impact of a*, the indicator of skin redness, was about a quarter of that of L*

or b*, and therefore should not be overlooked when skin brightness is concerned. There were some interesting differences between the results from American and Chinese panels, which might be due to the culture differences in the perception of skin brightness. A prediction model was also built based on the data. Overall, our study showed that the traditional metrics, such as L* and ITA, can be improved to better correlate with the perception of skin brightness if all three parameters in the L*a*b* color space are incorporated into a more comprehensive skin brightness metric. This quantitative skin brightness perception model is likely very beneficial for guiding better formulation and claim substantiations.

Plenary V: Claim Support and Science Based Marketing

Keynote Lecture: Cosmetic and Personal Care Product Claims: Facts and Fiction

K.P. Ananthapadmanabhan

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Introduction: The cosmetic and personal care product claims often range from simple skin mildness and moisturisation to far reaching claims such as antiaging, skin lightening, skin rejuvenation and even cellulite reduction. Significant amount of time, effort and funds are often needed in developing scientific support for such claims. Both self-regulation by the industry R & D and the constant scrutiny of claims by the competition often help keep the claims reliable.

In spite of such self-governance within the cosmetic and personal care industry, examples of claims can be found in the marketplace that are questionable and not fully supportable by scientific data/ evidence. Such situation often arises when the experimental data is stretched far beyond its limits to make conclusions that are not directly derived from the experimental results. There are also claims based on absence or removal of certain ingredients without fully understanding the underlying reason for their removal. Furthermore, often claims are made based on minor but statistically significant differences in certain measured parameters that may or may not be perceivable by or relevant to the consumer.

Methodology: In this paper, several examples of product performance claims, mostly implied claims, are presented and their validity is examined. One such example is the "incorporation" of actives like ceramides by skin to restore the skin barrier without regard to their potential to penetrate into deeper layers to deliver the type of "implied benefits" in the claim. Another example is around pH of the product in relation to pH of skin. For example, there has been a trend recently to formulate products at pH values identical to that of the stratum corneum to make "skin identical pH" claims. The implication is that a product formulated at skin pH will enhance such functions as lipid synthesis, corneum integrity and enhanced antimicrobial activity. While this argument may be valid for some leave-leave on products, extending this to low pH cleansers completely ignores the interaction of cleanser surfactants with skin under skin pH conditions.

Results and Conclusions: The examples provided in the presentation clearly will show the need for more rigor in developing claims and providing the right type of scientific data to support such claims. Such deeper analysis will even help in developing new and improved technology for skincare.

Cross polarized UVA Photography for the Imaging of Sunscreens

Dr Jonathan M. Crowther

JMC Scientific Consulting Ltd

Introduction: A novel method for visualizing the spreading and location of SPF ingredients based on cross polarized UVA reflectance photography is described providing new insights into the formation of final film morphology and how it correlates with *in vivo* SPF efficacy for a set of test products.

Methodology: High resolution UVA based images of sunscreen films spread onto PMMA plates were captured using a modified commercial SLR camera in a custom imaging system. Visual grading and

image analysis were used to describe the overall UVA absorbance and streakiness of the resultant films, and the data compared with both *in vivo* and calculated *in vitro* SPF scores for the products.

Results and Conclusions: A strong correlation was observed between the evenness of the resultant film as determined from the photographs and final *in vivo* SPF scores.

Cross polarized UVA reflectance photography is a valuable new method for assessing sunscreen distribution after spreading, and to differentiate products based on film morphology, as well as strongly correlating with final *in vivo* behaviour.

Removal of Contaminants with Hand Washing and Drying

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Introduction: Feclone, a safe hand contaminant simulant (simulates baby fecal material), is available with a fluorescent tag/probe, and may be applied to skin in known amounts. This contaminant can be observed and semi-quantified by image analysis. The objective of this study was to estimate the percent removal of Feclone contaminant from hands. This was based on percent change from baseline Feclone images at two time points: after washing and after drying.

Methodology: To provide a baseline of simulated contaminants, a standard amount of fluorescent Feclone (0.25 gms) was applied to a 1.5"x1.5" area on the palm of the hand and spread evenly. The Felcone area was imaged under UV light before washing, followed by a standardized hand washing process. A second image of the Feclone on the hand was taken after washing and before drying. After drying the hands with two paper towels, a third image of the Feclone on the hand was taken. All remaining Feclone was removed before repeating the above procedure with the other hand. All imaging was conducted using consistent and constant conditions so that different images could be directly compared. The images of the hands of 37 participants were later analyzed to measure the fluorescence intensity at each phase of the hand washing/drying procedure in order to estimate the amount of Feclone removed at each stage of the process.

Results and Conclusions: The average reduction of Feclone following hand washing was estimated to be approximately 50% of the baseline amount.There were no significant differences in performance between any of the paper towel products. The estimation of additional percent removal of Feclone combines all four commercial towels. Drying hands with paper towels further reduced the amount of Feclone after hand washing by an additional 28% on average.

The overall experience, washing hands with soap and water and drying with paper towels, reduced the amount of Feclone by approximately 78% on average. Just hand washing removed approximately 50% contaminants. Hand towels removed an additional 28%.

Improving Stratum Corneum Barrier Function through High Lipid Deposition from Rinse-off Cleansers: Skin Biomarker Measures

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Introduction: Dry skin is a common skin disorder that is reflected by reduced stratum corneum hydration, increased trans-epidermal water loss, and a loss of skin elasticity. The condition worsens during the dry winter season and upon repeated usage of regular non-emollient containing cleansing products. The purpose of this study is to develop a set of skin biomarkers as surrogate indicators of stratum corneum

barrier function and integrity, and to guide personal care formulation development to holistically improve skin conditions beyond conventional moisturization measures.

Methodology: Standard Leg Controlled-Application Test (LCAT) methodology was used. Treatment was conducted over a 3-week period during the winter season; women with dry leg skin had their legs washed once daily with the randomly assigned body wash products and water alone treatment as control. Typical moisturization measures were taken, including expert dryness grading, corneometer and TEWL. Ten successive D-square tapes were taken from virgin areas within each treatment site at baseline and at the end of each treatment week. The strips were then analyzed for biomarkers (IL-1α, IL-1ra, Keratin 1, 10, 11, involucrin, total proteins, and NMFs). Furthermore, the panelists returned during the summer season to determine baseline biomarker trends as skin conditions naturally improve.

Results and Conclusions: Results indicate that the emollient-depositing body wash delivers significant improvements in standard moisturization measures (dryness grades, corneometer hydration, and TEWL). For the first time in the rinse-off context, the advanced body wash is shown to significantly improve related skin biomarkers that are good indicators of stratum corneum barrier function and integrity. Importantly, the observed biomarker trends are all consistent with the seasonal effect as dry skin naturally improves. Conversely, regular body wash causes significant damages vs. water control as shown in both biomarker and traditional measures. Taken together, a set of skin biomarkers are developed to provide objective, non-invasive, and consistent measures of stratum corneum barrier function and health for guiding the development of superior personal care formulations.

Dynamic Thermal Imaging on Actinic Keratosis

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Introduction: Cutaneous premalignant and malignant lesions are commonly diagnosed by histopathological biopsy. However, this procedure is invasive and often results in functional or cosmetic defects. To avoid biopsy, actinic keratosis (AK) is frequently diagnosed only by its clinical findings. Unfortunately, this clinical diagnosis can be misleading. Therefore, a non-invasive diagnostic technique for AK is required to support clinical diagnosis. Dynamic thermal imaging is a non-invasive technique that quantifies the infrared (IR) radiation emitted by a subject after the introduction of external thermal stimuli.

Methodology: The experiment consists of two parts; mouse model and patient studies. In a mouse model study, forty hairless albino (Crl:SKH1-hr) mice were randomly selected as to the control group or the experimental group. The experimental group was regularly irradiated with UV (500 J/m2) five times per week for 16 weeks. The clinical photographs, immunohistochemical staining, and dynamic thermal imaging were compared between two groups. Twenty-six patients with histopathologically confirmed actinic keratosis (AK) were recruited in the patient study. The results of dynamic thermal imaging diagnosis of AK lesions and control sites were compared.

Results and Conclusions: In the mouse study, rapid thermal recovery to the baseline temperature after heat stimuli was significant on dynamic thermal imaging as photocarcinogenesis proceeded. Histopathological correlations were able to distinguish normal, premalignant, and malignant cutaneous lesions according to the thermal imaging results. CD 31 staining analysis showed that increased vasculature was a key change responsible for different thermal findings during the stage of photocarcinogenesis. In the patient study, it was possible to distinguish between AK lesions AK lesion and control sites by comparing thermal recovery patterns in dynamic thermal imaging results.

In conclusion, dynamic thermal imaging is useful for distinguishing normal, premalignant, and malignant cutaneous lesions in mouse model. The patient's actual AK lesions can be distinguished from normal skin. Increased vasculature is a key change responsible for different thermal imaging results.

Body Measurement, Possibilities & Limitations

Jean-Jacques SERVANT

Eotech, Marcoussis, France

Introduction: Body measurement addresses more and more product & treatment like modeling, reshaping, and slimming effect besides the more conventional cellulite treatment for dimples and nodules reduction. Nowadays, it concerns also other parts of the body than legs. Centimetric measurement is last century technique and non-contact 3D measurement is an obvious way to measure geometry, dimensional as well as volume changes on the body. My presentation will show objectives possibilities on body measurement and limitations as well. We have been involved in body measurement since 2011 and made some real progress in different applications like legs and cellulite, but also breast, waist, haunch and arm.

Methodology: We will compare different techniques and systems which can address body measurement. We will describe the real benefits of our approach where positioning and controls becomes critical. Our positioning bench is the core of body measurement.

Results and Conclusions: We have developed some specific analysis tools for the evaluation of body product effects. We like to present all the tip & trick for each application as well as study results obtained by our customer on such body measurement with their comments.

A Cross-Sectional Multiethnic Multicountry Study on the Prevalence of Visible Facial Skin Pores

Rong Kong, Beth Gyurke, Robin Thacker

Amway Corporation, Ada MI 49301

Introduction: Conspicuous facial skin pores are one of the top cosmetic concerns for many women and men around the world. Despite this high concern, our understanding of the prevalence of skin pores is still limited. Our goal is to use a large cross-sectional study to investigate facial skin pores and their associations with age, ethnicity and gender.

Methodology: The study was conducted on 7558 women and 1394 men between 16-80 years old from multiple countries (Mexico, Columbia, China, Japan, Korea, Taiwan, Thailand, India and USA) representing mainly three ethnic groups (Asian, Hispanic and Caucasian). Facial images were captured under controlled multiple lighting modalities using the VISIA-CR system (Canfield Scientific, Parsippany, NJ). Facial skin pore count and average size were quantified from the images using in-house image analysis software (Facial Analysis Computer Evaluation System or F.A.C.E.S.). The image analysis method for quantifying pores was validated by comparing the imaging results to expert visual grading in a separate set of subjects (N=57). The relationship between skin pore attributes (count and size) and population demographics (age, ethnicity and gender) was analyzed using MATLAB Statistics and Machine Learning Toolbox (Mathworks, Natick, MA).

Results and Conclusions: Our results showed that skin pore count and size both increased with age (count: $r^2=0.18$, $p<0.001$, size: $r^2=0.38$, $p<0.001$). Hispanics showed the largest average pore count and size (both $p<0.001$). There was no significant difference in pore count or size between Asian and Caucasian women. Interestingly, all three groups had almost the identical rate of increase in pore count and size with age. Within the Asian cohort, those subjects from Northeast Asia (China, Taiwan, Japan, Korea) showed significantly more and larger pores than those from Southeast Asia (Thailand) (count:

p<0.001, size: p<0.01). Finally, the results confirmed that men have larger pore count and size than women (p<0.001) at all ages across all ethnic groups. Overall, our large-scale study has shown the significant associations of skin pores with age, gender and ethnicity.

Investigate and Visualize the Skin Barrier Alterations Associated with Environmental Stresses (UV, Ozone) by FTIR Spectroscopy and FTIR Imaging Analysis

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TRI Princeton

Introduction: The skin is composed of two major layers: the epidermis, unvascularized epithelial layer, and the dermis, vascularized layer with a rich supply of capillaries, nerves, hair follicles etc. The epidermis plays a key role as it is our first protective barrier from environmental stresses like UV exposure, Ozone, exogenous contamination (Particulate Matter PM, heavy metals, etc.). Indeed, the superficial layer of the epidermis, the stratum corneum (SC) is permanently exposed to these external aggressions. Typically, the SC consists of ~ 10 to 20 layers of terminally differentiated corneocytes embedded in intercellular lamellar lipids. The skin barrier function is related to both the unique lipid composition of the SC and their complex and flexible structural organization. Modifications to the SC may result in significant changes of the skin barrier functions and therefore modify the skin permeability with regard to unwanted exogenous components. The objectives of the present study are to assess and visualize the impacts of UV exposure (10h-100h) and/or Ozone exposure on the skin barrier integrity.

Methodology: Both FTIR Spectroscopy and ATR-FTIR Spectroscopy Imaging were used to address these questions. FTIR spectrometer equipped with temperature-controlled transmission cell was used for studying SC lipids conformation and packing transition by analyzing the CH_2 peak positions between 2800 cm^{-1} and 3000 cm^{-1}. The accessory is able to create a gradual heating of the SC samples. In all the experiments the FTIR spectra were acquired as a function of temperature from 5°C to 95°C and recorded every 2 to 3C°. All spectra were collected with a spectral resolution of 4 cm^{-1} and 64 scans accumulation. By studying the frequency variation as a function of the temperature, the phase transitions under which the system goes from an ordered to a disordered conformation were observed. FTRI images were acquired with a Spectrum Spotlight 400 imaging system (Perkin Elmer Instruments, Shelton, Conn., USA) using a MCT (mercury-cadmium-telluride) detector. FTIR images were collected in the reflective mode at a spectral resolution of 4 cm^{-1} in the mid-infrared (MIR) region from 4000 cm^{-1} to 850 cm^{-1} with a spatial resolution of 6.25 x 6.25 µm or 1.55 x 1.55 µm

Results and Conclusions: Our data show that progressive UV and Ozone exposure significantly changed the lipid organization inside the SC. These changes could significantly alter the skin barrier proprieties and dramatically change the skin permeability. On the other hand, the FTIR images recorded on the skin surface allow us to visualize the oxidative steps that the epidermal lipids undergo during these environmental stresses. This study highlighted the pertinence of the FTIR Spectroscopy to visualize and investigate the impacts of the pollution on the skin.

In Vivo Breast Skin Imaging Using Multiphoton Tomography

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Introduction: Radiation therapy, typically used in breast cancer treatment, has as a very common side-effect radiation dermatitis. Multiphoton tomography (MPT) could be used assess the severity of radiation-induced skin damage and aid in the development personalized therapies.

Methodology: The breast and forearm skin of healthy volunteers was imaged using the certified clinical tomograph MPTflex. The influence of breathing during image acquisition was considered. Skin alterations induced by hormonal variations were also evaluated.

Results and Conclusions: The human breast skin was characterized at multiple depths based on tissue endogenous autofluorescence and second-harmonic generation. Several regions were imaged in a comfortable manner. The imaging parameters were optimized to minimize depth-related artifacts induced by breathing. In female volunteers, the thickness of the skin epidermis appears to the influenced by hormonal variations.

We demonstrate that the breast skin can be characterized using MPT. Skin evaluation during radiation therapy could lead to the design of novel and individual skin treatments which could reduce radiation dermatitis in patients.

Non-Invasive LED-Based Measurement of the Sun Protection Factor

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Introduction: For each new sunscreen formulation, the sun protection factor (SPF) values must be determined, causing sunburns on the backs of at least 10 human test subjects. Due to the invasiveness of this method, a non-invasive method applicable on volunteers to determine SPF values for new products is strongly requested.

Methodology: The measurement principle is the detection of the diffusely reflected light at different distances to the incident beam. As a light source UV-LEDs are used and a spectrometer or photodiodes detect the back scattered light. The fiber bundle enables the spatially resolved reflectance measurement principle at several measurement points on the skin.

Results and Conclusions: First measurements were performed on pig ear skin. Eleven different sunscreen formulations (lotions, sprays, creams, mixed or pure physical filters) of different manufacturers have been measured so far. The data from the test institutes correlate with the obtained SPF from the non-invasive method. First in vivo results on human volunteers back confirm the correlation with the reference values.

Using OCT to Detect Changes in Epidermal Thickness after Application of Topical Cosmetic Products

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Introduction: Optical Coherence Tomography (OCT) has emerged as a valuable, noninvasive tool for skin diagnostics and treatment guide. Its capability in detecting small changes that occur in healthy skin in response to topical cosmetic treatment remains to be tested.

Methodology: Two formulations were applied to the volar forearms of healthy females for 3 weeks, a 3rd site remained untreated. All sites were randomized. One formulation was also tested on the face using a randomized split-face design. OCT imaging of each test site was taken at baseline and week 3. Epidermal thickness was measured using A-scan trace.

Results and Conclusions: Statistically significant changes in epidermal thickness on the volar forearm was detected for the formulation containing retinol. Vehicle control and untreated sites showed no change. The changes were supported by data from a fluorescent spectroscopy measurement. Result from this pilot study demonstrates that OCT has the resolution to detect small changes in epidermis in response to topical cosmetic treatment. Results from the face will be discussed as well.

Friday May 4th

Plenary VI A: New Devices, New Models and New Metrics

Opening Lecture: Multiphoton Imaging of Stem Cells in Hair Follicles

Karsten Koenig

No abstract

The Dermal-Epidermal Junction - Revisited

Rainer Voegeli[1], Victoria L. Newton[2,3], Robert S. Bradley[4], Pierre Seroul[5], Marie Cherel[5], Christopher E.M. Griffiths[2,3], Anthony V. Rawlings[6], Michael J. Sherratt[7] and Rachel E.B. Watson[2,3]

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Introduction: The current knowledge about the dermal-epidermal junction (DEJ) is primarily derived from histological 2D sections. Thus, the aim of our studies was to understand age-related changes more precisely by the visualization and characterization of its 3D structure.

Methodology: Techniques for 3D modelling and characterization of the DEJ were developed using superimposed section images obtained by microcomputed tomography (µCT) from biopsies and *in vivo* from reflectance confocal microscopy.

Results and Conclusions: A novel approach was developed to evaluate rete ridges (RRs) and dermal papillae (DPs) more precisely. We placed a median plane through the DEJ and defined protrusions from the median plane to the skin surface as DP_m and those from the median plane towards the dermis as RR_m. This procedure revealed clear differences in the morphology of DP_m and RR_m and the 3D structures enabled us precisely to determine their volumes. However, a significant age-related difference was only found in the µCT reconstructions.

Based on the median plane approach we created network images, describing connectivity and branching of the DEJ. The networks provided a new type of illustration of the structure and ramifications of the DEJ. Young photo-protected skin had longer and more branched DP_m.

3D modelling enabled a more realistic view of the highly complex spatial structure of the DEJ and thus improved our understanding of ageing skin.

Multi-Scale Identification of Cutaneous Tension by a Folding Model and Elastic Wave Propagation

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Laboratory of Tribology and Dynamics of Systems. University of Lyon. UMR CNRS 5513. ENISE – ECL

Introduction: This work focuses on the characterization of cutaneous natural tension during aging.

Methodology: From the three-dimensional imaging of skin furrows measured by a confocal microscope in white light on skin replicas, we can identify the multi – scale distribution of skin folding, as well as a statistical strain anisotropy. The basic parameters for the calculation of folding rate are determined by the scale of width and depth of furrows. The morphological transformation of the cutaneous line network into a structure in the form of a tree of tension lines allowed us to study the multi-scale nature of folding in all directions. This quantification takes into- account the z position of folding.

To access to the anisotropy of the elastic modulus, we have developed a new contactless system based on the propagation of shear waves. This device makes it possible to measure the anisotropy of the elasticity modulus

Results and Conclusions: The results show a good coherence between the multi-scale anisotropy of the folding rate and the anisotropy of elasticity modulus as a function of aging.

Development and Validation of a Non-Invasive Spatially Resolved Spectroscopy Probe for Collagen and Water Determination

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Introduction: We report in this document the development and validation of an infrared spatially resolvedspectroscopy device for the in-vivo and non-invasive determination of the reduced scattering and optical absorption coefficients of the dermis. This integrated probe uses three LEDs and photodiodes pairs separated from a few millimeters to a few centimeters to light the skin at a wavelength range from 1200nm to 1650nm and to collect the backscattered beam. At these wavelengths and emitter-detector separations, the reduced scattering and absorption coefficients are respectively linked to the collagen fibers and the water concentration in the dermis. The determination of these two parameters relies on an embedded physical model that has been validated on phantoms.

Methodology: We present first the design of this new probe using dedicated Monte Carlo simulation takingespecially into account view angles, spectral responses and active sizes of the LEDs and photodiodes. The sensibility of the simulated optical power detected to the emission wavelength and source-detector separation is analyzed. Then, the development of fluid phantoms to reproduce the optical behavior of the dermis is described. These new fluid phantoms made of water, heavy water, pyridine and silicon dioxide spheres powder have shown a near-ideal optical behavior when compared with the one of the skin, with a direct quantitative correlation of all the detected optical powers. The performance of the probe is finally examined using the phantoms. Using the extrema of thereduced scattering coefficient and the water volumic concentration reported in the literature from 3.4cm-1 to 17cm-1 and from 50% to 85%), we have demonstrated a linear correlation with R^2 greater than 0.93 in all these ranges.

Results and Conclusions: These results have shown that this new hand-held probe is able to retrieve both scattering and absorption coefficients of the dermis, which are linked to respectively collagen fibers state and water concentration. This probe can thus be used to analyze skin-aging and keep track of skincare products effects.

The Evolution of Gender Effect on Static and Active Tactile Perception Over Age

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Introduction: Even if the human finger is the interface used for the touch process, very few studies used their properties to explain the tactile perception. The goal of this study is to understand the age and gender influences on the tactile perception (static, dynamic) via the density of mechanoreceptors solicited during the touch. This last can be calculated by using the real contact area and the finger size. We are also looking for an answer to the question "what is the effect of the mechanical properties anisotropy on the tactile perception and touch gestures?".

Methodology: To achieve our objective, *in vivo* studies on 40 subjects (20 of each gender) of four age groups of 26±3, 35+-3, 45+-2 and 58±6 yo have been performed. Two different systems based on a fingerprint reader and normal force sensor have been developed to measure each type of real contact area (static and dynamic). Then, the size of the finger is measured with a CCD camera and a contour detection algorithm.

Results and Conclusions: The results obtained gave a clear explanation about the evolution of gender effect on static and dynamic tactile perception over age. In addition, the tactile perception shown a significant difference for the used touch gestures.

Nanoscale Quantification of the Stratum Corneum Based on Optical Coherence Tomography Coupled With Statistical Decision Theory

Florian Formanek[1], Cristina Canavesi[2], Jinxin Huang[2], Changsik Yoon[2,3], Thomas Bornschlögl[1], Anne Potter[1], Jannick P. Rolland[2,3], Bernard Querleux[1]

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Introduction: Till now, the nanoscale characterization of the stratum corneum has been possible only using complex protocols mainly based on electron microscopy. We report in this present work on the first nanoscale quantification of the corneocyte mean thickness, intercellular lipid thickness, and the number of corneocyte layers, using Gabor-domain optical coherence tomography (GD-OCM) and statistical decision theory.

Methodology: GD-OCM system (*LighTopTech, West Henrietta, USA*) was used to acquire 3D images of isolated SC sheets and *ex vivo* skin samples. A multi-layer bio-optical SC model consisting of repeated alternating layers of corneocytes and lipids was devised.

TEM images were used as the reference method to quantify the 3 SC descriptors. Cosmetic treatments, such as hydration to predict an increase in the corneocyte mean thickness, and tape stripping to predict only the decrease in the number of corneocyte layers, were performed to assess the sensitivity of the method.

Results and Conclusions: Quantification of these 3 SC parameters whether through quantitative GD-OCM and TEM measurements were in good agreement, even for the intercellular lipid thickness of about 50 to 70 nm. Effects of cosmetic treatments were also clearly predicted by the method.

In conclusion, by coupling a numerical model with GD-OCM, we obtained a nanoscale description of the stratum corneum. Such results will offer new insights on its barrier mechanisms and its response to the application of topical pharmaceutical and cosmetic products on one hand, and on a second hand, afford some new knowledge on SC ultrastructure *in vivo* according to chrono- and photo-aging, ethnicity, and body sites.

Plenary VI B: New Devices Models and Metrics.

A Deep-Learning Strategy for Automated Detection and Localization of Skin Vasculature with Application to Basal Cell Carcinoma Classification

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Introduction: Vascular structures of the skin are important biomarkers in assessment and diagnosis of abnormalities. Detection and localization of cutaneous vessels provide critical information towards disease diagnosis and stage status. However, detection of skin vasculature is complicated by the variability in vessel size, color and architecture. Considering the variety of skin vasculature, conventional techniques lack the ability to detect different vessel types. Furthermore, such techniques highly depend on precise hand-crafted features which require expert knowledge, are time-consuming and computationally inefficient. To overcome these challenges, we propose a deep learning framework based on stacked sparse auto encoders (SSAE) for automated detection of cutaneous vessels and computer-aided BCC classification.

Methodology: Each training image is divided into patches of either containing or non-containing vasculature. Training patches are fed into SSAE to learn hidden representations of the data. The learned features are subsequently fed into a classifier that detects vasculature in each patch. After the unsupervised learning, we treat each of the learned kernel weights of the SAE as a filter. Convolving each filter with the lesion image yields a feature map. Feature maps are further integrated with patient profile information. The overall features are then fed into a softmax classifier for BCC classification.

Results and Conclusions: Over a test set of 200 images, SSAE demonstrated a superior performance of 93.26% detection accuracy of a wide variety of vessel patterns; outperforming other techniques by achieving the highest positive predictive value of 86.16%. For BCC classification, on a set of 1199 BCC images, the proposed framework achieved an area under the curve of 91.1%, while preserving clinical interpretation of the features. The proposed framework provides a non-invasive fast BCC detection tool that incorporates both dermoscopic features and patient information, without the need for complex handcrafted feature extraction.

Application of Reflectance Confocal Microscopy (Rcm) in the Evaluation of Skin Photoaging on Young Men

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Introduction: The differences on skin physiology and lifestyle between men can influence the damages of solar radiation on the skin. The aim of this study was to evaluate the skin changes resulted from solar exposure in young male using skin imaging techniques.

Methodology: The malar region of 20 men (18-28 years old) were evaluated by high-resolution images (Visioface®); Skin thickness and echogenicity by ultrasound of 20 mHz (DermaScanC®) and Morphological and structural skin characterization by Reflectance Confocal Microscopy (VivaScope® 1500).

Results and Conclusions: Pigmentation disturbs, telangiectasias and wrinkles were observed. A better echogenicity ratio on dermis and lower thickness of epidermis were found for participants with photoprotection habits. 35% showed an irregular honeycomb pattern, 40% polycyclic papillae, 35% coarse fibers of collagen and 10% huddles of collagen. All morphological alterations were observed for those who did not use sunscreen. The study brings relevant data of skin alterations for young men without photoprotective habits.

Poster Presentations

1. Automated Quantification of the Skin Aging Process Using In-Vivo Confocal Microscopy

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Introduction: Reflectance confocal microscopy (RCM) is a powerful tool to visualize the skin layers at cellular resolution. We propose new methods to automatically quantify the skin aging process on RCM images. We present clinical validation of our results.

Methodology: The study enrolls 160 subjects from 4 different ethnics (Caucasian, Hispanic, African and Asian). Image acquisition are carried out on the cheek.

Clinical annotations are performed on the regularity of the epidermal honeycomb pattern, the shape of the dermal epidermal junction (DEJ) and the collagen fibers type. Corresponding aging descriptors are automatically obtained using machine learning algorithms. Random Forest classifiers are used to create a predictive model for clinical annotations. We present the accuracy, sensibility and the specificity of the predictions.

Results and Conclusions:
Scores of the predictions are:
- Epidermis: 80% of accuracy, 81% of sensibility, 81% of specificity.
- DEJ: 83% of accuracy, 76% of sensibility, 81% of specificity.
- Dermis: 80% of accuracy, 63% of sensibility, 89% of specificity.

To our knowledge, these are the first results comparing a computer-based approach to a dermatologist for the assessment of skin aging using in-vivo confocal microscopy.

2. Image Analysis of Pigmented Spot Related to Age in Korean Female

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Introduction: Facial skin pigmentation is one of the visible features of skin aging, so it is important to evaluate the skin pigmentation in the cosmetic and esthetic fields. Several groups have investigated and developed the image analysis methods for skin pigmentation and some of the groups reported age-related changes of facial skin pigmented spot number and size. However, they didn't show the changes of pigment spot number and size by defined size, and there is no report for Korean female in regards to pigmentation. The aim of this study is to investigate the facial skin pigmentation number and size in Korean female with aging.

Methodology: Total of 194 Korean females aged 20~69 (48.97 ± 17.11 y.o.) participated in this study. Digital facial images were obtained using VISIA-CR and following pigmented spot analysis was performed by Image-pro premier 9.2. The facial images were cropped on cheek area and then applied various filters such as large high-pass filter and median filter for detecting and analyzing the pigmented spots number,

size and intensity. Statistical analysis was performed using SPSS 23.0 with a significance level of p < 0.05.

Results and Conclusions: There were significant correlations between age and total pigmented spot number (No.), size and intensity (I) (Pearson's r = 0.688, r = 0.645, r = -0.563) and the following values were significantly correlated with age [(2 ~ 4mm2' No, Size, I: r = 0.514, r = 0.516, r = -0.556), (4 ~ 10mm2' No, Size, I: r = 0.603, r = 0.599, r = -0.539),(10 ~ 20mm2' No, Size, I: r = 0.420, r = 0.417, r = -0.441), (> 20mm2' No, Size, I: r = 0.393, r = 0.377, r = -0.373)]. According to ANOVA results, there were significant differences in percentage of spot size of 2 ~ 4mm2 and > 20mm2 between 20s and 70s. These results indicate the facial skin will have more pigmented spot with age and increase in the large pigmented spots is most noticeable in Korean female.

3. Mild Industrial Hand Cleansers: Formulation Approaches and Clinical Assessment of Barrier Function

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Introduction: Hand washing is vital to ensuring the overall health of humans. The potential for it to negatively impact barrier properties provided by the skin is well known. This is particularly true in health care and industrial environments where the exposure to surfactants is higher to mitigate transmission of pathogenic organisms and the removal of foreign soils. The repetitive hand cleaning, common among these employees, removes lipids and native moisturizing factors that protect the skin.

Many of the hand wash products in the professional market rely on claims such as "contains moisturizers" and "mild ingredients" to create an association among consumers that the product promotes skin health.

The objective of the current research was to develop and evaluate a mild hand wash formulation for the industrial market that removes foreign soils without compromising skin health. In the course of this research, we evaluated mild hand soaps and industrial hand soaps with and without grit.

Methodology: An exaggerated arm wash study followed the Sharko method, well documented since 1992, with 4 wash formulations evaluated per study. Twenty subjects reported to the test facility for baseline Expert Grader assessment of dryness and erythema as well as transepidermal water loss (TEWL) and conductance measurements on their volar forearms. Each skin site was washed 4 times per day for 4 consecutive days. An expert grader evaluated the test sites for visible erythema and dryness prior to the first and third cycle daily. On day 5, two wash cycles were followed by visual and instrumental measurements.

Results and Conclusions: The first study showed that two mild personal care hand washes had significantly lower impact on skin barrier function when compared to a conventional industrial hand soap and a bar soap. Using the same study protocol, another study focused on how various cleaning agents (surfactants, solvents and grit) impacted skin barrier function.

Overall, surfactant selection and usage appears to be the critical factor influencing the impact wash products have on skin barrier function as measured by TEWL, conductance and expert grading of dryness and erythema.

4. Three-Dimensional Visualization of Internal Skin Structure by Amplification of Structure-Associated X-Ray Absorbance Micro Computed Tomography (ASAXA-µCT) - Discovery of a Skin-Aging Mechanism: Dermal Cavitation -

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Introduction: Aging causes loss of skin elasticity and sagging, but the mechanism is unclear, because skin contains complex internal structures and no method has been available to visualize them. We aimed to clarify the mechanism of skin aging, focusing on changes of the internal skin structure.

Methodology: To observe internal skin structure three-dimensionally, we established a micro computed tomography method combined with iodine staining (to amplify X-ray absorbance of appendages) and acetone pretreatment (to differentiate each appendage) (designated ASAXA-µCT).

Results and Conclusions: ASAXA-µCT revealed aging-related defects filled with subcutaneous fat at the bottom of the dermal layer ("dermal cavitation"). Further, sweat glands shrink upward with aging, which induces cavitation. Dermal cavitation (ultrasonography) was correlated with decreased dermal elasticity (cutometer) and increased sagging (photo-grading) at the cheek of 30 female volunteers.

Thus, we visualized internal skin structures three-dimensionally for the first time, and discovered a novel skin aging mechanism, dermal cavitation, caused by sweat gland shrinkage.

5. In Vivo Human Models for Evaluation of Skin Barrier Properties and Cutaneous Inflammation

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Introduction: Given the complexity of skin structure and function, studying the mechanisms regulating skin homeostasis and underlying skin diseases can be challenging. In order to investigate specific processes, in vivo skin models can be used.

Methodology: Bioengineering techniques provide objective and non-invasive measurements of skin properties. Other useful techniques are based on the interaction of light with various skin structures and on the subsequent measurement of the exiting photons, such as Raman microspectroscopy and in vivo reflectance confocal microscopy. Focusing on the non-invasive, objective and in vivo evaluation of skin barrier properties and cutaneous inflammation in vivo models were evaluated.

Results and Conclusions: Combining in vivo human models and these non-invasive techniques, offer novel possibilities for evaluation of skin barrier properties and cutaneous inflammation. In particular histamine iontophoresis can be used as in vivo model to elicit local and acute inflammation with minimal impact on the skin barrier, in contrast to tape stripping and topical application of irritants in which the skin barrier is disrupted either mechanically or chemically.

6. Use of the Novel Device, gpskin® for Measuring TEWL

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Introduction: TEWL(transepidermal water loss) is the parameter in evaluating the skin barrier function non-invasively. gpskin(Gpower) is a novel closed chamber type device for measuring TEWL. It covers TEWL range up to 80 g/m^2h, weighs just 40 g, and costs 25-144 times lower than existing devices.

The aim of this study is to verify the repeatability and reproducibility of the new device and compare with other devices.

Methodology: 10 Korean subjects in their 20s to 40s were enrolled. TEWL measurement was triplicated by 2 gpskins on the forearm. Correlation among the gpskin, Vapometer(Delfin), and Tewamter(Courage & Khazaka) was evaluated on the forehead, cheek, chin and forearm. Statistical analysis was performed using SPSS with a significance level of $p < 0.05$.

Results and Conclusions: The gpskin showed good repeatability and reproducibility. Correlation between it and others was about 0.8. The power of discrimination of the test sites was same as other two instruments.

In conclusion, gpskin worked consistently and the correlativity with other devices was high. Also it is lighter and more economical than any other devices evaluating skin barrier function.

7. Muscle Stiffness Impacts Skin and Subcutis Properties

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Introduction: Thrust is recognized as one major osteopathic technique able to decrease muscle stiffness and restore mobility and elasticity of muscles. The aim of this study was to evaluate secondary impacts of such treatment on skin and underlying tissues.

Methodology: 14 healthy subjects (> 18 y.o.) were enrolled in this clinical study, which was approved by the hospital ethics committee. Clinical exam through palpation, self-assessment by the subjects and instrumental evaluation by ultrasound imaging of skin, subcutis and muscle on the vertebral segment before and after a thrust were performed.

Results and Conclusions: As a primary effect of the osteopathic treatment, ultrasound elastography was able to quantify a decrease of muscle stiffness after the thrust in the 3 muscular plans versus control site. Clinical evaluation and self-assessment were also in good agreement to demonstrate an improvement of the paravertebral mobility.

Skin thickness was statistically increased after treatment and to a lower extend, similar thickening of the subcutis was measured. This study is another demonstration that reducing muscle stiffness can induce a decrease of the skin tension through interactions mediated by the superficial musculo-aponeurotic system. Apart from botulinum toxin, osteopathic practice or devices targeting muscle stiffness decrease could be noninvasive procedures for deep wrinkle and face sagging improvement.

8. Hyperspectral Imaging of Cross-Sectioned Human Hair for the Evaluation of Relipidization Process from Cosmetic Treatments

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Introduction: The hair penetration pathways are normally investigated using fluorescent dyes with classical optical microscopy or confocal laser scanning microscopy, however, there are some limitations specially related to the specificity of chemical interactions of fluorescent dyes and the wide classes of haircare ingredients with the hair fiber structure, making questionable the indirect inferences based on the intensity of fluorescence of the hair cross section before and after the haircare treatments.

FT-IR spectroscopic imaging analysis can provide rapid and specific chemical information at the molecular level, becoming a powerful tool in chemical analysis as it combines spectral and spatial information at the same time and may allow the distribution of vibrational modes on the surface to be visualized, which can potentially solve the problems of specificity described previously.

In our study, we applied the transmittance FTIR microspectroscopic imaging to study the efficacy of haircare products in delivering exogenous lipids to the inner part of hair fibers.

Methodology: Medium brown virgin hair was cleaned following a standardized procedure and then treated with hair rinse off mascaras containing different proportions of conditioning ingredients. A hair tress treated with SLES 10% served as control. Around ten hair fibers of each treatment were embedded in an LR White Medium grade resin for thin cross sections (≈ 5 µm thick) using a microtome diamond knife and deposited on a glass cover slip.

FTIR microscopy analysis of cross sectioned hair fibers were performed on a Perkin Elmer FTIR SpotLight 400 spectrometer coupled to an optical microscope. We used 120 scans, 4 cm-1 resolution. The system consists of a linear array of mercury-cadmium-telluride detectors and a high precision automated XY sample phase. The hyperspectral FTIR images of hair cross sections were obtained by monitoring the lipid peak at 2850 cm-1 after normalization. To calculate the multivariate distance values, the spectral range from 2850-2990cm-1 in the absorbance mode was utilized. CytoSpec software package (CytoSpec, Berlin, Germany) was used to generate the intensity maps.

Results and Conclusions: To be efficient, it is necessary for a hair care product to permeate into the layers of the hair fiber. We demonstrate the applicability of transmittance FTIR based hyperspectral imaging technique to extract spatially resolved chemical information, more specifically the lipid composition, to assess in a direct manner, the efficacy of different haircare treatments.

9. Phyla Diversity of Human Facial Skin Microbiota

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Introduction: The skin microbiota encompasses a diversity of microbes including commensal and potentially pathogenic bacteria that contribute to human skin health and disease. Several investigations have examined the skin microbiota on the forearm and axilla; however few have examined the diversity of

the skin microbiome on the face. The differential diversity on facial skin may aid in the development of cosmetic products designed to improve overall skin microbiome balance and ultimately skin health and beauty.

Methodology: 40 Subjects were enrolled in a prospective study. Data from baseline visits are reported in this abstract. Subjects sampled their own facial skin according to standard methods obtained from central laboratory (Corebiome, Minneapolis, MN), briefly, subjects successively sampled both cheeks utilizing a standard swab and transfer fluid able to maintain the viability and avoid proliferation to collect skin microbiota. Samples were immediately sent to central laboratory for analysis. Sample analysis: each colonial morphotype was identified by molecular methods based on 16s rRNA gene sequencing.

Results and Conclusions: Phylum Diversity in Facial Skin Microbiota is shown in table 1 and is compared to that found in published forearm data (1, 2). There is substantial differentiation between facial skin microbiota on the phylum level from that of forearm body skin. These data may have implications for product development for the balance of skin microbiomes in either facial or body cosmetic products.

Table 1. Phylum Diversity

Phylum	Facial Skin Percentage of Flora Study Group (N=40)	Forearm Percentage of Flora Published Data (1,2)	Difference
Proteobacteria	64.8%	51.8%	+13.0%
Actinobacteria	22.0%	24.4%	-2.4%
Firmicutes	10.8%	16.5%	-5.7%
Bacteroidetes	2.8%	6.3%	-3.5%

References:
1. Cosseau et. al – Proteobacteria from human skin microbiota- One Health 2016
2. Grice et. Al. – A diversity profile of human skin microbiota – Genome Research 2008

10. Test Method for the Evaluation of Skin Dynamic Topography and Skin Mechanical Properties During Facial Expression

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Introduction: In human skin, the dermis is a loosely bound tissue rich in water, and is regarded as the main layer underlying the formation of wrinkles. Meanwhile, the stratum corneum (SC), which is the outermost and most rigid sublayer of the skin, has a water content lower than the dermis and is believed to be responsible for the formation of fine wrinkle lines. These differences suggest that facial expressions have different impacts on the dermis and SC. Therefore, these two layers have different roles in the formation of wrinkles.

In this context, our challenge was to develop a method for assessing the effects of cosmetic preparations on the improvement of mechanical properties of the skin during facial expressions (skin's movement), establishing a standardized condition for recording and analysis of the skin during its movement.

Methodology: For the study, 60 female healthy volunteers aged between 45-70yo were enrolled after obtaining written informed consent. Six different skin moisturizers were studied in six sub groups of 10 volunteers, where they were acclimatized at 20°C and 50% RH for 30 minutes prior to the first "smiling" movie recording. After, 2mg/cm2 of moisturizer was applied topically to the wrinkles around the corners of

the right eyes and then they were acclimatized at 20°C and 50% RH for 2 hours prior to the second "smiling" movie recording.

The new test method demanded a standardized positioning of the volunteers by using a stationary table adapted with supports for the chin and control of the inclination of the head. Face orientation at 60 ° relative to the camera and lighting system and other controls that will be detailed in the poster. For recording the movement of regions of interest, volunteers were instructed to perform 10 to 15 eye blinking movements. At least three highspeed videos were recorded at each experimental time-point. For the imaging acquisition a high-speed camera was used (HighSpec 2G Color at 100 frames per second, resolution 784x628, fixed lens 35mm and lighting by LED Flash Triopo 65º TTV160 1020LM.

The statistical program MATLAB version R2009 and R2014a was used for of the mathematical / statistical model and the interfaces that together formed the tool to make the comparative image analysis of the parameters: dynamic roughness, dynamic texture and stress maps, evaluating the before/after of 5 different cosmetic products.

In terms of image processing, the methodology used to calculate the pixel displacement was the Particle Image Velocimetry (PIV), which is an optical method that allows the visualization and analysis of the movement of particles in fluids and can be adapted and extrapolated for other purposes, as we did in this study. The skin extensibility maps within the region of interest is a result of the PIV analysis. For the extraction of the dynamic texture parameters, we used the methodology of gray level co-occurrence matrix (GLCM) and the recognition and interpretation of the dynamic roughness from the image texture analysis were done from the Support Vector Machine (SVM), a kind of supervised learning model with associated learning algorithms that analyze data used for classification and regression analysis.

Results and Conclusions: Through the skin movement mapping was possible to extract mechanical parameters such as skin extensibility, dynamic texture and dynamic roughness, allowing to demonstrate positive effects after the usage of some cosmetic moisturizing products. The methodology demonstrates technical feasibility to observe improvements in the parameters of extensibility and texture even after just a single moisturizing product application.

11. Non-Invasive Clinical Imaging of Skin Erythema by Laser Speckle Imaging

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Colgate-Palmolive

Introduction: Skin erythema is the redness of skin that occurs with hyperemia of superficial capillaries. Current methodologies for measuring skin erythema include indirect measurement of skin irritation/ inflammation biomarker levels and subjective clinical scoring. Moreover, visualization of skin erythema by photography is limited by factors such as environmental lighting and dark/uneven skin tone.

Laser speckle contrast imaging (LSCI) is an advanced methodology that measures blood perfusion in real time as an indicator of skin erythema. In our study, we are using this advanced imaging tool to demonstrate the benefit of a cleansing personal care product with a proprietary blend of ingredients to mitigate induced skin erythema.

Methodology: Skin forearms were washed twice a day for seven days using a cleansing personal care product containing the proprietary blend (B) on one side and the placebo formulation (A) on the other side. Skin erythema was induced by consecutive tape stripping (TS) and was monitored by LSCI (Pericam PSI System, Perimed, Sweden) up to 24hr post. Both qualitative and quantitative data was recorded.

Results and Conclusions: After product use and following induced damage, the skin treated with test product showed less skin erythema than the skin treated with placebo product, as supported by both

images and blood perfusion data. LCSI is a fast, objective and sensitive methodology that provides both quantitative and qualitative measurements for real-time monitoring of skin erythema in clinical testing. In this study, we demonstrated that the test product with the proprietary blend reduced skin erythema that was induced by tape stripping.

12. Antera 3D Camera – a Review and Outlook

Mariotto Guido, Kantor Roman

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Introduction: Skin topographic, colorimetric and multi-spectral measurements are crucial in the field of dermo-cosmetic evaluation. We will discuss a literature review of the Antera 3D, a multi-purpose handheld camera, and present the new Antera software with enhanced features for skin image acquisition and data analysis.

Methodology: We will discuss sensitivity and specificity of multi-spectral and colorimetric measurements, and measurements' repeatability of topographic and colorimetric measurements, evaluated on samples of artificial skin.

Results and Conclusions: The Antera 3D has demonstrated its relevance for claim support and substantiation in the field of dermo-cosmetics. The new Antera software will open new possibilities for analysis of topographic, multi-spectral and colorimetric measurements.

13. Microalgae for Cosmetic Applications

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Introduction: Scarce data support the use of microalgae in cosmetic preparations. This study aims to probe the antioxidant capacity of formulations with *Neochloris oleoabundans*.

Methodology: The work was conducted in the forearm of human subjects. After pre-treatment with the formulations an erythema was induced by a nicotinate. Basal blood flow and continuous blood flow was registered for 15 min with Laser Doppler flowmetry. Onset time was determined.

Results and Conclusions: The microalgae extract decreased the intensity of the erythema. Longer onset times were noted in the treated sites. Nicotinates cause vasodilation by a mechanism of action involving release of prostaglandin and cyclooxygenase. This pathway cascade triggers the production of endoperoxides, leading to the production of ROS. Our findings are indicative of benefits in the application of formulations containing *Neochloris oleoabundans*, and confirm the applicability of the methodology to assess antioxidant activity.

14. Propolis as a New Antioxidant Ingredient for Topical Formulations.

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Introduction: Antioxidants from natural sources may provide new possibilities for the treatment and prevention of oxidative stress-mediated skin diseases.

Methodology: Two propolis extracts were assessed *in vitro* for their antioxidant and antimicrobial activity and tested (efficacy) in human volunteers. After 2h pre-treatment with propolis formulations under patch, an erythema was induced in each area, by an aqueous solution of ethyl nicotinate. Measurements of the cutaneous microcirculation of the individual test sites were then recorded continuously for twenty minutes with Laser Doppler flowmetry.

Results and Conclusions: Results demonstrated that propolis extracts have antimicrobial and antioxidant activity and showed that propolis formulations decreased the intensity of the erythema caused by ethyl nicotinate. Therefore, results suggest that propolis extracts can be used as a skin ingredient with an antioxidant activity,that also ensures protection in topical formulations.

15. Exploring Human Skin Anisotropy by 3D Plotting

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Introduction: The CutiScan® is a recent device apparently capable of quantifying skin viscoelastic behavior in a 360º topography. Here we propose a new 3D representation of CutiScan's data and parameters obtained from 13 healthy female foreheads (n=7 20.0±1.2 y.o., an n=6 50.2±2.4 y.o.).

Methodology: The CutiScan® quantified the height of displacement, after suction, for each of the 360 angles. From a 3D polar data representation (time-displacement-angle), several 2D plots were constructed, from which other parameters - stretchability, stretching speed, stretching time and rise time were calculated and graphically represented.

Results and Conclusions: These representations shown that older subjects display larger displacement heights, as well as larger height max slopes, higher rise and stretching times, irrespective of orientation. Results suggest that these new parameters are sensible and useful for the characterization of in vivo skin biomechanics.

16. Strengthening Photoplethysmography Value through the Wavelet Transform

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Introduction: Laser Doppler flowmetry (LDF) and photoplethysmography (PPG) are commonly used to assess microcirculation, displaying complex oscillatory properties. The wavelet transform (WT) was used to decompose these signals and identify each component in response to a 100% oxygen challenge.

Methodology: LDF and PPG signals were registered on toes of 10 healthy subjects (20.5±3.1 y.o., after informed consent, before, during and after breathing 100% oxygen. Signals were decomposed in their main components with the WT.

Results and Conclusions: Six components were identified in both signals (cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent and endothelial NO-independent) in identical spectral positions. The cardiac, respiratory and myogenic activities increased during hyperoxia in both signals, while endothelial activities gave different responses. These discrepancies may be attributed to different measuring depths and therefore, to different vascular phenomena.

17. Effect of Isometric Dorsiflexion on the Lower Limb Microcirculation, In the Standing Position

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Introduction: This study explores the influence of isometric dorsiflexion on peripheral microcirculation dynamics in the standing position. Six young healthy subjects (31±9 years old) were included after IWC.

Methodology: Perfusion changes were obtained by laser Doppler flowmetry (LDF) and by photoplethysmography (PPG), during 5min standing, 1min active dorsiflexion, and 5min recovery. Nonparametric statistics and a confidence level of 95% were adopted.

Results and Conclusions: During dorsiflexion LDF detected a perfusion increase (p=0,028) while PPG detected a decrease (p=0.028) and these probably results from the different penetration capacity of both technologies. A high contraction pressure is generated deeper in the tissue, compressing the vessels and displacing the blood for more superficial vessels. PPG seems to operate in deeper structures than LDF. These two conjugate technologies might help looking deeper into perfusion dynamics during movement.

18. The Dermatological Potential of Plectranthus Species against Extracellular Matrix Enzymes and Reactive Oxygen Species

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Introduction: Prevention of skin disorders has been a growing concern and traditional medicine has a high expertise using plants, such as *Plectranthus* spp. Since ROS activate ECM enzymes and melanogenesis, this work provides a screening of antioxidant/anti-ECM enzymes synergistic effect of *Plectranthus* spp. components.

Methodology: Ultrasonic extracts (acetone, methanol and ethyl acetate) and isolated compounds from *Plectranthus* spp. (di- and tri-terpenes and phytosterols) were evaluated with DPPH assay and enzymatic assays (tyrosinase, elastase and collagenase). All data were analyzed by one-way ANOVA followed by Tukey HSD test.

Results and Conclusions: Overall, the results showed powerful antioxidant activity of *P. grandidentatus*, *P. madagascariensis* and *P. ecklonii* extracts, and inhibitory action particularly of tyrosinase and elastase from isolated abietane diterpenes and triterpenes, supporting further search against skin sagging and hyperpigmentation.

19. Characterising the Acute Microcirculatory Changes Induced by Massage in the Human Hind Limb

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Introduction: Effects of massage on microcirculation are far from being fully understood. Here we assess the effects of the hind limb massage (upward-upMp and downward-dwMp) by noninvasive technologies such as, laser Doppler flowmetry (LDF) and photoplethysmography (PPG). 24 young healthy subjects (19.9 ± 1.7 y.o.) both sexes, were selected after IWC.

Methodology: Protocols included a 10 min baseline (phase I) register, a 5 min massage in one limb (phase II) and 10 min recovery (phase III) registers, in both limbs, the contralateral used as control. LDF and PPG were analysed with the wavelet transform showing different adaptations in both limbs.

Results and Conclusions: Both protocols increased circulation in the massage foot, but also affected perfusion in the contralateral limb, although LDF and PPG here shows opposite effects, probably because they work in different depths. Our study also reveals a circulatory cooperation between limbs that should be further explored.

20. Non-Invasive Quantitative Analysis System of Skin Melanin

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Introduction: Melanin is one of the most import pigments in human skin which relates to skin color, diseases or tumors. There exist no clinical devices that allow melanin quantitative analysis, diagnosis is mainly based on observation, and limits further investigations. We present a novel device for non-invasive, quantitative analysis of skin melanin, allowing monitoring, evaluation, and observation.

Methodology: The device has 2 separate parts: high quality acquisition system including hyper-spectral imaging sensor, lighting system, calibration and metric system, and control system; analysis system, consisting of emulation, simulation and optimization algorithms, for storage and analysis. Through multiple error reduce technologies, we can reconstruct the skin reflectance accurately and rapidly. A complete model for spectrum reconstruction allows the computation of 18 skin parameters, and controls the reconstruction error without adding more parameters.

Results and Conclusions: Skin spectrum simulation takes 0.15ms on average. Reversely, the analysis takes 0.1 seconds with error about 0.5‰. The device is evaluated with normal skin, vitiligo and nevus. The result shows great value in clinical grading, skin and cosmetic evaluation. The parameters may intrinsically relate to pathology and mechanism and may play important role in big data and AI technology.

21. Impact of Massage with Sunflower Seed Oil versus Mustard Seed Oil on Neonatal Skin Integrity in Rural Nepal

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Introduction: Research on emollient therapy to reduce neonatal mortality is a global priority, particularly in developing countries where nearly 3 million annual neonatal deaths occur annually. Skin-care based interventions, including topical emollient massage, have emerged as important strategies, particularly in premature birth. Innate immunity is conferred by the skin barrier at birth and postnatally. Strategies to protect the infant and maintain innate immune functions conferred by the skin barrier depend upon understanding of neonatal skin barrier development/maturation during the neonatal period. The choice of massage oil used for massage may be important. Topical application of sunflower seed oil among premature infants reduced nosocomial infections and mortality in hospital settings. This result prompted an investigation in the community setting in rural Nepal where high humidity and temperature conditions are common. We compared the potential mechanisms and effects of two emollients for routine massage in premature and full term infants in a cluster-randomized controlled trial.

Methodology: Neonates were randomized within clusters to receive either promotion of full body massage with either sunflower seed oil (SSO, intervention) or mustard seed oil (MO, standard

community-wide practice) in the trial from July 2012-May 2014. Biological measures of skin integrity were evaluated on days 1, 3, 7, 14 and over 28 days, and including: skin condition (erythema, rash, dryness), skin surface pH, stratum corneum (SC) cohesion via protein concentration, and transepidermal water loss (TEWL). Statistical analyses were conducted using STATA v14. Skin measures were compared using a bivariate random-effects model, accounting for the clustering and repeated measures.

Results and Conclusions: 500 and 495 live born neonates received repeated topical applications with MO and SSO, respectively. Mean relative humidity was 75.1% ± 13.2% and mean temperature was 30.0oC ± 4.5oC. In both groups, skin erythema, rash, and dryness increased during the first two weeks (worsening skin barrier), followed by a decrease from days 14 to 28, with very little dryness and no significant group differences. Mean skin pH and SC protein concentration decreased more quickly for sunflower than mustard oil, with rates of 0.091 (95% CI: 0.017-0.17) and 0.97 μg/cm2 /day (95% CI: 0.23-1.71), respectively, per day more quickly for SSO than MO over 28 days. SC protein concentration was significantly higher for SSO ($p < 0.001$). TEWL for both groups increased over time, with no significant group differences. Erythema, rash and dryness increased (worsened) over days 1-14 then decreased by day 28 with no significant group differences. TEWL increased over time, with no significant group differences. There were few significant differences noted among early (<34 weeks GA) and late (34-36 weeks GA) preterm and term infants in skin pH and protein.

These data provide some evidence that oil type/composition may contribute to differences in skin integrity in neonates massaged regularly with SSO versus MO during the first month of life. The generally poor skin conditions observed in both groups regardless of gestational age, especially during the first two weeks of life, may be due to high humidity conditions in this region. The more rapid acid mantle development and normalization of SC cohesion observed for SSO may be protective for premature and full term neonates in lower resource settings.

22. Age-Dependent Transformation of Skin Biomechanical Properties and Micromorphology during Infancy and Childhood

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Introduction: The objective of our work was to investigate the maturation of biomechanical function of skin postnatally and throughout childhood.

Methodology: A study has been conducted on 70 subjects aged 1 day to 35 years old. Skin properties were examined by cutometer and reflectance confocal microscopy (RCM) in vivo, and by immunohistochemistry on foreskin biopsy samples.

Results and Conclusions: Skin elasticity increased from infancy to 2 years of age and plateaued. In contrast, the viscoelastic component decreased from infancy to adulthood and total recovery was slightly higher at older ages. Interestingly, we observed by collagen fibers were fibrillar and showed a parallel orientation in newborns. Altogether, the analysis of both structural and biomechanical properties of the skin in the same subjects over a wide range of age is very informative and supports a contribution of mechanical forces to dermal maturation postnatally.

23. Assessment of Topographic Evolution of Skin in Newborns

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Introduction: We investigated the topographic evolution of the skin surface during postnatal development.

Methodology: The study was conducted in 6 age groups: (*n=6 to 9 /group, 5- 6 weeks, 6 months, 1-2 years, 4-5 years, 7-8 years, 10-11 years).* The 3D topography was measured by a confocal microscope in white light. As indicators of the geometric transformation of the topography, we have studied the developed area and the fractal dimension. To study the state of cutaneous tension, we developed a micro-mechanical model that uses the state of fold of the cutaneous surface to determine the mapping of the fold in the plane x-y. This model has been extended to a mapping of the folding rate from the top of the plateaux to the deepest furrows.

Results and Conclusions: The developed area and the fractal dimension of skin relief show the maximum evolution for children 4-5 years old. This result was confirmed by the evolution of the mechanical tension, which reaches the maximum of skin folding for the same age of children 4-5 years.

24. The Examination of Age-Related Changes by Visualizing and Measuring the Viscoelasticity of the Dermic and Subcutaneous Fat Layers Using Ultrasound Elastography

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Introduction: The viscoelasticity of the inner part of the skin which consists of many layers was not examined in detail. In this study, we visualized and digitized viscoelasticity of the skin depth wise using elastography, with the aim of ascertaining the characteristic age-related changes.

Methodology: The study included 140 women aged 20 – 69 years. The region of the face measured was the left cheek. For the elastography measurement, we used the Hitachi Noblus (Hitachi, Japan). The elastographic images were divided into seven areas in the depth direction. The mean measured value was calculated within each area.

Results and Conclusions: On measuring the dermic layer which was divided into two layers, in the only lower layer, viscoelasticity decreased statistically significantly with age. On measuring the subcutaneous fat layer, in the upper and middle layer, viscoelasticity decreased statistically significantly with age. In the lower layer, there were no clear age-related changes observed. The present study showed that, depth wise, for both the dermic and subcutaneous fat layer, age-related changes in viscoelasticity tended to differ.

25. In vivo Carotenoid Detection and Persistent Pigment Darkening as Markers of UVA and Blue Light Derived Depletion of Antioxidants in Human Skin

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Introduction: UV light, especially UVA, and even blue light from the sun trigger free radical formation in skin and therefore oxidation of skin own molecules. Carotenoids as a part of the antioxidant system of the skin protect the lipophilic molecules.

Methodology: We used multiple spatially resolved reflectance spectroscopy (SRRS, biozoom®) to measure carotenoid breakdown in the skin. UVA light and even blue light further provoke immediate pigment darkening of the skin. We measured the stable residue of this reaction the persistent pigment darkening (PPD).

Results and Conclusions: A standard antioxidant and a vehicle were applied to the back of 10 volunteers and irradiated with UVA or blue light. The carotenoid content of the skin was measured with SRRS. The PPD was measured with a Chromameter.

The investigated antioxidant led to effects of different magnitude regarding reduction of carotenoid breakdown and PPD inhibition.

In vivo measurements of carotenoid depletion and PPD formation in sun simulation experiments are promising methods to investigate the efficacy of antioxidants on human skin under realistic conditions.

26. Quantitative Relationship between Instrumental Measures, Plasma Biomarkers, and Facial Imaging in the Assessment of Factors Related to Skin Glycation

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Introduction: Antioxidative polyphenols contained in plant-based extracts have been shown to inhibit glycation reactions, including skin glycation. Finding a reliable technique for assessing efficacy in studying skin glycation changes after nutritional interventions is important as glycation may play a significant role in skin appearance. It is of interest to analyze the data derived in a clinical setting using different assessment tools. Therefore, the purpose of this research is to compare glycation assessed by two different methods, skin glycation as measured by auto fluorescence and plasma glycation biomarkers, and evaluate these measures against skin assessment using clinical instrumentation and facial imaging.

Methodology: The clinical study was conducted at Clinical Research Laboratories Inc., Piscataway, New Jersey, USA. 107 female subjects between the ages of 30-65 without diabetes, BMI 27-35, and skin glycation no less than 5% above the mean auto fluorescence value pertinent to the subject's age were recruited for the study. Upon recruitment, subjects were randomly assigned into one of three treatments as follows: 1) 200 mg Aronia extract (high polyphenol), 2) 130 mg Aronia and 122 mg of Licorice root extract (low polyphenol with flavonoids), 3) Placebo. The efficacy of treatment products was assessed with AGE Reader skin auto fluorescence (inner arm), plasma glycation biomarkers via liquid chromatography-tandem mass spectrometry (LC-MS/MS), elasticity via Cutometer, and facial imaging via

VISIA-CR with Mirror software by Canfield Scientific Inc. (Fairfield, NJ, USA), at baseline, and after 8 and 16 weeks of daily consumption. The Pearson correlation coefficients were analyzed and visualized using scatterplots or heatmaps. The data were analyzed using statistical software JMP by SAS Institute Inc. (Cary, NC, USA).

Results and Conclusions: We observed a positive linear correlation between chronological age and skin glycation (r = 0.6, p-value < 0.0001), indicating glycation increases with age which is consistent with the literature findings. We also found a positive linear correlation between skin glycation and plasma glycation biomarkers MG-H1 (r = 0.4, p-value < 0.0001) and PENT (r = 0.3, p-value = 0.0013), suggesting that as the plasma biomarkers increase, skin glycation also increases and supporting the notion that skin glycation and plasma glycation are related. Additionally, skin glycation has shown a significant correlation with elasticity (Cutometer readings Q1 – Q3, r ranges from |0.4 to 0.5|, p-value < 0.0001). Further, skin glycation was found to be positively linearly correlated with wrinkles (r = 0.4, p-value < 0.0001). These findings indicate skin glycation plays an important role in skin appearance. Treatment with antioxidative polyphenols showed measurable change to skin glycation over time. Accordingly, change in skin glycation and change in Q2 by cutometer measures showed negative association (r=-0.3, p-value = 0.0040), meaning as skin glycation decreases elasticity improves. These findings indicate that a plant-based nutrition intervention may affect both skin glycation and instrumental measures of skin appearance.

27. Efficacy evaluation of „Topipen" against Propionibacterium acnes

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Introduction: Acne is one of the most common diseases in current dermatological practice, almost any patient having a degree of acne in puberty and as a young adult. Although it does not endanger life, it can be extremely troublesome and can affect the appearance of a person in the long run, often triggering serious emotional disturbances (depression, low self-esteem, shame, anger, limitation of social life). „Topipen" (Courage + Khazaka, Cologne, Germany) is a prototype device that induces in the action area an electric field of constant current that change the acidity (pH) of the skin on a short-term basis. Acne vulgaris is considered to be a multifactorial disease occurring the sebaceous follicles of man. *Propionibacterium acnes* strains have been shown to produce a variety of exocellular enzymes that have a role in the production of the disease acne vulgaris.

Methodology: This study was performed on twelve voluntary subjects using an inoculum of Propionibacterium acnes ATCC 6919. The skin of the subject was previously wiping with a sterile compress with a 70% 2-propanol solution on one forearm, randomized.

On the subject's forearms, 4 sites for each forearm were established corresponding to baseline and 3 „Topipen" application times: 5 seconds, 10 seconds and 25 seconds. A corresponding rating score for each individual discomfort sensations, for each site was recorded.

From each testing site a sample was collected using the Williamson-Kligman scrub cup technique and then the count of *Propionibacterium acnes* ATCC 6919 from these samples was determined. The results were reported in Log_{10} CFU/mL.

Results and Conclusions: The mean Log_{10} CFU/mL of *Propionibacterium acnes* ATCC 6919 was significantly lower after 10-seconds (p=0.001) and 25-seconds (p<0.001) of „Topipen" application

compared to baseline. At 5-seconds (p=0.059) of „Topipen" application there was no significant difference compared to baseline.

The mean sensations of discomfort scores were 0.38, 1.43 and 1.90 for 5-second, 10-seconds and 30-second application, respectively.

„Topipen" was effective against *Propionibacterium acnes* ATCC 6919 at 10 seconds and 25 seconds application time.

28. pHascinating pHacts! New Insights Into the Host and Lifestyle Factors that Shape Skin Surface pH

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Introduction: The skin's surface pH is thought to play an important role in skin health and disease. The aim of this study was to better understand how skin surface pH varies with age, gender, ethnicity and body site in generally healthy people. We also set out to identify the skin biophysical and lifestyle factors associated with having an acidic or basic skin surface pH.

Methodology: The voluntary walk-up cross-sectional study enrolled 191 male and 259 female subjects, ages 9-78, of Caucasian (66%), African American (24%) or other (10%) ethnicity. Subjects' skin was not washed or prepared in any manner prior to skin surface pH measurement.   pH measurements were made using a Mettler-Toledo Seven2Go8 Pro equipped with an "InLab Surface" flat-surface electrode and calibrated regularly with pH 4 and pH 7 standard buffer (Mettler-Toledo, Columbus, OH). Other objective measures collected in the study included: forehead sebum secretion rate, cheek skin elasticity, facial wrinkling, hyperpigmentation, pores, red spots and porphyrins, forehead and scalp 16S microbiome diversity, heart rate variance, average heart rate, and the subject's height and weight. A structured questionnaire was used to collect lifestyle data such as lifetime smoking pack years and facial cleansing habits.

Results and Conclusions: Across the sample population, cheek and forearm skin pH varied over a wide range, from pH 3.1 to pH 6.8. However, within a subject, cheek skin pH was significantly correlated with forearm pH, i.e., subjects with high cheek skin pH tended to have high forearm pH, and vice versa (r^2=0.29, p<0.001). While there was no significant difference in cheek skin pH in males vs. females (mean ± SD: 5.29±0.43 for males and 5.24±0.46 for females), forearm skin pH was significantly lower in males compared to females (mean ± SD: 4.60±0.65 for males vs. 4.79±0.61 for females). Cheek and forearm skin pH increased slightly with age (p<0.001). There was no significant difference between Caucasian and African American cheek or forearm pH. Across the population, mean cheek and forehead skin surface pH values were significantly higher in the morning compared to the afternoon suggesting diurnal variation. A more acidic cheek skin surface pH was significantly associated with having less facial wrinkling, less hyperpigmentation, less visible pores, less red spots, less porphyrins, higher cheek skin elasticity, and lower forehead sebum secretion rate. Interestingly, having a higher average resting heart rate was significantly associated with having a more acidic cheek and forehead skin surface pH. Skin surface pH was not significantly associated with lifetime smoking pack years, BMI, daily caffeine consumption, heart rate variance, and forehead or scalp skin microbiome diversity. In summary, we observed high inter-individual but low intra-individual variability in cheek and forearm skin surface pH. The significant association of skin surface pH with several skin biophysical parameters suggests an important role for skin pH in overall skin health and appearance.

29 Clinical Efficacy of a Multifunctional Cosmetic Formulation for Mature Oily Skin

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Introduction: The skin may change due to factors as high temperatures, increasing sebum excretion and presenting oiliness and acne. These alterations can persist during the aging and provoke more changes that influence the use of cosmetics. The objective of this study was to evaluate the clinical efficacy of a cosmetic product developed for the mature oily skin.

Methodology: The clinical efficacy was evaluated on 30 participants aged between 39 to 55 years old with oily skin. The analyzed parameters were: stratum corneum water content, TEWL, sebum content and percentage, microrelief and dermis echogenicity. The analyses were performed on different regions of the face. A placebo formulation was also tested.

Results and Conclusions: The developed formulation improved the sebum content and percentage, skin microrelief in terms of skin roughness and desquamation and dermis echogenicity. The biophysical and skin imaging techniques utilized in this study were useful to test the clinical efficacy of an effective formulation for mature oily skin.

30. Evaluation of Young Skin Photoaging Using Biophysical and Imaging Techniques

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Introduction: Photoaging is associated to an intense solar exposure, thus the photoaging signs can be observed also in the young skin, mainly in countries with high UV incidence, like Brazil. The aim of this study was to evaluate the skin changes resulted from photoaging in Brazilian young skin in comparison to photoaged mature skin.

Methodology: Thirty participants were divided in two groups: the first between 18 to 35 years old and the second, 40 to 60 years old. Analyzes were performed on the randomized facial malar region. TEWL, stratum corneum water content, sebum content, high resolution imaging, echogenicity and dermis thickness, skin color and elasticity parameters were analyzed.

Results and Conclusions: The obtained results showed that sun exposure can cause changes even in the young skin, with the appearance of spots and the reduction of the echogenicity of the dermis, besides there were no significant differences between young skin and mature skin in most parameters. In conclusion, signs of photoaging may be frequent even in young skin.

31. Application of Reflectance Confocal Microscopy in the Evaluation of Skin Hydration

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Introduction: The Reflectance Confocal Microscopy (RCM) is an important tool to evaluate skin hydration. However, there is a lack of studies in the literature. This study evaluated the hydrating effects of different active ingredients using RCM.

Methodology: A carbomer gel added or not (vehicle) with Hyaluronic Acid (F1), Glycerin (F2), Hydrolyzed Rice Protein (F3) or *Kappaphycus alvarezii*&*Caesalpinia spinosa* Extracts (F4) was used. The anterior leg of 20 participants was utilized and a control was kept. Measures of TEWL, stratum corneum water content and interkeratinocyte reflectance, furrows size, morphology and skin surface irregularity were done before and after 2, 4 and 8 hours.

Results and Conclusions: Improvement of TEWL and stratum corneum water content was noted with F2 and F4. Furrows size, morphology and skin surface irregularity improved with F2 and F3. F1 and F2 showed an increase of interkeratinocyte reflectance. RCM is an efficient technique to evaluate morphological changes of skin hydration, showing the modifications of skin structures by alterations reflectance and morphology.

32. Air Pollution Intensifies the Effects of Age on Chinese Women Skin Tone

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Introduction: Skin colour results from a combination of various chromophores such as melanin, oxy-hemoglobin, or deoxy-hemoglobin. Several studies have shown that the skin is generally darkened with age in Asian women. However, there is no reports on the effect of air pollution on skin colour. The aim of this study is to determine the changes of the Chinese women skin colour as a function of age and air pollution.

Methodology: We involved 200 volunteers, age range from 20-59 in Guangzhou and Zhanjiang. Their skin colour and related chromophores on the skin covering the eye, cheek and the inner upper arm was measured via chromameter and hyperspectra camera, while full face images were captured by VISIA CR.

Results and Conclusions: Based on the images of VISIA-CR, the skin colour is darkened with age in both cities, however skin darkening is intensified in Guangzhou. In addition, the melanin index measured by the hyperspectral camera at the eye region is significantly lower in Zhang Jiang population. The a* value at the cheek and arm region is signficantly lower in Guangzhou population. These results suggest that air pollution steps up the effect of ageing on skin colour in Asian women.

33. Assessing Skin Barrier and Stratum Corneum Surface Integrity after Application of Skin Cleansers on Human Skin by Ex-Vivo Confocal Laser Scanning Microscopy

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Introduction: The physicochemical properties of the stratum corneum (SC) assures skin barrier integrity and can be affected by surfactants-containing cleansers by modifying skin pH and components. The aim of this study was to assess the effect of cleansers on the surface of human skin by confocal laser scanning microscopy (CLSM) in the fluorescence mode, by monitoring the labelling of acridine orange dye through the SC.

Methodology: First, we evaluated the application of SDS solutions (0.1-0.5-1-5%). We then compared 0.5% SDS solution with a body shower cleanser (product A) or an emollient shower oil containing Rhealba® Oat Plantlet extract for soothing properties (product B). SDS or test products diluted with acridine orange dye were applied on the surface of skin biopsies. After washes, CLSM imaging was proceeded with the Vivascope®2500 (MAVIG). Five VivaStack acquisitions were performed per condition. For quantification of dye penetration, the stack projections were analyzed using ImageJ and internal Octopus softwares. The experiments were repeated on n=3 donors.

Results and Conclusions: Untreated skin biopsies exhibited a low level of dye penetration and this was expected for a normal, unmodified skin barrier. In contrast, we observed a significant, dose-dependent-effect, increase of dye penetration through SC exposed to SDS (x4.1, x24, x48, and x46), revealing alterations of the skin barrier. Comparison of test products showed that 0.5% SDS and product A induced a significant increase of dye penetration (x11.3 and x6, respectively) while no significant modification was found for product B, demonstrating that emollient shower oil containing Rhealba® Oat Plantlet extract preserved SC and skin barrier. Our data showed that fluorescent dye penetration monitored with Vivascope®2500 CLSM imaging is a promising method to evaluate the effect of cosmetic products on skin barrier.

34. Multiphoton imaging of Stem Cells in Hair Follicles

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Introduction: The use of fluorescent proteins in combination with multiphoton imaging represents a powerful method to visualize and to track stem cells in hair follicles in mouse models.

Methodology: Green fluorescent proteins (GFP) as well as endogenous fluorophores (autofluorescence) in nestin--driven GFP transgenic nude and normal mice have been excited via a two-photon absorption process with a near infrared femtosecond laser multiphoton tomograph.

Results and Conclusions: We report on the visualization of hair follicle-associated pluripotent (HAP) stem cells in their natural environment. HAP stemhave been found to express nestin, reside in the bulge area and the dermal papilla and have the capacity to develop nerves, blood vessels, and smooth muscle. (FLIM). They have an oval-shaped 7 μm long body and dendritic-like arms. We were able to track moving stem cells over a 5hour period.

35. A Study on the Skin Characteristics According to Exposure to Repetitive Temperature and Humidity of Korean Summer Weather

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Introduction: During summer in Korea, there are large differences in indoor and outdoor temperature and humidity. The korean are repeatedly exposed to these temperature and humidity differences. This study aimed how skin properties change in these certain circumstances.

Methodology: Twenty-two female subjects (29.86±4.66 yrs) participated in this study. The subjects were exposed Korean outside summer weather (32±3°C, 75±5%, 30 min) and air-conditioned indoor environment (22±3°C, 35±5%, 30min) six times repetitively. Skin color, hydration, sebum, elasticity, temperature, translucency, and pore (size, number, area) on the cheek and forehead were measured.

Results and Conclusions: As a result hydration level did not change but the sebum secretion increased rapidly. The yellowness of the skin and hemoglobin were increased but elasticity was decreased under the sudden changes. In 30s, elasticity decreased after 2 times repetitions. And pore size and area didn't changed.

36. Impaired Perilesional Skin Integrity & Impaired Tissue Integrity Related To Altered Circulation in Patients with Vasculogenic Ulcers: Use of Ultra-Sliding Cream

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Introduction: Introduction: Vasculogenic ulcers represent a public health problem that requires an interdisciplinary approach emphasizing nursing. The focus of nursing on caring when this disease is present should be on diagnoses "impaired tissue integrity" and "impaired perilesional skin integrity", among others. Despite the high number of bandages made to the outpatient units of the Goiania Department of Health, the nursing staff has not conducted systematic approach and evaluation. In the presence of vasculogenic ulcers, there may be impaired tissue integrity, related to altered circulation. Commonly changes in the perilesional area, due to maceration (action of excess of exudate), small ulcerations, superficial, or abrasions (due to mechanical action of adhesives, chemical action of enzymatic debridants), configuring the "skin integrity of impaired perilesional area, related to chemical agents, moisture, mechanical factors. Used ultrasliding cream, a hypoalergenic, emolient product that provides intensive sliding of the hands during massage. Metabolic, hydrating, vasoprotetor, oxygenant and nutritive stimulant.

Methodology: Longitudinal clinical case study of intervention with three patients, carried out in a house with elderly patients. In the protocol the cream was used three times a day for sixty days, after the consent of the elderly. The study was carried out between November and December 2017.

Results and Conclusions: Among the related factors (FR) analyzed, the "altered circulation" was found in all participants. The three elderly patients presented ITB values between 0.5 and 0.8 as also features of venous alteration (edema, ocher dermatitis, lipodermatosclerosis, hyperkeratosis and / or erythema), making it appear to be related to venous and arterial circulation. The ultra-sliding cream was used with

components such as green tea and some herbs on the peri-lesional skin, a reduction in inflammation and a better hydration.

37. Multiphoton imaging of Stem Cells in Hair Follicles

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Introduction: The use of fluorescent proteins in combination with multiphoton imaging represents a powerful method to visualize and to track stem cells in hair follicles in mouse models.

Methodology: Green fluorescent proteins (GFP) as well as endogenous fluorophores (autofluorescence) in nestin--driven GFP transgenic nude and normal mice have been excited via a two-photon absorption process with a near infrared femtosecond laser multiphoton tomograph.

Results and Conclusions: We report on the visualization of hair follicle-associated pluripotent (HAP) stem cells in their natural environment. HAP stemhave been found to express nestin, reside in the bulge area and the dermal papilla and have the capacity to develop nerves, blood vessels, and smooth muscle. (FLIM). They have an oval-shaped 7 μm long body and dendritic-like arms. We were able to track moving stem cells over a 5hour period.

38. Study of the Effects of Pregnancy on Skin Properties: A Mechanical Approach

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Introduction: The aim of the study was to investigate skin mechanical properties of a highly stressed area (abdomen) and a less stressed area (thigh), in non-pregnant woman and in pregnant woman before and after delivery.

Methodology: Mechanical properties of skin have been measured with a Cutometer MPA580 on 15 non-pregnant women and 26 pregnant women at the 8th month of pregnancy and 4 months after delivery. Measurements were performed on the abdomen and the thigh area.

Results and Conclusions: On the abdomen results showed that the skin in pregnant woman is significantly less extensible and more viscous than in non-pregnant women. A decrease in skin tonicity is also observed in post-delivery skin condition when compared to non-pregnant and pregnant skin conditions. On the thigh, a decrease in tonicity is also observed during pregnancy and after delivery.

This study showed that skin mechanical properties significantly change during pregnancy compared to the non-pregnant condition and that these properties are still altered 4 months post- delivery.

39. Evaluation of Oily Hair and Skin: Comparison between Self Perception and Clinical Analysis Using Biophysical and Imaging Techniques

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Introduction: Excess of oiliness can cause skin changes such as acne and compromise the cutaneous physiology, affecting of both skin and hair. Thus, the aim of this study was to evaluate skin and hair alterations due to excessive amount of sebum using biophysical and imaging techniques.

Methodology: 100 participants (18 - 49 years), with oily skin and hair, were recruited. Skin was evaluated in terms of stratum corneum water content, TEWL, activity of the sebaceous glands, amount of porphyrins and pores. Scalp was evaluated in terms of sebum content.

Results and Conclusions: Participants were divided 4 groups: 1- Oily skin and hair (45,23%), 2- Oily skin and normal hair (10,71%), 3- Normal skin and oily hair (34,52%) and 4- Normal skin and hair (9,52%). The participants with oily skin presented activity of the sebaceous glands of 9.1 ± 1.1 surface (%), high amount of pores and presence of porphyrins, and scalp amount of sebum of 330,6 ± 9,8 µg/cm². Although all the panelists considered their hair and skin oily, they were classified differently, showing that the tropical weather can influence the self-perception and lead to a wrong treatment without the correct evaluation.

40. Application of Topical Formulations Containing Natural Origin Actives and UV-Filters in the Prevention of Photoaging in French and Brazilian Skin

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Introduction: The study of skin from different populations brings an essential knowledge to the development of skin treatments. The aim of this study was to evaluate the immediate effects of topical formulations using biophysical techniques and to compare the skin biology of the participants.

Methodology: 36 subjects, 18 French and 18 Brazilians, were enrolled. Transepidermal water loss, stratum corneum water content, skin viscoelasticity and skin brightness were evaluated before and 60 minutes after formulations application.

Results and Conclusions: Brazilian skin had a lower TEWL and less gloss on the skin surface when compared with French skin. There was no difference in hydration and viscoelastic profile. After 60 minutes, there was a significant increase in stratum corneum water content and skin brightness, a significant decrease in TEWL and no difference in skin viscoelasticity in both groups. In conclusion, biophysical differences were found on the groups and the formulations were effective in both populations.

41. pH Triggered Nanoparticles to Enhance and Control Cutaneous Drug Delivery - Investigated by EPR Spectroscopy and Laser Scanning Microscopy

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Introduction: With advancement in material design and engineering, nanoparticles (NPs) have been developed for dermal and transdermal drug delivery. NPs can improve skin penetration of drugs and can enhance activity and tolerability. Yet, the underlying dynamics of the drug release from NPs and the mechanism of enhanced penetration remain poorly defined. Furthermore, diseased skin shows altered pathologies, such as the permeability of skin barrier, water content and pH of the skin surface, which could influence the penetration and drug release of NPs. Here, differences in pH will be used to trigger NPs to provide selective treatment and controlled delivery.

Methodology: In the present study, electron paramagnetic resonance spectroscopy (EPR) and confocal laser scanning microscopy (CLSM) were combined to comprehensively investigate cutaneous penetration of probes loaded to Eudragit® pH sensitive NPs. NPs loaded with spin labeled glucocorticoid dexamethasone (DxPCA) was used in EPR measurement to quantify the drug penetration, determine the microenvironment of DxPCA and monitor drug release on porcine skin ex vivo. Both, intact and barrier disrupted skin which was established by removing stratum corneum by tape stripping was treated. NP loaded with nile red was applied for CLSM measurement to visualized the spatial distribution of probes and NPs in skin.

Results and Conclusions: EPR proved the pH triggered drug release of DxPCA from NPs on *ex vivo* skin and enhanced cutaneous drug penetration in comparison to a cream formulation. Compared with intact skin, twofold amount of DxPCA penetrated into epidermis and dermis of the barrier disrupted skin. Furthermore, the percentage of released DxPCA from NPs within 2 h on disrupted skin was 3.7 times higher than in intact skin. The preferential drug exposure on lesional / barrier disrupted skin by Eudragit® pH sensitive NPs and may allow to avoid adverse effects on healthy skin. Studying the lipophilic probe nile red, CLSM results are well in accordance. With NPs, the penetration of nile red reached a penetration depth of 160 μm, moreover, intrafollicular nile red related fluorescence indicates the transfollicular penetration of the pH sensitive NPs, providing a shortcut to deliver drugs into viable epidermis and dermis. The ability of EPR to trace the drug release is of clinical significance, because the drugs encapsulated in nanocarriers need to be released to exert pharmacological effect. EPR measurements can also guide the optimization of formulations to achieve a reasonable kinetics of drug delivery. The combination of EPR and CLSM utilized in this work shows the comprehensiveness to evaluate the cutaneous penetration of NPs, from the quantification to microenvironment, and from the drug release kinetics to the spatial distribution.