

ABSTRACTS
WEDNESDAY, 28 NOVEMBER 2012

FO1.01

**IMAGE ANALYSIS AND CORRELATES OF FACIAL WRINKLES IN A COHORT OF WOMEN:
PRELIMINARY DATA FROM A RETROSPECTIVE ANALYSIS**

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Background: Facial wrinkles result from natural aging taking place over a lifetime. Genetic and environmental factors are responsible for such changes. Research has attempted to find a correlation between different factors and the progression of wrinkles. Objective: The objective of this investigation was to analyze the characteristics and amount of facial wrinkles in women of different age groups and ethnicities. Methods: Image analysis and health information variables were used to determine correlations between different factors in different age groups and ethnicities. Clarity Pro™ was utilized for facial wrinkle analysis. A test population of 311 women identified from our database, who had previously undergone a 7 day “washout” was used for this research. Participants underwent facial image analysis (left profile) examining the crows feet area for total wrinkle count, fine line count, deep line count as well as wrinkle length, width and severity. Results were analyzed utilizing logistic and linear uni-variate regression with the variables age, body mass index (BMI) and Fitzpatrick skin type. Results: Significant positive correlations were observed with age and total number of wrinkles and each Clarity parameter measured. Conversely, negative correlations were observed between total wrinkle count and wrinkle severity with Fitzpatrick skin type and BMI regardless of age. Conclusion: These results are an initial step in characterizing the detail wrinkle progression in women and the factors associated with enhanced progression and/or prevention. Further work is underway utilizing numerous additional variables as possible correlates and stratification in a larger sample size.

FO1.02

**DETERMINATION OF THE SEBUM CAPTATION FACTOR OF THE SEBUMETER METHOD:
EFFECT OF APPLICATION PRESSURE**

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Introduction: The determination of the sebum casual level can be carried out using the photometric method (Sebumeter). The Sebumeter measuring probe (cassette) is applied with a constant pressure on the skin surface using a spring system. In the literature values ranging from 6.6 to 10 N are reported. The measured quantity of sebum is only a fraction of the real quantity of sebum present (captation factor). Reported captation factors vary from 0.40 to 0.60. It is the purpose of this work to evaluate the captation factor as a function of the applied probe pressure. Methods: The application pressure of 15 measuring cassettes was measured using a precision balance. Sebum repetitive determinations at respectively low (normal) pressure ($\pm 5-6$ N) and high pressure (10 N) allowing the calculation of the captation factor were carried out on the forehead of 15 subjects. The captation factor was calculated as the slope of the logarithm of the sebum quantity in function of the number of collections. Results: The pressure of application of the cassettes is reasonably constant 4.8 ± 0.3 N ranging from 4.6 to 5.4 N. The captation factor remains constant 0.73 ± 0.14 in function of the interinstrumental variation. No significant difference was detected between the captation factor at low pressure (0.71 ± 0.03) versus high pressure (0.73 ± 0.03). Conclusion: The measuring of the Sebumeter system is not influenced by variations in the application pressure of the probe.

FO1.03

BIOMECHANICAL ASSESSMENT OF THE SKIN USING MULTILAYER APPROACH

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Background: of the various dermatological evaluation methods involving touch, palpation is one of the most used to evaluate the skin properties. Indentation technique is the closest instrumental test to perform this type of clinical movement. Contact and non contact methods are now available for the assessment of cosmetic product effects. Non contact method has several advantages including no adhesion effect, no disturbance of the tested area and less positioning problem on complex shape. Basic interpretation of this kind of test only considers force or displacement values. First level interpretation considering semi infinite material can give an equivalent Young modulus of the tested area. As skin is a multilayer material, more complex model has to be used for the characterization of each layer. Purpose: The aim of this work is to evaluate the ability of biomechanical devices which use normal load to characterize multilayer skin properties. It can include classical contact indentation test as well as non contact test. Methods: Theoretical analysis and numerical simulation are used to investigate the optimal test settings including number of trials, loading, depth of penetration, thickness measurement. Rheological model with elastic or viscoelastic behavior are considered. Results: Sensibility of the assessment of the properties to each parameter is determined. Conclusion: inverse approach using the developed model in association with *in vivo* or *in vitro* measurement will be tested to extract multilayer skin properties.

Key words: skin mechanical properties, indentation, non contact test, multilayer.

FO1.04

ESTIMATION OF SWEAT PRODUCTION DURING EXERCISE AT TWO INTENSITIES USING THE AQUAFLUX INSTRUMENT

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Introduction: Determination of the sweat production during exercise is an important component for the development of (re)hydration strategies. Up to now sweat production is measured by weighing the amount of sweat in a cotton patch applied on the skin during the exercise or by the use of the cumbersome flow true cell technique. The condensation technology used in the AquaFlux instrument allows a high measurement capacity. In the present study, an adapted version of the AquaFlux instrument was used to measure sweat production during exercise. Methods: 15 healthy male subjects participated in the experiment. A bike test was performed at an intensity of respectively 55% and 75% of maximal heart rate during 20 minutes. An adapted AquaFlux measurement chamber (with increased measuring capacity) was used to measure the produced sweat before and during exercise (at 5, 10, 15 and 20 min) on different skin sites (shoulder, back). Sweat production was equally assessed using the cotton patch technology attached to the back of the subjects. Results: At an intensity of 55% AquaFlux measurements (in g/m².h) at 10, 15 and 20' predicted the amount of water in the patch on both skin sites (r values ranging from 0.79 to 0.84). At 75% prediction of the amount of sweat in the patch was possible at all measurement intervals during exercise (5, 10, 15 and 20' with r values ranging from 0.62 to 0.86). Conclusion: The AquaFlux seems to be a promising instrument for the determination of sweat during physical exercise.

FO1.05

ANALYSIS OF HUMAN ECCRINE SWEAT GLAND PATTERNS USING THE SKINCHIP

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Recently, we have become very interested in how the pattern of eccrine sweat gland activity in certain pathologic conditions such as cystic fibrosis may differ from those of normal individuals. Previous studies along these lines have utilized either painting the skin surface with a starch iodide solution, or casting silicon based impressions to reveal activated sweat glands. Unfortunately there are a number of problems associated with both methods and a more user-friendly approach is needed for monitoring sweat gland patterns in such patients especially in non-laboratory conditions. The primary purpose of this study was to determine if the SkinChip could be used to characterize the sweating patterns on the volar forearm of healthy human volunteers. This device is based on a

capacitance sensor that was originally designed for fingerprint imaging but, as shown by researchers at L'OREAL, can also be used to study hydration levels and microrelief of the skin surface *in vivo*. We have found that the SkinChip is ideally suited for these types of investigations because the activated sweat glands will appear as dark dots in a grey scale image which can be captured through the SkinChip software. The pattern of dots can be easily measured by conventional image analysis. This gives rise to two parameters: SGD, which is the density of activated sweat glands, and SGO, which represents the output of the individual sweat glands as revealed by the cross sectional area of the detected dots. Since a sequence of images can be placed in a registry, it is possible to follow the activity of an individual sweat gland over time. In this way we were able to determine changes in the number, distribution, and output of sweat glands on the volar forearm of healthy volunteers during dynamic exercise under normothermic conditions. Additional studies are currently underway to explore the use of the SkinChip as a novel method to study abnormal sweating patterns as found in thermal stress, cystic fibrosis and other pathological conditions.

The authors thank L'OREAL RECHERCHE who provided the SkinChip for investigating these severe and distressing pathologies.

FO1.06

NONINVASIVE ASSESSMENT OF HUMAN SKIN PLASTIC RESERVES

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Purpose: To determine the skin plasticity reserve in human different body parts, as well as to predict the skin stretching degree indirectly in on-line mode. Patients and Methods: The noninvasive assessment of skin mechanic-and-acoustic properties was performed in 35 females (25-59 years old). The limb skin area and that of the face was tested using ASA-4 acoustic skin analyzer and BLF-21 lazer Doppler flowmeter. A functional test was made-the flexion of limb joints and cervical spine (patent No. 2340277, RU). Results: The skin plasticity coefficient (SPI) in the two mutually perpendicular directions tested denotes the current level of integument tissue plasticity reserves. The skin integument plasticity reserve value below 0.9 was considered low. When 1.2 value was exceeded high skin plastic properties were considered. The range of face skin plasticity coefficient longitudinally in 20-29-year group was 0.98-1.39 (1.29 ± 0.09), while in 50-59-year group-1.07-1.46 (1.21 ± 0.03). In view of the marked individual differences in skin structure and type it is advisable to evaluate the biomechanical property reserve in every particular tested subject. Conclusion: The approaches proposed to reveal the skin biomechanical plasticity reserves allow to assess the skin integument mechanic-and-acoustic, as well as to predict the skin stretching degree indirectly when making body spatial voluntary movements.

FO1.07

OBJECTIVE SKIN SOFTNESS MEASUREMENT: ACOUSKIN METHOD

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Background: objective softness assessment of the skin is a very complex problem. In the cosmetic domain, the clinician usually touches the skin and tries to assess the softness of the tissue according to its own subjective sensations. Standardization and reproductive evaluations between different experimenters are also very difficult to obtain. Some objective methods have been developed to evaluate the tribological behaviour of the skin during a touch movement. Purpose: the aim of this work is to present the ability of a new technique based on the acoustic measurement of the sound induced by a touch movement on the skin to assess the variation of softness after cosmetic product application. Method: the sound is produced by the contact between the finger of an experimenter and the tested material. The measurement is performed by using a specially developed device called Acouskin®. It is composed of a rigid part which amplifies the sound, and a measurement part which records the acoustic signal. Treatment of this acoustic signal is then performed. The average sound level and the distribution of the sound in the frequency domain are calculated. Tests on inert materials and various *in vivo* tests have been done. Results: the tests performed show the correlation between subjective sensation perceived by clinician and objective measurement performed by the device. Products effects are well characterized for the entire performed test. Conclusion: the developed method is able to objectively evaluate the softness of the skin. Several applications can be developed.

Keywords: softness, acoustic measurement.

FO1.08

WETTABILITY AND FRICTION COEFFICIENT: IN VIVO AND IN VITRO STUDY

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Introduction: The frictional behaviour of the skin while contacting different materials plays a critical role in the skin sensory perception which is extremely important in cosmetic application such as antiaging cream and moisturizers. It depends on several parameters. Objectives: To show the hydrophobic/lipophilic balance of the skin and the effects of sebaceous lipids and saliva on the skin friction coefficient. Methods: Measured parameters : Friction coefficient (μ) and water contact angle (θ_w), measured by an especial device, designed for in vivo, ex vivo and *in vitro* studies. Wettability and friction coefficient: *In vivo* and *in vitro* study. Studied surfaces: Skin (forearm, forehead), hair, Tongue, Teflon®, glass, steel, Silflo® , resin (elastomer). Results and discussion: The friction coefficient in the forearm rise by increasing of the sliding surface hydrophilia: It changes from 0.18 of Teflon® (hydrophobic) to 0.74 of steel (hydrophilic). The same effect of the surface hydrophobia is also observed in vitro. The saliva reduces tongue surface hydrophobia, and decreases the friction coefficient by a factor of 1.6. Conclusion: A positive relationship has been demonstrated between water contact angle and friction coefficient. An important function of the salivary layer in the oral cavity is to play the role of a lubricant between oral tissues. Saliva like sebum, increases the hydrophilicity and reduces the friction coefficient.

Key words: friction, wettability, skin, sebum, saliva.

This work was published in Computer Methods in Biomechanics and Biomedical Engineering; Vol. 14, No. S1, August 2011, 159-160

FO1.09

FRICTION HEEL BLISTERS: THE EFFECT OF HYDRATION ON THE RISK OF BLISTER

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Objectives: Foot blister research has focused on the efficacy of treatments rather than exploring the underlying pathophysiology of the formation of blisters. The authors of this study have developed a laboratory-based blister creation model that applies controlled friction loads to heel skin. The critical events related to early signs of skin trauma, such as superficial inflammation and increased temperature, are monitored using infrared thermography. The effect of hydration of the skin on the risk of skin trauma is reported. Methods: The skin on one foot of each of the 20 healthy volunteers was hydrated using a water foot soak and subjected to a consistent load until an inflammatory response akin to that of skin trauma was evident. Load application was then ceased and temperature measurements taken at set times during the following 30 minutes. The contra lateral foot acted as a control, i.e. load was applied in a similar way but without pre-hydration of the skin. Results: The hydrated skin group demonstrated a significantly greater rate of inflammation (in response to friction) compared to the control group ($p=0.001$). There was a positive correlation between skin hydration and the rate of inflammation ($r=0.52$). Conclusion: This study has shown that the rate of inflammation, in response to friction application, increases with increased hydration of heel skin. However, these results are reported within the context of the experimental design. Further work is needed to confirm and apply the physiological and mechanical response of skin to blister treatment interventions.

FO1.10

ULTRASOUND EVALUATION OF THE EFFICACY OF ANTI-ALOPECIA FORMULATION

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Introduction: Androgenetic alopecia (AA) is a common cause of consultation and to date there are no reports on the use of variable frequency ultrasound for evaluating cosmeceutic products. Objective: To assess the anatomical changes of the hair follicles using a cosmeceutic formulation (madecassoside, aminexil®, arginine and vitamin B). Methods: A prospective study was performed in 28 patients (17-41 year-old). Inclusion criteria: male, AA (\geq type II Hamilton). Exclusion criteria: concomitant treatment, cutaneous/systemic diseases, hair implants. A sonographic examination was performed in T0 (basal) and T8 (8 weeks post treatment). Measurements (mm) included the right

frontal and occipital (control) regions (thickness of dermis, depth and transverse axis of the hair follicles, blood flow). An improvement ratio (IR) was created ($IR = \text{depth hair follicles (dhf)} / \text{thickness of dermis}$; dhf=mean of the depth in 6 hair follicles per area. Statistical analysis (student t test) was performed. Results: The hair follicles were recognized in all cases. A decrease in the number of hair follicles in the alopecia areas was observed. No significant differences were found in the vascularity. An improvement of IR ($p < 0.05$) was found in 32% of the cases ($n=9$) in the frontal region and in 46% ($n=13$) when comparing frontal vs occipital. In 50 % of the younger cases (17-30 year old, $n=12$) a significant increase of the transverse diameter of the hair follicles ($p < 0.05$) was founded. Conclusion: Ultrasound can assess the anatomical changes in the hair follicles of the scalp and the efficacy of a cosmeceutic formulation.

FO1.11

MULTIVARIATE ANALYSIS OF RAMAN CONFOCAL SPECTRA OF THE STRATUM CORNEUM FOR EVALUATION OF EFFICACY OF A MOISTURIZING COSMETIC PRODUCT

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Raman Spectroscopy has emerged as a useful tool to assess *in vivo* the molecular composition of the stratum corneum. This method is of high interest as it allows following water content as a function of depth. The objective of this study was to assess the molecular changes affecting the stratum corneum after application of a moisturizing product. Twenty four healthy women, with dry or very dry skin on the forearms, applied a moisturizing cream randomly for 1 week, twice a day, under in use conditions. The skin moisturization was assessed using a corneometer. Raman spectra (2500 – 3800 cm^{-1}) were acquired from the top of the stratum corneum up to a depth of 28 μm . Sparse Partial Least Square Regression (sPLS) was used to compare the treated forearms spectra with untreated ones. The water content as a function of depth was computed. The sPLS allowed discriminating between treatment groups and finding the range of the spectra that differed. The 3 first components of the sPLS highlighted the contribution of the water (3350-3550 cm^{-1}) and the lipid bands (2820-2900 cm^{-1}). A significant increase of the water content was shown from both the Raman water profile and the corneometer. In this clinical study, the analysis of the Raman Confocal Spectra demonstrated that both water and lipid contents were modified by application of a moisturizing cream. The multivariate statistical method is of interest to identify all peaks affected by product application and can be used for any other topical skincare treatment.

FO1.12

TEXTURE ANALYSIS OF COSMETIC/PHARMACEUTICAL RAW MATERIALS AND PRODUCTS

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Objectives: The purpose of this work was to quantify textural properties of cosmetic and pharmaceutical raw materials. Methods: Textural parameters such as hardness, consistency, cohesiveness, index of viscosity, stickiness, and resilience were evaluated. The measurements were performed using a Texture Analyzer (a tensile meter equipped with special probes in the form of acrylic cylinder and stainless steel sphere), which can penetrate the measured sample of a product recording the force, distance and time. The instrument simulates the action of a human finger touching the surface and probing the properties of an object. The set-up has been previously shown to quantify the rheological/textural properties of cosmetic and pharmaceutical products such as creams, lotion, and gels as well as rheological properties of human skin. Results and Conclusions: The results include the analysis of water, glycerin, as well as aqueous solutions of thickeners such as Acrylates/C10-30 Alkyl Acrylate Crosspolymer (Ultrez-20 obtained from Noveon), and Carbomer. Solutions of common surfactants and complex surfactant formulations such as shampoos have also been investigated. The results, in the form plots of force as a function of time or distance, resulting from slow bi-directional probe movement (submergence and de-submergence) in the analyzed fluid, were interpreted by considering buoyancy, drag, and viscous drag force given by the Stokes equation. The data can be used to correlate with tactile analyses of products by trained panel evaluations

FO1.13

ANTIPERSPIRANT WITH 20% ALUMINUM CHLOROHYDRATE EFFECTIVELY DECREASES SWEATING AND ODOR 48 HOURS AFTER USE

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Antiperspirants generally contain salts of metals, particularly aluminum, which form gel plugs that block the excretory ducts of sweat glands. Antiperspirants decrease sweating as well as diminish unpleasant odor, which can be further enhanced by supplementary deodorizing substances. The present study investigates the effect of an antiperspirant containing 20% of Aluminum Chlorohydrate in combination with a deodorizing substance, Triethyl Citrate. The assessment was done objectively by studying the amount of secreted sweat and subjectively by the subjects' own evaluation of sweating and odor. The study was double-blind and randomized. Fifteen volunteers used one test antiperspirant and one placebo for 3 days, once a day. Forty-eight hours after the last application, volunteers exposed themselves for moderate physical activity for about 30 minutes with absorbing pads fixed in the armpits. The weight of pads was recorded before and after the exercise. Moreover, volunteers were asked to evaluate odor and feeling of dryness under the armpits before the exercise, using a visual analog scale. The tested antiperspirant diminished secreted sweat 48 hours after the last application in comparison with placebo, with median of the decrease being 0,5 g ($p=0,027$), corresponding to a decrease by 23%. Odor was also less pronounced and armpits felt dryer than areas treated with placebo as assessed by volunteers. The tested antiperspirant effectively reduced sweating and odor 48 hours after the last use, which was verified by both objective and subjective measurements. Efficacy of antiperspirants is of great importance as perspiration and malodor are perceived as negative factors.

FO1.14

EFFECT OF MICROPARTICLES CONTAINING GREEN TEA EXTRACT ON FACIAL SKIN IMPROVEMENT

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Objective: To clinically evaluate an effectiveness of skin cream containing green tea extract loaded chitosan microparticles for facial wrinkle treatment. Method: Twenty-nine volunteers were randomly assigned to apply skin cream containing 1% green tea extract loaded chitosan microparticles (GT-Cs) and a placebo cream on each of their half faces for 8 weeks. Skin elasticity was evaluated by using Cutometer® and the photographs of each half faces were also compared. Skin moisture and skin irritation were determined by Corneometer and transepidermal water loss (TEWL) respectively. Results: GT-Cs cream significantly increased skin elasticity than the placebo cream after application for 4 weeks. The facial photographs obviously presented the wrinkle improvement. Surprisingly, skin moisture of the treated area was not different from the baseline. Moreover, fifty percent of volunteers presented decreasing trend of skin moisture in the half face applied with GT-Cs cream. However, skin irritation was not observed in all volunteers. Conclusion: Skin cream containing green tea extract loaded chitosan microparticles shows satisfied results. Volunteer's skin elasticity significantly improved in both microscopic and macroscopic properties. Visible wrinkle decreases can be noticed after applied chitosan microparticle containing green tea extract cream for only 4 weeks. There is no sign of skin irritation.

SS01.01

MOLECULAR ORGANIZATION OF THE SKIN BARRIER

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The skin barrier is fundamental to terrestrial life and its evolution; it upholds homeostasis and protects against the environment. Skin barrier capacity is controlled by lipids that fill the extracellular space of the skin's surface layer - the stratum corneum. Here we report on the determination of the molecular organization of the skin's lipid matrix in-situ, in its near-native state, using a novel methodological approach combining very-high magnification cryo-electron microscopy of vitreous skin section (CEMOVIS) defocus-series, molecular modelling and electron microscopy simulation. The lipids are organized in an arrangement not previously described in a biological system stacked bilayers of fully-extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety. This arrangement rationalizes the skin's low permeability towards both water and towards hydrophilic and lipophilic substances, as well as the skin barrier's robustness towards hydration and dehydration, environmental temperature and pressure changes, stretching, compression, bending and shearing.

SS01.02

HUMECTANTS REGULATE SKIN HYDRATION AND SKIN PERMEABILITY. HYALURONIC ACID, GLYCERIN AND UREA EX VIVO AND IN VIVO

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Humectants are used in moisturizing creams in order to diminish the clinical symptoms of skin dryness. Humectants may also change skin permeability by influencing the ambient humidity and by penetration into the skin. High molecular polymers (e.g. hyaluronic acid; HA, polyethylene glycol; PEG) stay on the surface after application, whereas low molecular weight humectants (e.g. glycerin, urea) penetrate the skin. In an ex vivo study the high molecular weight polymers were found to reversibly regulate the skin permeability of a model drug by varying the gradient in water activity across the skin. Similar results were found for the low molecular weight humectants glycerin and urea, but in addition these substances appeared to influence the permeability with another mechanism. *Ex vivo* studies with confocal Raman Spectroscopy demonstrated a higher tendency to formation of water rich domains within the stratum corneum after treatment with glycerin and urea in comparison to treatment with high molecular weight, hydrophilic polymers. *In vivo* data also showed higher transepidermal water loss (TEWL) after exposure to an aqueous solution of glycerin and urea, compared to exposures to aqueous solutions of high molecular weight polymer. The results highlight the importance of considering the water activity of the applied formulation and the fact that humectants influence the skin permeability with different mechanisms.

SS01.03

USE OF “CONNECTIVITY MAP” TO IDENTIFY NOVEL RETINOID-LIKE AGENTS WITH COSMETIC BENEFITS

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Retinoids have been used as therapeutic agents for numerous skin diseases, from psoriasis to acne. The same substances have also been recognized in the treatment of hyperpigmentation disorders such as melasma. Further studies have shown that, the clinical effects of retinoic acid include improvement of wrinkles, surface roughness, mottled pigmentation, and skin appearance as a whole when used on photo-damaged skin. Consequently, several independent studies now suggest retinoids to be the Holy Grail of anti-aging ingredients. However, many studies have also highlighted the limited skin tolerance of retinoids. The aim of the current study was to identify novel compounds that mimic the activity of retinoids. For this purpose, we used an innovative method, named the “connectivity map” (Cmap) which is a large public database containing thousands of gene expression profiles that can be coupled to the researcher’s gene profile. This method is currently used to identify therapeutic drugs for a wide range of carcinomas. We developed an innovative model for assessing the gene profile of retinoic acid-treated full thickness skin explants from female breast skin and correlated these 30,000 genes to the Cmap database. To our knowledge this is the first time that such approach has been taken to identify novel cosmetic agents. Using this method we have identified a set of compounds that were shown to exhibit retinoid mimicking activity and are currently under clinical investigation for anti-aging and photo-aging treatment. Moreover, most of them were shown to be well-tolerated when topically applied.

SS01.04

PENETRATION FOR EFFICACY AND TOXICITY OF COSMETICS

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Cosmetic studies focusing on active and/or excipient delivery- aimed at quantifying and maximizing safety and efficacy traditionally employ static *in vitro* diffusion chambers. This presentation enlarges the traditional approach to document fifteen (15) steps in penetration - many of which can be 'modeled'- and provides examples of clinical relevance.

Reference: Ngo M and Maibach H: 15 Steps to Percutaneous Penetration. American Chemical Society, 2012

FO2.01

DEVELOPMENT AND DIAGNOSTIC ACCURACY AND RELIABILITY OF A TELEDERMATOLOGY TOOL FOR OCCUPATIONAL SKIN SURVEILLANCE

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This study aimed to test the validity and diagnostic accuracy of a teledermatology tool using standardised photographic images for the assessment of hand dermatitis in the workplace. Methods: A photographic toolkit was developed to produce standardised photographs of the hands of workers for online assessment for signs of hand dermatitis. The criterion standard was visual inspection by a physician. The tool was tested in four different occupations: health care workers, engineering workers, hairdressers and nursery nurses. The visual inspection was done repetitively over a 7 month period and standardised photographs were taken at the same time. The photographs were assessed via a web-frontend for inter-rater and intra-rater agreement. The assessment used a categorical classification (healthy skin; dry skin; dermatitis). Agreement was calculated using (weighted) kappa and presenting Bangdiwala's Observer Agreement charts. Results: 332 participants were seen repeatedly over a seven month period generating, in total, 1212 reviews. A prevalence of about 70% for minor or major hand dermatitis was found in our study population. Kappa for 'healthy skin' and 'dry skin' against 'hand dermatitis' was > 0.79 (+ve likelihood ratio 7.4) for the intra-rater and a kappa of 0.3 for the inter-rater assessment. Conclusions: The toolkit has been shown to produce reliable and standardised photographs and the assessment of the photographs showed a very good intra-rater agreement to the criterion standard, which is easy to set up and use and would allow conducting skin surveillance with minimal interruption in the workplace and with reliable results from the assessment.

FO2.02

OPTICAL COHERENCE TOMOGRAPHY MONITORING OF NMSC UNDERGOING PHOTODYNAMIC THERAPY

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Objectives: To describe the Optical coherence tomography (OCT) morphology in NMSC lesions during PDT treatment and to assess how OCT morphology before, during and after treatment reflects the treatment outcome at 3 months follow-up. Non-invasive treatment of non-melanoma skin cancer (NMSC) with photodynamic therapy (PDT) requires pre-therapeutic tumor thickness and delineation assessments for optimal efficacy. Methods: A total of 20 patients diagnosed with NMSC at Department of Dermatology, Roskilde Hospital, Denmark, were monitored by OCT during PDT treatment. Images were taken before, at 3 time points during PDT treatment and at 3-months follow-up. BCC diagnosis was based on histopathology. The OCT system (Vivosight, Michelson Diagnostics, UK) is a low intensity, 1310nm laser light, non-contact, non-invasive real-time imaging device. Outcome was assessed clinically as well as by OCT imaging. Results: Both AK and BCC demonstrated disruption of normal skin layering before and during treatment. OCT imaging was able to identify residual lesions that were not clinically visible. At the follow-up visit all lesions that did not demonstrate normalization of layering and/or absence of dark globular structures in OCT images were histopathologically found to be recurrences. Conclusion: OCT can visualize skin structures in AK and BCC lesions *in vivo* to a depth of 2 mm. The OCT system is able to identify residual tumour tissue that is not clinically apparent. OCT imaging of non-melanoma skin cancer may therefore provide diagnostic and prognostic information. Further evaluation of the potential of OCT as a monitoring device during non-surgical NMSC treatment is warranted.

FO2.03

IMAGING TELANGIECTASIA USING OPTICAL COHERENCE TOMOGRAPHY

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Objectives: OCT is an optical imaging technology that provides micrometer resolution *in vivo* in skin. Images are cross-sectional. Telangiectasias are a common finding in e.g. patients with rosacea and often require treatment with laser or Intense Pulsed Light (IPL) Systems. Treatment effect can however only be assessed several weeks after treatment. Currently, High-frequency ultrasound (hUS) is used for describing larger vessels in the skin. This study explores the potential of OCT imaging of telangiectasias and other abnormal vessels in the skin that are too small to

be imaged by hUS. Methods: The OCT system used is a low intensity, 1310nm laser light, non-contact, non-invasive real-time imaging OCT device (Vivosight, Michelson Diagnostics, UK). The system can acquire both 2D and 3D images. Vessels were visualized before and after treatment with IPL. Results: Telangiectasias can be visualized using OCT, which provides data on their diameter and location within the skin (depth). Different treatment induced changes were identified, some of which appeared to have prognostic value regarding the ultimate effect of the treatment at an early stage after treatment. Conclusion: Monitoring and planning of laser and IPL treatments is a promising aspect of the findings in this study. OCT has the potential to measure the diameter and depth of cutaneous vessels. The data provided by OCT imaging may therefore be useful when deciding treatment intensity; and imaging immediately post-treatment may identify appropriate responses to therapy associated with positive long-term effects. The data suggest that further large-scale studies may be fruitful.

FO2.04

IN VIVO IMAGING OF SARCOPTES SCABIEI INFESTATION USING OPTICAL COHERENCE TOMOGRAPHY

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Objectives: To describe the Optical coherence tomography (OCT) changes in skin morphology due to scabies infestation, and explore the potential of OCT imaging to study scabies infestations in vivo. Methods: Five infested patients were OCT-scanned. The patients were collected from the Departments of Dermatology, Augsburg, Germany and Roskilde, Denmark. Mites were OCT-scanned ex vivo and identified by light microscope to confirm the diagnosis. The OCT system used (Vivosight, Michelson Diagnostics, UK) is a low intensity, 1310nm laser light, non-contact, non-invasive real-time imaging device. Results: OCT identified sarcoptes scabiei mites in all patients in vivo. Mites and burrows were visualized, and some detail on burrow content was provided. Conclusion: OCT can visualize sarcoptes scabiei mites in vivo, suggesting that it may be used to study the biology of the mites in vivo and provide early assessment of scabicide therapy. OCT is potentially able to visualize structures in the skin with an 8 µm resolution. This technology could therefore potentially allow rapid, non-invasive, in vivo diagnosis and analysis of infestations.

FO2.05

A HUMAN, IN VIVO, MINIMALLY INVASIVE WOUND MODEL FOR THE EVALUATION OF SKIN REACTIVITY

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Individual variability in skin reactivity and healing capacity after trauma are important clinical issues. The ability of the skin to react to trauma is innate and an integral part of subsequent healing. Naked eye and LDPI data from previous studies had shown that erythema remained for approximately 3 days after provocation (Varol et al. AODR. 2010;302:383-393). Polarisation spectroscopy (TiVi) was performed on wounds followed for 96 hours. The technology allowed rapid collection of data and the ability to perform up to 8 wounds on the skin area gave excellent potential for flexibility of experimental design. Previous study of erythema after microdialysis catheter insertion gave a purely dermal trauma compared to the whole skin trauma of the lancet provocation. Use of microneedle provocation gave a less pronounced trauma depending on the physical characteristics of the actual microneedle array. Use of high resolution TiVi allowed study of lower levels of trauma than the lancet trauma. Reactivity to trauma is, together with responsiveness to irritants and UVB, a non-microbial manifestation of the skin's innate immune system. This reactivity can vary even in health according to phenotype, with more pronounced abnormalities in responsiveness, such as pathergy, in disease states (Varol, et al. AODR 2010;302:155-168).

FO2.06

A STRUCTURED APPROACH TO UNDERSTAND THE MECHANICS OF SKIN

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The mechanical skin properties are not only depending on intrinsic factors like anatomical site, age and skin hydration level, but can also vary between measurement methods and the applied theoretical model. Reported elastic moduli vary from 4 to 5 orders of magnitude within a single layer like the stratum corneum (SC) and the

dermis, as well as for the global human skin response. If true, this would require an enormous adaptability of the tissue structure. Therefore, this study focuses on understanding the mechanical properties of the SC, the epidermis and the dermis and their interactions from a structural approach. The ideal experiment would require an *in vivo* measurement set up from which the behavior of separate layers and their interactions can be derived. Since this cannot be achieved yet, another approach is presented here. The first part consists of a literature review of *in vivo* studies using a numerical-experimental approach to assess the contributions of separate layers and attempts to correlate those with *in vitro* studies. Additionally, *in vitro* shear experiments using a well-controlled experimental set up have been developed for testing the three skin layers both separated and together. The observed deformations are correlated to the tissue structure using imaging and histology techniques. Results demonstrated that the linear strain regime of all layers is less than 1%, the role of the epidermis is declining by increasing strains because of its lack of elastic components and irregular tissue deformations at higher strains are observed within all layers.

FO2.07

DEVELOPMENT OF INTERVENTIONS TO REDUCE PEDIATRIC PRESSURE ULCERS

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Objectives: The aims were to determine the characteristics of pressure ulcers (PUs) in pediatric and neonatal patients, apply multiple skin imaging and biophysical techniques to identify early compromise and examine the efficacy of specific interventions to reduce them. **Methods:** Over two years, 2966 inpatients were examined every two

weeks for PUs. The skin condition of a subset was evaluated using digital color imaging for early detection of erythema, infrared thermography for indications of ischemia-reperfusion, three-dimensional (3d) surface scans for mask fit and hydration for skin surface moisture. **Results:** There were 200 PUs and 62% were due to medical devices. Face masks for non-invasive ventilation accounted for the highest proportion and 46% of patients had diagnoses associated with craniofacial anomalies. Skin compromise (non-blanchable erythema) occurred at the bridge of the nose, cheeks and forehead, and persisted throughout the day even when the mask was not being worn. Hydration was significantly higher at the mask/skin interface versus adjacent control sites. Three-dimensional scans of patient faces and of several commonly used masks demonstrated the impediments of fit and areas of risk for increased pressure and ischemia-reperfusion injury. Silicone foam and hydrogel dressings, designed to reduce mask pressure on the skin, each reduced hydration relative to no intervention. The skin hydration at contact points of a deformable mask of semipermeable cloth was comparable to control skin. **Conclusions:** Improved fit, reduction of contact pressure and maintenance of normal skin hydration are effective strategies for reducing the frequency and severity of mask-related PUs in pediatric patients.

FO2.08

BIOMECHANICAL, STRUCTURAL AND TOPOGRAPHICAL FEATURES OF THE SKIN OF PATIENTS WITH SPINE PATHOLOGY

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Aim: To study the regional details of skin integument structural and biomechanical properties in patients with severe congenital and idiopathic spine deformities. **Patients and Methods:** The noninvasive study of skin structural parameters and biomechanical properties has been performed in 25 patients (8-26 years old). The following zones studied: the hip anteroexterior surface and the femoral middle third anterior surface; the lower thoracic spine. Skin integument echography was used (Logic S-6 ultrasound unit, GE Co.). The characteristic of skin acoustic anisotropy was evaluated by measuring tissue acoustic wave spreading velocity, electrothermometry performance and capillary blood flow determination (BLF-21 laser Doppler flowmeter, USA). **Results:** The echographic patterns of skin stretch values had the irregular level of dermis echogenicity. The localization of skin stigma disemбриogenesis was different. The lower thoracic spine skin thickness was up to 1.25 ± 0.01 mm. The total skin thickness in femoral zone has been authentically lower than that of the hip ($P < 0.05$). Acoustic heterogeneity was characteristic of the femoral skin to a greater extent. The capillary blood flow of the right femur skin was 3.01 ± 0.27 ml*min./100 g, while that of the left segment 3.45 ± 0.24 ml*min./100 g. **Conclusion:** The techniques used have allowed to reveal some regional differences in skin integument structural and mechanic-and-acoustic properties in patients with axial skeletal pathology; the informative potential of diagnostic techniques for skin structural-and-mechanical condition has been demonstrated.

FO2.09

EMPTYING OF THE DICLOFENAC STRATUM CORNEUM RESERVOIR IS INFLUENCED BY THE MODE OF APPLICATION

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Introduction: The information concerning the efficacy and the penetration profiles of substances used in the physiotherapy setting is scarce. The aim of our study was to estimate the emptying of the diclofenac SC reservoir after respectively a passive, occlusive and electrically assisted application of diclofenac (DF). Methods: In total 5 different groups, of healthy volunteers (n= 60, 24 male and 37 female) participated in this study. A 1% DF (Voltaren Emulgel®, Novartis) formulation (12mg) was applied on the volar forearms on randomized defined circular skin areas of 7cm². DF was applied for 20 minutes under 3 different conditions at the same time; passive diffusion under a semi occlusive sponge, an application without occlusion and an iontophoretic application. To estimate the bioavailability of DF in the skin, methyl nicotinate (MN) responses (Minolta Chromameter a* parameter) at the different moments post initial DF application (respectively 1.5, 6, 24, 32, 48, 72, 96, 120 hours) were compared (ANOVA procedure with Bonferroni corrected post-hoc tests). Significant level was set at 5%. Results: At 1.5 hours after the initial DF application a significant decreased response was detected for the occluded and iontophoretically delivery. After 48 hours the iontophoretic DF delivery was no longer significantly reduced (p=0.296). At 72 hours post DF application only the response after the passively delivered DF remained significant (p=0.038). After 96 respectively 120 hours no difference between the MN responses was detected. Conclusion: The formation and emptying of a DF SC reservoir is influenced by the DF application mode.

FO2.10

CUTANEOUS MICRODIALYSIS: INTERPRETING CYTOKINE PRODUCTION IN INNATE SKIN REACTIVITY OF CYTOKINES AFTER CATHETER INSERTION

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Cutaneous microdialysis is used in pathogenetic research, product development and potentially clinically. 3 recent cytokine studies Sjögren et al (Acta Derm Venereol 2009;89:459-465, AAPS Journal 2010;12:741-9 and JAAPS 2012 DOI: 10.1208/s12248-012-9331-z) show that the technique is well tolerated, can be performed in a clinical research situation, that actual insertion trauma is a relevant provocation of the innate immune system, that the chronology of mediator response can be followed up to 30 hours, that “end point biopsy” couples microdialysis to histological findings and that mediator levels are often higher (more accessible) than in peripheral blood. We suggest viewing the response to catheter insertion in regard to cytokines in 3 phases: an initial 2 hours in which demonstration of cytokines indicates constitutive presence; a 6-8 hour period in which reactivity occurs and a subsequent period of about the same length in which cytokines fall as the (normal) tissue recovers. Abnormalities can occur in these phases (all subsets of a “pre-equilibration” period). Detection of cytokines not usually seen in “normal” skin can imply complicity in pathogenesis. In the design of experiments the performance of a particular target molecule needs to be determined in a reference (“normal”) population for comparison with results from normal looking (“uninvolved”) skin as well as lesional skin in the subject population. Chemometry statistical multivariate analysis can be used to compare a cytokine fingerprint in individual test cases with the performance of the reference set. Combination of microdialysis assessment with other bioengineering assessments can be incorporated into the multivariate analysis.

FO2.11

PROTECTIVE EFFECTS OF TAMARIND SEED COAT EXTRACT IN UVA-IRRADIATED HUMAN SKIN FIBROBLASTS

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The present study was focused on the possible role of tamarind seed coat extract in slowing the effects of skin cell aging. The extract of seed coat of tamarind (*Tamarindus indica* L.) showed stronger antioxidant activity (DPPH inhibition, EC₅₀= 12.5 µg/ml) than L-ascobic acid (EC₅₀= 22.9 µg/ml) and tocopherol (EC₅₀= 29.3 µg/ml). The

extract (200µg/ml) inhibited cell death that occurred in the fibroblasts after acute UVA exposure (5-40 J/cm²), 85% and 84% of cells were viable upon pre-treated and post-treated UVA irradiation, respectively. Moreover, the reduction of cell cycle arrest in S and G2/M phase and progression of G0/G1 phase exhibited with the extract treatment. The fibroblasts treated with the extract resulted in prevention of UVA-induced depletion of antioxidant enzymes defense molecules such as glutathione peroxidase and the level of glutathione. Intracellular total glutathione of UVA-induced cells treated with the extract increased to 10-25% compared to the UVA untreated group at 24-72 h cultivation. Also, the extract significantly decreased UVA-induced MMP-1 secretion and prevented the decrease of UVA-modulated dermal collagen synthesis by fibroblasts. Finally, the contractile of fibroblasts embedded with in collagen lattice was studied. Our findings indicated that tamarind seed coat extract reversed the fibroblast deficiencies in reorganization of collagen and may underlie a wrinkle treatment. This is the first report shows that tamarind seed coat improves skin cell aging. Further developments may show their benefits as active ingredient with multifunctional activities.

FO2.12

THE PLATFORM SKINEXPLORERTM FROM FULL-SIZE OVERVIEW TO DYNAMIC SKIN VISUALIZATION IN S3D ENVIRONMENT

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The platform SkinExplorerTM is a relevant way to explore the skin. Numerical geometry and serial histological sections are tools used to reconstruct the skin components into 3D models and to visualize the relationships between these components in stereo three dimensional (S3D) environments. The objective is the 3D interactive visualization for apprehending the real skin world, depicting the complex elements and enlarging on acquisition, 2D-3D treatment and navigation. The combination of immunohistochemistry potentialities and acquisition of images using a confocal microscope has brought information on biomarkers localization corresponding to proteins expression. This combined technique applies favorably to the skin exploration with a high quality of visualization and allows generating massive data up to 40 GB for a final model. Some attributes are developed to increase the 3D perception as color channels overlaying and post-treatment as channel filtering, sphere mapping, ambient occlusion, halo and smoothing effect. For all these parameters, robust algorithms are necessary to manage the huge quantity of data, which requires more conversions to optimize real-time renderings and accelerate the processing steps. Once 3D models are reconstructed, the platform advantages are numerous: fast visualization using “Ray Tracing” method on the GPU for displaying data, easy use of many viewpoints in the scene which provides an instrument to understand skin organization, a variety of renderings, and an intelligent interactivity in terms of managing big volumes of data by the GPU. Finally, the action of biophysical devices is simulated on numerical designed models using deformation vector fields that schematize the skin behavior.

SS02.01

NON-INVASIVE IN VIVO ASSESSMENT OF THE ANTI-OXIDATIVE STATUS OF HUMAN SKIN

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Oxidative stress is among the major causes of premature skin ageing and cutaneous diseases including cancer. To combat the adverse effect of reactive oxygen species (ROS), the human skin has developed an antioxidant defence system consisting of vitamins, carotenoids, enzymes, and other substances. Being known as effective quenchers of ROS, a reduction in the cutaneous carotenoid concentration could be directly associated with the amount of ROS generated. The carotenoid concentration in human skin should be monitored by non-invasive methods. Resonance Raman spectroscopy and reflection spectroscopy are well suited to analyze the carotenoid concentration in human skin in vivo. Using these two non-invasive methods, the kinetics of carotenoids in human skin was determined. The results showed the relatively fast degradation of cutaneous carotenoids after exposure to stressors, e.g., irradiation, inflammatory diseases, smoking, sleeplessness. Recovery turned out to be a prolonged process and depended on an antioxidant-rich nutrition. Topical application of antioxidant-rich formulation gives rise to an immediate increase in the carotenoid concentration in the stratum corneum. The protection strategy against the action of ROS could rely on the systemic and topical application of antioxidants. Topically applied carotenoids are stored in the skin only short-term, as desquamation, textile contact, washing and environmental stress causes rapid depletion. Other than

topically applied carotenoids, those systemically applied are stored in the fatty tissue and slowly released onto the skin surface with sweat and sebum. Combined topical and systemic application of antioxidants is optimally suited to protect the antioxidant network in human skin.

SS02.02

MATHEMATICAL MODELLING - NEW MEANINGS FOR ESTABLISHED VARIABLES

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Complex dynamical variables, even if expressed in absolute values, are often difficult to understand. This is the case for well known transcutaneous (tc) variables that, in our opinion are far from being adequately explored, regarding human physiology and pathophysiology. Here we gather different mathematical strategies designed for that purpose. Results were obtained from different groups of human volunteers, according with specific inclusion criteria, respecting the Declaration of Helsinki and respective amendments. Compartmental models were developed from pharmacokinetic concepts, and applied to tcpO₂ and LDF (laser doppler flowmetry) signals obtained *in vivo* after hemodynamical maneuvers (Group I n= 54). A oxygen elimination rate constant was obtained as the quantitative descriptor. Another model, adjusted to TEWL desorption curves (Group II n= 33), provided water dynamical descriptors such as Evaporation half-life times and Dynamic Water Mass (DWM) . LDF measurements from perfusion restriction experiments (Group III n=18) were treated with a combined approach of Wavelet (WA) and Detrended Fluctuation Analysis (DFA) . Compartmental models allowed to describe the flow related tcpO₂ changes in Group I, where the Oxygen elimination rate was used to characterize the microcirculatory function of all individuals. In group II, the kinetical parameters, allowed to detect significant “barrier” differences never revealed before by simple TEWL measurements. Finally WA-DTA analysis proved to be useful to separate the different physiological components of LDF signals – cardio-respiratory, myogenic, sympathetic and metabolic . Thus, mathematical modeling is a rigorous and sensitive easy-to-use instrument that may be developed to look further into multiple cutaneous functions.

SS02.03

CLINICAL SCALES, SCORES AND INSTRUMENTS: ASSESSMENT OF DISEASE SEVERITY IN DERMATOLOGY WITH ADVANCED OPTICAL METHODS

Joachim Fluhr

Abstract not available at the time of printing.

SS02.04

NEW INDICATIONS OF OCT AND CONFOCAL MICROSCOPY: MORPHOLOGY AND FUNCTIONAL ASPECTS

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Optical coherence tomography (OCT) and confocal laser microscopy (CLSM) are noninvasive imaging techniques. Using OCT, thicknesses of layers as well as architectural details within the epidermis and dermis can be determined and evaluated, whereas CLSM provides a cytological diagnosis of epidermal changes. OCT is used clinically to diagnose actinic keratoses and basal cell carcinomas, to determine the tumor thickness and the lateral dimensions of the lesion and to control treatment efficacy after photodynamic therapy or imiquimod. Inflammatory diseases, wound healing and treatment effects can be monitored and quantified over time using OCT. Melanocytic lesions can be assessed using CLSM, which allows a microscopic diagnosis of nevus nests or pagetoid spread of atypical single melanocytes in malignant melanoma. Dynamic changes like blood flow or wound healing can be monitored in real time. Using the fluorescence mode, different fluorescent dyes can be applied topically or intralesionally for investigation of the penetration of substances as well as of the skin barrier function.

SS02.05

MUCOSA: PHYSIOLOGY AND PENETRATION PATHWAYS

Christian Surber

Abstract not available at the time of printing.

SS02.06

EFFECTS OF TISSUE-TOLERABLE PLASMA ON CHRONIC WOUND TREATMENT COMPARED TO A MODERN CONVENTIONAL LIQUID ANTISEPTIC

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Recently, it was reported that tissue-tolerable plasma (TTP) is highly efficient in the reduction of the bacterial load of the skin surface. However, these studies were mostly performed using either cell culture assays or animal skin *in vitro*. We aimed to compare the antiseptic efficacy of tissue-tolerable plasma and an octenidine dihydrochloride-based wound antiseptic on chronic wounds. Sixteen patients suffering from chronic leg ulcers were treated with either tissue-tolerable plasma (TTP) or octenidine dihydrochloride 3 times a week over a time period of 2 weeks. The rate of wound healing during the study period was monitored. Moreover, the bacterial colonization of the wound surface was investigated by determination of the density of colony forming units in the bacterial culture. Wounds treated with either TTP or octenidine dihydrochloride showed comparable healing rates underlining its low cytotoxicity as suggested by previous studies. The reduction of microbes was slightly lower within the TTP-treated wounds compared to the octenidine dihydrochloride-treated wounds. However, technical challenges with an early prototype TTP device could be held responsible for the slightly reduced antiseptic properties of the TTP compared to a standard antiseptic solution since the manual treatment of the skin surface with a small beam of the TTP device might have led to an incomplete coverage of the treated area. Here we report, that TTP is an innovative and new antiseptic approach for the treatment of chronic leg ulcers with an antiseptic efficiency compared to one of the most efficient and biocompatible liquid antiseptic.

SS02.07

ELASTICITY AND VIABILITY OF CELLS TREATED WITH SURFACTANTS AND PROTEINS

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Objectives: Studies of the elastic properties of cells are important for the understanding of many physiologic processes. We would like to examine whether the stiffness of epidermal keratinocytes measured by atomic force microscope (AFM) can be correlated with viability test. Methods: For the studies presented here we used the spontaneously immortalized HaCaT cell line ensuring high intra- and interlaboratory reproducibility. MTT assay was applied to determine the survival fraction of keratinocytes treated with sodium lauryl sulphate (SLS) and hydrolyzed collagen (HK) solutions of various molar ratios. The AFM measurements of the keratinocytes stiffness and the visualization of actin filaments were carried out immediately after the exposure of cells to the SLS and HK respectively. Results: The concentration-dependent cytotoxic effect of SLS was determined for HaCaT cells. This effect can be inhibited by addition of hydrolyzed collagen. AFM study showed decrease in the cell stiffness for cells treated with SLS. Fluorescence microscopy revealed that the interior of SLS-treated cells undergoes remodeling of actin filaments. On the other hand SLS:HK mixture treatment resulted in mechanical stiffness close to untreated cells. Conclusion: These results provide possible correlations between mechanical properties and viability of keratinocytes. This approach enables us to track chemically-induced changes in cell elastic properties under physiological conditions.

SS02.08

EFFECTS OF POLYOLS ON HUMAN KERATINOCYTES: BEYOND THE PHYSICAL-CHEMICAL PROPERTIES

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Polyols (e.g. glycerol), due to their hygroscopic nature, are implicated as key molecules in providing the proper water-holding capacity of the epidermis and thereby contributing to the formation of the epidermal physical-chemical barrier. In the current study, we assessed the effects of Glycerol and Xylitol on the gene expression profile of cultured human epidermal keratinocytes. The polyols (Xylitol: 0.045 – 0.45%; Glycerol: 0.027 – 0.27%) exerted differential effects on the expression of certain molecules involved in various epidermal processes. Xylitol markedly upregulated the expression of filaggrin, a key molecule of the epidermal barrier. In addition, it effectively prevented the actions of bacterial lipopolysaccharide (LPS, activator of toll-like receptor TLR4 to mimic Gram negative bacterial infections) to induce synthesis of the pro-inflammatory interleukins IL1 β and IL6. However, Xylitol did not affect the pro-inflammatory actions of lipoteichoic acid (LTA, TLR2 activator, Gram positive bacterial infections) and polyinosinic:polycytidylic acid (poly-IC, TLR3 activator, viral infections). Interestingly, Glycerol did not affect filaggrin expression; yet, it exerted profound anti-inflammatory actions; it completely blocked the effects of TLR2 and TLR3 (but not TLR4) activation to upregulate the expression of HLA-DR and the poly-IC -induced upregulation of IL1 β . Importantly, polyols did not alter cellular viability, cell volume, or intracellular calcium concentration. These intriguing data suggest that combined applications of Glycerol and Xylitol exert complex and robust anti-inflammatory and barrier-regenerating actions which are most probably independent of their physical-chemical properties. Therefore, polyol-containing formulations may hold therapeutic advantages in the management of inflammatory conditions with impaired and/or damaged epithelial barrier.

THURSDAY, 29 NOVEMBER 2012

KL01

STRATUM CORNEUM BIOMECHANICS: FROM PROPERTIES TO FUNCTIONS

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SC has to be easily stretched and compressed to allow humans to move, eat, sleep, express their feelings etc...certainly the most important mechanical function of SC. It must also be cohesive enough to resist to friction or other mechanical trauma. Protective function against environment is also essential. SC must also permit humans to sense their surroundings for efficiently grasping objects or to feel and perceive subtle tactile contacts. SC can fulfill these functions thanks to its peculiar viscoelastic properties, which are now globally well described. These properties come from both the composite structure of this membrane, much more complex than the "brick-wall" model may suggest and the nature of its inter-related components and with the water molecules. SC is not a flat membrane independent of the other layers which both support it: the living epidermis, dermis and hypodermis. Skin micro relief constitutes in fact a "deformation reservoir" which facilitates SC deformations when skin is stretched. New findings come to illustrate this SC behavior. Several papers have already addressed these different points without answering however numerous questions about the role of water molecules, the influence of the SC components on its viscosity and extensibility, for example. Another question relates with the influence of SC itself upon the entire skin mechanical properties. A computer model based on the minimization of the elastic energy of a 3 layer model which describes the influence of the SC rigidity on the skin mechanical properties has recently been published (1). Another experimental approach of SC mechanical properties comes from the use of micro and nano-methods. The different possibilities offered by Atomic Force Microscopy (hardness, elasticity, friction), for example, allow the description of the very local SC properties. It might permit to explore more deeply the relationship between the fine structure of SC and its global properties. As said above, measurement of the SC friction coefficient is also important. New methods were proposed in this field. As a practical example of these new approaches, the important and rather vague cosmetic concept of skin smoothness may now be objectively quantified.

(1) Lévêque JL and Audoly B, *Skin Res J*, 2012 (under press)

SY01.01

AN EPIDERMAL TURNOVER MODEL PREDICTS UNEVEN SKIN TONE WITH AGING

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Background and Objectives: The appearance of the skin is known to become uneven with aging, mostly because of the non-uniform distribution of melanin. At the tissue level, skin color is determined by a balance of melanin synthesis, the transfer of melanin granules to keratinocytes, melanin degradation and epidermal turnover. In this study, to elucidate the effect of epidermal turnover on uneven skin tone, we developed a computational epidermal turnover model using the particle method. **Methods:** The model was initially designed by setting proliferative keratinocytes and non-proliferating melanocytes in a basal layer. In the model, keratinocytes undergo mitosis at a designated frequency, and the daughter cells are pushed and moved toward the skin surface by an inter particle force. A single melanocyte is inserted among every three keratinocytes to define the pigmented region and among every five keratinocytes as the normal region. Each melanocyte produces melanin and transfers it to neighboring keratinocytes at a steady rate. Transferred melanin within keratinocytes is degraded at a constant speed. The intensity of skin color was then calculated as the sum of melanin within keratinocytes in a skin area of 10 x 10 mm². **Results and Conclusion:** As keratinocyte mitosis speed slowed, the value of L* decreased and the coefficient of variation of L* in the total simulated area increased, indicating that skin color becomes darker and the contrast of the skin color is enhanced. This result suggests that increasing epidermal turnover time is one cause of aged skin features such as an uneven tone.

SY01.02

CHARACTERIZATION OF DRY/SCALY SKIN AND QUANTIFICATION OF SCALINESS

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Objective: The objective was to present a new method of visualizing and quantifying scaliness using a videomicroscope equipped with a narrow-angle illumination system. Background: A healthy skin is well hydrated, soft and smooth in contrast with dry/scaly skin in which the surface is covered with scattered clumps of corneocytes (scales) of varying sizes and patterns. Methods: The scales are brighter than the rest of the skin and can be considered as “highlights” in terms of Image Processing. We developed a new *in vivo* method using a side-lighted videomicroscope. The colored Image obtained, showed enhanced appearance of scales. By using an image processing method of tonal adjustment, the mid-tones were suppressed, resulting in a dramatically highlighted image of scales on the skin surface. An Image Analysis program was developed to calculate a Scaliness Index (SI) based on the area covered by the scales and the mean luminance of the highlighted areas. Results: Marked reductions in Scaliness Indices were observed after treatment of dry/scaly skin with a moisturizer product. Inclusion of an emollient in an alcohol-based hand disinfectant, showed significant reduction in scaliness. Conclusion: This method can be used for studying the disruptions of stratum corneum which result in dry/scaly skin. These disruptions can originate in a variety of ways e.g. physical and chemical trauma, excessive use of soaps as well as a number of dermatoses. The method is fast and easy to use and can detect subtle changes in scaliness.

SY01.03

IN VITRO EVALUATION OF ISOTRETINOIN LOADED MICROEMULSIONS WITH ATR-FTIR SPECTROSCOPY AND TAPE STRIPPING

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Isotretinoin, a derivative of retinoic acid (13-cis-Retinoic acid) has multiple biological effects in skin and it is used commonly for the treatment of severe acne and other dermatological diseases. It has obvious adverse side effects by oral administration and the conventional topical preparations show significant skin irritation. Therefore it is necessary to improve skin accumulation of isotretinoin and reduce its adverse side effects by using a novel topical formulation. A novel approach to deliver high contents of isotretinoin and to increase its partition onto skin could be the application of a colloidal carrier system such as microemulsions. Microemulsions are thermodynamically stable colloidal systems composed of oil, water, surfactant and co-surfactant, and their globule size is between 5-100 nm. In our study, isotretinoin loaded o/w type microemulsions was developed. An *in vitro* study combining ATR-FTIR spectroscopy and tape stripping was performed to investigate the biophysical changes in skin barrier function at the molecular level and to determine isotretinoin in upper and lower stratum corneum (SC) layers qualitatively and quantitatively. Our results showed that microemulsion systems enhanced the delivery of isotretinoin into the lower SC. The C-H asymmetric and symmetric stretching vibrations shift to significantly higher frequency values in upper and lower SC compared to the untreated control skin ($P < 0.05$). The interaction of microemulsion components with SC intercellular lipid domain played a significant role in the enhancement effect.

SY01.04

NEW BARRIER FUNCTION ESTIMATION BY FLUORESCENCE CONFOCAL MICROSCOPY

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Background: *In vivo* Confocal Laser Scanning Microscopes, like Mavig Tri Laser Vivascope, permit stratum corneum (SC) surface imaging thanks to fluorescence. Fluorescent dyes like fluorescein highly improve signal around corneocytes. Despite that great quality, confocal distinctiveness implies low depth of field images and truncated information about the SC. Purpose: Confronted with the lack of existing solution, we developed a novel image processing algorithm which, from multiple depths images, automatically computes a solely image containing the whole SC information. Above all, the algorithm takes into account volunteer shifts, which usually weaken interpretation. Methods: 42 healthy volunteers applied during 7 days twice daily a solution of 10% SLS miming altered barrier function, confirmed with TEWL measures. Confocal acquisitions were realized with 488nm laser, after applying fluorescein solution. 7 mosaics of 16 horizontal cross-sections were acquired between depth 0 μ m (epidermal surface) and 18 μ m. For each stack of the mosaic, layers were automatically (Qt, OpenCV Library) compared one by one to evaluate the translation that occurred between them. Finally, for each pixel, maximum intensity among all layers was retained corresponding to focal point information despite skin topological variations.

A 4 expert's trained panel scored the reconstructed images according to cellular organisation (shape, arrangement). Results: Resulting images are of impressive quality compared to original ones. Expert's notation and TEWL measures present good correlation (68%, $p < 0.0001$). Conclusion: Quality of native confocal observations has been automatically improved permitting a more complete analysis of the SC. Algorithm is useful for qualitative and quantitative SC evaluation thanks to TEWL validations.

SY01.05

IMAGING TOPICAL NAIL DELIVERY USING STIMULATED RAMAN SCATTERING (SRS) MICROSCOPY

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Efficient drug delivery into the nail is important for the effective treatment of diseases such as onychomycosis and nail psoriasis. However, due to the architecture and composition of the nail-plate, drug uptake following topical administration is often too low to be effective. Formulation optimization is on-going to improve drug penetration through the nail to the infected site. At present, to quantify the amount of drug inside the nail after application of a formulation, the extraction method used typically involves destruction of the treated sample, and does not provide any information on the real-time disposition of the formulation components nor the depth of their penetration into the nail. In this project, Stimulated Raman Scattering (SRS) microscopy is being used to image drug disposition and pharmacokinetics in the nail following application with excipients typically used in commercialised formulations. SRS microscopy is a recently-developed, label-free imaging technique that allows the acquisition of high resolution three-dimensional images of multiple chemical components of a formulation, as they penetrate the nail, without destructive analysis (Saar, B.G., Contreras-Rojas, L.R., Xie, X.S., Guy, R.H., *Imaging Drug Delivery to Skin with Stimulated Raman Scattering Microscopy*. 2011. *Molecular Pharmaceutics*, 8, p.969-975). It uniquely provides information on the precise penetration depth, as well as mechanistic insight into the penetration pathways. It may also provide quantitative information to facilitate the design and optimization of formulations and delivery vehicles for topical nail delivery.

KL02

DEVELOPMENT OF INSTRUMENTATION FOR ASSESSMENT OF SKIN

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Over the last 4 decades many new methods and instruments for assessment of the skin have been developed and brought to the market. In an attempt to categorize, one can identify these developments as one-point methods, 2D imaging methods (top view or cross-sectional) and, more recently, full 3D imaging methods. Some methods further allow for the study of dynamic changes in skin parameters with the use of both time plot graphs and real time or compressed time scale video-clips. Most methods are non-invasive in nature and incorporate both contact and non-contact measurement technologies. This lecture explores the various steps in the development of skin testing methods and instruments and discusses in further detail common aspects such as 1) the origins of innovations, 2) theoretical considerations, 3) prototype development, 4) bench testing, 5) in-vivo evaluation and 6) paths for commercialization of new devices.

SY02.01

MULTIPHOTON/CARS MICROSCOPY FOR SKIN IMAGING

Karsten König

Abstract not available at the time of printing.

SY02.02

MEASURING SKIN CELL STIFFNESS

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Changes in mechanical properties are an essential characteristic of the aging process of human skin. Previous studies attribute these changes predominately to extracellular aspects as alterations in polymerization and crosslinking of collagen and elastin. In this context, age-related changes in protein synthesis and production of

extracellular matrix components are well known but less is known about the mechanical characteristics of individual cells. To determine the viscoelastic properties of single cells the optical stretcher technique was applied to examine the deformability of fibroblasts. The microfluidic optical stretcher is an optical trap that allows trapping and controlled deformation of suspended cells by using two counterpropagating laser beams. The global stress applied to the cell surface permits the measurement of whole cell elasticity that characterizes the integral effects of molecular changes on the cytoskeleton. The use of optical deformability as a sensitive cell marker has already been demonstrated for characterization of individual cancer cell lines as well as for cancer diagnosis by mechanical phenotyping of oral keratinocytes by Guck et al (2005, *Biophys. J.* 88(5):3689–3698). We have analyzed, if optical deformability can also serve as a sensitive biomarker of aging on the level of individual dermal cells. We found that individual dermal fibroblasts exhibit a significant increase in stiffness during aging.

SY02.03

ELECTRON SPIN RESONANCE SPECTROSCOPY TO EVALUATE BIOPHYSICAL PARAMETERS OF THE SKIN

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Electron paramagnetic resonance (EPR) spectroscopy is able to measure paramagnetic substances such as radicals in the skin and can help us learn more about the following biophysical parameters of the skin:

1. It can provide data about the antioxidative capacity of the barrier.
2. The extent of induced oxidative stress can be measured by measuring the radicals formed in the skin.
3. Stable nitroxides can be used as spin probes or labels to investigate the penetration behaviour of substances or carriers as well as drug release.

Investigations have shown that the radical scavenging capacity increased after oral intake of natural extracts and radical production after irradiation in the visible and infrared range could be reduced after topical application of creams rich in antioxidants as well as after supplementation with natural extracts. The nitroxide PCA is well suited for the detection of irradiation induced ROS production. However, due to its hydrophilic properties skin penetration is poor but could be enhanced when applied within carriers such as liposomes and core multi shell nanotransporters. The nitroxide TEMPO reacts well with the cutaneous antioxidant system and can therefore be used to investigate the stabilizing effect of nanocarriers. It could be shown that the lipophilic nitroxide TEMPO is mostly located within the lipid matrix of nanostructured lipid carriers or liposomes, which protects TEMPO from being reduced by cutaneous antioxidants after application to the skin.

KL03

OPTICAL METHODS FOR ASSESSMENT OF SKIN

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Optical methods now provide 3D structural and functional imaging *in vivo* at clinically relevant speeds and depths with or without labels. Indeed, photonics promises to bring healthcare to the next level, as it is the only *in vivo* means to image layers, sweat ducts, capillaries and to map cells and molecules in small, accessible, low cost and safe imaging systems. Recently high resolution label-free imaging of the skin at clinically relevant depths and has begun to move from research labs to commercial reality. There continues to be great innovation in this area. For example, the number of papers on the microcirculation published in physical sciences doubled between 2006 and 2008 representing a jump from the long stable 5% of all microcirculation papers to 15% and has maintained this level to date. This paper will present the current status of methods for structural and functional imaging on the skin and some results from our own work.

SY03.01

FEVER SCREENING BY THERMAL IMAGING OF THE FACE

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Pandemic Influenza virus infections have caused increasing concern, especially in air travel. The International Standards Organisation has produced two documents (2008 & 2009) to define the optimal instrumentation needed for infrared thermal imaging and its implementation for screening for fever. This study in Warsaw on a group of 402 children using an infrared imaging system (FLIR SC640). There were 192 male and 210 females age range from 1yr to 16.5 yrs. All were seated and examined using clinical thermometry (axilla), tympanic membrane

radiometry, and infrared thermogram of the frontal face at close up, to measure forehead mean temperature and inner canthi (i.c.) of both eyes. Results: 350 Children(85%) were free from fever and 52(15%) had definite fever. Temperatures from the non-febrile group: Eyes i.c. mean. C 36.48 (SD 0.49) Forehead 36.44 (SD 0,65) Axilla 36.34 (SD 0.59) Ear 36.12 (SD 0.71). Fever group: Eyes i.c. mean C 38.9 (SD 0.84), Forehead 34.7 (SD 0.86), Axilla 38.9 (SD 0.68), Ear 37.4(SD 1.41). The highest correlations were found between the Axilla thermometry and Eye i.c. In airport screening , many installations of infrared cameras are sited to survey moving crowds. The ISO standard requires 9x9pixels to correctly obtain the temperature of the i.c. i.e a stationary subject imaged at close range. Conclusion. Fever can be detected by thermal imaging of the eye regionwith ISO criteria. More studies, especially in adults are needed to increase the validity of these data.

SY03.02

QUANTIFICATION OF BLOOD CONTENT IN SKIN DURING THE CAPILLARY REFILL TEST HIGHLIGHTS GENDER DIFFERENCES IN SKIN MICROCIRCULATION REACTIVITY

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Observation of the return of blood flow to an area of pressure blanched skin is termed the capillary refill test (CRT). Video mode polarisation spectroscopy also known as tissue viability imaging (TiVi) allows the detailed, objective, quantitative study of the chronology of the return of blood content to an area after CRT. The response includes a return to baseline level, a subsequent hyperaemia and then a slower return back to baseline values. Study of the CRT at five sites (finger pulp, dorsal finger, volar forearm, sternum and forehead) in 24 healthy individuals (10 males and 14 females) showed gender variability, with a greater difference between men and woman at the finger pulp, forehead and volar arm. Protocol standardisation is required in performance of the CRT. The detailed data made available by the TiVi technology opens possibilities for vastly improved clinical and research applicability of the CRT. This technique can in the future earlier add information affecting treatment by non-invasive quantitative measurements and monitoring of patients at high risk of going into circulatory collapse.

SY03.03

IN VIVO MULTIPHOTON MICROSCOPY ASSOCIATED WITH 3D IMAGE PROCESSING: NON INVASIVE, QUANTITATIVE CHARACTERIZATION OF HUMAN SKIN.

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(3) Service De Dermatologie -Hôpital St. Louis, Paris France

In the past decade, multiphoton microscopy has emerged as a promising non-invasive skin imaging technique. It allows skin characterization with sub-cellular resolution by taking advantage of intrinsic multiphoton signals from cells, elastic and collagen fibers . However few image processing methods have been developed to extract quantitative parameters. 3D image processing algorithms adapted to multiphoton images of skin were developed to assess whether multiphoton microscopy coupled with specific image processing tools could provide new insights into the organization of different skin components and age related changes or modifications induced by topical treatment .Accordingly, a clinical trial in 15 young (18-25) and 15 aged (70-75) human female volunteers was designed focusing on the ventral and dorsal sides of forearm. Results from quantitative evaluation of skin aging show that *in vivo* multiphoton microscopy enables several skin aging alterations to be evidenced: morphological changes in both epidermis and dermal-epidermal junction, modifications in the amount of melanin and in the amount and organization of the collagen and elastic fiber networks. The same process applied for studying skin alterations induced by topical corticosteroids showed its ability in monitoring the effects of cutaneous treatments. In conclusion, multiphoton microscopy coupled with specific 3D image processing software appears to be a powerful tool for both dermatological and cosmetic investigations.

SY03.04

FLUORESCENCE LIFETIME IMAGING AND MULTIPHOTON TOMOGRAPHY FOR THE STUDY OF SKIN TUMORS

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Multiphoton Laser Tomography (MPT) is a recent non-invasive imaging technique, which gives access to the cellular and extracellular morphology of the skin, based on the combination of autofluorescence and second harmonic generation signals coming from the intrinsic tissue fluorophores when they are excited by a near-infrared laser source. Fluorescence lifetime imaging (FLIM) is an additional technique, implemented to the MPT, enabling the characterization of endogenous molecules and their surrounding medium by measuring the decay rate of fluorescence emission. MPT/FLIM has been introduced in the study of skin tumors with the aim of increasing diagnostic accuracy with respect to traditional imaging techniques. In preliminary studies MPT-FLIM descriptors for basal cell carcinoma (BCC), nevi and melanoma (MM) were identified in training sets comprising these skin tumors and healthy skin. Subsequently, sensitivity and specificity of these descriptors were assessed on test sets. For BCC, 3 epidermal descriptors, i.e. detached cells with enlargement of intercellular spaces, cells with irregular contours and random arrangement, and 6 BCC descriptors, namely, aligned elongated cells, double alignment of monomorphous cells, palisading cell islands surrounded by fibers, sheets of cells intermingled with fibers, phantom islands and blue cells were identified. MM was characterized by 30 descriptors referring to atypical short-lifetime cells present in upper MM and lower MM layers and lesion architecture. Sensitivity and specificity of these descriptors enabled the diagnosis of both BCC and MM with a diagnostic accuracy higher than that of dermoscopy. MPT/FLIM also provides quantitative data characterizing and differentiating BCC cells from MM cells.

KL04

APPROACHED TO TODAY AND FUTURES NEEDS IN WORLD OF CLINICAL CHANGE

Howard I Maibach

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Bioengineering techniques in dermatologic research and clinical practice - initialed with transepidermal water loss measurements by Malten and Thiele a half century ago- have rapidly developed in the research domain but have not been widely accepted as a clinical tool. This presentation focuses on recent innovative research related insights made possible practical by these techniques and will end with what's mandated to morph into standard clinical practice.

SY04.01

FOUNDATION OF INSTRUMENTAL MEASUREMENTS IN FORMULATION DEVELOPMENT & CLAIMS SUBSTANTIATION OF SKIN CARE PRODUCTS

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Measurements of various biological and physical facets of skin can now be achieved using relatively affordable and easy to operate instrumentation. The application of biophysical measurements ranges from basic research to technology/methods development to optimization of treatments or products, often leading to product performance claims. The focus of this presentation is not detailed methodology, but rather the process to design a readily defensible protocol leading to reproducible, scientifically valid instrumental data to qualify methods/formulations or to support claims believed to be key to the commercial success of a personal care product. A distinction between research and substantiation will be developed relative to instrumental measures. In each case, it is imperative to understand the specific objective of the testing. While basic research/formulation development seeks to discover if something is true, claims support testing is intended to demonstrate to a relevant authority that a specific claim is true and adequately supported. A foundation will be provided for the appropriate use of biophysical instrumentation across the range of testing emphasizing fundamental building blocks such as instrument knowledge, validation, and study design. For claims substantiation, details of product application, measurement, timing and control must be based on the advertised claims, consumer use instructions, and practical considerations of the measurement method. Failure to properly design the substantiation study can lead to rejection of a study and loss of a desirable, compelling claim. This is particularly critical since external reviewing authorities range in their expectations and in how deeply the study details will be scrutinized.

SY04.02

TOPICAL 5-ALPHA REDUCTASE INHIBITORS MAY EFFECTIVELY REDUCE SKIN SURFACE SEBUM PRODUCTION

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Many individuals are distressed about having excessively oily skin and seek topical remedies for this condition. Skin having a high water content and low sebum secretion is considered to be highly desirable (moisturized and hydrated but without visible sheen). Sebum lipids are primarily a product of follicular sebocytes, and synthesis is believed to be positively modulated by androgens. The transformation of precursors to androgens such as testosterone is dependent on the enzyme 5-alpha reductase. Inhibition of 5-alpha reductase is believed to be a useful target in androgen-dependent disorders such as acne and alopecia. We therefore investigated the use of several botanical extracts to determine, first, if these extracts could inhibit 5-alpha reductase in vitro, and second, if inhibition of 5-alpha reductase would correlate with inhibition of sebum production in human subjects. A clinical study was conducted to determine whether topical treatment formulations containing the effect of various botanical 5-alpha reductase inhibitors could reduce skin surface sebum after 4 weeks of use. Results show the variability in the activity of the different test materials.

SY04.03

FRICITION AND ADHESION OF PRODUCTS AND MATERIALS ON HUMAN SKIN: PRODUCT DEVELOPMENT RESEARCH IN KIMBERLY-CLARK

*Martha Tate, A Wright, R Zepp, K Zwick
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Friction and adhesion are important variables in the development of personal products. Most of the products in our portfolio touch the skin of humans. Their soft feel and ability to stay put or release when needed, are important to the user as well as to the function of the products. Tensile testers have been adapted for friction and adhesion. Products evaluated for friction against skin include diapers, medical face masks, wet wipes, and facial and bath tissue. Adhesion of various marketed medical tapes will be reviewed, along with the effect of temperature on patient warming pads used in health care surgical applications. The softness of a product is perceived based on the contact and friction between the product and the skin. Products are perceived as softer when there is less variation in the friction force to tug and stimulate the skin. Products with high stick-slip friction are perceived as rough and abrasive. Stick-slip can be reduced by treating the skin or the product with a lubricating layer. The overall level of friction force as well as the variation in friction force should be kept low to avoid abrading the skin and dislodging corneocytes. Adhesive tape must adhere for as long as needed. Medical tapes, such as adhesive pads, may be placed over first aid lotions and ointments, which can alter the adhesion of the tape. Patient warming pads are used to maintain core temperature during lengthy surgeries, and they are intended to relay heat to the body through the skin.

FRIDAY, 30 NOVEMBER 2012

SL01

OCT - TECHNIQUE AND INDICATIONS IN DERMATOLOGY

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Optical coherence tomography (OCT) is a noninvasive imaging technique of superficial skin layers *in vivo* and real time. Thicknesses of layers as well as architectural details within the epidermis and dermis can be determined and evaluated. In the time domain technique, a scanning reference mirror is used to detect the reflectivity profile along depth. In the frequency or spectral domain technique, the entire spectrum is analyzed simultaneously with respect to a static reference mirror, leading to an increased sensitivity and a higher image acquisition speed. OCT is used clinically to diagnose actinic keratoses and basal cell carcinomas, to determine the tumor thickness and the lateral dimensions of the lesion and to control treatment efficacy after photodynamic therapy or imiquimod. A visualization of infectious diseases like scabies, changes of the nails and blistering skin diseases is possible. Inflammatory diseases, wound healing and treatment effects like steroid atrophy can be monitored and quantified over time using OCT.

SE01.01

OPTICAL COHERENCE TOMOGRAPHY IMAGING OF SKIN TUMOURS AND INFLAMMATORY SKIN DISEASES

Mette Mogensen

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Optical coherence tomography (OCT) is a non-invasive optical imaging technology that provides cross-sectional or 3D images of skin with a penetration depth of approximately 2 mm. OCT works in analogy to ultrasound; the reflection of infrared light (instead of acoustical waves) from the skin is measured, and the signal strength is mapped as a function of position. The OCT probe is applied directly to the skin. In the last decade OCT has been widely investigated for imaging of non-melanoma skin cancer (NMSC). This overview will present some of the most recent advances in OCT imaging of skin tumours. A break-up of the characteristic layering of normal skin is found in both OCT images of NMSC and malignant melanoma lesions. However, this disruption of layering is also seen in various benign lesions as seborrheic keratosis and benign melanocytic nevi but certain clues in OCT images can suggest malignancy. Furthermore, OCT images of inflammatory skin diseases - granulomatous, bullous and sclerodermic are described and interesting preliminary results from on-going OCT studies will be presented. OCT imaging can potentially improve diagnostic accuracy in both skin tumours and inflammatory skin diseases and serve as a tool for treatment planning in patients with NMSC.

SE01.02

HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY IN THE DIAGNOSIS OF NON-MELANOMA SKIN CANCER

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Background: Optical coherence tomography (OCT) allows real-time, *in vivo* examination of non-melanoma skin cancer. An innovative high-definition (HD)-OCT with a horizontal (en-face) and vertical (slice) imaging mode offers additional information in the diagnosis of non-melanoma skin cancer such as actinic keratosis (AK) and basal cell carcinoma (BCC). Objectives/Methods: To define the characteristic morphologic features of AK (20) and BCC (22) by using HD-OCT in the two imaging modes compared to histopathology as gold standard. Furthermore, to compare the diagnosis of ex vivo BCC (20) in HD-OCT with the findings of frozen-section histology in micrographic surgery. Results: The preoperative in-vivo diagnostics of AK showed the following features in the en-face imaging mode of HD-OCT: Disruption of stratum corneum, architectural disarray, cellular/nuclear polymorphism in the stratum granulosum/spinosum, and bright irregular bundles in the superficial dermis. In the HD-OCT imaging of BCC in the en-face mode the following features were described: lobulated nodules, peripheral rimming, increased vascularity, epidermal disarray and variably refractile stroma. A total of 80 HD-OCT images of 20 BCCs were evaluated and in 45% HD-OCT correlated exactly in all 4 section margins with the histology. Conclusion: HD-OCT with both the en-face and slice imaging mode offers additional information in the diagnosis of AK and BCC compared to conventional OCT and might enhance the possibility of the non-invasive diagnosis of non-melanoma skin cancer. Additionally, HD-OCT allows the postoperative identification of BCC ex vivo, but has still limitations in the recognition of tumor margins in comparison with the micrographic evaluation of frozen-sections.

SE01.03

HISTOLOGICAL CORRELATES OF OPTICAL COHERENCE TOMOGRAPHY IN NON-MELANOMA SKIN CANCER

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Non-melanoma skin cancer (NMSC) is now the most common malignancy occurring in white populations, accounting for 70% of the cost of managing skin cancer. Optical coherence tomography (OCT) has the potential to improve diagnostic accuracy and help delineate pre-surgical margins in NMSC. Its widespread clinical acceptance awaits the accumulation of evidence from studies of direct histological comparisons. Method: In this study, seventy-eight subjects presenting with skin lesions, including 28 NMSCs, were imaged using the VivoSight® OCT scanner and a biopsy taken. Haematoxylin and eosin stained histology sections were compared with the OCT images. The depth of superficial basal cell carcinoma (BCC) lesions (<1 mm) can be measured accurately using OCT. A low-strength OCT signal at the periphery of the cell nests seen in superficial and nodular BCC is identified as corresponding to cellular palisading. A weak inverse linear correlation ($r^2 = 0.3$) is found between the optical attenuation coefficient measured on OCT and the nuclear-cytoplasmic ratio (N/C) of cells determined from histology. OCT has clinical value in providing accurate dimensional measurement of superficial BCC and in identifying the presence of peripheral palisading in nodular BCC.

SE01.04

HAIR SHAFT ANOMALIES IN ALOPECIA AREATA EVALUATED BY OPTICAL COHERENCE TOMOGRAPHY

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Background: Contrary to histology and electron microscopy, optical coherence tomography (OCT) has the advantage to demonstrate *in vivo* highly reproducible measurements of hair shaft thickness, diameter, cross sectional surface area and hair shape. Aim: The aim of this study was to evaluate whether OCT is suitable as a standard method to determine hair shaft abnormalities in alopecia areata (AA). Methods: Using OCT, in 9 patients (n=9) with AA measurements were performed in 50 hairs from the border of an alopecic area and in 50 hairs from an area without hair loss. The hair parameters determined were cross section (CS) and form factor (FF). Using a fixed measuring distance from the scalp surface, the ratio between the maximal and minimal hair diameters determined the form factor (d_{max}/d_{min}). Results: In all cases, the CS of hairs from an AA patch was significantly lower as compared with hairs from an unaffected area. The FF, however, did not indicate any hair growth disturbances. Conclusions: In active lesions of AA, structural abnormalities of hair shafts are demonstrated in all patients. OCT is a valuable non-invasive technique for *in vivo* studies of the pathogenesis of AA.

SE01.05

OPTICAL COHERENCE TOMOGRAPHY OF NMSC UNDERGOING IMIQUIMOD THERAPY

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Optical Coherence Tomography of NMSC undergoing imiquimod therapy. Objectives: To describe the OCT morphology in NMSC lesions during imiquimod treatment and to assess how OCT morphology before and during treatment reflects the treatment out-come at 3 months follow-up. Non-invasive treatment of non-melanoma skin cancer (NMSC) with imiquimod implies pre-therapeutic tumor thickness and delineation assessments for optimal efficacy. Optical coherence tomography (OCT) is an optical imaging technique that can potentially diagnose and estimate basal cell carcinoma (BCC) and actinic keratosis (AK) thickness *in vivo*. Methods: A total of 17 patients diagnosed with NMSC/AK at Department of Dermatology, Roskilde Hospital, Denmark, were monitored by OCT during imiquimod treatment. Images were taken at baseline, twice during treatment and once at 3-months follow-up. BCC and AK diagnosis was based on histopathology. The OCT system (Vivosight, Michelson Diagnostics, UK) is a low intensity, 1310 nm laser non-contact, non-invasive real-time imaging device. Results: Both AK and BCC demonstrated disruption of normal skin layering before and during treatment. At follow-up OCT made it possible to differentiate cancerous structures from non-cancerous structures though the latter was clinically interpreted as recurrences. Conclusion: OCT can visualize skin structures in AK and BCC lesions *in vivo* to a depth of 1 mm. This pilot study indicates that OCT imaging may increase the diagnostic specificity of suspected

recurrences/residual tumour. OCT imaging of non-melanoma skin cancer may therefore provide diagnostic and prognostic information. Further evaluation of the potential of OCT as a monitoring device during NMSC treatment with imiquimod is warranted.

SE01.06

OPTICAL COHERENCE TOMOGRAPHY OF HUMAN SKIN UNDERGOING CRYOTHERAPY

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Objectives: To describe OCT morphology of normal skin treated with cryotherapy, and to examine the potential of OCT in monitoring cryo-treatment of Actinic keratoses (AK). AK are dysplastic skin lesions that are commonly treated with cryotherapy. To reduce the potential risk of adverse effects it is important to monitor the extent of freezing. Optical coherence therapy (OCT) is a non-invasive optical imaging technique that may be useful as a monitoring tool during cryo treatment. Methods: Normal ex vivo skin and *in vivo* AK lesions were examined using is a low intensity, 1310nm laser light, non-contact, non-invasive real-time imaging OCT device (Vivosight, Michelson Diagnostics, UK). Cryotherapy was applied and OCT images were acquired at baseline, during and at defined point after cryosurgery. The OCT morphology was described and ex-vivo images were compared to OCT images of *in vivo* skin images. Results: Cryotherapy treatment produced an opaque iceball, and it was therefore not possible to monitor the freezing depth by OCT. In 82% of the lesions, vesicle formation could be identified in OCT images shortly after the treatment. Vesicles were not clinically visible at the time of the OCT scan. Conclusion: OCT cannot monitor the freezing depth during cryotherapy. However, OCT visualization of vesicles in AK lesions shortly after the cryo procedure indicate that OCT imaging may predict outcome and therefore be valuable in evaluating the efficacy of the cryotherapy treatment.

SL02

COMBINING EXPERIMENTS AND SIMULATIONS FOR A BETTER UNDERSTANDING OF SKIN PROPERTIES

Bernard Querleux

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During a long time, skin properties were evaluated through palpation and visual control. If clinical exam remains the reference approach for individual diagnosis, it has also shown its limits in reproducibility and accuracy for quantifying skin properties in clinical studies aiming at characterizing chronological and photoaging, or for evaluating the efficacy and safety of dermatological and cosmetic products. Taking advantage of the accessibility of the skin *in vivo*, non invasive methods were developed for about 40 years, offering nowadays for instance accurate measurements of the skin color through optical methods, firmness and elasticity measurement through biomechanical devices, and even direct measurements of skin functions as the excretion, the trans epidermal water loss, the perfusion, and the barrier function. Skin imaging has also appeared these last years and gives us more and more information on the skin structures from the microscopic to macroscopic levels. However, we should admit that the mechanisms involved in these properties are still partly understood due to their multidomain (biological, biochemical, and biophysical domains) and multiscale dimension (cellular and below to tissular and beyond). In many scientific domains, including biomedical engineering, numerical modelling is nowadays recognized as a key-actor for improving our knowledge, and there is no reason that it could be different for skin science. This short lecture will illustrate how numerical simulation of the skin considered as a multilayer structure coupled to experimental data can give new insights of the impact of skin components on its optical and mechanical properties.

SE02.01

MOVING BEYOND THE ESTABLISHED TEST LABORATORY: MEETING THE CHALLENGES

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Usually studies with biophysical measurements are conducted in established test laboratories. There conditions, e.g. environment, laboratory set-up and space, are optimized for the conduct of these studies. However, in order to have access to certain subject/patient populations, e.g. ethnic groups or skin diseases, it may be necessary to conduct studies in the field where the infrastructure is not established and the investigators/study personnel are not yet trained in the use of devices. This presentation will address how to meet this challenge, allowing for collection of reliable data for skin function and structure in other laboratories and practices. First and foremost, suitable investigators must be identified who are motivated to participate in demanding skin physiology studies. During site qualification visits important issues such as availability of subjects, environmental conditions in the laboratory,

configuration of outlets and furniture in the available rooms, GCP training of study staff and amount of time which can be dedicated to the study should be clarified. Device training for the entire study staff should be held using laboratory manuals covering all aspects in the practical conduct of the biophysical measurement methods, including guidelines for plausibility checks and data transfer/backup. If multiple devices are used, the sequence of each measurement series should be set in advance. Methods should be chosen carefully, with particular attention to ease of use and potential for incorrect use. Finally, monitoring is important to ensure high quality and correctness of data collection, especially as a tool to identify potential problems early in the study.

SE02.02

COMPARISON OF ODOUR TEST METHODS BASED ON EXPERIMENTAL DATA

Björn Maxeiner

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The VDA 270 is an established method for odour evaluation in the automobile industry for years. Since 2012 a new method for the building product industry exists. The name of the standard is Determination of odour emissions from building products using test chambers (ISO 16000-28:2012). The description of the measurement for odour intensity is considered here. Beside those standards the Odournet GmbH has an established in-house method for the evaluation of odour emission. 3 different sample materials were investigated according all 3 methods. The data analysis shows significance differences in the accuracy of the methods.

SE02.03

A NOVEL METHOD FOR DYNAMIC ASSESSMENT OF SWEAT GLAND ACTIVITY

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Objective: Skin perspiration is composed of two parts – water vapor diffused through the epidermis and water secreted through sweat glands. While transepidermal water loss (TEWL) measurement systems quantify a combination of both parts, they are incapable of accomplishing separate measurements of the latter. The objective of this study was to develop a method of discreet and dynamic assessment of the number of active sweat glands. Methods: A digital camera was fitted with a zoom-in objective, magnifying lenses, polarizing filters and a ring of 15 light emitting diodes (LEDs). Thus equipped, the system produces co-polarized photos of skin surface structures at a lateral resolution of about 5 micrometers per pixel. During the course of imaging, as sweat glands secrete water, the white light from the LEDs create reflections of 15 small white dots on the surface of each droplet. If photos are captured consecutively and replayed in rapid mode, the discharge of sweat drops can be seen as expanding rings of dots which gradually disintegrate with evaporation. Results: A computer software algorithm detects the appearance of dotted rings of varying size, identifying the location of active sweat glands. With the placement of markers at the rings' centers, a computer generates a series of maps which display the dynamic appearance and disappearance of water droplets. Data derived after placing markers on the integrated maps, yield time curves representing the number of active sweat glands.

SE02.04

FLUORESCENT FIBRE-OPTIC CONFOCAL IMAGING OF LESIONAL AND NON-LESIONAL PSORIATIC SKIN COMPARED TO NORMAL SKIN IN VIVO

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Background/aims: Fibre-optic confocal imaging (FOCI) allows non-invasive visualization of live skin in vivo. A contrast agent, a fluorophore, is injected in the dermis. FOCI images are optical sections from a horizontal (en face) view. The aim was to study epidermis and the cellular structure of keratinocytes of psoriatic plaques and adjacent non-lesional with healthy skin as a reference. Methods: Twelve patients with stable plaque psoriasis were studied and compared to a control group of eight healthy individuals. Fluorescein sodium was used as fluorophore. A hand held fibre-optic laser scanner (Stratum®, Optiscan Pty., Melbourne) was used. The study included morphometric analyses. Results: The confocal *in vivo* images demonstrated characteristic features of epidermis and keratinocytes in lesional and non-lesional skin versus healthy skin. Morphometry based on FOCI demonstrated an approximately 30 % increased width of keratinocytes of psoriatic skin versus healthy control, and the number of keratinocytes per viewing field was reduced. FOCI allowed non-invasive visualization of cell nuclei and parakeratosis of psoriatic epidermis. The horizontal width of dermal papillae of psoriatic skin was increased by approximately 50 % as

compared with healthy skin, and the flow of erythrocytes in the papillar vessels could be observed in real-time. Conclusions: FOCI can directly visualize essential epidermal structures of plaque psoriasis *in vivo*, in real-time and with cellular resolution without the need of taking biopsies and thus without disturbing the natural state of the skin. FOCI is a versatile future tool for non-invasive microscopic diagnosis and therapy follow-up of psoriasis.

SL03

SAFETY EVALUATION OF COSMETICS IN EUROPE WITHOUT ANIMAL TESTING

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Cosmetic products are developed to be applied to human skin and external mucosa by the general public. Their safety is traditionally guaranteed by animal experiments. Side effects, both local and systemic, however, may occasionally occur. Their regulatory safety evaluation in Europe is based on Directive 76/768/EEG, which is now being replaced by Regulation 1223/2009 (RECAST). Considerable progress has been made in the development of alternatives to animal experimentation to detect undesirable effects and this methodology has been fully taken up in RECAST. In particular, alternatives detecting local and short-term effects exist. Validated replacement methods are available for skin corrosivity and irritation, dermal absorption, mutagenicity/genotoxicity and phototoxicity. For eye irritation internationally accepted screening procedures for severe irritants exist. Validated reduction/refinement alternatives, still using animals, exist for acute toxicity and skin sensitization. No validated alternatives have been developed successfully for systemic and long-term toxicity, including repeated dose toxicity, carcinogenicity, reproductive toxicity and toxicokinetics. Human-based bioengineering is only to a limited extent taken up in safety assessment of cosmetics and their ingredients. Although it is clear that human volunteer testing cannot replace animal tests in safety assessment of cosmetics, skin compatibility tests on the contrary may be needed scientifically and ethically. They can confirm that no harmful effects occur when applying a cosmetic product for the first time to human skin or mucous membranes. Therefore, bioengineering methods can contribute substantially to the safety of cosmetics, by non-invasive and objective evaluation of skin compatibility in carefully monitored clinical studies.

SE03.01

COSMECEUTICALS - “AN ANNOYING ITCH THAT WON’T GO AWAY”

Chris Gummer

Cider Solutions Ltd, Research & Development, Chilworth, Surrey, UK

For a product category that doesn't exist, Cosmeceuticals generate a lot of debate. Are they cosmetics with increased activity or pharmaceuticals that don't work very well? It is clear from the directives that a product must be either a cosmetic or a pharmaceutical although, as with all things in life, it is rarely always black and white. Cosmeceuticals are the Advertiser's dream product. Even the name implies efficacy yet these products typically go to market on the back of minimal data and curious in-vitro claim support. In fact they often promise a lot and deliver little. Cosmeceuticals illustrate an inability of the industry and regulators to deal with high levels of cosmetic efficacy. In parallel they demonstrate the pharmaceutical regulators restrictions on rapidly developing actives and efficacious cosmetics. Cosmeceuticals are simply a distraction and should cease to exist. However, the space they try to occupy will itself disappear under increased advertising regulation unless we combine the efforts of cosmetic companies, raw material suppliers and measurement scientists to show that cosmetics really do work.

SE03.02

NANOPARTICLES IN COSMETICS: PROSPECTS AND SAFETY ASPECTS

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The requirements on nanoparticles for use in cosmetics are very different. While nanoparticles widely applied in sunscreens, like TiO₂ and ZnO, shall remain on the skin surface or in the upper cell layers of the stratum corneum, nanoparticles intended for drug delivery shall penetrate through the skin barrier to the target structures in the living cells. At the Charité - Universitätsmedizin Berlin various methods are used to investigate the penetration and storage of nanoparticles in the skin, hair follicles being in the focus of attention. Human hair follicles are ideal target structures for drug delivery. Hosting both the stem and dendritic cells, they are surrounded by a dense network of blood vessels. Investigating nanoparticles of different size and materials, it was found that particles of approximately 600nm diameter penetrate most efficiently into the hair follicles and can be stored there for

maximally 10 days. Their retention time in the hair follicles exceeds that in the stratum corneum by almost one order of magnitude. No experiment had shown, however, that particles of 40nm-1µm in diameter penetrated from the hair follicle into the living tissue if the skin barrier was intact. This is plausible as the hair follicle has its own barrier. Only if the barrier was disturbed artificially or by illness, nanoparticles of 40nm in diameter had penetrated into the living tissue. Penetration through the intact skin barrier can be excluded for the investigated particles. Nevertheless, nanoparticles are well suited to deliver drugs into the hair follicles for subsequent release.

SE03.03

SATISFYING THE EUROPEAN CONSUMERS. THE NEW REGULATION AND CHALLENGES TO THE INDUSTRY

Gerald Renner

Cosmetics Europe - The Personal Care Association, Brussels, Belgium

The manufacture and marketing of cosmetic products in Europe has been covered by strict safety legislation since 1976 – the European Cosmetics Directive (76/768/EEC), as amended. From July 2013 the Directive will be replaced by the new Cosmetic Products Regulation (EC) No 1223/2009. The main premise to only place safe product on the market remains the same. However there are a number of new requirements, and the deadline for implementation is fast approaching. Cosmetics Europe has been involved with all of the Commission groups working on major areas of change and guidance to industry for the past two years. The presentation will give an overview of the basic changes introduced by the new Regulation and highlight key areas in its implementation for which companies need to get ready.

- Roles and Responsibilities along the supply chain
- Product Notification – Electronic Cosmetic Product Notification Portal
- Cosmetic Product Safety Report
- Reporting of Serious Undesirable Effects
- Nanomaterials
- General criteria for claims

SE03.04

PRECLEARANCE OF TV ADS FOR A HAIR LIGHTENING PRODUCT - AN EXAMPLE OF INSTRUMENTAL MEASUREMENT, VISUAL, AND CONSUMER DATA IN CLAIMS SUBSTANTIATION

Karen Lammers, J Woodford, S Sherman, W Billhimer

Kao USA Inc, Cincinnati, OH, USA

This presentation will focus on a specific example of how method development, colorimetric, and consumer measurements were used to support the development of hair lightening product claims. Various instrumental, physical, and color measurements were made using relatively affordable and easy to operate instrumentation. The application of technical measurements for claim support range from basic research, to technology/methods development, to optimization of treatments or products. These unique methods and results of consumer questionnaires may lead to novel product performance claims. A summary of the data used to substantiate television claims in the U.S. and United Kingdom will be presented for the hair lightening product. The process used to design a readily defensible protocol leading to reproducible, scientifically valid instrumental data and other methods used to successfully support claims will be discussed. The preclearance submissions for US television and UK television advertising will be compared and contrasted in this case. Specifically, scripts will be discussed including the claims, submissions to each agency (ABC Network in the U.S. and Clearcast in the UK), and final commercials will be included.

SE03.05

HOLD OF DECORATIVE COSMETICS DETERMINED BY LAY AND EXPERT RATING AND IMAGE ANALYSIS

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Since prehistoric ages and early cultures, women have decorated their faces and bodies to improve their appearance and for expressing their social status. Nowadays, decorative cosmetics are widely used and it was shown that their use leads to an improvement in the quality of life of the users. Products like mascara, make-up and lipsticks are promoted with long-lasting claims and covering powers. To be able to substantiate these claims, we have developed

a standardized evaluation of clinical photographs by either lay rating, expert rating and/or image analysis. The clinical photography set-up provides optimal repositioning and optimized color reproducibility so that the obtained images can be perfectly assessed or analysed. To assess the hold of different types of make-up products, make-up and lipstick were applied by a beautician to the faces of 12 female subjects, the mascara products were applied by the subjects themselves. Before application, 10 minutes, 1, 3, and 6 hours after application clinical photos were taken from the subjects' faces. The images were rated by 6 trained experts and by the subjects themselves on defined parameters to assess the hold of the products over the investigated period of time. Image analysis was performed on the images additionally. Statistical comparisons show the advantages of one or the other method of evaluation. In conclusion, the hold of decorative cosmetics can be determined in a reproducible way by subjective lay rating, semi-subjective expert rating and objective image analysis of high-quality clinical photos. A comparison of these methods is given.

SL04

MICRODIALYSIS SAMPLING IN HUMAN SKIN: A VERSATILE METHOD FOR HUMAN, ANIMAL AND EX VIVO APPLICATION

Eva Benfeldt

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Over the last decades microdialysis sampling has been developed further and the use has been extended to new applications. Examples of how the method can assist in evaluation of penetration in diseased or barrier damaged skin, in small and compact studies of bioequivalence and bioavailability and finally in achieving new information on human-animal correlations and in vivo-in vitro/ex vivo correlation will be given, all with an emphasis on studies of topical drugs and formulations.

SE04.01

PIG SKIN VS. ARTIFICIAL MEMBRANES IN PENETRATION INVESTIGATIONS

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Background: In vitro methods used in the research of transepidermal transport of active substances generally rely on the penetration rate of test compounds through standard membranes. Models typically used in penetration experiments are specially prepared human or animal skin samples or synthetic membranes. Objective of this study was to establish if the test results for an artificial liposome membrane can be extrapolated to determine the actual bioavailability of active substances. Methods: Tests were conducted in a side-by-side diffusion cell. As model membranes, a liquid-crystal lipid membrane (LM), phospholipid membrane (PM) and pig skin sample were used. The test compounds were eight synthetic dyes used in hair colouring products. Results: The transport rate of a dye through the LM is several times higher than that through the pig skin, which points to lower barrier properties of the tested synthetic membrane. Nevertheless, the LM has better barrier properties in comparison with the PM. Observed correlation between the results obtained for porcine skin and the LM was linear ($R=0.95$ and $R=0.93$ in the presence of a 1% SLS in donor system). Correlation between penetration coefficients through the PM and pig skin was not significant ($R=0.82$). Conclusion: Research findings reveal that membranes composed of lipids, identical to those present in the epidermis provide a close approximation of the actual bioavailability of active substances. Unlike biological membranes, intercellular cement does not contain phospholipids. The experiments confirm that the PM constitutes a less credible model for the studies of transepidermal transport in real life conditions.

SE04.02

LOCAL DISTRIBUTION OF INSULIN IN PORCINE SUBCUTANEOUS TISSUE RELATED TO HYPODERMIC NEEDLE DIMENSIONS - EVALUATED BY 3D CT-SCANNING AND HISTOLOGY

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(2) Novo Nordisk A/S, Denmark

(3) Bispebjerg University Hospital, Denmark

Smaller needles for subcutaneous administration of therapeutic peptides are continuously developed to decrease injection discomfort. However, little is known about local drug delivery in relation to needle length and thickness. Present study investigated local distribution and deposition depth in the subcutaneous tissue related to three hypodermic needle sizes of 3mm 34G, 5mm 32G, and 8mm 30G. Ex vivo experiments were carried out in six pigs receiving a total of 72 randomized injections, i.e. 24 injections with each needle type. 400 µL was injected including 70% NovoRapid® (Novo Nordisk A/S, Bagsværd, Denmark) and 30% Xenetix® (Guerbet, Villepinte, France) contrast medium mixed with 1 mg/mL Alcian blue. Surgical biopsies of injection sites were sampled and CT-scanned in 3D. Volume graphical software, VGStudio MAX 2.1 (Volume Graphics, Heidelberg, Germany) was used to evaluate the 3D CT-scannings in terms of depth deposition and local distribution. A sample of the biopsies were prepared and sliced to evaluate deposition in comparison to the CT-scanning findings. CT-scanning (n = 57) and histology (n = 10) showed that, regardless of injection depth, the bulk of the injection was in the subcutaneous tissue and did not propagate from subcutis into dermis. The volume deposition peaks were related to needle length and compared well with histology estimations for the individual biopsies. Furthermore, CT-scanning and histology confirmed that local propagation and distribution of the injection followed a route of less mechanical resistance as determined by connective tissue barriers preset in the site of injection, aiming for systemic uptake.

SE04.03

THE USE OF MINIPIGS IN WOUND HEALING RESEARCH

Peter Glerup

CiToxLAB Scantox, Ejby, Denmark

The skin of pigs and minipigs shows many similarities to human skin. In drug development the most appropriate animal species should always be used for non-clinical safety testing, and for dermal pharmaceuticals it is therefore difficult to justify not to use the pig for these studies. But also in development of wound care pharmaceuticals and devices, the pig constitutes a much better model than all other animal species. The wound healing process consists of the same phases as in humans and for both humans and pigs, wound contraction occurs quite differently as compared with loose skinned animals. In addition, the sensitivity of the skin of pigs is more similar to human skin, whereas exacerbated reactions are often seen in the rabbit. This presentation will focus on the use of minipigs in wound healing research for efficacy and safety testing. Scientific as well as regulatory and practical aspects will be discussed and methods used for evaluation of wound healing will be described. In addition to this, abraded skin models for use in regulatory toxicity testing of dermal products and other modified wound healing models will be presented.

SL05

IMAGING TECHNIQUES, WHY, WHEN AND WHICH?

Klaus-Peter Wilhelm

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Imaging techniques have become an indispensable tool in clinical dermatology as well as in dermatologic research. Technological progress over the last two decades has been tremendous. Today there is a wide array of imaging methods available to study not only morphological but also functional parameters of skin *in vivo*. Ultrasound, Confocal Microscopy, Optical coherence tomography, Nuclear Magnetic Resonance (NMR), multiphoton microscopy to name just a few, allow *in vivo* imaging of skin morphology. Skin surface profile parameters are obtained with fringe projection or 3D-imaging techniques. Skin blood flow and skin temperature are visualized and quantified by laser Doppler principle and IR-thermography respectively. Last but not least has the introduction of affordable high-resolution digital photography in combination with advancements in automated image analysis programming provided new options not only for the documentation but also for quantification of disease states. As with all techniques imaging techniques also have their limitations and shortcomings. One major drawback of most imaging techniques is the high dependency upon the investigator. This holds true for obtaining images in the proper way, selecting of the “right” detail and last but not least for making the appropriate interpretation. This review will focus on recent developments in skin imaging techniques and try to provide guidance regarding the strengths as well as limitations of the various techniques.

SE05.01

COMMON APPLICATIONS OF ULTRASOUND IN DERMATOLOGY

Ximena Wortsman

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Background: In recent years there has been a growing use of ultrasound in dermatology. Objective: To assess the usefulness of variable frequency color Doppler ultrasound in common dermatologic conditions. Methods: Analysis of the normal sonoanatomy, the technical considerations and the sonographic morphology of frequent skin lesions will be performed. This review includes benign (vascular and non vascular) tumors, malignant entities, inflammatory diseases and nail abnormalities. Clinical, sonographic, surgical and histological images will be provided for highlighting the session. Conclusion: Participants will have an opportunity to develop a better understanding of the requisites and common applications of this non- invasive and real time imaging modality in their daily practices.

SE05.02

ULTRASOUND EXAMINATION OF SKIN: WHAT IS IMAGED AND WHAT IS MEASURED?

Jørgen Serup

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The ultrasound texture of skin is mainly composed of the entrance echo, the reflections of the collagen fibre network of the dermis, and the interface towards fat. Disturbances and pathologies such as inflammatory changes, tumours and keloids occupying the dermis are mostly seen as echo-lucent patterns delineated by the echo-rich dermis. Accumulation of oedema especially takes place in the very outer dermis often imaged as a sub-epidermal band, the width of the band indicating the severity of the oedema. *In vivo* distance including skin depth is measured assuming ultrasound velocity in skin at 1580 m/sec. Echo-density can be analysed by image analysis with the low-echogenic window defined at 0-30 on a 1-255 scale. Data on reproducibility of measurements and inter-observer variation are presented. Automatic border detection is described. Ultrasound images the undisturbed skin and has advantages relative to punch biopsy because change of dimension due to biopsy cutting and histologic preparation is avoided, and advantages relative to other methods of imaging such as confocal microscopy and optical coherence tomography since these methods only image the epidermis and the very outer dermis, while ultrasound images the full-block skin including the subcutaneous space. With recent development of ultrasound equipment resolution has been significantly improved and the application field widened, and ultrasound is today positioned as a validated and widely used method in dermatologic research including non-invasive diagnostic evaluation of skin pathologies

SE05.03

High Sensitivity Rate of Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC) using the Berlin Morphology Criteria for Lymph Node Metastases significantly reduces Need for Surgical Sentinel Node (SN) Staging in Melanoma

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(4) Institut de Cancérologie Gustave Roussy, Villejuif, France

Background: US-guided-FNAC prior to surgical SN staging is emerging as a possible cost-effective addition to the staging of melanoma patients (pts). Formerly, sensitivity (sens) rates of lymph node US in melanoma were disappointing (20–40%). The introduction of the Berlin Morphology Criteria has significantly improved sens rates for US-FNAC (*J Clin Oncol* 2010;28(5):847-52). The aim of the current study was to report on 1000 patients the sens, specificity (spec), positive (PPV) and negative (NPV) predictive value rates of US-FNAC from our prospective database with prolonged follow-up. Methods: Since 2001, >1000 stage I / II consecutive melanoma pts have undergone US-FNAC prior to SN. All patients underwent lymphoscintigraphy. Peripheral Perfusion (PP) (Fig 1A & 1B), Loss of Central Echoes (LCE), Balloon Shaped (BS) were the Berlin Morphology Criteria which were registered. FNAC was performed in case of presence of any of these factors. SN tumor burden was measured according to the Rotterdam Criteria. All patients underwent SN or LND in case of positive FNAC. Results: Mean/median Breslow thickness was 2.56 / 1.57 mm (0.2 – 44 mm). Mean/median follow-up was 39 / 32 months (0 – 115). Ulceration was present in 24 %. SN positivity rates were 20 % (202 / 1000). Sens was 51 %. Spec, PPV and NPV were 99%, 91% and 89%. Sensitivity was highest for T4 tumors (77%). PP, LCE, BS had sens of 69%, 24%, 25%. SN tumor burden > 1 mm in largest diameter according to the Rotterdam Criteria was identified by US-FNAC in 86%. Threshold for positive FNAC was 0.4 mm in maximum diameter. Survival curves of US-FNAC positive patients mimic the survival rates of SN pos patients. Conclusions: The new criterion of Peripheral Perfusion is of key importance to achieve the high sensitivity of US-FNAC according to the Berlin Morphology Criteria (Voit C et al. *J Clin Oncol* 2010; 28(5): 847-852) to identify lymph node metastases. Sensitivity of US-FNAC with use of the Berlin Morphology Criteria (51%) was significantly higher than other reports in literature (20 – 40%). PP had high sensitivity rates, but low PPV (it was an early sign of metastases, but still often negative). BS and LCE had a high PPV, but low sensitivity rates (late sign of advanced metastases). This was confirmed with the high detection rate in T4 tumors and large metastases (> 1 mm). Therefore, US-FNAC might be useful to detect lymph node metastases in advanced melanoma patients. Especially for T4 patients and in patients with advanced SN tumor burden it can reduce significantly the need for surgical SN staging. The EORTC Melanoma Group will launch the prospective validation study, USE FNAC, in 2013.

SE05.04

ULTRASOUND ASSESSMENT OF TATTOO REACTIONS

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Tattoos are trendy and paralleled by an increasing number of adverse reactions ranking from minor to serious. Particulate pigment in or just above the nano-range is installed in the outer dermis. Pigments may cause allergic reaction, foreign body reaction with granuloma and various special events including toxic reactions and photosensitivity. In total, 73 patients with adverse tattoo reactions were studied, using the Dermascan C 20Mhz ultrasound scanner of Cortex Technology, Denmark. Mean skin thickness was 1.91 mm (SD 0.62) in reactions contra 1.18 mm (SD 0.27) in adjacent normal skin, $P < 0.01$. A sub-epidermal echo-lucent band was consistently observed. The thickness of this band correlated with skin thickening. Accordingly, the echo-density (0-30 low-echogenic band) of the outer dermis of reactions was reduced versus normal skin. A total of 58 punch biopsies had been taken from the tattoo reactions. Pathologists' ranking of cellular infiltration levels (1 outer, 2 middle and 3 lower dermis) correlated with the thickness of the echo-lucent band by ultrasound. Representative ultrasound images of inflammatory reactions including pseudolymphoma and granulomatous reactions/sarcoid granuloma are shown. It is concluded that ultrasound has a vital role in the diagnostic assessment of tattoo reactions, displaying the undisturbed micro-anatomical structure of the entire dermis, measuring depth and invasion level of the tattoo reaction prior to dermatome shaving.

SE05.05

SEBACEOUS GLAND SIZE INFLUENCES VISCOELASTICITY OF THE SKIN - COMPARISON OF THREE-DIMENSIONAL ULTRASOUND MICROSCOPE IMAGING AND BIOMECHANICS MEASUREMENTS

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Objectives: Connective tissue components in dermis such as collagen and elastin may contribute to skin viscoelasticity. However, little attention has been paid to small vessels or sebaceous gland (SG) in dermis. The purpose of the present study is to compare SG morphology and biomechanical properties of the skin. Methods: 3D ultrasound microscope with the central frequency of 120 MHz was used for visualization of SG which showed low intensity echo. Sebum level of the skin surface was measured with Sebumeter® and viscoelasticity of skin was measured with Cutometer®. The parameter Ur/Uf was used as the representative value. Twenty one healthy male subjects with written informed consent were involved and the cheek and forearm were evaluated in each individual. Results: The SGs were distributed approximately 800 μm beneath the skin surface in most cases. Thus the following parameters were measured at 800 μm ; average size of SG, number of SGs /mm² and SG-occupying ratio. Surface sebum level and the number of SGs were significantly higher and Ur/Uf was lower in the cheek compared with the forearm. However, surface sebum level was not correlated with SG size, SG number or SG-occupying ratio. Ur/Uf was significantly correlated with SG size and negatively correlated with SG number but no significant relation was found with SG-occupying ratio. Conclusion: 3D ultrasound clearly showed 3D structure of SG. The comparison with biomechanical properties indicates that the SG size is more important than sebum content in the dermis to determine the skin viscoelasticity.

SL06

NON-LINEAR SPECTRAL IMAGING OF SKIN

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Fluorescent molecules can be excited by the simultaneous absorption of two or more Near Infra Red photons. The non-linear dependency of the multi-photon excitation (MPE) probability on the excitation intensity results in the confinement of the excitation volume and can be employed for 3-D imaging. MPE affords excitation of autofluorescing molecules with one photon absorption bands in the UV and enables high resolution imaging deep inside tissues. Here, multi-photon spectral imaging was employed for imaging the intrinsic emission of skin. This results in 'optical signatures' of the tissue that are potentially interesting for characterizing skin and diagnosing diseases. Both mouse and human skin were investigated. Different layers of skin could be clearly distinguished based on both their spectral signature and morphology. The images contain signals from native chromophores such as NADPH, elastin, collagen, flavins and melanin. In addition a Second Harmonic signal generated by collagen is observed. The different signals can be separated using linear unmixing techniques, including the recently introduced spectral phasor method. MPE offers potential for the diagnoses of diseases such as skin cancers. The

skin of UV exposed mice reveal spectral changes as well as morphological changes that can be associated with tumor formation. An important step is to reduce the size of non-linear microscopes and make them compatible with clinical applications. We are now working on miniaturizing non-linear microscopy with spectral detection capabilities. A mobile prototype has been constructed that is equipped with a fibre coupled miniaturized scan head. First results will be presented.

SE06.01

IN VIVO MICROSCOPIC EVALUATION OF DERMAL CHANGES OVER AGE WITH REFLECTANCE CONFOCAL MICROSCOPY (RCM)

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Reflectance Confocal Microscopy has recently been used to assess age-related parameters in the skin. New confocal descriptors such as different collagen fibers patterns have been identified as markers of age-related changes. Four different collagen configurations named thin reticulated collagen, coarse collagen, huddled collagen and curled bright structures were found. The objective of this study was to assess the ratio variations between these 4 types of collagen with age. Fifty nine healthy women belonging to different age groups (group I aged 20-24 years, group II aged 36-44 years and group III > 60 years) were enrolled in the study. Images of confocal microscopy were collected on the forehead, the cheek, the inner arm and the inner forearm at the dermal level. A paired Student t-test or Wilcoxon test was done to compare groups of age for each dermal descriptor and each body part (significance level $p \leq 0.05$). Whatever the body part measured, the dermal descriptors showed organizational changes over age. Younger groups presented thin fibrillar collagen forming weblike pattern which decreases progressively in the elderly in favor of coarse and huddled collagen. Curled fibers were observed in age group III, corresponding to elastosis. In this study, the reflectance confocal microscopy allowed to quantify dermal age-related skin changes successfully. The advantage of this non invasive method is to be able to follow up, *in vivo*, at histological resolution, a wide skin area at the dermis level.

SE06.02

SKIN AGING: RCM SCORING SYSTEM. APPLICATION TO COSMETICS' RESEARCH

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In vivo confocal microscopy has been discovered as a noninvasive tool with a nearly histologic resolution. Distinct morphologic confocal aspects on facial skin have been described and correlated with the histopathologic counterparts. More specifically, RCM showed the presence of more irregularly shaped keratinocytes and areas with unevenly distributed pigmentation as well as increased compactness of collagen fibers in aged skin. Moreover, in elderly, thinning of the epidermis, marked keratinocyte alterations and huddles of collagen and curled fibers, corresponding to elastosis, were readily identifiable upon RCM. Combining these RCM features, three different semi-quantitative scores can be calculated: - epidermal disarray score (irregular honeycombed pattern + epidermal thickness + furrow pattern); - epidermal hyperplasia score (mottled pigmentation + extent of polycyclic papillary + epidermal thickness; - collagen score (curled fibers, 2 for huddles of collagen, 1 for coarse collagen structures, and 0 for thin reticulated collagen). The epidermal disarray score showed a stable trend up to 65 years and a dramatic increase in the elderly subjects epidermal. Hyperplasia score was characterized by an ascending trend from younger subjects to middle age. The total collagen score showed a progressive trend with age with a different proportion of distinct collagen type. To sum up, RCM can be successfully applied to identify *in vivo* skin changes occurring in aged skin both at epidermal and dermal level at histopathologic resolution and permit to microscopically quantify the aging signs and to test cosmetic efficacy.

SE06.03

CUTANEOUS RESPONSE TO ULTRAVIOLET RADIATION EXPOSURE

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Background: UV radiation (UVR) represents the main risk factor for skin cancer and the use of sunscreens recommended for prevention of acute and chronic effects of UVR. Reflectance confocal microscopy (RCM) represents an innovative technology allowing for the visualization of in-vivo effects at cellular resolution. Objective: We aimed analyse morphological induced by acute and chronic UV-exposure by the use of RCM. Results: RCM allows the detection of morphological changes induced by UV including spongiosis, sunburn cells, micro-vesicles, blood vessel dilatation and pigmentation. Conclusion: RCM seems to be beneficial for the non-invasive evaluation of dynamic changes following acute UV exposure. Signs of acute as well as of chronic photo damage can be visualized by RCM and efficacy of sunscreen may be tested on a cellular level.

SE06.04

CONFOCAL LASER SCANNING MICROSCOPY IN THE FLUORESCENCE MODE

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Confocal laser scanning microscopy (CLSM) is a high resolution imaging method. Based on the reflectivity of different structures within the epidermis and upper dermis, microscopic details are visible. In addition to the reflection mode, fluorescence of topically or intralesionally applied dyes can be detected using different lasers and filter systems. The fluorescence dyes get chosen in respect to the excitation and emission wavelength, the specificity of staining and the toxicity. Sodium fluorescein as well as indocyanine green can be applied in vivo. These dyes can be used to investigate the penetration of substances as well as the skin barrier function. For excised tissue, acridine orange and Nile blue showed the best results with specific staining of nuclei and elastic fibers, whereas collagen bundles showed only a weak fluorescence. Compared to the reflection mode, the contrast between epithelial structures and connective tissue is higher using fluorescence dyes. Therefore, ex vivo CLSM in the fluorescence mode can be used for rapid surgical pathology, especially for micrographic surgery of basal cell carcinomas.

SE06.05

INFLAMMATORY SKIN CONDITIONS AND THEIR DIFFERENTIAL CONFOCAL FEATURES

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In-vivo reflectance confocal microscopy has been already used for several inflammatory skin conditions microscopical features definition, as in psoriasis, contact dermatitis, lupus erythematosus and lichen planus, disclosing interesting and promising clinical applications. A methodological and systematic approach to the skin analysis using confocal microscopy needs the identification of major as well as secondary confocal criteria in order to define specific patterns and distinctive features for single entity identification. In this presentation specific features of the three main groups of inflammatory skin diseases (interface dermatitis, hyperkeratotic/acanthotic diseases/spongiotic dermatitis) will be described, correlate to optical histology and evaluated in correlation with the secondary microscopical criteria defining the real value of confocal microscopy in single entity (lichen planus vs Lupus erythematosus) evaluation.

SE06.06

SKIN HYDRATION, SKIN BARRIER DAMAGE AND INFLUENCE OF TOPICAL PRODUCTS ON KERATINOCYTE BIOLOGY EVALUATED BY REFLECTANCE CONFOCAL MICROSCOPY

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Background: Skin hydration and barrier function play a crucial role in maintenance of skin homeostasis. Their in-vivo assessment is of great interest for clinical perspectives and research, in particular for the study of irritants and cosmetic products. In-vivo Reflectance Confocal Microscopy (RCM), due to noninvasive visualization of epidermis and upper dermis at nearly histologic resolution, enables to determine skin microstructure and kinetics after the application of topical products. Here we define RCM features associated with dry skin and barrier damage, and the microscopic effects of different products on hydration and barrier damage prevention. Skin hydration: the most relevant RCM parameters for skin dryness and moisturiser efficacy were 'Furrow's size and regularity', 'Scales', 'Skin surface irregularity' (uneven stratum corneum reflectivity), and 'overall inter-keratinocyte reflectance' (contrast between keratinocytes' borders and cytoplasm at granulosum-spinous layer). Application of moisturisers determined a rapid reduction of furrow's size, a more regular morphology of furrows and a more even surface, with consistent reduction of scales. Inter-keratinocyte reflectance increased with a 30-60 min delay. RCM

demonstrated that moisturizers affect not only skin surface (corneocytes) but also intra-epidermal keratinocyte layers, probably influencing the composition of the interkeratinocyte space with a delayed mechanism and increasing cell-to-cell cohesion. Skin barrier damage: the most relevant RCM parameters identified after 24h of 5% SLS occlusion consisted in 'detached corneocytes', 'tragetoid keratynocytes' and 'irregular keratinocyte architecture' in the granulosum-spinosum layers, and 'sheer bright keratinocytes' in basal layer. Application of Vitamin-E 30 minutes before SLS showed a marked reduction of all RCM 'skin barrier' parameters.

SE06.07

IMAGING DRUG DELIVERY TO THE SKIN USING COHERENT RAMAN SCATTERING MICROSCOPY

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The effective treatment of dermatologic diseases requires efficient drug delivery to the skin. At present, a widely-used approach to examine this involves removal of the SC by sequential adhesive tape stripping at specific time points, post-application of a formulation. This enables determination of the concentration profiles of the active and key formulation ingredients; however, this method is both invasive and labour-intensive. Our objective is to use coherent Raman scattering microscopy (CRS) to image drug disposition in the skin following application with excipients typically used in dermatological formulations. This label-free imaging tool enables the acquisition of high resolution 3D images of multiple chemical components of a formulation, as they penetrate the skin, without destructive analysis. Preliminary studies have demonstrated different rates of drug penetration via hair follicles compared to intercellular pathways across the SC, in addition to the direct visualization of drug crystal precipitation at the skin surface [Saar B.G., Contreras-Rojas L.R., Xie X.S., Guy R.H. *Mol. Pharm*, 2011, 8: 969-975]. CRS microscopy was also used to observe the distribution of particles applied to skin which had been pre-treated with a poration device. Samples were imaged using coherent anti-Stokes Raman scattering microscopy (CARS) to reveal the architecture of the skin, in conjunction with either two photon fluorescence (TPF) or stimulated Raman scattering (SRS) to visualize fluorescent or deuterated particles, respectively. The images obtained offer visual information and chemical specificity, and are uniquely able to reveal information, which would not be attainable from techniques such as tape stripping.

SL07

OPTICAL DIAGNOSTICS FOR DOCUMENTATION AND ANALYSIS OF SKIN ATTRIBUTES

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The skin is perceived optically (visually) and its condition has been evaluated by professionals for centuries by sight and touch. To our knowledge there have been many attempts to characterize and quantify the visual information obtained from skin instrumentally since the 1920's. The advent of compact imaging instruments, powerful compact computers and almost unlimited digital storage capacity have brought about the capability of easy non-invasive documentation of skin attributes. Along the way there have been many misconceptions of what may be recorded through easily accessible instrumentation -such as 'skin color', and the amount of data generated in these attempts has been increasing making it difficult to process effectively. On the other hand a great deal of work has been carried out in single point measurements and the quantification of specific chromophores or fluorophores in skin. Of course the analysis is limited by the penetration depth of the probing radiation be it visible, ultraviolet or infrared. Thus besides images that resemble visual perception with appropriate filters at the excitation and emission sites maps of concentration and distribution of many chromophores may be generated. Spectral imaging, imaging at specific bands, has been used to provide valuable information on skin attributes. The number of endogenous to the skin molecular species that may be thus probed is greater than 12. These specialized imaging tools may be used to document skin attributes from the microscopic to the macroscopic level in cosmetic and in clinical applications. In recent years microscopic imaging with specialized optical tools has yielded exciting imaging information *in vivo* - these include reflectance confocal microscopy, two-photon fluorescence and second harmonic generation, confocal Raman and CARS imaging. Optical diagnostic methods for assessing skin attributes will only expand with time in number and in capabilities as the photonic technology expands with demands from the communications industry and computing.

SE07.01

ASSESSING FACIAL WRINKLES: AUTOMATIC DETECTION AND QUANTIFICATION

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As people mature, their skin gradually presents lines, wrinkles, and folds that become more pronounced with time. Skin wrinkles are perceived as important cues in communicating information about the age of the person. Nowadays, documenting the facial appearance through imaging is prevalent in skin research, therefore detection and quantitative assessment of the degree of facial wrinkling can be a useful tool for establishing an objective baseline and for assessing benefits to facial appearance due to various dermatological treatments. However, few image-based algorithms for computationally assessing facial wrinkles are present in the literature, and those that exist have limited reliability. In this work, an algorithm for automatic detection of facial wrinkles is developed, based on estimating the orientation and the frequency of elongated spatial features, captured via digital image filtering. The algorithm is tested against one set of clinically validated 11-point wrinkle scales present on the face. Furthermore, the algorithm is employed for assessing the presence of forehead furrows on a set of 100 clinically graded facial images. The proposed computational assessment correlates well with the corresponding clinical scores. We find that the results are in better agreement with clinical scoring when the wrinkle depth information, approximated via filter responses, is combined with the wrinkle length information as opposed to the case when the two measures are considered separately.

SE07.02

MANIPULATION AND ANALYSIS OF MASSIVE IMAGING DATA

Greg Paynok

Abstract not available at the time of printing.

SE07.03

EVALUATION OF FACIAL AGING BY SPATIAL FREQUENCY ANALYSIS OF DIGITAL IMAGES

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Current technologies enable us to analyze various parameters of facial aging, including wrinkles and sagging. The objective of this study was to examine a methodology designed to evaluate age based on an image of the entire face, rather than a specific part of it, using spatial frequency analysis (SFA) technology. With the digital images created, we performed SFA using MATLAB software. We used linear regression analysis and then multiple regression analysis to study frequency band and its relationship to aging. The multiple correlation coefficient, R, was 0.945, indicating a high correlation among these variables. We then performed cluster analysis of power values of SFA to classify the characteristic frequency; this frequency was a power value that was classified into five domains. We created an image of each domain from the original image, and estimated character of each domain. Domain 1 was related to brightness and firmness; Domain 2 to sagging, facial lines, and facial features; Domain 3 to deep wrinkle formation; Domain 4 to fine wrinkles and rough skin; and Domain 5 to the texture and smoothness of the skin. In conclusion, we classified the frequency of facial characteristics into five domains by evaluating complete facial images of women using SFA. From the power value of each domain, we established a method that could be very useful for estimating the age of a woman based on her facial appearance. This new integrated approach using SFA resulted in a more objective and accurate evaluation of facial aging than those previously available.

SL08

SECTOR EXPANSION AND ELLIPTICAL MODELING OF BLUE-GRAY OVOIDS FOR BASAL CELL CARCINOMA DISCRIMINATION IN DERMOSCOPY IMAGES

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Blue-gray ovoids (B-GOs) are a critical characteristic of basal cell carcinoma (BCC). They differ in size, shape and color and therefore other benign lesions may show similar characteristics. This is why it is important to analyze these structures in order to accurately detect them and also to provide knowledge for automatically detecting them. This study uses a total of 199 dermoscopy images. 68 of these images contain biopsy proven BCCs with B-GOs and 131 are dermoscopic images of benign lesions which contain B-GO mimics. This study used a segmentation method to expand the masks applied to the B-GOs and B-GO mimics. For logistic regression analysis 22 features were used; 21 color features and 1 size feature. Blue chromaticity showed to be the most significant feature for defining B-GOs. In order to optimally discriminate B-GOs a best-fitting ellipse was applied to the expanded B-GO masks. As a result the logistic regression analysis was able to discriminate expanded B-GO structures from B-GO mimics with a classification rate as high as 96.5%. This analysis result shows that color features are significant for accurate expansion of B-GO masks and the best-fitting ellipse modeling allows high discrimination of B-GOs from benign structures.

SE08.01

MODELLING SKIN COMPLEXION RADIANCE FOR QUANTITATIVE MEASUREMENT OF BETTER LOOKING SKIN

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Objectives: Skin complexion radiance is influenced by factors such as color, skin tone evenness, translucency, and surface texture. Currently, there has not been a comprehensive parameter which objectively measures skin complexion radiance. This study was aimed to develop such a method. Methods: A clinical study was conducted with a panel of 34 human volunteers who were directed to use a topical radiance-enhancing product 2–3 times a week for 6 weeks. Polarized facial images were captured at baseline and after product treatment at the end of study. In-house developed image analysis algorithms were used to measure each of the individual skin parameters listed

above. A model of skin complexion radiance index (CRI) was established as a function of those measured parameters, and a consumer perception study was conducted to help determine the functionality of CRI. Seventy Asian female panellists were asked to pick the better looking skin from each of the 34 pairs of blinded clinical images of before and after product treatment and results were correlated with CRI. Results: The CRI thus obtained yielded values within a 0–100 scale. When these results were compared with that of the consumer perception study, there was a 88.2% agreement between two methods in detecting skin complexion radiance changes after product treatment. The CRI value showed a 23.3% average improvement over baseline, with a statistical significance of $p=0.0022$. Conclusion: The novel parameter of CRI captures the basic properties of skin appearance and can describe changes in complexion radiance meaningfully.

SE08.02

AN INSTRUMENT FOR THREE DIMENSIONAL MEASUREMENT OF SKIN SURFACE TOPOGRAPHY

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A digital imaging device has been developed for the non-contact three dimensional measurement of skin surface topography using stereo photogrammetry. The optical measurement method differs from some other devices in that it does not require structured light or lasers so can be safely used in the proximity of the eye. The topographic map of the skin is calculated from the disparity between matched micro features in the left and right images of a stereo pair. The device originally developed for monitoring changes in the volume of chronic wounds has found applications in maxillofacial and cosmetic surgery to forensic recording of injuries. A ‘micro’ version of the device has recently been developed specifically for the measurement of fine wrinkles, small moles and melanoma. The instrument is compact and hand held and can be used in domiciliary applications. Data is stored in a format that can be transmitted electronically and can be viewed in with appropriate three dimensional technology for remote diagnosis. Data is presented on the calibration process which required the development of a motor controlled optical bench. A set of three dimensional verification targets were developed in conjunction with the National Physical Laboratory (UK). These targets were used to verify the accuracy and precision of the instrument; accuracy 1.0%, precision 2% of the measured volume. Examples will be presented of the assessment of volume changes before and after treatment for chronic wounds and also for cosmetic treatments with facial fillers.

SE08.03

EYE OPENING ANGLE: A NEW PARAMETER TO ASSESS ANTI-AGEING SKINCARE PRODUCT EFFICACY ON ASIAN PANEL

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With ageing process facial volumes become less balanced. Eye area presents ones of the most important clinical criteria for lack of firmness evaluation. Among several parameters the sagging of eyelid is an essential target for anti-ageing efficacy. On this purpose a photographic scale was developed and published to allow clinician to evaluate in an objective way the visible impact of cosmetic application. Methods: Here we introduce the “Eye Opening Age” which translates clinical evaluation to a linear, continuous and universal parameter. This new assessment method was used in a clinical study on 50 healthy asian women who applied a cosmetic product. We evaluate the clinical sign before and 20 minutes after application. We demonstrated a strong correlation between atlas quotation and angle based on clinical typology studies. It consists in producing visual supports which correspond exactly to the average values of the panels we previously obtained with clinical scale at each time of evaluation by using interpolation algorithm known as morphing software. From the images produced, we calculate a new parameter by measuring the angle between two lines which represent the aperture between outer and upper eyelids. Results: By comparing the angle observed between T0 and T20mins we are able to demonstrate an increase in “Eye Opening Angle” corresponding to a significant 8° addition in aperture. Conclusions: By supplying precise clinical information about skin firmness on face this study gives a better understanding of local variation and their impact in global perception of the apparent age.

SE08.04

OPTICAL METHOD TO ASSESS FACIAL PORES VISIBILITY IN ASIAN POPULATION

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Enlarged or highly visible facial pores are involved in the perception of shininess and homogeneity of skin by consumers. Indeed impact of product on relief homogeneity is a key part of whitening, as well as anti-oiliness and anti-aging product evaluation. This work presents conception and demonstration of the accuracy of a new evaluation method which combines in-house software with Dermascore® device. We describe the principle of detection algorithm and the correlation of the threshold (250µm) with expert evaluation which validates the detection of pore visibility on Asian skin. After this validation phase we analyze the potential of this new evaluation method to assess visible facial pores on Chinese volunteers according to age and season influences. 400 healthy Chinese women aged 18 to 80 years were recruited in Shanghai in August 2007 [summer] and Jan. 2008[winter] and split in 5 years old groups ; Dermascore® with parallel-polarized light and pore analysis software were used to characterize pore distribution. Results show that the younger group has a lower density and smaller visible pores, which was validated by the Sensory Expert Panel evaluation. All age groups present a stable density of visible pores with smaller pore size than in summer. The new method allows us to obtain objective measurements of visible pore surface and density for a more accurate evaluation of the visible effects of cosmetic products.

SE08.05

AN INTEGRATED PROCESS FOR CLINICAL PHOTOGRAPHY

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Clinical photography is a widely used and well accepted parameter in clinical trails. Images used for documentation, scoring or automated analysis need to be of high quality. However skin provides some challenges because of low contrast and partial translucency. Additionally movement of subjects and safety aspects are practical issues in high quality imaging. The technical quality of images has increased due to development of new cameras and better imaging equipment. However, excellent clinical images depend not only on the technical equipment. At least as important are technical tools e.g. positioning tools or capture software that simplifies image capturing. It was shown that the post-processing of digital images is also essential for the overall quality. Clinical images of high quality can only be generated if the complete process from image taking to final evaluation whether this is grading or automated image analysis is designed accurately. We have initialized a program focused on designing a validated process for obtaining images of the skin in clinical trails. Every single working step was analyzed and embedded into a defined process. Within this program defined cameras and objectives have been chosen, consistent software was established, a fundamental training in general and clinical photography for technicians and all staff working with images as well as completely integrated post-processing has been established. Carefully chosen technical standard tests allow the study team to capture high quality images in individual study designs. All these single steps are enclosed in a documentary system to ensure validated images of high quality.

SE08.06

RANKING OF PAIRED STANDARDIZED FACIAL PHOTOGRAPHS TO EVALUATE ATTRACTIVENESS AND SKIN RADIANCE

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Ranking or scoring of highly standardized facial photographs is the method of choice to assess parameters like attractiveness, which can not be quantified by technical measurement of the images. A blinded presentation of pairs of photographs of the volunteers in which everything is kept identical except for the treatment effect enables a very sensitive scientific proof of cosmetic effects on parameters like attractiveness, facial radiance and other attractiveness correlated parameters. On highly standardized color photographs of 12 female stimulus persons the effect of decorative cosmetic on facial attractiveness was assessed by 6 trained raters and the volunteers themselves. The decorative cosmetic products were applied by beautician. Images were taken before and up to 6 hours after cosmetic treatment. Pairs of different points of time were presented to the assessors on color calibrated monitors for rating. As the substantivity of the decorative cosmetic was not given over a whole day, it was assumed, that product dependant effects of increased attractiveness would fade away during the 6 hours after

application. It was shown that the use of the decorative cosmetics had a clearly positive effect on the perceived attractiveness. The study results further revealed time dependant effects on attractiveness in accordance with the assumed fading of the products.

POSTER ABSTRACTS
THURSDAY, 29 NOVEMBER 2012

FP01

ELASTICITY OF FACIAL SKIN: A PRECISE THREE DIMENSIONAL MAPPING

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The assessment of skin mechanical properties using suction techniques is well known for decades. However most of the studies are designed with few measurement areas which gives only local information. The aim of the present work was to achieve a precise mapping of the skin elasticity on the face. Methods: Skin elasticity was evaluated on the face using a Cutometer MPA580® (Courage & Khazaka, Köln, Germany) with a 2 mm diameter opening probe. This device allows measuring various mechanical parameters and more particularly the ratio Ur/Ue (mm/mm, parameter named also R5) related to the skin "elasticity". For this study, 13 women (between 50 and 65 years old) have been recruited. For each woman 24 testing areas (on one half-face) were defined for the measurements. Results: Mean values of the skin elasticity were calculated on each of the 24 measurement points and were projected on a 3D geometry representing a female face. Using standard interpolation techniques and based on the mean values of the 24 points, a continuous elasticity mapping of the face has been obtained. Additionally, a statistical analysis was performed and led to split the face into 6 homogeneous areas of elasticity. Conclusions: By supplying precise information about the skin elasticity on the face this study gives a better understanding of local variation and can help in the design of studies dedicated to the efficacy of cosmetic products.

FP02

VISIBLE AND MEASURED FIRMNESS: A NEW GLOBAL APPROACH TO LINK ACTION ON BIOLOGICAL TARGET

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Lack of firmness is key parameters in age perception whatever the population. These manifestations could be seen both by people and entourage and perceived or perceived by touch. The challenge we want to achieve with these investigations is to be able to demonstrate link between tissues behavior and clinical assessment and so highlight the impact of changes in depth in visible appearance on surface. Methods: We use DermalTorqueMeter (DTM) on Cheek and developed and published clinical scales for firmness of lower part of the face to evaluate ageing process among Caucasian and Asian populations. After we adapt same protocol to study in 40 healthy Caucasian women the efficacy of cosmetic product before and after two months of twice-daily application. Results: We are able to demonstrate same evolution of clinical signs and data describing mechanical behavior. In addition the more impact of lack of firmness observed with clinical scales in Asian populations after 50 years old seems very link to a difference in DTM measurements which present highest values for Caucasian women. Regarding product efficacy assessment we are able to observe evolution in both internal and external parameters. Conclusions: With these new data in two different populations we show the visual and tactile impact of cosmetic products by acting on biological targets. By this way we establish new fields of communications of cosmetic efficacy.

FP03

IMPROVEMENT OF IMAGE ANALYSIS OF DERMATOLOGICAL IMAGES BY UTILIZING INTELLIGENT SCISSORS

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Quantitative image analysis has become an important evaluation procedure in dermatological studies: wound-healing is assessed by the visible wound size on standardized photographs or teeth-whitening product by intensity and color distribution. To compute meaningful parameters, the object-of-interest as wound border or tooth has to be located exactly. Although fully automated segmentation is desirable this is possible only for some specific tasks. Manual verification and refinement of the result is typically required but can be even more time consuming than

manual segmentation alone. However, manual segmentation is often inaccurate with high inter- and intra-operator variability. Interactive image segmentation provides the best of both approaches: Simple regions are automatically segmented and complicated regions by the operator who also verifies the segmentation result. We have evaluated the "Livewire"-algorithm (or "Intelligent Scissors") which is an interactive algorithm widely used in analysis of CT or MRI for extracting object contours. The operator selects a point on the objects boundary. As the mouse is moved, the "shortest path" to that point is computed in feature space– typically along the object boundary. The next mouse click confirms the path and starts the process again. This approach allows the segmentation with as few mouse clicks as needed: Along strong edges, contours are automatically detected; in complicated regions the operator provides the input. The poster provides overview of applications of the livewire algorithm in dermatological studies. We show how the evaluation of wound sizes and tooth-color data can be improved by interactive segmentation both in precision and required time.

FP04

COLOR-CONSISTENT CLINICAL PHOTOGRAPHY IN DERMATOLOGICAL STUDIES

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Study parameters based on digital images are well accepted parameters in dermatological studies. The accurate and sensitive reproduction of color information is a mandatory prerequisite for correct scoring and image analysis and, therefore, product evaluation. Skin redness and pigmentation can only be assessed if the color is correctly reproduced over the whole photographic time series AND the whole image processing chain – from acquisition to the displayed image. The color-consistent image processing starts with a study-specific setup of our Unit for Standardized and Reproducible Clinical Photography to ensure standardizes illumination and color capturing. Color-standards are captured in the same position as the volunteer and further color charts are placed inside the photographed area. The main step in image processing is the conversion of the camera specific RAW data into a digital image format. Conversion parameters as color temperature and exposure compensation are manually set to adjust the L*a*b*-values of captured color-standards to their standard values. Conversion of each image is verified by a software that automatically detects the color chart and compares the L*a*b*-values against the color-standard image. This image processing workflow provides color-consistent photographic time series for visualization and analysis of small color changes – thus facilitates more sensitivity in expert scoring and color parameter evaluation. The poster describes the improved image processing for an attractiveness study. We show results on color measurements at lips and skin over time with and without color-consistent image processing. We found that a carefully designed image processing workflow is crucial for product evaluation.

FP05

CHINESE PANEL BIOMECHANICAL PROPERTY INVESTIGATION BY CLINICAL TOOL DENSISCORE

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Aging induces change in skin structures in the dermis and cause modification of mechanical properties. The objective of this study is to evaluate the skin mechanical properties with the aid of instrumental Densiscore and clinical measurements with age on Chinese panel and validated the tool accuracy for cosmetic efficacy evaluation. Densiscore allows standard compression of 42% in the plane of the skin. It comprises of two blocks measuring 40x20mm affixed to the skin by means of double-sided tape. Clinical evaluation is mainly based on determination of the width of skin folds. During study 220 healthy Chinese woman aged from 20-80 was recruited and with 10 years old as subgroup. External upper arm were chosen as investigated area during study. During cosmetic clinical study, totally 40 volunteer was recruited with age from 40-65 years old. Product was used twice per day and with duration of 2 months time. The result shows skin folds on external upper arm change lineally starting from 20 years old and quick increase after 60 years old. During cosmetic clinical study, compare with untreated area the skin with product significantly increase skin mechanical properties after 2 months product application measured by densiscore.

FP06

GLOBAL EVALUATION PLATFORM FROM LOCAL TO GLOBAL ASSESSMENT ON PORES

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Dilated pores are one of major aesthetic concerns for Chinese women. The aim of this study is to highlight new instrumental platform which allow us to explore pore characterization from depth to surface for global assessment. Study was carried out in Jan 2012 with 120 Chinese female volunteers from 20 to 80 years old with 5 years as subgroup. Three devices were chosen to build a global instrumental evaluation platform on selected pores from papillary dermis through epidermis and finally surface. First of all study was done in an ultrasound approach with a 25mhz ultrasound imaging system plus an in-house software for analyzing dermis textures from 150um to 750um with 200um in depth. DermaScore an internal device well-described and published allow us to measure visible pores features at the surface of the cheek. DTM was used to determine the skin's mechanical properties through a torsion test with 3mm guard ring, the U_r/U_e & U_d/U_r was chosen as the marker on face. Result show that visible pores covering index has significant correlation coefficient with biomechanical properties and dermis texture parameter. The more visible pore severity, the less firmness. And following the duct associated to visible pore by consumer we could notice rearrangement of dermis fibers around the duct but also epidermis structure. These new global platform allow us to characterize completely the pores phenomena which help to evaluate product efficacy in more accurate way & biological targets.

FP07

MOLECULAR MECHANISM OF STRATUM CORNEUM ELASTICITY: INVESTIGATION BY RAMAN SPECTROSCOPY

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The elasticity of *Stratum Corneum* (SC) is important for skin suppleness and appearance. Different physiological phenomena can induce skin stress which generates feelings of discomfort and tightness. Up to date, the behavior of SC in strain and tension is not well known. To highlight the molecular mechanism of SC elasticity, we investigated the molecular modifications due to the strain of SC by using Raman spectroscopy. Raman spectroscopic measurements were carried out to determine, firstly, the effects of strains on the conformational order of the SC components; secondly, the effect of SC lipids extraction on the SC elasticity. To induce stresses, normal SC and lipid free SC samples were stretched with a step-by-step strain of 0.5%. Moreover, normal SC and lipid free SC fixed onto glass slides and unfixed ones were compared. Our results show that, on one hand, the lipid conformation (among others C-C stretching bands: 1130 cm^{-1}) and the keratin secondary structure (Amide I band: 1600-1710 cm^{-1}) have been found to be sensitive to the induced strains. On the other hand, skin integrity (presence of lipids) seems to have influence on the elasticity of the SC. This work proved the potential of Raman spectroscopy for the skin structure investigation by providing the link between SC elasticity and Raman features related to lipid i.e. skin barrier function and keratin conformation.

Keywords: *Stratum Corneum*; skin strains; skin elasticity; skin barrier function; Raman spectroscopy.

FP08

NATURAL MODULATORS OF VASCULAR INFLAMMATION AND INNATE IMMUNITY: A NEW GENERATION OF ANTI-REDNESS SKIN CARE

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Objectives: A new Anti-Redness (AR) skin care has been formulated with 5 patented active principles from natural technology, to modulate PAR-2, restore skin barrier function, decrease aspecific and neurogenic inflammation, and to regulate innate immunity and vascular factors. *In vivo* efficacy has been investigated. Methods: A single-blind, not comparative study, has been conducted under dermatological control. Thirty women, aged 18-50, with sensitive skin and erythemato-telangiectatic rosacea applied the AR skin care on the whole face twice daily for 55 days,

followed by a placebo for 15 days (remanence). New generation Laser Doppler performed on skin redness area vs healthy skin, cross polarized digital photographs, stinging test, clinical and tolerance evaluations have been performed at day 0, 27, 55 and 69. Results: Laser Doppler: Whereas a significant difference in perfusion unit is observed at T0 between skin redness area and healthy skin, at T+55 days no such significant difference was observed, which confirms an effect of the Cross polarized digital photographs: A decrease in the mean intensity of the vascularization is observed at T+27 days, T+55 days and T+69 days statistically validated. Stinging test: A significant decrease in the mean score (treated-control) is observed at T+27 days, T+55 days and T+69 days. Clinically, diffuse erythema, telangiectasia and sensitive skin sensations were statistically. Tolerance was evaluated as Very Good. Conclusion: Thanks to its active principles specifically dedicated to control molecular pathways involved in sensitivity and redness skin troubles, a new formulation has demonstrated a significant improvement of the redness of the face.

FP09

AGING-INDUCED CHANGES OF THE DERMAL PAPILLA STRUCTURE AND DERMAL FIBER-LIKE STRUCTURE IN FACIAL CHEEK REGIONS

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Objective: Changes in the inner skin structure with aging may alter the elasticity and color of the skin. Therefore, a precise understanding of the inner structure of the skin is important for the beauty and cosmetic industry. Although there are some studies of the alterations of the dermal papillae and dermal fiber structure with aging, the sample sizes are small, and the data are obtained from different sites. Thus, the alterations of the facial inner skin structures with aging remain unclear. In this study, we investigated the effects of aging on the facial skin dermal papilla and fiber structure. Method: Images of the inner skin structure were obtained from ninety Japanese women, ranging from twenty to sixty years, using *in vivo* confocal laser-scanning microscopy (Vivascope 1500 plus, Lucid, USA). The amount of dermal papillae structures were extracted from the images using various classification standards, and the characteristics, including the number and cross-sectional area of the dermal papillae, were analyzed. The characteristics of anisotropy and clarity in the reticular layer of the dermis were analyzed using the image analysis method of frequency analysis. Results and Conclusion: The results demonstrated that the number of dermal papilla was reduced and that the cross-sectional area of increased with age. Regarding the dermal fiber structure in the reticular layer, the clarity of the images decreased and anisotropy increased with age. These changes of the inner skin structures might relate to the skin surface characteristics of elasticity and skin tone.

FP10

STRATUM CORNEUM STRUCTURE AND COMPOSITION EVOLVE WITH AGE IN ADULTS

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Objective: To study the effects of aging on *Stratum Corneum* (SC) structure and composition as they affect the water barrier function. Methods: This study was conducted on 40 French women volunteers without history of skin diseases. They were divided into 4 groups of 10: 20-30, 30-40, 40-50 and 50+ years of age. Measurements were done on the cheek and on two skin sites on the arm (one relatively protected and one exposed). SC composition (water, lipid/protein ratio, cholesterol, and ceramides) was measured by Raman confocal microspectroscopy, skin surface hydration by skin conductance, and water barrier function by trans-epidermal water loss (TEWL). Results: Skin conductance does not change significantly and TEWL decreases slightly with aging. Water concentration profiles show a decrease of water content only very close to the skin surface (an observation that is missed by skin conductance). SC thickness increases significantly with age and the increase is faster for the arm sites compared to the face. Lipid composition appears to be constant and changes only for the protected site showing a decrease with age. The SC on the cheek has significantly higher TEWL, water and lipid content but is less thick compared to the arm sites. Exposure also affects water and lipid content and composition. Conclusions: Skin aging as well as body site and exposure to the environment (e.g. to the sun) can have a profound effect on the SC composition, structure, and water barrier function.

FP11

A NEW APPROACH TO EVALUATE *IN VIVO* BIOMECHANICAL PROPERTIES OF NAILS

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Objective: Recently, a lot of new products claiming hardener, moisturizing or whitening effects about nails are developed. In according to requirements introduced by the 1223/2009 Regulation, it is mandatory to find and validate instrumental methods able to verify product effects. The aim of this work is to present a versatile quantitative approach in which measurements of thickness and water content are associated to a compression test for *in vivo* evaluation of flexibility and elasticity of the nails. Methods: The method set up was performed using artificial nails. Afterwards, 20 healthy women participated to the study and two kind of experiments were carried out. Nail flexibility was determined measuring the force required to bend nail by a flat punch. The biomechanical properties of nails were assessed measuring the reaction force against a compressive force applied. Tip punches with different shape and contact angle were used for this purpose. Results: Parameters as nail shape, asymmetrical structure, curvature and strain position on distal nail zones were investigated. All these measurements required a thorough validation process in order to ensure the data reproducibility. As the thickness, the nail flexibility and hardness varied throughout its distal part. Biomechanical properties were very dependent from water content of the nail. Conclusion: The evaluation of nail biomechanical properties can be very useful to study nail conditions; moreover, the measurements of flexibility and elasticity of the nail, before and after cosmetic treatment, can be successfully employed to evaluate the effectiveness of hardeners or reinforcing cosmetic products.

FP12

IMAGING HAEMANGIOMA USING OPTICAL COHERENCE TOMOGRAPHY

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Objectives: OCT is an optical imaging technology that provides micrometer resolution *in vivo* in skin. Images are cross-sectional similar to ultrasound images but OCT images with infrared light instead of sound waves providing a micrometer resolution. Haemangiomas are benign vascular tumours occurring in early childhood, and while a majority regress over time some require therapy. This study explores the potential of OCT imaging of haemangiomas as a prelude to monitoring lesions for early signs of spontaneous resolution or positive response to therapy. Methods: The OCT system (Vivosight, Michelson Diagnostics, UK) is a low intensity, 1310nm laser light, non-contact, non-invasive real-time imaging device. The OCT system acquires both 2D and 3D images. Results: OCT characteristics of haemangiomas are presented as a first step towards creating a diagnostic guide that positively identifies these tumours and suggests morphological changes predictive of evolution or response to therapy. Conclusion: Monitoring and planning of propranolol treatment is a promising aspect of the findings in this study. During the past 5 years it has been demonstrated an effective treatment for problematic haemangiomas. OCT has the potential to measure the diameter and depth of cutaneous vessels, and to identify characteristic hallmarks of haemangioma ultrastructure. The data suggest the need for further clinical studies of the predictive value of these hallmarks during treatment.

FP13

MILIA AND OTHER SCARS IN OPTICAL COHERENCE TOMOGRAPHY

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Objectives: If OCT is used to non-invasive imaging of non-invasive treatment of skin cancer, it is necessary to correctly identify scars and other consequences of tissue destruction by e.g. immune-mediated therapies. In order to enhance the utility of post-treatment imaging scarring and other changes must be differentiated correctly from residual neoplasms. The OCT appearance of different scars has not previously been published. Methods: A low intensity, 1310nm laser light, non-contact, non-invasive real-time imaging OCT device (Vivosight, Michelson Diagnostics, UK) was used on an available sample of scars in vivo. Scar tissue OCT was compared to imaging of normal adjacent skin. A catalogue of different scars is collected and presented. Results: OCT characteristics of various scars are presented as a first step towards creating a diagnostic guide that positively identifies non-neoplastic residue after non-invasive therapy. Conclusion: OCT appears to identify scars, and therefore be of potential use in the assessment of out-comes following non-invasive therapy of skin cancer. Additional studies are needed to identify the specificity, sensitivity and positive predictive value of the suggested hallmarks of scars and other non-neoplastic residue following therapy.

FP14

A QUANTITATIVE MODIFICATION OF MASI SCORE USING UV IMAGE ANALYSIS FOR PIGMENTARY SKIN DISEASES

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Background/Purpose: Generally, severity of pigmentary skin diseases are evaluated subjectively by visual scoring methods such as MASI. Therefore, it is necessary to develop a more scientific evaluation system for evaluating severity of melasma. Methods: All of the images were taken in a dark room in order to minimize the effect of surrounding visible light and maximize the contrast between the subject and surroundings. To modify the conventional MASI, we actually measured the area surface of the face. New MASI score was calculated under our optical image system. To reduce the curvature error in facial contours, gradient mask was used. Darkness and homogeneity were measured by our UV image analysis system. Four patients with melasma were analyzed by new MASI score and conventional MASI score evaluated by five experienced dermatologists. Result: The new MASI score measured by UV optical imaging system can objectively measure the severity of melasma. The new MASI score is capable of quantifying the slight severity difference among patients making it more detailed than the numerical scales of conventional MASI score. Discussion: This study introduced a new MASI score using UV optical imaging system for objective analysis of severity in melasma. Based on the results, this optical imaging analysis system could be used as a valuable tool to assess the severity of various pigmentary skin diseases including melasma.

FP15

QUANTITATIVE ASSESSMENT FOR EXTRAMAMMARY PAGET'S DISEASE USING ULTRAVIOLET IMAGING SYSTEM AND PHOTODYNAMIC THERAPY

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Background/Purpose: Extramammary Paget's disease (EMPD) is a rare adenocarcinoma of apocrine gland-bearing skin affecting mainly the genitalia and axilla in elderly patients. The recurrence after surgical therapy is common due to indefinite margin of the lesion and the multifocal nature of the tumor. Methods: A 69-year-old Korean female visited dermatology department with erythematous, non-pruritic confluent eczematous patch on perivulvar area. The patient had a history of EMPD which was treated with wide vulvectomy and topical imiquimod by gynecology department in 2009. She revisited our department due to suspicious recurrence, and recurrence of EMPD was diagnosed by biopsy. We perform PDD to clearly visualize the margin of the lesion. Topical 5-aminolevulinic acid (5-ALA) was applied under occlusion. Six hours later, we removed 5-ALA and illuminated the lesion with ultraviolet (UV) light. Under UV illumination, we marked the margin of the fluorescing

area. Result: Previously ill-defined tumor margin was clearly delineated by the fluorescence emitted by 5-ALA. The lesion was subjected to PDT using intense pulsed light (IPL). Follow up biopsies from three different sites were taken after 1 month. All sites showed no sign of EMPD. Discussion: Photodynamic diagnosis (PDD) is a non-invasive diagnostic method that can detect surface tumor by applying photosensitizer and scanning it with fluorescence examination. With PDD, the margin of the lesion can be more accurately clarified. It seems that PDT with assistance of PDD to be a useful alternate treatment method of EMPD.

FP16

IN VIVO NIR SPECTROSCOPY AS AN OBJECTIVE AND NON-INVASIVE MEASURE OF PSORIASIS SEVERITY

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Accurate documentation of disease severity is a prerequisite for clinical research and the practice of evidence based medicine. Quantification of skin diseases such as psoriasis currently relies heavily on clinical scores. Although these clinical scoring methods are well established and very useful in quantifying disease severity, they require an extensive clinical experience and carry a risk of subjectivity. The aim of the present study was to explore the correlation between local clinical scores and non-invasive *in vivo* NIR (Near-Infrared) spectra of the skin in psoriasis patients. A further objective was to build a predictive PLS (Partial Least Squares) regression model for objective measurement of the severity of a given psoriatic plaque based on NIR spectroscopy. *In vivo* NIR spectra were recorded from a total of 31 psoriasis patients. For each patient two spectra were obtained from a psoriasis plaque and one spectrum was obtained from clinically uninvolved psoriatic skin serving as a regional control. Furthermore, each measured lesion was assigned a clinical severity score based on an assessment of erythema, induration and scaling. These components are used to score the severity of plaques in standard score systems such as PASI (Psoriasis Area Severity Index) and each parameter was scored 0 – 4. A PLS regression model was used to analyze and predict the severity scores on the NIR spectra of psoriatic and uninvolved skin. The correlation between predicted and clinically assigned scores was $R^2 = 0.89$ (RMSE = 1.01), suggesting *in vivo* NIR provides accurate clinical quantification of psoriatic plaques.

FP17

COLOR DOPPLER ULTRASOUND CHARACTERIZATION OF GLOMUS TUMORS OF THE NAIL

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Background: Glomus tumors (GTs) arise from the neuromyoarterial apparatus commonly on the nails. They usually are very painful and the treatment is surgical. Recurrence in patients without imaging support has been described in up to 20% of the cases. Objective: To assess the sonographic morphology of GTs. Methods: Retrospective study of the GTs cases sonographically diagnosed and histologically confirmed (2010-2012). Results: 22 GTs cases (82% female/18 % male, mean age: 47 year-old; range:21-80) were studied. 95% of the cases involved the fingernails, 5% toenails. 68.1% affected the thumb, 13.6 % index finger, 9% ring finger. Left side : 59% and right side: 41%. 100% were single, oval shaped and hypoechoic. Location in the nail bed was: proximal : 64%; distal: 27 %,middle third: 9%. Mean transverse axis was 4.1 mm (range 2.4-7.4); depth axis was 2.9 mm (1.5-5.7) and longitudinal axis was 5.7 mm (range 2.9-10). 59% of the cases (n=13) showed a depth axis < 3 mm. Mean thickness of the tumor blood vessels was 1 mm (range 0.6-1.4) and peak systolic arterial velocity was 11.3 cm/sec (range 4.7-15.7). 86% presented scalloping of the bony margin of the distal phalanx. Conclusion: Color Doppler ultrasound provides relevant anatomic presurgical information in GTs which includes tumors < 3 mm.

FP18

SONOGRAPHIC APPEARANCE OF GLOMANGIOMAS

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Background: Glomangiomas (GMs), also called glomuvenous malformations are rare soft tissue anomalies that differ from glomus tumors in their clinical presentation. Commonly GMs affect children and young adults, show a non-ungual location and are asymptomatic, sometimes with a multiple and linear pattern. Objective : To assess the sonographic morphology of glomangiomas. Methods: Restrospective study of the morphology of the GMs sonographically diagnosed and histologically confirmed (2010-2012). Results: 15 cases (73% female/27% male; mean age 37 year old (range: 2-67). Solitary : 73% and multiple: 27%. Right side: 53% and left side: 47%. 100% were oval shaped, hypoechoic and showed low flow arterial vessels. 13.3% presented low venous flow. Hand location:26.6% (25% hypothenar eminence, 25% index finger, 25% middle finger and 25% little finger); leg: 13.3%; lumbar region: 13,3% thigh: 13.3; knee : 6.6%; foot: 6.6%; arm: 6.6%; forearm: 6.6%; scalp:6.6%. 93% were located in the subcutaneous tissue. 7% presented scalloping of the underlying bony margin. Multiple GMs were located in the hypothenar eminence, arm, thigh and lumbar region. Mean transverse axis: 5 mm (range 0.9-7.8 mm); 4.5 depth axis (range 0.7-12.3) and longitudinal axis: 5.2 (range 1.3-9.9). Mean thickness of the lesional vessels was 1 mm (range 0.8-1.2) and peak arterial systolic velocity: 12.8 cm/sec (range 2 - 31.1 cm/sec). Conclusion: Color Doppler ultrasound is an adjunct tool that shows presurgical anatomical information of GMs. Arterial low blood flow vessels, which included submillimeter vascularity, were detected in all lesions. Venous flow was rarely detected in the tumors.

FP19

SONOGRAPHIC CHARACTERIZATION OF PILOMATRIXOMAS

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Background: Clinical diagnosis in pilomatrixomas may be difficult and misdiagnosis rate in has been reported in up to 56% of the cases. Objective: To assess the sonographic morphology of pilomatrixomas. Methods: A retrospective study of the cases of pilomatrixomas sonographically diagnosed and confirmed by histology (years 2010-2012). Results: 105 cases were obtained (62.7% F/ 37.2%M; mean age 17 year old ; range 5-82 year old). 92% single and 7.5% multiple (≥ 2). Location: 48.5% face; 19% upper extremity; 10.3% neck; 8.5% lower extremity;13.7% rest of the body. 64.6% involved dermis and subcutaneous tissue; 32.6 % only subcutaneous tissue; 2.8% only dermis. Echostructure was: target pattern (hypoechoic rim and hyperechoic center) in 66.3%; hyperechoic 21.2%; heterogeneous 8.7% and hypoechoic 3.8%. In 3.8 % anechoic cystic areas were detected within the tumor. Mean transverse:5.98 mm (range 0.5-19 mm); mean thickness: 3.59 mm (range: 0.3-12 mm); mean longitudinal :6 mm (range 0.5-19 mm). Mean area: 157.13 cm² (range: 0.07 – 2148.44 cm²). 68% showed vessels, 95.8% low flow vessels(<15 cm/sec). Vessels distribution was 79.4% peripheral, 2.9% intralesional and 17.7% mixed. Mean thickness of the vessels: 0.94 mm (range: 0.5-1.7 mm). Main flow was 61.4% arterial, 2.8% venous and 35.8% mixed. Maximum arterial peak systolic velocity was 10.11 cm/sec (range 5.3-23.1 cm/sec). 88.5% presented calcium deposits and 26% showed posterior acoustic shadowing artifact.1.9% presented extrinsic compression of the underlying muscle. Conclusion: Ultrasonography can provide detailed anatomical information in pilomatrixomas which may support the diagnosis and surgical planning.

FP20

THE POSSIBILITY OF MONITORING USING THE HIGH-FREQUENCY ULTRASOUND IN PATIENTS WITH THE PRIMARY CUTANEOUS T-CELL LYMPHOMA

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Objectives: Mycosis fungoides (MF) is the most common form of the primary cutaneous lymphoma, characterised by a malignant proliferation of CD4+ cells. Narrow-range UVA1 irradiation at wavelengths of 365-375 nm is used in the treatment of skin diseases aetiologically linked to T-cells, such as atopic dermatitis. Methods: We present a case report of six patients with histopathologically confirmed MF. Before introduction of UVA1 phototherapy, the following examinations were conducted: physical examination with lymph node assessment, full blood count, chest

X-ray and abdominal ultrasound. UVA1 irradiations were conducted using GP-24H Cosmedico device (Germany). A high-frequency ultrasound was conducted in this case, using a linear probe (20MHz; Dermoscan C Cortex, Denmark). The examination was performed at least twice: prior to the treatment and after it was concluded, each time in the same location. Results: The patients were treated with 1630-2710 J/cm² UVA1 given in 29-40 fractions and the effect was assessed by clinical examination and by high-resolution ultrasound. After termination of UVA1 irradiation complete clinical remission was observed in all cases. Biopsies were taken in all of the patients before and after UVA1 therapy. Histopathological remission was observed. A low-echogenic band beneath the echo entry (subepidermal low-echogenic band - SLEB) was observed on ultrasound. During the treatment it gradually became thinner, down to complete disappearance in 5 out of 6 presented cases. Complete clinical remission was achieved in all cases. Conclusion: The presented case shows the possibility of monitoring in patients with mycosis fungoides. High-resolution ultrasound examination can confirm the clinically observed improvement in dermatological condition.

FP21

HF-USG AND CUTOMETER IN MONITORING OF SCLERODERMOID CGVHD PATIENTS WITH JOINT CONTRACTURES

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The purpose of this study was to illustrate the skin sclerotic lesions of two chronic sclerodermoid GvHD patients using two noninvasive methods. Two adult patients (23 year-old female and 52 year-old male) suffering from chronic sclerodermoid GvHD (cGvHD) were involved in the study. Typical sclerotic skin lesions affected their upper and lower extremities leading to dermatogenic contractures and difficulty in walking. Patients were treated with the medium dose regimens of UVA1 up to total dose 1000J/cm² and 1380J/cm² respectively, delivered by GP-24H device (Cosmedico, Medical Systems, Germany). Skin elasticity was determined using a suction skin elasticity meter, Cutometer MPA 480 (Curage & Khazaka, Cologne, Germany). Skin thickness and density were established by 20 MHz high frequency ultrasonography (HF-USG) before and immediately after phototherapy (Derma Scan Cortex Technology, Denmark). Clinical improvement after phototherapy resulted in increased skin elasticity: a small fold of the skin could be lifted as well as the range of lower leg joints movement was wider. The value of skin mechanical parameters before and immediately after UVA1 treatment in both patients revealed noticeable improvement of skin elasticity. In HF-USG with 20 MHz linear probe performed prior to and after the treatment of skin of the forearm and dorsal part of the foot. Marked decrease of echogenicity as well as reduction of skin thickness in comparison with baseline values was noticed. HF-USG and cutometer seems to be a useful, non-invasive and repeatable device in monitoring patients suffering from various sclerotic skin disorders such as cGvHD.

FP22

ULTRASOUND STAGING IN HIDRADENITIS SUPPURATIVA WITH CLINICAL CORRELATION

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Introduction: Hidradenitis Suppurativa (HS) is a chronic inflammatory disease of skin. To date the diagnosis and staging is based on clinical criteria. Objective: To correlate the clinical and ultrasound findings in HS and assess the role of ultrasonography in disease staging. Methods: A retrospective study of the ultrasound examinations of the HS cases was performed (January 2010- May 2012). The patients were clinically classified (Hurley) by 3 dermatologists through teledermatology. A radiologist performed the ultrasound examinations and scorings (Hurley- adapted ultrasound scoring). A double blind analysis of the clinical/ultrasound findings and stages was performed. The statistical analysis included the mode of the clinical classification using bivariate analysis of categorical variables. A kappa test was used to evaluate concordance (95% confidence interval). The study was approved by the Institutional Review Board. Results: 34 HS patients were evaluated, 80% female, mean age 26.7 ± 10 years. The most frequent locations were: axillae, 82.4% (n=28), groin 26.5% (n=9), axillae and groin 17.7% (n=6). Other locations included the inframammary, retroauricular and thoracic regions, 3% for each one. A significant correlation (bivariate analysis p < 0.05) was observed between the clinicians. When comparing the clinical and the sonographic staging underestimation of the severity and staging by the clinical examinations was founded (K= 0.343, p=0.009). Conclusion: Clinical examination can underestimate the severity and stage of HS. Ultrasound may be a diagnostic tool to assess extension, type of lesions and disease stage. The provision of this information may support a better and early management in these cases.

FP23

DIET WATER SEEMS TO INFLUENCE SKIN HYDRATION AND BIOMECHANICS

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The feeding habits of a given population were studied, specially regarding its daily regular water intake (diet and beverages) and tried to relate with those skin biometrical variables. This transversal study involved forty healthy volunteers, female, (mean 26,45±7,95 y.o.), after informed written consent. All procedures respected Helsinki principles and respective amendments. A Feeding Frequency Questionnaire (FFQ) previously validated for the Portuguese population was applied. Transepidermal water loss (TEWL, Tewameter TM300), epidermal hydration (Corneometer CM825) and skin's biomechanics (Cutometer CM575) were the cutaneous variables chosen. Measurements took place in several anatomical regions (face - zygomatic, forehead; ventral forearm and hand; leg external face). Statistics involved a one-way ANOVA and a Tukey test adopting a confidence level of 95% . The FFQ has shown fairly heterogeneous consumption habits regarding the daily water intake (and these may be related with the significant differences detected. The forearm hydration is different between the age groups under 30's and 40's y.o. They also differ for the total deformation recovery at the end of stress-off period (Ua) of the hand. Significant differences were also observed in total extensibility (Uf) and total deformation recovery at the end of stress-off period (Ua) of the forearm, hand and zygomatic , viscoelasticity index (Uv/Ue) and total deformation recovery at the end of stress-off period (Ua) in hand, and biological elasticity in the zygomatic area. No significant changes were detected regarding the barrier function. These differences seems to depend primarily from volunteers's feeding habits suggesting new interesting directions for the present research.

FP24

ANTIOXIDANT POTENTIAL AND SAFETY OF PURE GREEN COFFEE OIL

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The majority of active ingredients in cosmetics are plant extracts, vitamins, proteins and minerals. Natural oils, in special green coffee oil, can be used as active ingredient or adjuvant because of its rich composition in free fatty acids. Green coffee oil presents proved efficacy on skin hydration and can absorb UVB radiation (Wagemaker et al., Ind Crop and Prod, 2011, 33, 469-473). Additionally, it is cited in the literature as showing anti-inflammatory properties (Dias et al., J Agric Food Chem., 2010, 58, 88-93). Thus, the aim of this study was to evaluate the safety and the antioxidant potential of pure green coffee oil and several stable formulations containing 2.5, 5, 10 and 15% of this oil. The toxicity was evaluated by the MTT assay in human keratinocytes cell line HaCat and the brine shrimp assay. The DPPH assay was used to evaluate the antioxidant capacity. The means were compared by Tukey test ($p < 0.05$). The green coffee oil presented a weak antioxidant potential with IC50 value of 42% while the IC50 BHT value under the same conditions was 0.002%. The MTT and brine shrimp assays suggested that the pure green coffee oil, as well as the tested formulations containing this oil, are devoided of relevant toxicity.

FP25

TEXTURE ANALYSIS OF SKIN MAGNETIC RESONANCE IMAGING DATA: A FEASIBILITY STUDY

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Magnetic resonance imaging (MRI) is a non-invasive imaging technique that has high soft tissue contrast and spatial resolution, making it especially interesting for the study of skin. Texture analysis (TA) is a method for characterizing regions in an image by their texture content as provided by variations in pixel gray levels. Here, the feasibility of using TA to the study of MRI skin images was tested. The wrist skin of 4 male volunteers (23, 31, 46 and 62 years old) was imaged using an 8-channel wrist coil and a 1.5T MRI scanner. A 2D T1-weighted spin-echo sequence (SE) was used to plan for image acquisition. A 2D high-resolution T1-weighted SE sequence was used to

image skin layers (sequence parameters: TR/TE=500/20ms; flip angle=90°; number of averages=2; number of slices=1; in-plane resolution=110x110 μ m², slice thickness=3.0 mm; scan time=6:48). Images were visualized and the epidermis, dermis and hypodermis were segmented in Osirix software. TA of the segmented skin layers was done using entropy and gray-level co-occurrence matrix (GLCM) functions of MATLAB's Image Processing Toolbox. Mean values of entropy and GLCM-derived parameters (contrast, correlation, energy and homogeneity) were calculated and compared for each skin layer. TA analysis showed that the hypodermis had the highest entropy, correlation and homogeneity values and also the lowest contrast. Conversely, the epidermis showed the lowest entropy and correlation but the highest contrast. Finally, the dermis showed intermediate values. This work showed that TA is able to differentiate skin layers; therefore it could provide new quantitative parameters for skin characterization.

FP26

WAVELET AND DETRENDED FLUCTUATION ANALYSIS OF SKIN MICROCIRCULATION: ASSESSMENT OF PERFUSION RESTRICTION IN HEALTHY SUBJECTS

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Laser Doppler Flowmetry (LDF) signals of skin microcirculation are complex and hold information regarding different physiological components: heart, respiration, myogenic activity of the vessel wall, sympathetic activity and endothelial metabolic activity. Here, a combined approach of Wavelet Analysis (WA) and Detrended Fluctuation Analysis (DFA) was tested for its ability to detect physiological changes to restriction of blood perfusion. 18 female healthy subjects were studied: 9 young subjects (20.3, 44.0 years) and 9 older subjects (50.0, 64.1 years). LDF measurements were made at the ankle site during 30 min: 10 min resting; 10 min during perfusion restriction of the ankle with a cuff, and 10 min after cuff release. Signals were sampled at 32 Hz and segmented in 3 phases: resting, transient response immediately after cuff release and recovery. Data were analyzed using MATLAB's Wavelet toolbox and DFA algorithm. Wavelet amplitude ratios (AR) and DFA-derived alpha parameter were calculated for each physiological component and compared between phases and groups. It was observed that, immediately after cuff release, there was a decrease in sympathetic and an increase in metabolic ARs. This could be explained by reactive hyperemia following reperfusion. Additionally, it was observed that older subjects showed a metabolic alpha parameter significantly decreased in the recovery phase with respect to the resting phase, while in younger subjects the alpha parameter returned to resting values. This could be related to changes in NO release from the endothelium in older subjects. The use of WA + DFA shows potential for studying the regulation of skin blood-flow.

FP27

EFFICACY ASSESSMENT OF COSMETIC FORMULATIONS BY DYNAMIC TEWL ANALYSIS

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The dynamic approach based on the mathematical modelling of TEWL values following a Plastic Occlusion Stress Test (POST) has been recently refined, since the conventional 30 minutes evaluation is time consuming. The aim of this work is to confirm that a reduction in the time of data collection has enough sensitivity to assess the efficacy of a moisturizer. Fifteen female healthy volunteers participated in the study. On D0, an occlusive patch was applied in the volar forearm of each volunteer for 24 hours. After patch removal, TEWL was measured continuously for 15 minutes. Subsequently, each volunteer applied a moisturizer in a defined site in the volar forearm, twice a day, for 15 days. On D15 a new occlusive patch was applied for 24 hours and after patch removal a new set of TEWL data was acquired. Data analysis was performed by fitting a previously developed bi-compartmental model to the TEWL data sets. The most relevant parameter ($t_{1/2}$ evap) was compared with a pool of results obtained from similar protocols performed with 30 minutes experiments. Results show that in the site treated with cream TEWL was maintained at high values, which induced a high $t_{1/2}$ evap. No statistical differences were established in the parameters obtained with the longer protocols, which shows that the experimental time can be significantly decreased. The work confirms the possibility of optimization of the methodology, which enables a reduction in costs and an improvement in the comfort of the volunteers.

FP28

PLECTRANTHUS ECKLONII EXTRACTS AS NEW BIOACTIVE INGREDIENTS

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Plectranthus ecklonii is traditionally used in Africa for various ailments, including skin diseases. However, to properly understand the interest of this plant as a source of bioactive compounds for dermocosmetic products, investigations on efficacy and safety are still needed. We characterized two *P. ecklonii* extracts in terms of antibacterial and antioxidant properties, chemical composition and cytotoxicity. Aqueous extracts were prepared by microwave and decoction extraction methods with high yields (22.9 and 27.9 mg dry extract/mL, respectively). The antimicrobial activity was evaluated by the microdilution method and the minimum inhibitory concentration (MIC) value against *Staphylococcus epidermidis* was 10 µg/mL, for both extracts. The extracts were also investigated for their antioxidant activity, using DPPH method (IC50 values of 129.5 and 160.5 µg/mL for microwave and decoction methods, respectively). The main components were found to be rosmarinic, chlorogenic and caffeic acids, as quantified by HPLC-DAD. The potent antimicrobial and antioxidant activities of the extracts might be related to their polyphenolic content. The cytotoxicity profile of the extracts was characterized in the human keratinocyte cell line HaCaT, using the MTT assay. For the microwave extract, no cytotoxic effects were observed (up to 500 µg/mL, 24 h-incubation). For the decoction extract, the concentration of 500 µg/mL decreased cell viability to 82.3% of controls, while lower concentration were devoid of considerable cytotoxicity. The *P. ecklonii* extracts studied herein exhibited antibacterial and antioxidant activities and were not markedly cytotoxic for human keratinocytes. These extracts should thus be further studied towards their potential use as dermocosmetic ingredients.

FP29

SUB-PRODUCTS OF PRUNUS CERASUS LIQUOR AS NOVEL INGREDIENTS FOR TOPICAL FORMULATIONS

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Objectives: By-products of the sour cherry (*Prunus cerasus* L., *Rosaceae*) used in the Obidos liquor “ginjinha” from Portugal, were screened for industrial exploitation by determination of their phenolic content and antioxidant activity. This study aims to demonstrate their potential as novel bioactive dermocosmetic ingredients, mainly as substitutes for synthetic antioxidants. Methods: The sour cherry by-products (stems, leaves and the pomace) were extracted by maceration with different solvents (acetone, ethanol, methanol and water). All the solvents were evaporated from the extracts, which were then suspended in glycol or water. The Total Phenolic Content was determined with the Folin-Ciocolteau method. The antioxidant activity was confirmed by determination of their free radical scavenging activity through DPPH and FRAP assay. Results: Stem extracts showed a higher concentration in polyphenols and antioxidant activity than those obtained from leaves and pomace. The best extraction solvent was methanol, which provided the highest phenolic content for stems (30,84±0,51mg GAE/g dry plant), leaves (21,36 ±0,18 mg GAE/g dry plant) and pomace (279±0,15 µg GAE/g plant). The antioxidant activity of methanolic and ethanolic stems extract was similar to that obtained for the synthetic antioxidant BHT in the same conditions. Conclusion: The tested sour cherry pomace and the green waste (stems and leaves) are a promising agro-industrial by-product to be employed as low-cost antioxidant source. Further studies will be conducted to address their potential use as functional dermocosmetic ingredients.

FP30

AN IN VIVO, MINIMALLY INVASIVE RODENT MODEL TO ASSESS SKIN REPAIR

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Skin healing pathophysiology is addressed by a micromodel designed to study cutaneous “barrier” recovery in the rat. The model uses a well known contact challenger-sodium lauryl sulphate (SLS). In small concentrations it evokes inflammation, edema and barrier impairment without any relevant histological changes. This study aimed to establish the minimal concentration of topically applied SLS able to evoke barrier impairment in the rat’s skin. Four contralateral areas (2cm²) were marked in Wistar rats dorsum (n=7, weight - 520±60g), 2 in each median line side, after hair removal (24h pre-testing). Rats were previously sedated (inhaled ether ~10min) to avoid additional stressing stimulus. Patches with different SLS concentrations (2; 3.5; 5% v/v) were randomly applied, 1 site serving as control for 24h. The experimental procedure included clinical (visual scoring-scale) and biometrical evaluations. Specially relevant variables were transepidermal water loss (TEWL) (Tewameter TM300), erythema (Chromameter®) and local microcirculation (LDF-Periflux®). Measurements took place under controlled conditions (22±2°C, 50±5% humidity), before patching, 1h, 24h and every day after patch removal until full recovery using TEWL as the statistical end-point. A Two-way ANOVA (GraphPad Prism 5.0) was applied and a confidence level of 95% adopted. Results seems to suggest that 2% and 3.5% are the adequate SLS concentrations altering rat’s epidermal barrier without other clinically relevant modification, and correlates well with human data obtained with this same challenger. This minimally invasive animal model maybe explored to look further into these complex domains, including comparative patch and dressings efficacy.

FP31

STUDYING THE IMPACT OF AGE IN THE RAT’S SKIN PHYSIOLOGY

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Animal models have been useful to study specific mechanisms affecting human skin. It is the case of ageing and the micromechanical changes determining wrinkle in UV irradiated mice. These models allowed to perceive that ageing involved many peculiar mechanical responses that cannot be explained by homogeneous deformation of the skin. Nevertheless, the different life span of these species also affects the processes and this is a major aspect to consider. This project aimed to compare the skin properties of two Wistar rats groups with different ages – young-adult rats (n=7, 20–24 week-old, weight 379±30g) and old-adult rats (n=5, 48–72 week-old, weight 520±60g). Non-invasive measuring approaches involving transepidermal water loss (TEWL, Tewameter TM300), superficial hydration (MoistureMeter), and biomechanis (Reviscometer, and cutometry by MPA80) were taken, under controlled room conditions (22±3°C, 50±5% humidity) and procedures. Rats were previously sedated (inhaled ether ~5min) to avoid additional stressing stimulus. Opposite dorsal areas (neck, bottom) were marked after hair removal (24hours before testing). Measurements took place daily, during 5 days. Statistical comparisons between groups were performed using Unpaired t test (GraphPad Prism 5.0) and a confidence level of 95% adopted. Results revealed no significant differences for TEWL, firmness (reviscometer) or viscoelasticity between the two groups. However, a significant hydration decrease, near 40%, was noted on the adult-old rats group. These results, although preliminary, should be specially considered when the study of hydration dependent processes such as healing (and patch dressing efficacy) takes place in rat.

FP32

BODY MASS INDEX AND ASSOCIATION WITH *IN VIVO* SKIN PHYSIOLOGY

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Although poorly documented, obesity seems to impair normal skin's physiology. In fact modifications in skin's basic functions involving the "barrier" and epidermal hydration balance, skin biomechanics and repair mechanisms seems to be consistently present in these patients. The aim of this work is to evaluate how the body mass index (BMI) correlate with these skin indicators. This study involved 51 female volunteers, aged between 20 and 46 (mean 29+7) years old, with no relevant pathologies except the overweight or obesity. All procedures respected Helsinki principles and respective amendments. The Quetelet index (BMI) was calculated for each volunteer and a single measurement of skin hydration (CorneometerCM825), barrier function (Tewameter TM300) and biomechanical behavior (Cutometer MPA580 and Reviscometer) was performed, under controlled conditions, in different anatomical areas (face;breast;and abdomen). Statistics involved Pearson's correlation test adopting a confidence level of 95%. A significant ($p = .05$) moderate (coefficients between .312 and .379) negative correlation was found between BMI and hydration in all anatomical regions, except forehead. A positive correlation was found for TEWL in all anatomic regions (between .282 and .601), while, regarding the biomechanical behavior (U_a/U_f , U_r/U_e , U_v/U_e , U_a , RRT_{max}/RRT_{min}), the strongest correlations were found in the abdomen region (p between .377 and .534). Interestingly, this study allowed to evidence a clear association between BMI and skin hydration and biomechanics, with a special emphasis in the abdomen a well known problematic area in pathologies related with obesity and overweight. Nevertheless, further studies are still needed to validate these results.

FP33

LOOKING FOR A GLOBAL INDICATOR OF OBESE SKIN FUNCTION

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The impairment of water balance and biomechanical behavior of the skin seems to be consistently present in obesity, and probably related with most frequent signs and symptoms. The present work aimed to search for a global body mass index (BMI) related indicator for this functions. 51 female patients, aged between 20 and 46 (mean 29+7) years old, with no relevant pathologies except the overweight or obesity were involved. All procedures respected Helsinki principles and respective amendments. The Quetelet index (BMI) was calculated for each volunteer. Measurements took place under controlled conditions, in different anatomical areas (face;breast;and abdomen) and included skin hydration (CorneometerCM825), barrier function (Tewameter TM300) and biomechanical descriptors (Cutometer MPA580 and Reviscometer). Statistics involved Pearson's correlation test and a confidence level of 95% was adopted. Variables were normalized by the z-score's transformation and, when necessary scale-inverted. Global measures of hydration and elasticity were calculated, for each anatomic region, by the arithmetic mean between these normalized indicators. For assessing the sensibility of such global indicators, its association with BMI was studied. Negative correlations were found for the global indicator of hydration (involving capacitance and barrier measurements) and BMI for all anatomical areas, except breast area, but no significant correlation was found for the global indicator of biomechanics. Thus, under the present conditions, normalized measurements of hydration allowed to build up a global indicator of skin function, sensible to different levels of BMI suggesting that this research may be relevant to help to characterize obese skin.

FP34

LOW-ENERGY HELIUM-NEON LASER THERAPY INDUCES REPIGMENTATION AND IMPROVES CUTANEOUS MICROCIRCULATORY DYSFUNCTION IN SEGMENTAL-TYPE VITILIGO

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Using laser Doppler flowmetry and iontophoresis, we had demonstrated that cutaneous blood flow and its adrenoceptor responses are abnormally increased in segmental-type vitiligo (SV). Low-energy helium-neon laser (He-Ne laser) has therapeutic efficacy on SV. Forty patients with facial and/or neck SV were selected for this study. Assessments of cutaneous blood flow and its responses were performed in six patients with marked repigmentation (>50%) following He-Ne laser treatment. Cutaneous blood flow was significantly higher at SV lesions (2.9 ± 0.6 times) as compared to contralateral skin, but this was normalized after He-Ne laser treatment (1.5 ± 0.2 times). SV lesions were given iontophoresis with phenylephrine (α -agonist), clonidine (α_2 -agonist) and propranolol (β -blocker). The average decreases in blood flow were $20.5 \pm 7.8\%$ (phenylephrine), $46.4 \pm 13.0\%$ (clonidine) and $32.3 \pm 9.8\%$ (propranolol) before He-Ne laser treatment. At the repigmentation sites, the average decreases were $19.6 \pm 10.2\%$ (phenylephrine), $18.1 \pm 11.6\%$ (clonidine) and $24.0 \pm 11.4\%$ (propranolol). The mechanisms by which He-Ne laser treatment induces repigmentation and improves the dysfunctions of cutaneous microcirculation in SV are still unclear. We had reported that He-Ne laser treatment induces primitive pigment cell differentiation and melanocyte migration. In addition, He-Ne laser irradiation induces nerve growth factor (NGF) release from keratinocytes. NGF plays an important role in the regeneration of peripheral nervous system. Our study suggests that the therapeutic effect on SV by He-Ne laser treatment may be mediated in part by a reparative effect on sympathetic nerve dysfunction.

FP35

THE CHARACTERISTICS OF SKIN BARRIER DAMAGE OF FACIAL DERMATITIS

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Background: Facial dermatitis, led by numbers of causes, recently has a growing tendency. But there was rare reported about the skin barrier condition of the disease and the patient's quality of life. In this study we tried to find the relationship between the quality of life and the skin barrier damage for these patients whose faces were suffering from hormone-dependent dermatitis (HDD), facial recurrent dermatitis (FRD), adverse reaction of cosmetics (ARC) or peeling syndrome (PS). Methods: After washing faces with clean water, patients would take a 60-minute rest in a temperature-controlling room, during this period, we graded their disease severity and quality of life (questionnaire of dermatology life quality index). And then their faces' skin barrier parameters, namely, hydration, sebum, pH, trans-epidermal water loss (TEWL) and blood flow were measured. Results: Most of the patients with facial dermatitis have the history of misuse of topical steroids and cosmetics. Skin barrier parameters including HDD, FRD, ARC and PS all showed evident of barrier function's impairment, but no significant difference among 4 diseases was observed. A statistically significant positive correlation has been found between skin barrier damage and quality of life in the patients. Conclusion: Generally patients' subjective discomfort were obvious and the life quality appeared a mild to moderate descend, while their skin barrier was damaged, especially their sebum showed in the sharp drop. Our data suggests that vasoconstrictor and skin care products containing physiological lipids should be regularly.

FP36

QUANTITATIVE EVALUATION OF ADHESION AND COVERAGE EFFECT OF FACIAL BASE MAKEUP

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This study was conducted to evaluate the adhesion, spreadability and coverage effect of facial base makeup products using the "oscillation applicator" (A group), device for makeup, and by hand (H group). It is important to wear makeup using basic makeup cosmetic such as foundation or powder that has tight adhesion and even spreadability effect on facial surface. Also, the main function of makeup is because of somberness of skin tone and cover up skin defects. Previously, there was no reported about the evaluation for efficacy of the facial base makeup. Thus, we demonstrated a method for quantitative assessing the effect of adhesion, spreadability, coverage and coverage lasting of the foundation used in base makeup during 9 hours. 20 volunteers spread the foundation on

their face and forearm, using the oscillation applicator and by hand. We assessed adhesion effect on forearm by tape-stripping and we analyzed digital images on face to assess spreadability, coverage and lasting effect. In the evaluations of adhesion, spreadability and coverage, A group showed significantly twice as higher effect as H group. Both A and H group showed a tendency to decrease the coverage lasting effect, but the reducing effect in H group was more higher than A group at all measurement time. We discovered the distinction between using oscillation applicator and by hand in evaluation of the adhesion, spreadability, coverage and lasting effect. It is expected that tape-stripping and image analysis assessment would be a useful reference for the evaluation of the facial base makeup.

FP37

MORPHOLOGICAL PATTERN AS WELL AS QUANTITATIVE GRADE OF SKIN WRINKLE CAN BE ANALYZED USING FRINGE PROJECTION

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Background: The skin wrinkle increased in accordance with aging. There are many reports for the quantitative change of skin wrinkle such as depth and number of wrinkles, but the report of morphological change was limited. A clinical assessment was able to evaluate the depth and number of wrinkles, but morphological change was difficult to assess by it. However the analysis of skin roughness and wrinkle using the fringe projection might be able to evaluate the morphological change as well as quantitative change. Method: The clinical score on the crow's feet area of healthy Asian females were assessed according to photographic scale. The roughness parameters of same area were measured by fringe projection. The correlation between clinical assessment and roughness parameters was analyzed. The pattern of roughness parameters analyzed according to clinical score and age. Result: The Rt and SA parameters, representing wrinkle depth, were highly correlated with the clinical score. On the other hand the LR and SR parameters, representing length and area of wrinkle were not changed regardless of clinical score and age. Even the same clinical score, the ST parameter, represented maximum depth, was increased with age. Conclusion: The analysis of skin wrinkle using the fringe projection was well correlated the clinical assessment, furthermore the fringe projection was able to distinguish morphological pattern of wrinkle which unable to distinguish the clinical assessment.

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A NOBLE METHOD TO EVALUATE CLEANSING EFFECT OF CLEANSING PRODUCTS USING A DIGITAL IMAGING SYSTEM

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Objects: There is no test method to evaluate the efficacy of cleansing the sunscreen products. We could not assess this effect using most commonly used colored dirt model because of little color. The aim of this study was to develop a method for evaluation of properties to cleanse sunscreen products using a non-invasive digital image captured under UV light. Methods: Fifteen healthy subjects participated in the test for sunscreen products. Cleansing efficacy was assessed by Facial stage UV-capture mode and Spectrophotometer. 50