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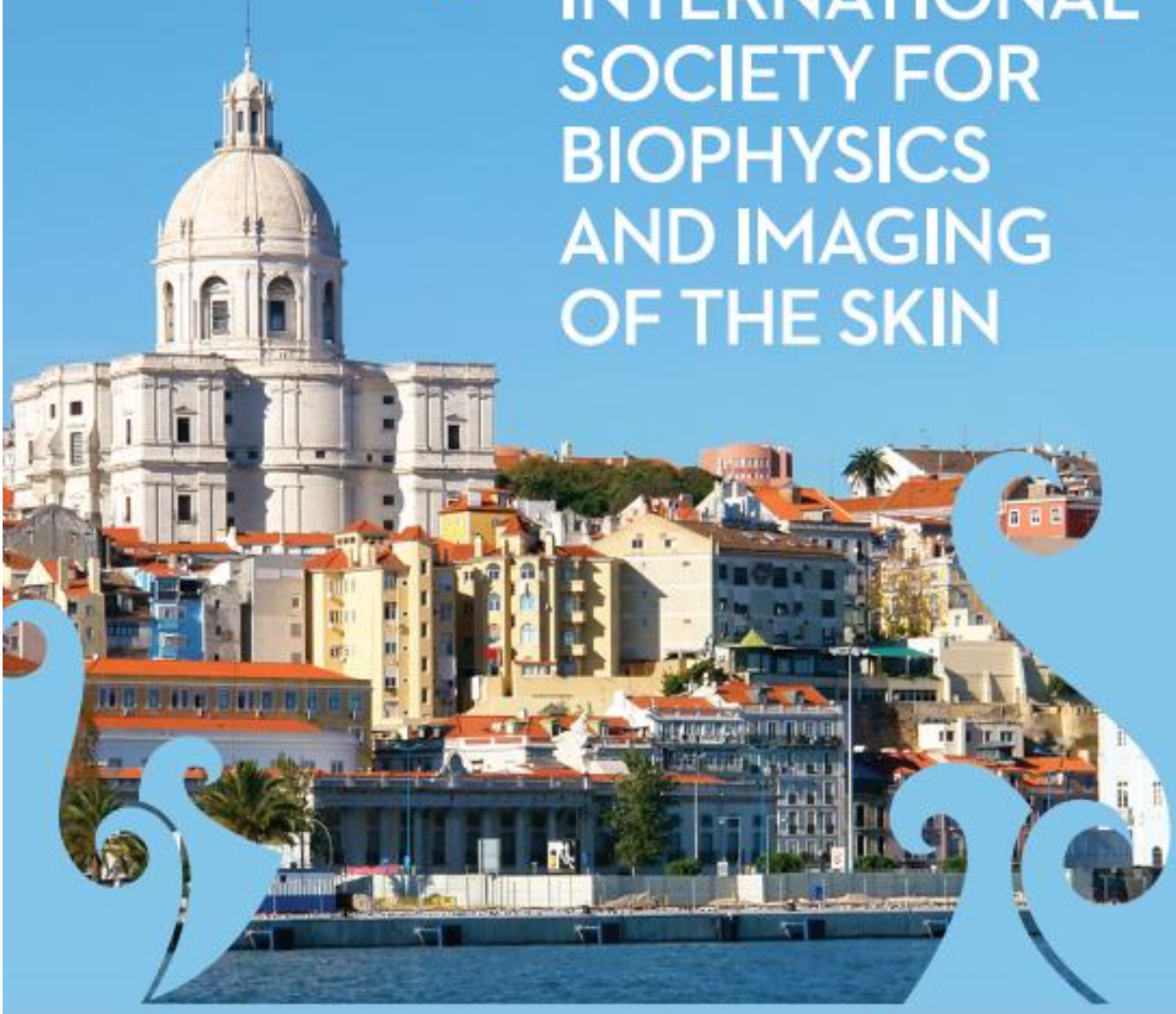

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ORAL COMMUNICATIONS

June 1st

Skin's functional variables and assessment

C01 Age and gender effect on human tactile perception

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The human finger is exquisitely sensitive in perceiving different materials. This perception is totally dependent to skin deformation as resulting of finger and object's contact. The objectives are to better understand the age and gender effects on contact and mechanical properties of the human finger with *in vivo* systems.

Two *in vivo* studies on the finger of 40 subjects (20 of each gender) of four age groups of 26±3, 35+-3, 45+-2 and 58±6 have been performed. An indentation method is used to measure the finger contact properties. The penetration depth of a rigid indenter is recorded as a function of the applied normal force, during a loading/unloading experiment. Main assessed parameters are Young's modulus and adhesive force. An innovative approach is proposed to characterize the dynamic finger mechanical properties. An air blast device included a linear laser has been developed to measure human skin mechanical properties. Main obtained parameters are wave propagation speed ***s*** and Young's modulus ***E***.

The indentation test permits to understand the finger and object contact. The calculated adhesive forces characterize the physicochemical properties of this contact, are varied between 3 to 12mN. Significant differences of the main dynamic parameters of air blast system are shown with ageing and gender. The comparison between male and female groups reveals that the parameters are higher of 9% for speed and 17% for Young's modulus, for women. The measurements show also an increase of the speed and Young's modulus with ageing (young men: ***s***=3.2+-0.2m/s; old men: ***s***=3.6±0.3m/s).

C02 Effects of ageing on tactile acuity: Experimental and numerical approaches

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Tactile acuity of 40 young and old subjects was assessed by two-point gap thresholds at the lower surface of the forefinger. Consistent with previous studies, tactile acuity thresholds in the finger averaged higher in the older subjects than in the younger subjects. The age-related decline in tactile acuity was the aim of this research. Ageing reduces the elasticity and extensibility of the skin throughout the epidermis, dermis and subcutaneous tissues, which greatly affects mechanical properties as well as layer thickness. These changes lead to a decrease in the overall Young modulus of skin and change the viscosity parameters. The effects of ageing on tactile acuity are studied by using two approaches:

As an experimental approach, we have developed the air flow system to identify rheological properties of the skin by using two tests. The first test is the creep test to calculate the viscosity parameters and the second test is the impact test to calculate damping parameters and the Young modulus of young and old subjects.

As a numerical approach, new 2D finite element models of a viscoelastic multilayer finger are developed under ABAQUS environment. These models simulate the test of tactile acuity using the experimental mechanical properties of the skinfor and old and young subject. The decline in tactile sensory capacity in older subjects has been evidenced. Our results proved why the tactile acuity decrease with the age.

C03 The relationship between human skin elasticity and seasonal changes as well as the biological aging considerations

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Skin elasticity is an important parameter for skin healthy and aging indications. Seasonal changes could affect the skin elasticity parameters as well as skin aging devlopment. In order to well understand whether seasonal skin elasticity parameter changes affected by environmental factors and/or by skin physiological conditions, a total of 8 human subjects were studied on face with temperature ranged from 1 degree celsius to 36 degree celsius and relative humidity ranged from 20 percent to 100 percent in a 365 days period, of which biophysical parameters of skin elasticity (CK580 Cutometer) and skin surface hydration (CK Corneometer CM825) were conducted. Another 30 human subjects from 20-50 year's old were studied to further understand skin elasticity parameter changes under biological aging status with a series of biophysical parameter measurements of skin elasticity (CK Cutometer), skin surface hydration (CK Corneometer SM825), skin ultrasound (Cortex ultrasound probe), skin glossines (CK Glossymeter) and skin melanin levels (CK Maxemeter). The studies results showed that different skin elasticity parameters correlated with environmental conditions and biological agings in a various patterns, as well as the skin ultrasound parameters in skin thickness, intensity score and age band. Our studies indicated that major skin elasticity parameters were correlate with environmetal temperatures, skin thickness and biological aging. These findings could further help us to develop beter skincare product evaluation protocals when environment conditions and biological aging are the major targets.

C04 The Fibrous structure of the skin and its relation to the elastic wave propagation

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On all body sites, the skin tension is gretaer in the direction of Langer's line. These are responsible for the anisotropic deformation of incised or excised skin. This phenomenon is the source of the Young's modulus anisotropy, whose distribution angle shows a maximum in the Langer's lines axis. Thsese data favor a similar orientation of the elastic fibers involved in the skin elasticity.

The objective of this study is to characterize the skin tension forces by a new non-contact measuring device, based on the propagation of an elastic wave. The excitation source is an air pressure of 2 bar and with a duration of 2 ms. This pulse initiates the propagation of an elastic wave at a speed which depends on the structure and the anisotropy of the collagen and elastin fibers. Measuring propagation velocity in eight directions allowed us to quantify the modulus of elasticity and skin tension forces during aging. From the data in different directions, this new approach is used to determine elastography and tensiography of the skin surface.

This opto-mechanic device was validated on 60 subjects from 20 to 65 years. The results clearly show the decrease tension forces and Young modulus over 30% between younger and older subject. With its non-contact nature, this device allows to also determine a velocity anisotropy mapping and tension forces to different sites of the body. The study of healing and some rare diseases caused by abnormalities in collagen is a major objective for this approach in the future.

C05 A numerical-experimental methodology for characterization of human skin mechanical properties by suction test

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Human skin is a complex biological soft tissue, which exhibits non-linear viscoelastic mechanical behaviour. Skin mechanical properties are influenced by internal and external biological factors such as: age, life style, health, etc. Knowledge on skin mechanical behaviour is crucial in many scientific and industrial fields such as: medicine, cosmetics, robotics and haptics. In this paper, an experimental-numerical methodology to assess skin mechanical properties is proposed. It takes into account the individual skin layers as well as the viscoelastic behaviour of the skin. Although crucial, this aspect is often neglected in the scientific literature for simplicity. In the first part of the paper, the results of skin suction tests, performed in vivo on the volar forearm using the Cutometer® device, are presented. A sample of healthy subjects representing different age groups was tested with different pressure settings. In the second part, an automated inverse modelling framework is presented. The framework is composed of a numerical simulation and of parameter estimation process. The former is carried out by finite element method where hyperelastic behaviour is modelled by the Yeoh function and the viscous behaviour is modelled by the Herschel–Bulkley law. The parameter estimation is performed by an optimization algorithm taking into account the sensitivity of skin displacement with respect to arbitrary material parameters. Numerical simulation is validated by the comparison with the experimental results. The proposed methodology is able to capture the viscoelastic nature of the human skin and it opens future perspectives for relating mechanical properties to general biological factors.

C06 Three-dimensional Epidermo-dermal Segmentation in In Vivo High-definition Optical Coherence Tomography: Redefining the Pathology and Treatment of Miliaria Profunda

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Background
The high-definition optical coherence tomography (HD-OCT) is a recently-developed non-invasive imaging modality for the skin. Miliaria profunda (MP) is an uncommon and potentially-fatal dermatosis in which blockage of sweat ducts inhibit sweating.

Methods
We developed a novel software algorithm to automatically segment the skin in HD-OCT images into the epidermal and dermal layers in a 3-dimensional manner. To evaluate its accuracy, the tool was applied to 5 image volumes from 5 normal individuals (512 images in each volume/individual) and compared to the standard laborious method of manually marking out the layers.

Results
The epidermal thicknesses determined from both computerised and manual methods were closely similar, with the mean of differences being 4.9 pixels. The tool was subsequently applied to the HD-OCT images of 5 patients with MP. The lesions of MP were found to be limited to the epidermis with the location of obstruction occurring at the sweat orifice, contrary to current belief. A novel treatment using isotretinoin to unblock the keratinaceous obstruction at the sweat orifice was provided for 2 patients, resulting in resolution of their diseases.

Conclusion
We demonstrated novel methods in producing *in vivo* HD-OCT images 3-dimensionally in high resolution and in image processing to segment the layers of skin. With these aids, we demonstrated that the skin lesions in MP are intra-epidermal in nature and the hyperkeratotic plugs at the sweat orifices are the likely cause of obstruction. Based on this new knowledge, we were able to institute a novel effective treatment.

C07 Histamine iontophoresis as *in vivo* model to study human skin inflammation with minimal barrier impairment. Results from application of the model to a sensitive skin panel

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[1]*Radboud University Medical Center, Nijmegen, The Netherlands,* [2]*Philips Research, Eindhoven, The Netherlands*

Introduction and aims: The delivery of substances to the skin by iontophoresis is a frequent research topic in pharmacology and dermatocosmetic sciences, offering a means to enhance permeation while minimizing interferences with the skin barrier. We applied iontophoretic delivery of histamine as *in vivo* model to study locally-induced human skin inflammation with minimal skin barrier impairment, and we evaluated its potential to identify pathomechanisms involved in sensitive skin, a widespread condition in the Western world for which no consensus on definition has been reached yet.
Methods: Eighteen healthy volunteers with sensitive (n=9) and non-sensitive (n=9) skin determined by a multifactorial questionnaire were included. Histamine iontophoresis was performed on the lower back with current 0.4 mA for 2.5 minutes. Transepidermal water loss (TEWL), a* value and epidermal thickness (HE histology) were measured up to 72 hours after stimulation.
Results: TEWL increased of 3.5 $gm^{-2}h^{-1}$ at 5 minutes then recovered, while a* value increased markedly over time at 5 and 30 minutes (2.45- and 2.72-fold, respectively). Sensitive skin subjects showed a trend towards slower clearance of erythema at 60 minutes (p=0.082). Epidermal thickening was detected at 60 minutes (p=0.065), 24 hours (p=0.055) and 72 hours (p=0.016).
Conclusion: Our data confirm minimal interference with the skin barrier. The epidermal thickening at 60 minutes is caused by spongiosis, while that at 24 hours and 72 hours could be due to a proliferative response, which will be further investigated. Next, the data support a hypothesis about involvement of an altered inflammatory response in sensitive skin.

C08 A Two Phase Study to Examine the Efficacy and Absorption of a Topical Anti-Aging Product when used in Conjunction with an Ultrasonic Facial Device

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BACKGROUND

The effects of “at home” Cosmetic ultrasound (US) devices are under described as are the utility of these at home devices in conjunction with typical topical formulations; therefore we undertook two clinical studies to evaluate the JeNu Infuser™ (Hermosa Beach, California, USA) with microsphere conducting gel in conjunction with Lancôme Advanced Genifique Yeux Youth Activating Eye Concentrate.

METHODS

Phase I was aimed to determine if the use of an at home Cosmetic US device would result in the ability of the device to “drive” the compound (and Vitamin C) further into the skin. Phase II compared the anti-aging topical products efficacy with or without the use of this device.

RESULTS

Phase I of the study revealed the amount of product found at the lower level of the skin was 6 times as concentrated with the use of the device ($p<0.001$). Additionally, quantitative HLPC was able to determine ten times the amount of vitamin C when the device was used compared to the product alone ($p<0.001$).

Phase II of the found that those surface areas treated with the combination of the topical anti-aging product and concomitant use of the Cosmetic US device was shown to have statistically significant improvements in the majority of parameters measured after four weeks of use including crow’s feet lines/wrinkles (p0.043).

CONCLUSIONS

The use of a Cosmetic US device results in improved absorption of topical formulations and common ingredients (Vitamin C) and enhanced product efficacy.

C09 Biomechanical properties of the skin: a non-contact measure of skin deformation by air blow and fringe projection

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A non-contact bioengineering technique (Dynaskin-4DTM, OrionTechnoLab - Eotech) was investigated to measure in vivo skin biomechanical properties.

Repeatability of the device was demonstrated for the 3 parameters, measured at a 2 day-interval in the same panel (n=51 subjects, p>0.1, Student t test): "volume" (in mm3), "area" (mm2) and "maximal depth" (mm) of the skin deformation.

A statistical difference between a 1.0 and 1.6 g. calibrated air blow was shown for the 3 parameters (n=51, p<0.001), the 1.6 g. power was found to be the best correlated with age (Pearson coefficient) for "volume" and "depth" (p<0.001, n=51, 21-69 years old subjects panel). A significant correlation (p<0.001) was also shown between age and "volume" (Pearson coefficient: 0.377) or "depth" (Pearson coefficient: -0.483), in a panel of 456 subjects aged from 20 to 70 years.
Data collected with a CutometerTM (Courage+Khazaka) in a total of 320 subjects of this same age range, from 20 to 70 years old, showed Uf, Uv/Ue, Ur/Uf and Ua/Uf were also very well correlated with age (Pearson coefficient; p<0.001).
A comparison of Dynaskin-4DTM and CutometerTM measurements performed in a same panel of subjects showed "volume" and "depth" (Dynaskin-4DTM) were correlated to Uv/Ue and Ur/Uf of the CutometerTM (n=98, p<0.01), but not to Uf.

Presentation will also include DynaskinTM results coming from about 20 clinical studies performed with cosmetic products tested on a period of 4 to 8 weeks or after a single application, with a positioning of the magnitude of effects for each parameter.

C10 Compared biomechanics of aging skin assessed with different optical-based instruments (Cutometer®, Reviscometer® and Cutiscan®)

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Skin anisotropy is due to differential fibre alignment, resulting from prolonged exposure to a surrounding stress state. This property is important in experimental studies about the skin biomechanics and in experimental dermatology.

Several devices were developed to study the cutaneous biomechanical behaviour. The Cutiscan® CS100 is the first to provide information on the elastic and viscoelastic properties, as well as on anisotropy and directionality of the skin.

The aim of this study was to further investigate the changes in the biomechanical properties of human skin due to age, combined with a study on the opportunities and limitations of the Cutiscan®. Additionally, it aimed to compare this equipment with two devices that are commonly employed to assess skin viscoelasticity and anisotropy - the Cutometer® and the Reviscometer®- and correlations between the variables of provided by the three devices were attempted.

20 volunteers participated in this investigation. Measurements were conducted in three different anatomical sites (forehead, forearm and leg) with the three devices.

Results showed that the devices were able to identify significant differences with skin aging, but not with the same magnitude in all the skin sites. Significant correlations were established between V1 and Uf, V2 and Ua and RRT and V3.

The Cutiscan® enabled a more complete assessment of skin's biomechanical properties, since it provides real-time images and 360º elasticity curves that allowed simultaneously to study the viscoelasticity, and fully characterize anisotropy.

Unmet needs for skin research

C11 In vivo assessment of the efficacy and safety of O/W photoprotective emulsions containing tea as external phase

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Formulations containing UVA and UVB filters require additives that enable photostabilization, such as antioxidants.

Teas contain several compounds with antioxidant activity. O/W formulations with avobenzone and octyl methoxycinnamate and green or black tea as the external phase were developed. This study aimed to further investigate these formulations, assessing in vivo their safety, biocompatibility, antioxidant activity and photo protective efficacy.

Different combinations of tea and filters were assessed. The control had water as external phase. Twelve volunteers participated in this study. Basal measurements were made of skin hydration, TEWL and erythema. Sites in the volar forearm were treated with a different formulation, twice-daily for 7 days, and another site was used as untreated control. Skin measurements were repeated after this period. Additionally, an ethyl nicotinate solution was applied for 60 seconds on each site, after which skin perfusion was measured for 15 minutes with laser Doppler Flowmetry. Onset time, AUC and the slope of the curve on the hyperemic phase were used to establish antioxidant capacity. SPF testing was conducted with 10 volunteers.

No erythema or significant differences in hydration and skin barrier were observed in the treated sites. Onset time and AUC results revealed higher antioxidant activity exhibited by the formulations with green tea. Both green and black teas were able to enhance the SPF of UV filters.

In conclusion, the results confirm the efficacy and safety of green and black tea formulations, and are indicative of the added value of combining these ingredients with UV filters.

C12 Pore Evaluation: Two Approaches to a Single Cosmetic Phenomenon

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The reduction of visible facial pores is a common aim of cosmetic products. Cosmetics can diminish the visibility or volume of pores according to different treatment principles. Exfoliating procedures or pore filling can reduce the pore volume, while camouflage effects can change the visibility of pores.

For claim support two accepted instrumental methods were used to evaluate pores in the face: 3D measurements using fringe projection for determining pore volume and standardized clinical photography to evaluate pore visibility by raters. Further, pores were assessed by image analysis.

We used the AEVA (EO-Tech) system to measure pore volume and the VISIA CR (Canfield) for photography. Pore density and pore area were assessed by image analysis and reduction of visible pores by blinded image comparison as performed by six trained raters.

Our results revealed that the different techniques may lead to contradictory results on the efficacy of products in pore reduction. In one case for a decorative cosmetic product we found that although the measured pore volume tended to decrease, the visibility of the pores increased. We assumed that the deposition of product pigments unfortunately enhanced the visibility of the borders of pores instead of masking it.

Our results obviously show that the measurement of pore volume as the only approach is not sufficient to support the efficacy of cosmetics in pore reduction. Only additional knowledge of the visibility prevents from misinterpretation and product failure in claim support.

C13 Complexity of facial skin hydration of different ethnic groups visualized by novel 3D and 2D color mapping approaches and the effect of a moisturizer

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It recently has become obvious that single point measurements are insufficient to describe the complexity of facial skin hydration and a novel 2D color mapping approach provides greater detail. To further develop this methodology 3D imaging and corresponding 2D maps were used to evaluate the effect of a moisturizer. Of 32 female subjects (22.5±1.7 years old) from three different skin ethnicities (Black Africans (Type V/VI), Indians (Type III/IV) and Caucasians (Type II/III)) stratum corneum capacitance was measured on 30 pre-defined sites on the left-hand side of the face and 3D digital images were taken before and after four weeks of use of a moisturizer.

A model was computed to link the capacitance data to the corresponding facial positions. Between each measuring site, capacitance values are interpolated to obtain a value for all pixels of the 3D image. Red/blue color maps, representing capacitance, were generated to continuously display facial skin hydration. For objective comparability the 3D images faces were then flattened to 2D images by converting Euclidean to cylindrical coordinates.

The complexity of facial skin hydration and the effect of a moisturizing cream were clearly visualized. The baseline images on day 0 revealed subtle differences on different areas of the face and gradients were distinctive in the different ethnic groups. All groups showed poor hydration around the nasolabial fold area but these areas showed the most pronounced treatment effect. Our approach pinpoints the complexity of facial skin hydration and areas of facial skin that need special attention in different ethnic groups.

C14 New investigations on antiperspirant action of Aluminum salts using microfluidics

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Aluminium Chlorohydrate (ACH) is widely used to control excessive sweating for personal hygiene purposes. Its commonly admitted mechanism of action as antiperspirant involves a physical plugging of eccrine sweat pores by aluminium hydroxide gel. Reported here is a microfluidic device that mimics sweat ducts specially designed to allow real time study of the interaction between sweat and ACH in controlled realistic hydrodynamic conditions leading to channel plugging. Key capabilities of this device include precise control of channels geometry, flow rates, ACH concentration, and sweat composition. This approach is especially helpful to address the issue of the diffusion of aluminium polycationic species in sweat counter flow. Small Angle X-Ray Diffusion Studies (SAXS) were performed to determine the mechanism of sweat pore plugging by ACH. Our results show that the pore occlusion occurs as a result of the aggregation of sweat proteins by aluminium polycations at specific locations of the channel, where both hydrodynamic and diffusive flows allow the formation of large and dense aggregates. This occupied volume grows exponentially by capturing species provided by the flow and becomes gradually denser to constitute an obstacle to a free flow. Despite their low concentrations, sweat proteins, which are negatively charged at sweat pH, are essential to build the plug. On either side of this location, excess polycations or polyanions prevents the growth of macroscopic aggregate. Altogether, these results give further credit to a purely physico-chemical plugging mechanism for ACH antiperspirant activity and open perspectives to find new antiperspirant agents with an improved efficacy.

June 2nd

News from Skin Imaging

C15 Ex vivo and in vivo quantitative 3D molecular cutaneous imaging in human skin using coherent Raman and 2photon microscopy

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Non-linear label free optical microscopy techniques are here used to image small molecule penetration through human skin over time. Vibrational signature of deuterated active molecular compounds is obtained with Coherent Anti-stokes Raman Scattering (CARS) and combined with the auto-fluorescence signal of the skin. The use of near infrared pulsed lasers provides deep tissue penetration and permits 3D sample sections and reconstruction. Skin layers from the stratum corneum up to the dermis are clearly distinguishable. This allows us to map the active molecule distribution and follow its evolution within the different skin layers with respect to time and up to several hours.

We first concentrate on ex-vivo human skin samples and show in-depth concentration together with penetration pathways for an active cosmetic molecule (LR2412), glycerol and water [1]. We then report, for the first time, in vivo experiments on human arm, following the cutaneous absorption of glycerol. Penetration pathway, concentration over time and depth will be presented and compared to ex vivo samples. Such results highlight the importance of the tissue active metabolism to understand the penetration of exogenous molecular compounds in skin. Our non-invasive quantitative methodology can easily apply for investigating any substance interaction with skin; provided possible deuteration of the element of interest. Effect of pharmaceutical active ingredients on the skin morphology as well as morphological changes due to skin cancer could also be explored.

[1] X. Cheng et al. J. Control. Release 200, 78 (2015).

C16 Shadow-Enhancing Facial Imaging and Age Perception

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Johnson & Johnson, Skillman, NJ, USA

Shading and shadowing on faces serve as cues to the brain about human age, however most modern imaging systems use diffuse, full illumination that eliminates these relevant cues. Our research combines imaging and human perception testing, to investigate the impact of imaging faces with enhanced shading and shadowing, on age perception in young (20-30 years), middle-aged (35-45 years), and mature (50-65 years) female faces. We have designed an imaging system consisting of a high-resolution SLR camera, coupled with 5 visible light sources positioned at specific angles relatively to the face, to ensure that facial shadows are enhanced. We assembled a database of face images by imaging 178 individuals, ranging in age from 20-65 years, under both diffuse (DI) and shadow-enhanced illumination (SEI). The first perception study had 127 observers estimate the ages of 30 Caucasian female faces, photographed either under DI or SEI. While young (20-30) and mature (50-65) faces' age estimations were not affected by illumination type, we observe that DI induces significant age underestimation for 35-45 year old female faces; in contrast, SEI leads to significantly more accurate age estimations for this age bracket. We executed a second perception study with 126 observers to prove that this increased accuracy under SEI was not attributable to the facial shadows changing perceptions of health and attractiveness. Our results prove that illumination has a significant effect on a person's age perception, with major implications for the use of imaging in clinical grading and product effectiveness studies, especially for women aged 35-45.

C17 Image analysis of skin pigmentation by image color channel separation

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The measurement of skin pigmentation which is one of the very important technics in cosmetic evaluation and it usually was performed by visual examination. However, this examination is very subjective. In this study, we propose the new easy pigment detection method which reducing non pigment region detection at facial image using commercial image analysis program.

Fourteen volunteers aged 40-50 year, healthy Korean females were participated in this study. Facial photos were taken by VISIA-CR and image analysis was performed by Image-pro plus. Our image processing was performed by image color channel separation, RGB color images. To compare standard method, 9 grade scales, visual assessment for degree of matching for pigmented skin area was performed by two cosmetics efficacy researchers. The statistical analysis was performed by using SPSS 20.0 software.

The standard and our methods showed 28 images pigmented skin area ratio is 16.9 ± 2.9 % and 15.5 ± 2.2 %, respectively. The visual assessment of matching score of each method showed 4.1 ± 1.3 points, and 6.0 ± 1.3 points, respectively. There was significantly difference at analyzed pigment area between two methods ($p = 0.049$). Our method showed significantly higher score than previous method at visual matching score ($p < 0.001$).

Our image analysis is more suitable than standard method because this method could match the pigment area by reducing over detection caused by face shadows.

C18 Topographical and structural properties of aged skin by skin imaging analysis

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Skin imaging techniques are valuable tools to evaluate skin ageing and clinical efficacy of cosmetics with anti-ageing purposes. The aim of this study was to evaluate the topographical and structural properties of aged skin and correlate the parameters obtained by skin imaging analysis using of Reflectance Confocal Microscopy (RCM), Fringe projection Technology (FP) and High Frequence Ultrasound (US). For this purpose, forty-one healthy French female volunteers aged between 40-65 years old with healthy skin on the face participated in the study after giving written informed consent. The structural and morphological characteristics of the epidermis and dermis were analyzed by RCM (Vivascope® 1500), dermis was evaluated by US (Dermascan® C) and topographical properties by FP (AEVA-HE®) . The correlation between ageing and the skin imaging parameters were evaluated. In addition, the correlation between structural properties of dermis and epidermis were correlated with results obtained by FP. The results showed that ageing is correlated with wrinkling, skin roughness and loss of dermal epidermal junction and presence of huddled collagen. Subepidermal hipoechogenical band was correlated with increase of length and area of furrows and huddled collagen. In conclusion, structural and topographical properties of aged skin are correlated and skin imaging techniques (RCM, US and FP) provide complementary information regarding skin structure, morphology and topography in aged skin.

C19 Fluorescent fibre-optic confocal characterization of *in vivo* epidermal changes in atopic eczema

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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 into the dermis. FOCI images are optical sections from a horizontal (en face) view. The aim was to study *in vivo* epidermal changes and the cellular structure of keratinocytes in moderate to severe chronic atopic eczema (AE) and adjacent non-lesional skin with healthy skin as a reference.

Methods: Twelve patients diagnosed AE with lesions on forearms were EASI scored, studied and compared to a control group of six healthy individuals. Fluorescein sodium was used as fluorophore. A hand held fibre-optic laser scanner plus confocal microscope 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 in AE versus healthy skin. Present results indicate non-invasive visualization of cell nuclei and *parakeratosis* in AE. Other histopathological correlates such as *acanthosis* and *spongiosis* could also be observed. Epidermal edema, furrows and microvesicles were visualized. Final results of morphometry based on FOCI and statistical analysis will be presented at the congress.

Conclusions: FOCI can directly visualize essential epidermal structures of atopic eczema *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 could be a versatile future tool for non-invasive microscopic diagnosis and therapy follow-up of atopic eczema

Keywords: confocal imaging - atopic eczema - in vivo - keratinocyte - parakeratosis - morphometry

C20 Non-invasive short-term assessment of two retinoids effects on human skin *in vivo* using multiphoton microscopy

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Background: Multiphoton microscopy is a noninvasive imaging technique compatible with *in vivo* 3D investigations of human epidermis and superficial dermis. We have recently developed 3D image processing tools that automatically segment the skin layers and extract quantitative parameters that characterize the skin[1,2].

Objective: To demonstrate that *in vivo* multiphoton microscopy, associated with a patch test procedure, could be used as short-term non-invasive screening of anti-ageing and whitening agents.

Methods: Twenty women (50-65 years) were enrolled. Two anti-ageing gold standards (Retinol 0.3% (RO) and Retinoic acid 0.025% (RA)) were applied to the dorsal side of their forearm under occlusive patches for 12 days. A patch alone was applied to a third area as control. Evaluation was performed at day D0, D12, D18 and D32. Epidermal thickness, normalized area of the dermal-epidermal junction (DEJ) and melanin density were estimated in 3D.

Results: Main results are[3]:

- (i) Epidermal thickening at D12, D18 and D32 with RO and at D12, D18 with RA vs. both baseline and control.
- (ii) Increased DEJ undulation at D32 with RO and at D12 with RA vs. both baseline and control.
- (iii) Decreased melanin content with RO (at D12 and D18 vs. baseline and at D32 vs. both baseline and control) and with RA (at D12 vs. baseline).

Conclusions: Short-term patch test protocol, combined with multiphoton microscopy and specific 3D quantification tools, allows anti-ageing and whitening effects to be accurately detected and quantified over time. This innovative approach could be extended to the evaluation of other pharmaceutical or cosmetic ingredients.

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C21 The effect of intrinsic ageing and photo damage on skin dyspigmentation: an explorative study

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The skin melanin system is affected by ageing, leading to pigmentation heterogeneity, clinical and psychosocial consequences. However little is known about changes in skin pigmentation in fair skinned subjects and on sun protected skin.Aim of this study was to investigate age-related changes in skin pigmentation and dyspigmentation in subjects of skin phototypes II–III and to develop and test parameters for quantifying dyspigmentation by means of image processing.

Twenty four volunteers of three age-groups were included in this explorative study. Skin colour was measured by Mexameter and Chromameter on the cheeks, forehead, dorsal forearm, volar forearm and the upper inner arm of the subjects. Skin dyspigmentation was measured by clinical evaluation and newly developed image-processing parameters. The reliability of the evaluations was investigated by intraclass correlation coefficients, and the validity of the dyspigmentation parameters on the face was analysed by bivariate correlations with related measures.

Skin lightness decreased with increasing age on the face, overall skin colour estimates were not different on the other sites. Clinical dyspigmentation indices showed positive associations with chronological age. RBX®-Brown transformation-based hyperpigmentation and hypopigmentation indices increased with age (correlation coefficients up to 0.74). We observed an increase in dyspigmentation at all sites, including sun protected skin areas, already in young adulthood. The image analysis-based parameters showed strong associations with the age, clinical scores and related measurements.

Dyspigmentation parameters seem to be better biomarkers for UV damage than overall skin colour measurements. The high reliability of the dyspigmentation parametrs supports their use in clinical research.

Emerging techniques and methodologies

C22 *In vivo* assessment of optical properties of melanocytic skin lesions and differentiation of melanoma from non-malignant lesions by high-definition optical coherence tomography

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One of the most challenging problems in dermatology is the early detection of melanoma. Reflectance confocal microscopy (RCM) in combination with dermoscopy improves considerably diagnostic accuracy in the hand of experts.

High-definition optical coherence tomography (HD-OCT) appears to offer additional information on melanocytic lesions complementary to that of RCM. However morphology analysis by HD-OCT does not permit diagnosis of melanoma.

The aim of this paper is first to quantify in vivo optical properties such as light attenuation in melanocytic lesions by HD-OCT. The second objective is to determine the best critical value of these optical properties for melanoma diagnosis.

The technique of semi-log plot has been implemented on HD-OCT signals coming from three successive skin layers (epidermis, papillary dermis and superficial reticular dermis). This permitted the HD-OCT in vivo measurement of skin entrance signal (*SES*), relative attenuation factor normalized for the skin entrance signal (μ_{raf1}) and half value layer ($z_{1/2}$).

The diagnostic accuracy of HD-OCT for melanoma detection based on the optical properties, μ_{raf1}, *SES* and $z_{1/2}$ was high (95.6%, 82.2% and 88.9% respectively). High negative predictive values were found for these properties (96.7%, 89.3% and 96.3% respectively) compared to morphologic assessment alone (89.9%), reducing the risk of mistreating a malignant lesion to a more acceptable level (3.3% instead of 11.1%).

HD-OCT seems to enable the combination of in vivo morphological and optical property analysis of tissue scatterers in melanocytic lesions. In vivo HD-OCT analysis of optical properties permits melanoma diagnosis with higher accuracy than in vivo HD-OCT analysis of morphology alone.

C23 Use of Optical Coherence Tomography to assess epidermal thickness changes induced by topical treatments

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In a quest to study human skin at various depths, in vivo, a few interesting high resolution optical techniques have evolved in recent years, e.g. Optical Coherence Tomography (OCT), Confocal Scanning Laser Microscopy (CSLM). Optical Coherence Tomography which obtains a crossectional image of skin, has a much higher resolution than the crossectional image of a 20mhz ultrasound device. OCT clearly reveals the epidermal dimension. OCT is based on an optical interferometry principle. The depth profiling is done by changing the path length. Derrmal and epidermal structures are visualized. We have stressed the skin in a variety ways using topical products and have followed the changes by OCT. We have observed thinning of epidermis after corticosteroid use as well as stratum corneum swelling after short exposures to dimethylsulfoxide (DMSO). OCT is commonly used to measure epidermal thicness changes. However, there are challenges to make accurate measurements. Epidermal boundaries at the basal layer are often vague and the presence of hair follicles and other microstructures makes it more difficult to measure thicness with reasonable accuracy. We have used a combination of image processing and image analysis to show reasonably good changes in epidermal thickness. The measurement of stratum corneum thickness is less feasible, however, gross swelling of stratum corneum can be observed after certain chemical applications. With some enhancements in methodology OCT can be a reliable technique to measure thickness of epidermis and other microstructures. This is a useful non-invasive technique to study various dermatoses and the effects of topical products on skin.

C24 Computer-assisted diagnosis of skin cancer: an overview of methods and applications in the 'Big Data' age

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Nowadays, we are witnessing unprecedented access to ever-larger amounts of digital information, so-called 'Big Data', and concurrently to ever-larger computational power, either via our personal computers, mobile devices or the cloud. This trend is observed globally and in various fields, including healthcare.

One of the challenges of the 'Big Data' age is the extraction of meaningful information from data that can help professionals to make optimized decisions. In healthcare, these decisions could correspond to establishing a correct and timely diagnosis. That is more so when dealing with life-threatening situations such as malignant skin cancers, namely melanoma.

Various computer applications have, in recent years, been developed to assist the diagnosis of skin cancer. These are based on the analysis of large databases of skin cancer images, by resourcing to machine-learning algorithms and high computational power. Using this approach, diagnostic accuracies that can surpass those of the physician alone have been claimed.

Here, an overview of the methods and algorithms used and the various applications, including mobile apps, will be described. Additionally, advantages and challenges in computer-assisted diagnosis of skin cancer will be discussed.

C25 MR molecular imaging for melanoma research

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Background: Molecular imaging, a type of *in vivo*, real time imaging, is a rapidly emerging technology that fusion science the fields of molecular biology, chemistry, pharmacology, medicine, genetics, biomedical engineering and physics.

Purpose : Molecular imaging has some advantage to the achievement of following important goal. 1) assessment of the effects and shortening the developmental periods in new drugs development, 2) rapid, repeated and quantitative in vivo imaging in same individuals over whole experimental periods. This study aimed to development the advanced preclinical efficacy evaluation methods of new drug for melanoma using MR molecular imaging.

Method : For development of animal melanoma model for MR molecular imaging, we construct a lenti -viral vector encoding a heavy chain ferritin gene (hFTH). To generate stable B6 melanoma cell line that express hFTH, gene were transduced with lenti-viral vector. B6 melanoma cell containg ferritin gene was subcutaneously inoculated in dorsal side of C57BL/6J mice.
New developed antimelanoma drug(oregonine) and DTIC were intraperitoneal injection to animal melanoma model for MR molecular imaging. Then, T2 weighted images were acquired by 9.4 Tesler animal MRI (Bruker ^{R})

Result : Efficacy of oregonine, new antimelanoma drug, are shown the comparable effeteness with DTIC. Oregonine plus DTIC are shown more higher efficacy than oregonine only. So oregonine could be use as additive drugs with DTIC for melanoma management.

In conclusion, melanoma animal models for the MR molecular imaging could be use a valuable tools in new drug development with highly clinical significances and more precisely preclinical efficacy evaluation.

C26 In vivo characterization of murine and human components of (laser Doppler) flow during hyperoxia

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Laser Doppler flowmetry (LDF) is a reference technique to quantify in vivo microcirculation, but It provides a highly variable signal, that limits its use. Several strategies have been dedicated to simplify it, but also to look deeper into the information included in those oscillatory components. This study shows how the wavelet transform can decompose the LDF signal into its main components (frequency ranges) both in human and animal models in order to identify the respective LDF components' frequency ranges. The LDF signal was recorded on the lower limb of 60 healthy subjects, selected after informed written consent, both genders, grouped by age (group 1: N=35, 22.1 ± 3.7 y.o.; group 2: N=25, 49.2 ± 7.0 y.o.) and of 15 male C57BL/6 mice (group 3, 14 weeks old) under ketamine-xylazine anesthesia. All procedures involving animal experimentation were ethically supervised. In both cases, the provocation test involved breathing a saturated O2 atmosphere. Wavelet-derived periodograms are useful to perceive how human and animal react differently to hyperoxia - on groups 1 and 2 the only significant change observed was the increase in respiratory activity. On the other hand, mice responded with a decrease in respiratory activity, together with an increase in both sympathetic and endothelial activities. These results show, for the first time, the murine LDF frequency ranges, and also highlight the usefulness of the wavelet transform for the characterization of microvascular reactivity.

C27 Elucidating the spatial distribution of both native and topically applied compounds within skin tissue using ToF-SIMS

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This work presents the use of time of flight secondary ion mass spectrometry (ToF-SIMS) as an emerging tool for skin analysis, offering the ability to perform chemical histology and monitor the distribution of xenobiotic compounds. Our work demonstrates that both 2D and 3D spatial distribution profiles of analytes within skin are achievable for both topically applied compounds following permeation and inherent compounds present in native tissue. By conducting analysis of native ex vivo porcine tissue we were successfully able to detect and spatially map chemical biomarkers of both the stratum corneum and underlying epidermis. In addition, the 3D distribution of analytes throughout the epidermis could be visualised for both pharmaceutical and cosmetic topical products following Franz cell experiments. This work has also demonstrated the ability to analyse individual tape stripped layers of human stratum corneum, both native and following application of a topical pharmaceutical. The permeation of diclofenac, a nonsteroidal anti-inflammatory, could be detected and mapped from individual tape strips, offering the potential for permeation information to be gained in vivo using a non-invasive procedure. The sensitivity of the technique has also enabled the detection of analytes from native tape stripped samples. We have highlighted differences in the lipid composition of the stratum corneum relating to both intrinsic and extrinsic aging effects. In particular, a significant increase in the presence and a localised spatial distribution was observed for cholesterol sulfate, which has been shown to play a key role in desquamation.

C28 Quantitative and simultaneous non-invasive infrared spectroscopic measurement of skin hydration and sebum levels

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Skin sebum and hydration levels are considered important factors in skin health and integrity. Disrupted balance in sebum – hydration results in a defective skin barrier function and is an indication of different skin disorders found in skin diseases like such as eczema, psoriasis, ichthyosis vulgaris.

In spite of many technological developments throughout the years, until now no non-contact devices and methods have been reported for the quantitative and simultaneous measurement of skin hydration and sebum levels.

To facilitate quantitative and simultaneous measurement of skin hydration and surface lipids, we developed a short wave Infrared Optical Spectroscopic set-up using differential detection between three wavelengths 1720, 1750, 1770 nm corresponding to the lipid vibrational bands that lie “in between” the prominent water absorption bands.Initially, we measured the absorption properties of sebum and water in the spectral range 400-2000 nm and identified the lipid vibrational bands around 1720 nm lying “in between” the prominent water absorption bands. We built an experimental set-up that was employed to couple these three wavelengths to the skin and the backscattered light was detected by Ge photodiode. The sebum and hydration levels estimated using our experimental set-up showed good correlation with Sebumeter and Corneometer.

June 3rd

What irritates the skin?

C29 Refining the STARS (Skin Trauma After Razor Shave) Bioassay

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Although repetitive tape stripping remains among the more popular ways to mechanically disrupt the stratum corneum barrier we have found that the STARS bioassay provides a more suitable approach for evaluating the effects of topically applied products on traumatized skin. By "dry shaving" the mid volar forearm with a disposable razor without the benefit of any skin preps, lubricants, shaving cream, etc. one can produce a very uniform test bed that is the equivalent of a superficial brush burn. Since water loss levels are elevated due to the mechanical disruption of the SC barrier, we can objectively monitor the restoration of normal barrier function by computerized evaporimetery. We have also found that if left non-treated the skin surface will eventually appear drier over time and the extent to which this occurs can be objectively evaluated using either the C+K Corneometer or the DermaLab Moisture Meter which are respectively based on either capacitance or conductance values. Capacitive imaging using the C+K Moisture Map has also proven to be very useful in monitoring changes in skin surface hydration levels. Thus the STARS Bioassay provides a standardized wound healing model in which the effects of various topical products can be readily assessed using a variety of well characterized biophysical changes. Various factors such as timing of treatments that need to be considered when designing these types of experiments will also be presented.

C30 Evaluation of cutaneous irritation and discomfort sensations induced by the repeated friction of cellulose tissues on the skin

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In daily life, human skin often rubs against other external surfaces, which causes a lot of irritations and friction injuries to the skin. The purpose is to characterise in vivo tribological behaviour, mechanical irritations and discomfort sensations of human skin due to repeated friction of cellulose tissues of different qualities.
A specific rotary tribometer, which enables the control of normal force, contact length and speed, has been developed to conducted in vivo friction tests on the forearm. During the test, a quantification of the friction force is realised. The repeated friction of the tissues induces skin irritation which is evaluated with different common quantitative parameters: the trans-epidermal water loss (TEWL), the hydration level and the local microcirculatory blood perfusion. The original concept of this study is the evaluation of the skin mechanical properties after the appearance of cutaneous irritation. For this purpose, an innovative device has been developed to measure the Young modulus of the skin in vivo. The device generates an air blast solicitation without any contact on the skin area and measuring its dynamic response (evaluation of Young's modulus) with a linear laser.
Two tissues of different softness have been tested on 40 healthy men and women, aged from 22 to 62. The repeated friction shows a decrease of hydration and an increase of TEWL, blood perfusion and skin Young's modulus. Furthermore, the quality of tissues has a direct effect on the irritation because it produces a different friction. The paper with the highest friction displays higher irritation.

C31 The load tolerance of skin during impact on artificial turf

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Background: Understanding the mechanism of sliding induced skin injuries on artificial turf is important for development of preventive measures. Latest findings, by studying the landing phase of sliding tackles revealed that high peak loads during the impact on knee and thigh are critical for inducing skin injuries.. Unfortunately, skin failure data under combined impact load is lacking.

Purpose: To develop an ex-vivo model, biaxial load applicator and loading protocol to investigate the load tolerance of skin to impact on both artificial turf and natural grass.

Methods: A special designed biaxial load applicator, consisting of a mulitbody mass-spring construction is used to apply varying combinations of impact loads. Nine different impact conditions were intended in varying both the vertical and horizontal impact velocities in the range of 0.5 and 1.5 m/s in total. Ears of rabbits are used to study the skin morphology after impact.

Results: The different impact conditions deliver combined normal-shear peak stresses levels ranging from 9 $N \cdot cm^{-2}$ up to 75 $N \cdot cm^{-2}$. The critical normal-shear stress combination at which skin breakdown occurred on artificial turf was 10 and 20 $N \cdot cm^{-2}$ The stratum corneum thickness was found to be correlated ($r = -0.730$, $P=0.01$) to the shear stress. Skin breakdown on natural grass only occurred at a combined normal-shear stress of 144 and 60 $N \cdot cm^{-2}$.

Conclusions: With the aid of a newly developed load applicator it was found that skin damage on artificial turf in contrast to natural grass is strongly related to the magnitude of the impact load.

C32 The role of the topical antioxidants in the prevention of skin inflammation

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Skin inflammation is a fundamental and beneficial response to initiate the healing process aiming to restore skin homeostasis. During the early inflammatory phase, Reactive Oxygen Species (ROS) are generated and can induce severe tissue damage. Thus, the aim of this study was to evaluate the influence of topical formulations containing antioxidants in the prevention of inflammation using Biophysical and Skin Imaging Techniques (BSIT) and Reflectance Confocal Microscopy (RCM). Ten Japanese subjects (27-47 years old) were enrolled. Nine test areas were defined on each volar forearm of the participants: one control area (C), one positive control (no antioxidant treatment - NT) and, seven antioxidant formulations and vehicle (V). The areas were treated with 15µL of the formulations without (V) or with 1% of six antioxidants: Green tea polyphenol (GTP), Resveratrol (R), Ascorbyl tetraisopalmitate (AT), Magnesium ascorbyl phosphate (MAP), Coenzime Q10 (CO) and Zinc glycinate (Zn). 15µL of 5% Sodium Dodecyl Sulphate (SDS) solution was applied during 30 minutes for four consecutive days. BSIT and RCM measurements were taken before (baseline) and after SDS treatment. Each antioxidant treatment was compared to the NC, NT and the V using Student's t test ($p \leq 0.05$). On the initial phase of inflammation, the antioxidant-based formulations can help to prevent the damage caused by ROS generation and attenuate inflammatory signs. Zn-based formulation showed the lowest skin redness and MAP presented the lowest dermis thickness and, vehicle showed no significant difference to the NT for all parameters. Antioxidant-based formulations can prevent skin inflammation signs in a different way.

Clinical Application of non-invasive measurements

C33 Infant Skin Maturation: Color Features and Biomechanical Properties Over the First Years of Life

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Background: Newborn infant skin transitions from aqueous surroundings and adapts to drier conditions for some time after birth. The majority skin structural, histological and compositional research is on fetal versus adult skin and epidermal barrier changes. We further quantified skin maturation by evaluating lightness, color and response to mechanical stress.

Materials and Methods : A prospective observational study was conducted among 102 subjects of1.3 - 38 months. Standardized photographs of normal skin were separated into dark-light, red-green and blue-yellow images. Biomechanical properties were measured with a suction device. Outcomes were determined using general linear models with $p < 0.05$.

Results: Significant results were: Skin was lighter at 14.6 versus 2.2, 4.5 and 7.6 months. Red color was higher at 2.2 than all others. Yellow color was lower at 2.2 and 4.5 versus 10.6 and 14.6 months and at 7.6 versus 14.6 months. Biological elasticity was lower at 2.3 versus 14.7, 22.7 and 57.6 months and 4.6 versus 14.7 and 22.7 months. Elastic recovery was lower at 7.5 versus 14.7, 22.7, and 57.6 months. Viscoelastic creep and residual deformation were higher at 2.2 months. Biological elasticity was higher for abdomen versus back, arm, face and head and for leg than arm, face and head.

Conclusions: Skin adaptation/maturation continues for at least 24 months after birth. Increasing elasticity may reflect increased collagen. Decreased viscoelasticity may reflect increases in elastin fiber structure and function. The findings have implications for patient care, to prevent skin compromise, e.g., pressure ulcers, associated with mechanical forces.

C34 Nail and skin wettability "treatments effect"

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Introduction: Water changes the properties of human keratin fibers in skin, and nails. Examples are the sorption of water by stratum corneum and the spreading of water or lipidic liquids which influences skin absorption... Frequent washing increases brittleness of the nail. Chronic wetting and drying of nails may also cause lamellar dystrophy (Onychoschizia). Brittle nails affect about 20% of the population and women are affected twice as frequently as men. Different treatments effects were tested on skin and nails wettability.
Material and Method: Water contact angle theta between skin and nail was measured in vivo using a system developed for in vivo wettability measurement. Contact angle was measured from the tangents of the profile drop using dedicated software.
Results and Discussion:
Skin is a hydrophilic surface (theta = 57°-73° on forehead) on sebaceous area, while it is hydrophobic on non-sebaceous area (theta = 80°-91° on forearm). Degreasing with an organic solvent increased the skin hydrophobia (theta = 100°). The suppression or the alteration of this layer increased the skin hydrophobia.
Nails are hydrophilic surfaces with a theta = 67°; as on skin surface, degreasing with detergent increase hydrophobia. No significant difference was found in relation to ethnicity or sex.
Conclusion: Nail and skin affinity with water is a major parameter in biology and in cosmetology. These data should also be used to guide the formulation process and emulsions discrimination for a better spreading on skin or nail, and to improve therapeuticals and cosmeceuticals.

Key words: nail, skin, wettability, solvent effect

C35 Biometrology can help the dermatologist in his daily practice

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Introduction & Objectives:
Skin research saw during the last 30 years strong improvements in knowledge and developments.
The aim of the presentation is to try to look forward how the future dermatology consultation will look at, and to link it to the biometrology knowledges.

Materials & Methods:
Dermatologists are now aware of specific terms like "hydration index", "trans-epidermal water loss", "wettability", "visco-elasticity" or "chromametry"... New imaging technologies allow even to see under the skin, in live view, in different slice plane, and 3D, in a non-invasive way. Added to classical photography and dermoscopy methods, it brings to the dermatologists complete and high-tech equipment. Clinical cases will illustrate the diagnosis and follow-up being made possible.

Results:
Parameters collected around patients'skin provide a better understanding of its pathology. Impressive before/after treatment cases will show the important relation needed between the clinical expertise, and the biometrological results, guided by the dermatologist'eye, but quantified thanks to scientists.

Conclusions:
Multi-modal evaluation of the patient is now accessible. Clinical exam and skills are still in the center of the dermatologist approach, but patricians will be more and more surrounded with engineers and scientists during their patients' examination. Dermatologist of the future is a manager of a real research team, and integrator of a numerous of data increasingly accurate, in order to provide an ideal treatment.

Key words: biometrology, dermatology, quantification of the effects

C36 Unwelcome guests involved in inflammatory diseases: how to track these parasites?

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Introduction:
Some skin inflammation could be caused by the presence and proliferation of Demodex Folliculorum parasite, infesting hair follicles on human faces. Reflectance confocal microscopy (RCM) is a new method of diagnosis of this mite.

Materials & Methods:
Patients concerned with asymmetrical persistent skin face inflammation and suspected for demodicosis were examined by RCM. This skin imaging instrument performs non-invasive direct exploration of skin surface, up to 250µm depth, by using a laser light and the reflectance properties of the tissue. Thanks to its cellular resolution level, hair follicles can be investigated in details, researching possible unwanted hosts.

Results:
The presentation illustrates through follow-up of some clinical cases the importance of detection of parasites on different part of the face. Oriented by dermatologist exam, available tools bring the possibility to explore and quantify individually the causes of inflammation: RCM allows confirming the diagnosis of mites' presence, quantifying the infestation per surface unity, while standardized photography control and illustrate the interest and benefit of the treatment over time.

Conclusions:
New technologies offer a better understanding and follow-up of skin surface microflora and its consequences when deregulation or infestation. Diagnosis becomes certain without any invasive procedure, and accurate quantification of some pathology causes is now accessible. Thus this new kind of methods will involve not only dermatologists, but also engineers, and permit more and more an optimization of pathology appreciation and patients' care in dermatology departments.

Key words: demodex, parasite, skin, inflammation

C37 Visual and mechanical properties of striae distensae on Chinese women

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Background:
Stria distensia (SD) is well clinically characterized on Caucasian women. The objective of this study was to investigate the optical and mechanical properties of SD lesions on Chinese women and to compare newly developed SD to more installed one.

Methods:
The study involved 110 Chinese women aged between 20 and 45 yo. Sixty volunteers presented recent and inflamed SD while sixty showed old and scarred one. Morphological characteristics and anatomical distribution of the SD was evaluated by a trained expert.
Visual contrast, color homogeneity and skin anisotropy were computed from parallel and cross polarized images. The color spectrocolorimetric data were used to estimate the chromophors concentration.
Finally, mechanical properties of the SD were assessed using the cutometer and image analysis of the microrelief pattern was performed to evaluate the mechanical strain of the SD.

Results:
Most of the volunteers (90%) claimed that their SD appear after a pregnancy. They were mainly located on the abdomen (93.6% of women), the thighs (72.7%) and the hips (83.6%).
The old SD appeared significantly lighter than the recent one (L*) who were significantly redder (a*). This color difference was related to a higher concentration of skin chromophores on the recent SD.
The cutometer results showed consistent trends for both recent and older SD, while the mechanical stains highlighted two different categories of SD: elastic and plastics.

Conclusion:
The mechanical behavior of the SD is either plastic or elastic, which may suggest different care strategy for these two types of SD.

C38 Automatic and consistent detection of facial hyperpigmented lesions and its application to efficacy evaluation of drug and quasi-drug spot treatment products

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Efficacy evaluation of hyperpigmented lesion treatment requires accurate measurement of lesion area and darkness. Currently, the lesion severity is evaluated primarily by clinical grading due to the lack of a standard objective method. In this study we report a comprehensive image analysis method which automatically detects the lesion area and intensity eliminating the subjectivity of clinical grading or the error-prone manual analysis. Images were obtained by using either Canfield VEOS DS3 for distinct spots or VISIA-CR for melasma. The color images were transformed into CIE-LAB and HSV color spaces, and the histograms of pixel intensity were regressed to fit Gaussian distribution for dynamically calculating the threshold while taking into account the color of the normal skin adjacent to the lesion area. The outputs are the lesion area and color parameters for describing the specifics of facial hyperpigmentation.

Efficacy evaluation was conducted on two products, a drug category chemical peel for treating melasma and a skin lightening essence of a daily skincare regimen. In each clinical study, images from multiple check points were combined into a stack and the lesion areas were aligned using image registration if necessary. Three key parameters were reported: the reduction of lesion intensity in the same lesion area as at the study baseline, the change in lesion area, and the change of color contrast (DE) in lesion and non-lesion areas. Statistically significant improvements were detected from these studies. The method would add values to hyperpigmentation severity assessment in dermatology and the cosmetic industry.

POSTERS

Clinical Application of non-invasive measurements

P01 *In vivo* evaluation of an anti ageing cream using multiphoton tomography on the structural properties of collagen network and of dermo-epidermal junction

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The main objective of the study was to qualify and quantify the long-term effects of a skin care product on structural properties of collagen network and of dermo-epidermal junction with a non-invasive method. The study was carried out on 11 volunteers during a 8-week period using Olympus Fluoview 1200 MP two-photon microscopy. Two-photon images were obtained simultaneously in two modes: autofluorescence (AF) and second harmonic generation (SHG).

The results showed a significant collagen network and dermo-epidermal junction restructuring, as evidenced by a number of measured parameters:

- - a significant increase in the mean frequency of observations with homogenous images of collagen by 80.5% after 4 weeks ($p=0.04$) and by 100.2% after 8 weeks of treatment ($p=0.02$), indicating a skin-rejuvenating effect,
- - a significant increase in the mean frequency of images with fine network pattern (117.6%, $p<0.05$) after 4 weeks, indicating a collagen network-refining effect of the test product,
- - and a significant increase in the density of dermal papillae by 64.8% after 8 weeks of treatment.

Moreover, we observed a tendency towards increase in the papillary surface area after the treatment. More sensitive algorithms could be used to finalize this analysis.

Another in use test was performed with more classical methods (macro and micro relief analysis with replicas, ultrasound measurement of the dermis, cutometric and dynaskin measurements...) which also confirmed these strong results on skin restructuration.

P02 A method for measurement of efficacy of fermented green tea on peripheral skin temperature

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Background
This study was aimed at assessing the therapeutic efficacy of green tea on peripheral skin for cold hypersensitive subjects, who had the feeling of cold hands and feet at cold temperatures, one of the most common complaints in Asian women.

Methods
This randomized and placebo-controlled clinical study included 60 Korean female subjects who had the feeling of cold hands and feet at cold temperatures. The subjects were randomly assigned into two groups to receive fermented green tea or a placebo.

Results
The skin temperature of the hands and feet was measured using digital infrared thermography at the baseline and at 15, 30, 45, and 60 min after the oral administration of the tea or placebo. The skin temperature of the hands and feet of the fermented green tea-administered group was significantly higher than that of the placebo-administered group. The temperature difference between the finger and the dorsum of the hand was significantly lower in the fermented green tea-administered group than in the placebo group.

Conclusions
This is the first clinical study to evaluate the efficacy of fermented green tea on peripheral skin in subjects having the feeling of cold hands and feet at cold temperatures by infrared thermography and we could assess the effect of orally administered fermented green tea. Therefore, this is a good method for the evaluation of the therapeutic efficacy on peripheral skin in human subjects.

P03 The use of high-definition optical coherence tomography imaging in the diagnosis of toxic erythema of chemotherapy presenting as miliaria crystallina and follicular eruption induced by pegylated liposomal doxorubicin

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Toxic erythema of chemotherapy (TEC) is a term introduced to collectively describe several overlapping clinical entities post-chemotherapy that share the similar histopathological features of interface dermatitis. It is characterized by burning pain and redness affecting various parts of the body secondary to a dose-dependent administration of chemotherapeutic drugs, which is likely due to damage to the cutaneous cells. We describe a patient with metastatic peritoneal carcinoma who, apart from experiencing classical palmoplantar erythrodysesthesia and intertrigo-like eruption after pegylated liposomal doxorubicin, also developed a diffuse keratotic follicular eruption and miliaria crystallina. Three-dimensional reconstruction of high-definition optical coherence tomography (HD-OCT) images with cross-sectional view demonstrating the surface topography of a vesicle and a hyperkeratotic papule illustrates that such lesions were intraepidermal in nature and hyper-refractile keratin was apparent in the epidermis, further aiding the diagnosis of miliaria crystallina and follicular eruption as a manifestation of toxic erythema of chemotherapy and defining the exact site of cellular damage. Recognition of these manifestations, with the aid of HD-OCT, will enable prompt appropriate management to be instituted.

P04 Diminished sweat gland activity is associated with X-linked hypohidrotic ectodermal dysplasia in dogs

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X-linked hypohidrotic ectodermal dysplasia (XLHED) is a congenital disorder which has been described in humans, mice, cattle and dogs. In affected individuals, structures of ectodermal origin may be absent or abnormally formed. Various defects such as sparse hair and malformed or missing teeth have been very well characterized in a breeding colony of dogs with XHED and as in humans found to be associated with mutations in EDA. In affected XLHED children, there is significant morbidity and mortality due to hyperthermia, caused by their inability to sweat. Although defective sweat glands have been noted on the paws of affected dogs, studies have been hampered due to a lack of a convenient method for assessing sweating. In the present investigation, we have utilized several imaging methods to monitor the functional response of the sweat glands to orally administered pilocarpine. These include the CK Moisture Map which is a capacitive imager like the SkinChip pioneered by L'OREAL and the "Paw Print" which is a novel optoelectronic sensor being developed in-house. Both devices have allow us to quickly capture images that provides at least semi-quantitative data which clearly identifies XLHED affected pups from their normal littermates and the effects of various therapeutic interventions performed both pre- and post-natally. Hopefully, these devices will also prove useful in upcoming human clinical trials to determine if ED1200 can help reverse some of the developmental defects that occur in XLHED infants as a result of not having the functional EDA protein.

P05 Mechanical Properties of the Skin of ATS Patients, Preliminary Data

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Arterial tortuosity syndrome (ATS) is a rare hereditary, autosomal recessive connective tissue disorder attributed to mutations in the SLC2A10 gene. As its name suggests, ATS is characterized by blood vessel abnormalities, particularly abnormal twists and turns (tortuosity) of the arteries that carry blood from the heart to the rest of the body. The majority of these patients also have congenital involvement of the skin which is typically described as being hyper-extensible. In order to get a better understanding of the bio-mechanical properties associated with this genetic syndrome, we studied a number of ATS patients and their unaffected siblings/ parents who attended the 1st International ATS Conference hosted by the A Twist of Fate-ATS Foundation. The elastic properties of their mid volar forearm were assessed using 2 different suction cup devices, namely the CK Cutometer and the DermaLab Elasticity Probe. Skin thickness measurements were obtained with the DermaScan C High Frequency Ultrasound unit.

In these preliminary studies, we found that both devices provided related but not identical values due to differences in cup geometry and suction settings. Both devices clearly showed that the skin of the ATS patient was indeed very hyper-extensible and under suction extends very easily but quickly snaps back when released. Considerable variation was found to exist in this regard among the ATS patients and these differences in viscoelastic behavior will hopefully provide suitable biomarkers for establishing genotype-phenotype correlations within this genetic disorder.

P06 Two-photon microscopy of the skin and the eye

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We report on the feasibility of two-photon microscopy to characterize tissues with different optical properties: the eyelid and forearm skin, the sclera, and the cornea. Image acquisition was performed using the multiphoton tomograph MPTflex.

The sclera and cornea, contiguous at the limbus region, compose the most external layer of the human eye. The sclera is a connective tissue with white coloration responsible for maintaining the shape of the eye globe. The cornea is the transparent portion of the globe. The eye globe protection is accomplished anteriorly by the eyelids. The eyelid's skin epidermis is one of the thinnest in the human body. It is approximately 50 µm thick, while in the forearm is about 75 µm thick. These tissues, skin (eyelid and forearm), sclera, and cornea, have different optical properties. Namely, the skin and the sclera are highly scattering tissues while in the cornea light scattering is minimal.

We were able to characterize all layers of the cornea, sclera, eyelid and forearm skin based on their autofluorescence intensity, lifetime, and second-harmonic generation signals. Differences in cell's morphology and metabolism were assessed using the metabolic co-factors autofluorescence. The structural organization of collagen fibrils from skin dermis, sclera, and corneal stroma were determined by second-harmonic generation.

With the MPTflex tomograph, it was possible to characterize tissue at multiple depths, with subcellular resolution. This demonstrates that two-photon microscopy is a robust imaging modality for tissue characterization, despite their different optical properties.

P07 In vivo analysis of photothermolysis of melanin by reflectance confocal microscopy after Q switched Nd:YAG laser

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Several lasers were used in the treatment of melasma based in selective photothermolysis. Clinically, Q-switched neodymium-doped yttrium aluminum garnet (Nd:YAG) laser has shown the good efficacy for the melasma. In zebrafish skin, subcellular selective photothermolysis was proven after Nd:YAG laser. But clinical results confirmed after several sessions, the change of melanin in each skin layer during selective photothermolysis could not be investigated in human skin. In this study, we analysed the brakedown of melanin after one session of Q-switched Nd:Yag laser by reflectance confocal microscopy. Female subjects with hyperpigmentation symptom were included in this study. Subjects treated with 0.4J or 1.26J by Q-switched Nd:Yag laser and measured with Mexameter® MX18 (C+K, Germany) and Vivascope® 1500 (Lucid, USA) before, 30 and 120 minutes after laser treatments. We observed that melanin intensity following laser treatment decreased compared to baseline. This result will be helpful to understand the change of melanosome during laser treatment in human skin.

P08 Noninvasive measuring apparatus for the investigation of nail mechanical properties

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The NCA (Nail Complete Analyzer) is an new apparatus based on a recently patented technology, developed for the evaluation of nails in terms of thickness, compressive strength and deformation lengthwise and crosswise.

The evaluation of the thickness is useful in testing all treatments for nail care and it can be performed in a timely manner and with high accuracy.

The assessment of the resistance to compression measures the cohesion of the nail matrix, while the evaluation of the resistance to transverse deformation measuring the response of the nail plate to a force imposed. These parameters are normally used together and they are useful for evaluating treatments of nail reconstruction and reinforcing products. The results are expressed as an index of cohesion and as an index of transverse resistance and they are strictly related to nail thickness and curvature.

Furthermore the apparatus is able to assess the resistance to a longitudinal deformation applied to long nails and it measures the flexibility of the nail plate in response to an imposed force; this parameter is useful to assess products whose claims are both the protective action and the increase of nail resistance against external physical and chemical stresses. The results are expressed as longitudinal resistance.

The full analysis of the nail is carried out with the acquisition of digital images for the evaluation of the curvature of the nail.

The NCA represents an innovative apparatus for the safety and efficacy evaluation of nail products in several fields: cosmetics, pharmaceuticals and medical devices.

P09 Optical biopsy for new exploration of the skin

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Introduction:
Clinicians can use since few years "optical biopsy" tools in their routine practice. Publications, books, atlas present the way to compare images to histology patterns. The presentation will review the opportunity and challenges that these devices - with agreement for clinical use - provide for the researchers interested in skin exploration.

Materials & Methods:
Optical biopsy devices allow seeing under the skin without being invasive, bringing new diagnostic, evaluation and comprehensive aids: applying a probe on the skin surface during few seconds, a living view of skin layers is directly obtained, thanks to tissues answer to light, ultrasound, acoustic waves...

Results:
Reflectance confocal microscopy, ultrasound, optical coherence tomography (OCT), Raman spectroscopy, multiphoton microscopy are the new tools of the skin explorers. Current barriers represented by the technologies and their cost will be soon removed, as many teams are now working on the subject. The challenges lies in helping the clinician and searcher to better understand and analyze the images, or even to become actor and preset the device in function of their questions, before examination.

Conclusions:
Optical biopsy will help the researchers to better understand skin physiology, and to complete clinical and biometrological exams. Associated to new predictive factors (genetics), it will participate to the optimization of skin analyzes.

Key words: skin exploration, imaging, future

P10 Hyperoxia evokes opposite hind limbs vascular responses in the murine model of ischemia

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The murine model of hind limb ischemia is one of the most popular models to study the vascular disease, including new therapies testing. However, published information regarding the murine vascular function regulation and its comparison with human is scarce and, in some cases inexistent. This study evaluates the vascular reactivity of a murine ischemic hind limb model to hyperoxia. Ischemia was induced on the left hind limb of nine male C57BL/6 mice (11 weeks old) by excision of the superficial femoral artery, the right hind limb serving as control. All procedures involving animal experimentation were ethically supervised. Distal perfusion was assessed in the paw by LDF before (day 0) and on postoperative days 4, 6, 8, 12, 15 and 21 under anesthesia (ketamine-xylazine). During measurements, mice were subjected to a 100% oxygen breathing for 10 minutes. The perfusion change from baseline to provocation on both paws was assessed with the Wilcoxon signed-rank test ($p<0.05$). No differences in perfusion between paws were found on day 0 in the preischemic period. A significant decrease in perfusion was observed on postoperative days 4 and 8 for the control, with no significant differences on the remaining days. By opposition, for the ischemic paw, an increase in perfusion was consistently observed in all postoperative days, only significant on days 6, 12 and 21. These results suggest a change in microvascular reactivity of the ischemic hind limbs to hyperoxia, probably due to the underlying neo- and revascularization processes that occur during the tissues recovery to ischemia.

P11 Studying the Venoarteriolar reflex as part of the in vivo regulation mechanism of peripheral circulation

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Postural changes are often employed as dynamical tests to quantify peripheral microcirculation. The leg lowering maneuver has been studied in the past in both health and disease, but many questions remain open. Our objective was to quantify the peripheral vascular response to a leg lowering maneuver on both extremities. Ten subjects (both genders, 26.0 ± 5.0 years old), were selected after informed written consent. The maneuver, which consisted of three phases - 10 min baseline, with both legs at heart level; 10 min, with one leg being lowered 50 cm below heart level (provocation); 10 min recovery with both legs in the initial position. Blood flow was quantified with laser Doppler flowmetry on both feet and pO_2 was measured by transcutaneous (tc) gasimetry on the lowered foot only. Leg lowering produced a significant decrease in local blood flow on both feet (54% in the lowered foot vs 21% in the non-lowered), while a significant increase in $tcpO_2$ (8%) was noted. These results suggest that leg lowering induces a bilateral vasoconstrictor response. By lowering the leg, blood pools in the venous territory, initiating the venoarteriolar response and reducing perfusion. Skin heating is required to measure $tcpO_2$, which in turn ablated the venoarteriolar response, causing the $tcpO_2$ increase. On the contralateral limb the perfusion decrease may be attributable to more centrally-mediated reflexes. This study seems to confirm the usefulness of this protocol as a model to assess peripheral microcirculation.

P12 Application of the wavelet transform to photoplethysmography to assess the cardiorespiratory activity in the anesthetized dog

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Photoplethysmography (PPG) is a noninvasive, easy to use, cost effective technique useful to monitor several cardiovascular parameters, including heart rate and blood pressure. Recent evidence suggested that PPG signal can also provide information regarding the respiratory activity which would widen its interest and applicability. In the present study, the goal is to detect the frequency ranges of cardiac and respiratory activities of anesthetized dogs by decomposing PPG signals with the wavelet transform. Data was collected from 4 mongrel dogs (2 males, 2 females, 9.0 ± 4.1 years old, 20.2 ± 6.9kg), at the university's vet hospital, submitted to minor surgical procedures under anesthesia (propofol+isoflurane). All procedures involving animal experimentation were ethically supervised. PPG signals were sampled at 100Hz and recorded for 3 min on a randomly chosen ear, 5 min after the anesthesia induction. A wavelet-derived periodogram was then created for each PPG signal and mean ranges were determined. On all periodograms two main high frequency bands were visible - one ranging from 2.1 to 1.3Hz, consistent with the cardiac activity, and another ranging from 0.4 to 0.2Hz, consistent with the respiratory activity. This study further reinforces the potential interest of PPG to monitor cardiorespiratory performance in a veterinary setting.

Drugs vs. Cosmeceuticals

P13 New Effective Retinol/ Emu Oil Formulation for Photoaging and Photorejuvination

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New Effective Retinol/ Emu Oil Formulation for Photoaging and Photorejuvination

Objective; Cosmetic grade retinol has been shown significantly improve photoaging and induce photorejuvination.[1] These authors reported that emu oil has anti-inflammatory, noncomedogenic skin penetrating and collagen stimulating properties.[2]This study was designed to investigate whether emu oil can improve retinol efficacy and prevent retinoid dermatitis.

Methods; Double blind split face study. Half of subjects received 1% retinol in emu oil base (Nirlana serum) to be applied to one side of the face ; other subjects received 1% retinol in neutral base (retinol base) also applied to one side of the face . Results were objectively analyzed by subjects' satisfaction numerical evaluation (1 [lowest] to 5 highest] scale), photographic data and by US board certified dermatologist. No financial compensation was provided to participants of this study.

Results; Nirlana serum was statistically better than retinol base in " making facial wrinkles less noticeable" ($p < 0.05$), was not associated with any side effects including retinoid induced dermatitis observed in 50% of subjects using retinol base. These results were confirmed by photographic data and clinical examination.

Conclusions; Nirlana serum was more effective than retinol base in reducing photoaging and clinical appearance of wrinkles and prevented retinoid induced irritation/dermatitis.

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Emerging technologies

P14 Targeted Strategies for Treatment of Cutaneous Melanoma: Photothermal Therapy *versus* Chemotherapy

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Treatment of cutaneous melanoma has improved over the last thirties years; however, without demonstrating significant increase on survival of patients with advanced melanoma. Some of those treatments show several limitations, such as reduced target specificity, severe adverse effects and cases of drug multiresistance. Hybrid nanoparticles made of polymers or natural substances like modified polyesters, lipids, polysaccharides and proteins, hold multiple functionalities and a promising role in localized cancer therapy. The main objective of this work is the study of alternatives as adjuvant treatments, more efficient and less aggressive (minimal non-invasive), for cutaneous melanoma. Aiming the application of nanosystems as targeted anti-tumor platforms, two strategies have been developed: 1) development of gold nanoparticles, coated with natural polymers and peptides, with absorption at the near infrared range, for photothermal therapy; 2) development of lipidic and polymeric nanoparticles, for encapsulation of anti-tumor compounds, coated with natural polymers and peptides, capable of a local chemotherapy at the tumor site. Physico-chemical behavior and stability of both nanoparticles were investigated for each application. Internalization of nanoparticles and *in vitro* cytotoxicity on melanoma and non-melanoma cell lines were assessed and *in vivo* assays were conducted on SCID mice. Both nanoparticles were able to internalize the cells overexpressing specific cancer receptors and reduce melanoma cells viability on *in vitro* cytotoxicity assays. *In vivo* experiments showed that both photothermal and chemotherapy were able to inhibit tumor growth. Developed nanosystems appear to be promising platforms for melanoma treatment, presenting the desired structure and a robust performance for targeted anti-tumor therapies.

P15 New Trends of Artificial Intelligence in Dermatology: Generalizing Skin Image Analysis

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Introduction: Pattern analysis is a critical task in diagnosis and assessment of skin disorders. Valuable efforts have been made to improve skin pattern understanding by incorporating computer-assisted image analysis techniques. Classical computer-assisted skin analysis consist of extracting generic low-level features along with disease-specific patterns from skin images. However, there are major shortcomings in such methods. Generic low-level features do not provide meaningful clinical insights, while clinically meaningful disease-specific patterns are restricted in generalizability, highly depend on precise hand-crafted features and require annotated data. This motivates designing frameworks capable of learning directly from data. This study investigates the feasibility of applying deep learning techniques in computer-assisted skin analysis.

Method: A Stacked Sparse Autoencoder, was designed. This sub-category of deep frameworks consists of multiple layers of sparse autoencoders, each of which automatically learn features from unlabeled data. Outputs of each layer are connected to inputs of successive layer to generate higher-lever attributes.

Results: The technique was implemented on 1000 dermoscopy images comprising of BCC and non-BCC lesions. We demonstrated the proposed architecture learns high-level clinical features such as telangiectasia, pink color, pigmentation and scales in an unsupervised manner. As a validation, the method was capable of successfully detecting BCC vs. non-BCC.

Conclusion: A deep framework was proposed to systematically learn lesion representations from skin image data and provide clinically interpretable and generalizable results. Our preliminary data showed that learned features were in agreement with the expected clinical attributes; thereby uncovering great potentials of deep architectures as an enhancement to skin pattern understanding.

P16 New topical lipidic-based formulation with antimicrobial activity

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The emerging problem of multidrug-resistant organisms requires the development of new topical formulations with antimicrobial agents. One of the main problems is the transcutaneous absorption of the active principles. The modern research is confronted with the need of findings means to facilitate the passage across skin of active products. One of the most promising strategy is lipidic-based formulations. Phytosomes are complexes obtained by reaction of a pure phospholipid and pure active principles in specific stoichiometric amounts. These advanced delivery systems of herbal products are better absorbed comparing to free active.
Plectranthus madagascariensis is reported to have use in treatment of scabies and small wounds and abietane diterpenes have especially antimicrobial activity. In previous studies, 6,7-dehydroroyleanone, isolated from the essential oil of this plant, has demonstrated activity against *Enterococcus faecalis*, Vancomycin-resistant *Enterococcus* and *Mycobacterium smegmatis*, which are responsible for skin or soft-tissue infections.
Phytosomes containing this last abietane diterpene were formulated. The 6,7-dehydroroyleanone-phytosome complexes were further analyzed. The average size was 150.7±86.6 nm with a polydispersity index of 0.630. Zeta potential presented a value of -1.36 mV and the encapsulation efficiency 74.9%.
These results showed that this phytosomal formulation reveals a great potential to deliver natural compounds, from botanical extracts, through the skin. Further permeation studies are ongoing.

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Skin Imaging – what's new?

P17 The Quantitative Method of Hair lusters by Confocal Laser scanning Microscopy

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Introduction
Hair lusters have been calculated by glossmeters, goniophotometers and visual scoring. But analysis methods for one strand of hair lusters were rarely exists. Confocal laser scanning microscopy(CLSM) provides high-resolution images, roughness, thickness and angle but couldn't obtain optical data such as transparency and gloss. The object of this study was analysis hair lusters by confocal laser scanning microscopy.

Method
The hair samples were cuttings collected from volunteers living in China. Before cutting hair, all volunteers were washed with a shampoo and air dried. The hair images and thickness were obtained by OLS4100 (Olympus, Tokyo, Japan). The pixel number of reflected light of hair was obtained by Image-Pro Plus 7.0 (Media Cybernetics, Silver Spring, USA) and divided from thickness for correction. Hair lusters were obtained before and after treated hair oil.

Result
These principles of this method were based on the reflection of light. The new quantitative method of hair lusters were compared with hair roughness using 100 Chinese hair samples. The pearson's correlation between hair roughness and pixel number of reflected light of hair is -0.303($p<0.05$). So new method is suitable for measuring lusters of hair. And we confirmed the pixel number of hair lusters was increased from 38406 to 117620 after treated hair oil, change rates was 41.02%.

Conclusion
The new method of hair lusters by CLSM can obtain optical images and quantitative data using one strand of hair. This method might be useful for assessment of clinical hair research.

P18 Three-dimensional visualisation and analysis of the dermal-epidermal junction in young and aged skin

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The dermal-epidermal junction (DEJ) is a three-dimensional (3D) structure which is remodelled in aged skin. Current understanding of DEJ structure derives primarily from 2D histological sections. We therefore compared the ability of *in vivo* and *ex vivo* imaging modalities to characterise the 3D structure of the DEJ in young and aged skin.
Extensor forearm skin from Caucasians (aged 18-30 or ≥65yrs; *n*=6/group) was imaged *in situ* by reflectance confocal microscopy (RCM). Biopsies (6 mm) from the imaged site were then processed for imaging using micro-computed X-ray tomography (microCT). In RCM and microCT data sets, epidermal and dermal compartments were segmented, with RCM images reconstructed in 3D using a newly developed algorithm that distinguishes dermal papillae.
For the first time microCT reconstructions demonstrated that rete ridges form long interconnected elevations and RCM highlighted the more discrete nature of dermal papillae projections. Rete ridge height profiles and the volumes of dermal papillae and rete ridges on microCT reconstructions were significantly reduced in aged skin compared with young ($p<0.001$ & $p<0.05$). However, differences in volumes were not observed using RCM.
Both RCM and microCT approaches allowed reconstruction of DEJ 3D structure, but age-related differences were only quantifiable using microCT. *In vivo* RCM avoids sample preparation artefacts and enables longitudinal studies, but, potentially due to limitations in dermal papilla resolution, in our hands only the *ex vivo* X-ray imaging approach was capable of resolving age-related differences in DEJ structure. Crucially, both methods indicate that rete ridges and dermal papilla differ in their 3D morphologies.

P19 The OLÉ Imager: A Novel Imaging System with Open-Air, Overhead Lighting Environment (OLÉ) for Clinical Assessment of Facial Skin Aging Attributes

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Visualization and measurement of facial aging attributes requires images captured using specific lighting modes with directional and uniform illumination. Historically, facial skin imaging is accomplished using closed imaging environments for illuminating the subject with uniform diffused lighting. However, natural lighting under which a subject's face is typically perceived is overhead and directional.

To fill the unmet need for a portable and application-driven open imaging environment tuned for visualization and measurement of facial aging attributes, we present the OLÉ (Overhead Lighting Environment) imaging system.

The OLÉ Imager combines a high-resolution digital color camera with application-controlled Xenon flash sources for standardized facial imaging. The imaging system canopy provides overhead lighting that illuminates the subject's face with vertical and oblique raked light that mimics natural lighting environments and enhances topographical skin attributes. With no secondary reflections, the OLÉ Imager also provides better axis-based polarized lighting for analysis of pigmented spots. Industry standard perspectives of the face (frontal and oblique views) are captured by rotating the canopy around a stationary subject.

The software application driving the OLÉ Imager presents a protocol-specific, wizard-driven workflow, and locks all capture settings to secure repeatable performance for clinical trial imaging. Real-time color and intensity analysis of the captured images ensures consistency in image quality. Measurement of facial skin aging attributes is achieved post-capture with an integrated and scripted image analysis application.

With a combination of novel design, advanced system control application, and image analysis algorithms, the OLÉ Imager will provide improved clinical imaging and assessment of facial skin attributes in clinical studies.

P20 Qualification of a New Digital Imaging System for the Assessment of Facial Aging Attributes

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Background: Digital imaging is an effective tool for detecting and assessing aging related facial attributes such as wrinkles and hyperpigmentation. As such, it can be a valuable methodology for determining and quantifying the effectiveness of facial anti-aging products at improving these attributes. A new digital imaging system combining a high resolution camera, an open lighting environment utilizing Xenon flash sources optimized to enhance targeted skin attributes, and advanced software for precise control of the camera and lighting, was used in conjunction with advanced image analysis algorithms to assess the efficacy of products designed to improve the appearance of facial wrinkles and hyperpigmented spots.

Materials and Methods: The ability of the new imaging system to detect changes in the appearance of facial fine lines, wrinkles and hyperpigmented spots was evaluated in two large-scale clinical studies in the United States and China. Treatment with products containing anti-aging ingredients including niacinamide, retinyl propionate and undecenoyl phenylalanine, were tested against vehicle control treatments for effectiveness in improving the appearance of facial aging attributes.

Results: At the completion of each study, significant improvements ($p \leq 0.05$) in the visual facial attribute of interest was detected using images from the new system for the treated subjects versus those treated with vehicle.

Conclusions: Through rigorous qualification testing, a new digital imaging system was shown to detect anti-aging treatment related improvements in the appearance of facial attributes such as wrinkles and hyperpigmented spots. This imaging system will have great utility in the clinical testing of cosmetic anti-aging products.

P21 New method for extracting and analyzing fine lines & features on the skin

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After having presented multi scaling perspectives, by having high resolution device for measuring the skin in 3D over the full face or body parts, detecting small or large features on these large scales is always challenging. Base on local curvature detection, this new tool provides some nice potential to detect and extract those small features and can be applied for measuring skin hydration, skin smoothing, anti-ageing effect, and reshaping too. Detecting small features like pores, fine lines & wrinkles required that the local surface has to be flattened before detecting these objects by threshold method. On a complex shape surface like face and body parts, this is very difficult to find a method to flatten it, therefore we developed a curvature algorithm to calculate local curvature for each measured point on the 3D data. The curvature is defined by its radius and its concave or convex shape. By selecting concave and a curvature interval, we can define a range of small curvature which represent pores, fines line & wrinkles and detect them with a high sensitivity. This detection can be used to calculate a density of features by calculating the area covered by the detected features or as a new detection method for pores, fine lines and wrinkles on topographies for further areas, volume and depth evaluation. It can also be used for evaluating smoothing properties on the skin by looking to the curvature of the skin network lines. By selecting convex, we can analyze dimples, acne or scars on any part of the face or body. We will present the method, some results from studies on the face, evaluation on smoothing properties and more future potential.
In conclusion I will describe the advantages and limitations of such a method, and future prospective for testing efficacy of cosmetic product or for skin ageing studies.

P22 The image processing technique for modern cosmetic industry

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The image processing technique was used for studying the condition of cells within the stratum corneum - corneocytes (4 donors, age 30-35 years old) after day and night cream treatment for 14 days. Cells were sampled using a special adhesive tape. We can observe differences in morphology and cells organization before and after cosmetic treatment. Image processing and measurements provide automatic, objective and precise analysis of the corneocytes. Due to wide range of grey level recognition and proper process algorithm, application of the image processing and analysis method gives set of valuable, quantitative information describing morphometric features of analyzed objects. Analysis was performed using two set of SEM images (with and without "nutrition treatment") in different magnification: 35x and 200x, saved in BMP format. First set of images with lower magnification were applied for global analysis, which derive information about the area of the cells, number of the cells per area unit and volume fraction of the cells in analyzed images. Assessment of those parameters are a standard in stereological analysis and its results give quantitative characteristic of the samples, which is essential for comparative study of analyzed samples with and without "nutrition treatment". Second set of SEM images (200 magnification) were used for texture analysis, which gives an answer about the differences on cells surface. Obtained result shown significant differences between tested samples in global analysis. An ambiguous results of texture analysis confirmed that changes on the surface of the cells are subtle and difficult to quantitative characteristic

P23 Dynamic Infrared Thermography of Infantile Hemangiomas and Infant Skin

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Background: Infantile hemangiomas (IH) are warm due to increased proliferation perfusion then involute with apoptosis and reduced perfusion. Objective quantitation of treatment response is critical. We examined IH status and uninvolved control skin over time using dynamic infrared (IR) thermography.

Materials and Methods: The preliminary prospective study was conducted among twenty-five subjects (< 19 months) over 59 clinic visits. Infrared images of IHs and control sites and color and three-dimensional images were acquired. Tissue responses to application and removal of a cold stress were recorded with video thermography. Outcomes were areas under the curve during cooling (AUCcool) and rewarming (AUCrw) and thermal intensity distribution maps.

Results: AUCcool and AUCrw were significantly higher and cooling rate slower for IHs versus controls indicating greater heat, likely from greater IH perfusion and metabolism. Significant changes in IH thermal activity were observed in the difference (AUCcool – AUCrw), with 6.2 at 2.2 months increasing to 37.6 at 12.8 months. IH cooling rate increased with age, indicating slower recovery, reduced proliferation and/or involution. For normal control sites, AUCcool was significantly greater at 2.2 months than all others and 4.5 versus 7.6 and 10.1 months.

Conclusions: Dynamic thermography was an effective, quantitative imaging method for the clinic, particularly when structural changes, i.e., height, volume, color, were not easily detected. It may assist in monitoring progress, individualizing treatment and evaluating therapies. The findings results indicate greater thermal activity resulting in lower susceptibility to cold stress and greater capacity to recover in younger versus older infants.

P24 New approach to measure the skin aging

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The C.E.R.CO is expert since many years in the cutaneous relief evaluation and is at the origin of RmD (Micro-Depressionary Network) characterisation. Various 3D techniques are existing to measure RmD or the wrinkles. Based on an single zone evaluation (crow's feet, forehead, nasolabial fold, lip contour), these techniques give a very high resolution thanks to a reduced surface for analysis but representing only one little part of the face wrinkles. As a consequence, the question is : do all the face wrinkles react the same way ?
Improvements in the measurement techniques and the use of profilometry with fringes projection have paved the way for the development of measurements without direct contact on the volunteer with larger measurement zones.
It is now possible to visualize and measure in only one image, the whole of the face wrinkles and their evolution, while keeping a high resolution ; this measurement of a whole face is then much more representative of what we see when looking at a face.
This major step forward in in-vivo measurement will allow to value differently the new cosmetic developments and their efficiency and to have a more global approach of the skin ageing.
Indeed, the topographic analysis of the "density of the wrinkles" of a face makes possible to take into account all the fine wrinkles and wrinkles, verticals and horizontals, whatever they are of expression and/or genetics. This global measure represents one of essential dimensions of ageing study.

P25 Skin surface analysis thanks to non-invasive OCT imaging

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Introduction:
Optical Coherence Tomography (OCT) is a non-invasive method originally used in ophthalmology; its use in dermatology is rather recent: it allows observing sagittal views of layers of the skin, until 1-2mm depth. Thus, morphological description and characterization of healthy or pathological tissues can be performed. Our research focused on the study of skin surface roughness thanks to this method.

Materials and Methods:
The current way to explore and quantify the roughness of the skin is based on clinical scoring, self-assessment questionnaire, photography analysis (Skin Surface Analyser), fringes projection, or Visioscan® (Courage+Khazaka electronic GmbH). A specific module developed by Michelson Diagnostics Ltd permitted to extract parameters from skin volume obtained with Vivosight device.

Results:
Objective, illustrative, and quantifying results will be provided. The presentation will show interesting results taking into account the specificity of each method (influence of contact, analysis from 2D or 3D images...), the correspondence between the parameters furnished, and comments about the relevance, interest or complementary of the devices.

Conclusion:
Imaging techniques offer new visualization of what happen under the skin surface in a non-invasive way. In our project we propose to evaluate the added value on skin surface exploration. More widely, it could be interesting to work on research projects aiming to confront new imaging methods against historical biometrology references.

Key words: skin roughness, imaging, non-invasive

Skin Molecular Research novelties

P26 Effect of anti-aging peptide on age-reletaed changes in N-glycosylation process in fibroblasts and skin elasticity *in vivo*

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Aging results mainly in the loss of dermal collagen and the accumulation of unorganized collagen and elastin fibers in the dermis, however it is also contributed to glycoproteins and proteoglycans deficiency. Protein N- and O-glycosylation of eukaryotic secretory and membrane-bound proteins, is fundamental protein modification, serving many intra- and extracellular functions. The group of disorders caused by alteration of this process display severe phenotypes which can affect also skin tissue, like skin laxity, severe wrinkling, dryness or psoriatic lesions. So far changes in the activity of protein glycosylation during skin aging were not reported. Thus the aim of the study was to evaluate the efficacy of anti-ageing peptide on protein N-glycosylation process and impact of cosmetic formulation containing this peptide on skin elasticity *in vivo*. The activity of Dpm1 (dolichylo-phosphomannose synthase) was checked before and after anti-aging peptide treatment in the fibroblast derived from individuals in different age. The *in vivo* study of cream was conducted on 26 volunteers. Skin condition was analyzed after 4 weeks of product usage by VISIA and Cutometer. The *in vitro* assay showed decrease of Dpm1 activity in older cells. This effect was reversed by addition of anti-ageing peptide. In the *in vivo* studies we observed that skin elasticity improved by 108% (R0 measurements). In addition to this we observed improvement in skin elasticity displaying the level characteristic for younger subjects. This result suggest that N- and O-glycosylation might be altered with age and select cosmetic active compounds may reduce symptoms of skin aging.

P27 Novel antimicrobial abietane cationic amphiphiles for dermatological applications

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Skin diseases can be caused by viruses, bacteria, fungi or parasites. The most common bacterial skin pathogens are *Staphylococcus aureus* and group A β-hemolytic streptococci. In terms of virus, Herpes simplex is the most common viral skin disease. The dermatophytic fungi, *Trichophyton rubrum*, is the most prevalent cause of skin and nail infections. Medicinal plants have been extensively used for dermatological applications, and their isolated compounds applied in topical formulations.

Dehydroabietic acid (DHA), an aromatic diterpenoid, has been extensively studied and has displayed a wide spectrum of biological activities such like antiproliferative, anti-inflammatory and antimicrobial. Previous studies demonstrate that DHA has antimicrobial and anti-biofilm activity, but its efficacy and spectrum augmentation must be enhanced.

The aim of this study was to improve DHA antimicrobial activity through a synergetic effect between an antimicrobial synthetic molecule, namely a new abietane cationic amphiphile (ACA), and an encapsulation polymer, alginate.

The ACAs antimicrobial efficacy was screened in some reference strains (*S. aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Candida albicans*) by the well diffusion test and the two-fold microdilution method.

ACAs showed to be a potential strategy for antimicrobial application. Future studies will focus on the polymeric encapsulation of the ACAs derivatives with improved efficacy, following a screening of their antimicrobial properties and their incorporation in topical formulations.

Skin's functional variables and assessment

P28 Tape stripping and non-invasive reflectance confocal microscopy: an *in vivo* model to study skin damage

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Background and purpose: Disruption of the skin barrier function, epidermal growth and concurrent immune response can be studied in an *in vivo* sellotape stripping model. However, to evaluate the (immuno)histological and cell biological changes in the skin, an invasive skin biopsy is required, which makes this model inappropriate to study skin damage over time. *In vivo* reflectance confocal microscopy (RCM) might overcome this limitation and can build a bridge between clinical techniques, research and the industry. Therefore, we evaluated the use of the tape stripping model in combination with RCM to provide morphological data on skin damage and recovery.

Methods: In 12 volunteers an acute and in 13 volunteers a chronic tape stripping stimulus was applied. The skin was imaged with RCM during 1 week and 3mm punch biopsies were obtained.

Results: Strong correlations between epidermal thickness measurements by RCM and conventional histology were found. RCM thickness measurements correlated also well with epidermal proliferation. The 10x or 15x chronic tape stripping resulted in skin damage similar to acute stripping. Mild chronic tape stripping showed no signs of skin damage.

Conclusion: it was demonstrated that non-invasive RCM in combination with tape stripping can be used as model to obtain morphological and cell biological data on skin-material interactions.

P29 A Randomised Controlled Trial to evaluate the effect of a new skin care regimen on skin barrier function in those living with podoconiosis in Ethiopia

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Background: Podoconiosis is a non-infectious neglected tropical disease. It is treatable but not curable. In Ethiopia, it affects over 3 million people. Alkaline volcanic soil and microbes enter feet via breaches causing an inflammatory reaction, lower leg lymphoedema and skin changes. It was hypothesised that adding the humectant glycerine to the current daily skin care regimen and reducing the amount of NaOCI would significantly augment and repair stratum corneum (SC) barrier function improving clinical outcomes.

Objective: To develop and test an effective evidence-based low-cost sustainable skin care regimen for those with podoconiosis.

Methods: An RCT was conducted in two podoconiosis clinics in Ethiopia over 3 months (n=193). The control group used the current treatment, including washing feet/legs in soapy water, soaking in water with added NaOCI, air drying and application of petroleum jelly. Anti-fungals were used if required. The experimental group added 2% (v/v) glycerine to 1/6 of the amount of soaking water containing 2/3 the amount of NaOCI. The primary outcome measures were proxy measures of SC barrier function, determined by trans-epidermal water loss (TEWL) and SC hydration.

Results: Results indicated that the addition of 2% glycerine with 0.008% NaOCI significantly ($p<0.05$) improved measures of skin barrier condition and clinical outcome.

Conclusions: The addition to the existing regimen of 2% glycerine with a reduced amount of NaOCI was a very effective successful intervention. The saving in precious water was also important. This regimen may prove valuable in other lymphoedematous skin diseases where skin barrier is impaired.

P30 Comparison of biophysical and biological measurements in an in vitro skin equivalent model following urine and/or fecal irritant exposure

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Background: In infants, postnatal skin adaptation and exposure to bodily excretions pose challenges on skin maturation. There is an important need to develop an *in vitro* tool to evaluate skin damage to overcome limitations associated with clinical testing in this sensitive population.

Methods: Skin impedance-based measurements, including capacitive reactance and transepithelial electrical resistance (TEER), were performed in skin equivalents upon urine and/or fecal stimuli using customized instruments designed to fit the transmembrane inserts. Biophysical readouts were compared to changes in skin morphology and to skin inflammatory cytokines.

Results: The feasibility to use biophysical measurements for evaluation of skin barrier *in vitro* was demonstrated. A significant increase in skin capacitive reactance and a significant decrease in TEER were found in an in vitro skin model upon exposure to a mixture of urine and fecal irritants, compared to urine simulant or saline exposure alone. Consistent with biophysical measurements and histological data, higher skin inflammatory cytokines, including IL-1a, IL-8, and GM-CSF were only detected in skin exposed to urine and fecal irritants.

Conclusion: These results suggest that biophysical measurements, such as capacitance and TEER, can be adapted for use in an *in vitro* setting to assess skin barrier function, thus providing a valuable tool that can be used to evaluate products that target sensitive skin populations.

P31 Effectiveness of a Natural Formulation containing Cotton Extract to Protect Skin from Irritants & Enhance Barrier Function of Sensitive Skin: Evidence from an *ex vivo* model and an *in vivo* clinical study

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OBJECTIVE: To evaluate a natural facial lotion containing cotton extract for 1) protection from surfactant stress *ex vivo* on skin biopsies, and 2) for tolerability and effectiveness in women with sensitive skin when compared with a leading dermatologist recommended commercial lotion.

METHODS: *Ex vivo* biopsies obtained from two human donors, were treated for 48 hours with cotton extract containing natural facial lotion and a placebo, followed by exposure to 1% SLS for 24 hours and involucrin immunostaining. In a clinical study, 2 groups of 20 female subjects each with self-perceived sensitive skin used either cotton extract containing natural facial lotion (Test) or Commercial lotion (a leading recommended brand for sensitive skin) on their treated forearms 2X daily for 5 days followed by a 24 hour 1% SLS patch. Changes in blood flow by laser Doppler velocimetry, erythema by chromameter and barrier function by evaporimetry were compared.

RESULTS: In *ex vivo* study, quantification by Volocity* software showed a characteristic increase of involucrin expression in skin after SLS stress. Applied 48 hours before SLS aggression, the natural facial lotion containing cotton extract did not show significant involucrin up-regulation indicating that the facial lotion protected skin barrier function. There were no significant differences in perfusion, skin redness and barrier function between the Test and Commercial lotion in following SLS induced irritation.

CONCLUSIONS: A natural day lotion containing cotton extract reduced the potential for skin irritation and was well tolerated in sensitive skin populations comparable to a leading recommended brand in a 1-week barrier protection study.

P32 Skin surface pH does not correlate with skin pigmentation and TEWL

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The importance of an acidic skin pH for antimicrobial defence is well recognised and some groups found that stratum corneum (SC) barrier function, recovery and desquamation was impaired at neutral/alkaline pH's. A lower skin pH on volar forearm and dorsal hands of more pigmented subjects has been reported to be correlated with a superior functioning SC. It is hypothesized, by some, that pH-regulated mechanisms account for pigment-related differences in SC barrier function and that the superior barrier function of pigmented skin can be attributed to the lower pH of the outer epidermis. However, this is disputed by others.
Our aim was to investigate the relationship between skin surface pH and transepidermal water loss (TEWL) of different ethnicities (Caucasian (n=12), Black African (n=12) and Indian (n=8) female subjects (22.5±1.7 years old)) together with body site (facial sites, volar forearm and dorsal hand).
We observed generally lower skin surface pH values on the Caucasian subjects which were significantly lower compared with the Black African subjects on all sites except the forearm and all sites except the jaw, forearm and hand regions compared with the Indian subjects. TEWL was also generally lower in the Caucasian cohort. Moreover, we could not find any significant correlation of pH and TEWL. Thus, although all measured sites had an acidic pH, our results do not support a lower skin surface pH for more heavily pigmented ethnic subjects. Indeed the lower pH values were observed for the least pigmented subjects who also had the better barrier function.

P33 Concomitant, non-invasive measurement of inflammatory markers and antimicrobial peptides by transdermal patch analysis following acute barrier disruption

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Introduction: It is known that acute skin barrier disruption results in the release of cytokines from the epidermis, leading to an immediate irritant reaction subsequent induction of barrier repair mechanisms. Nonetheless, data on how levels of cytokines chance following acute barrier disruption, *in vivo,* are scarce.

Objective: To explore the feasibility of detecting skin surface cytokine levels, following acute barrier disruption, using FibroTx TAP, a novel and non-invasive technology for measuring protein biomarkers directly from the skin surface.

Methods: Tape stripping was performed on the volar forearm of healthy volunteers (n=27), divided into four groups. In each group, a different pool of four cytokine biomarkers was assessed using TAP technology, either before (baseline) or 30 min, 24 hrs and 72 hrs after stimulation.

Results: The inter-individual variation in skin surface cytokine levels was substantial. However, levels of the inflammatory cytokines Il-1α and IL-1RA, antimicrobial peptides (AMPs) hBD-1 and hBD-2 and the neutrophil-attracting chemokine CXCL-3 were clearly detectable at baseline. Il-1α and IL-1RA showed an immediate upregulation at 30 min, followed by a rapid decrease to baseline at 24 hrs. The dynamics of hBD-1, hBD-2 and CXCL-3 was delayed, with a slighter immediate upregulation at 30 min and a slower recovery to baseline. In contrast, chemokine IL-8 (or CXCL-8) was not found at baseline, but found upregulated at 24 and 72 hrs.

Conclusion: Non-invasive measurements of cytokines *in vivo*, using TAP, holds great promise for unraveling the roles of cytokines in processes related to skin barrier disruption and repair.

P34 Convex structures at the bottom of the dermal layer function as "anchoring structure" contributing to maintenance of skin firmness and morphology

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The dermal layer contains abundant extracellular matrix, and contributes to skin firmness and morphology. These functions require that the dermal layer should be properly anchored, but little is known about the mechanisms involved.

First, we observed the three-dimensional structure of the dermal layer in thick facial and body skin specimens, using micro X-ray computed tomography. This revealed characteristic convex structures at the bottom of the dermal layer in facial skin. They were connected to form honeycomb-like structure. Histological observation showed the usual dermal components (collagen and elastic fibers) in this structure. But, scanning electron micrography showed that the collagen fibers are directed vertically to the skin surface, in contrast to the bulk dermal layer, where collagen fibers are directed horizontally. Elastic fibers showed similar orientations. Thus, the convex structures may serve to retain the dermal layer vertically.

Ultrasonography showed that when facial skin is pulled up vertically, the dermal layer was retained tightly on subcutaneous tissues through the convex structures. The retaining force, measured with a cutometer (-Uv/Ue, 6-mm probe), was significantly positively related to the depth of convex structures in the cheek of female volunteers. Furthermore, facial sagging severity was significantly negatively related to the depth of these structures. Convex structures significantly decreased with aging, and the decrease was significantly related to facial sagging.
Therefore, these convex structures appear to serve as "anchoring structure" to retain the dermal layer, contributing to skin firmness and superficial morphology. They could be a key to understanding the function of the dermal layer.

P35 Biomechanical properties of stretch marks assessed with the CutiScan

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Stretch marks (SM) are a common skin condition characterized by localized manifestations of linear dermal scar and epidermal atrophy.

This work aims to test a recently introduced equipment, the CutiScan CS100®, in the characterization of stretch marks, as well as in the efficacy assessment of a cosmetic product designed for this condition. Its performance was compared with that of other similar devices.
20 female volunteers with normal skin or with SM in the gluteal region participated in this study. Measurements with the three devices were conducted in all volunteers with the CutiScan CS100®, Cutometer® and Reviscometer®. The volunteers with SM participated in the second part of the study, which consisted in the application of a relevant commercially available formulation, twice daily for 15 days, after which measurements were repeated.

Results obtained with the CutiScan CS100® show differences in the viscoelastic behaviour of normal skin and SM. However, no statistically significant differences were established for the anisotropy. The Cutometer® showed that the skin with SM was less firm, less elastic and less deformable. Lower RRT were observed in SM with the Reviscometer®, and no significant differences were established in the anisotropy. After two weeks of application of the cosmetic formulation, significant changes in the viscoelasticity and anisotropy of the skin were only detected by the Reviscometer® and the CutiScan CS100®.

In conclusion, the CutiScan CS100 is a useful tool in the study of the skin elastic and anisotropic behavior, as well as in the efficacy assessment of cosmetics.

P36 Determination of the relative weight of the different aging attributes of the eyes area for perceived age

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Background
The eyes contour is an area of the face particularly affected upon aging. It is also important since it is a major zone one focus on to determine the age of a person. Therefore, our goal is to determine the relative importance of skin aging attributes in the eyes area of Caucasian women (aged over 50) for perceived age.

Method
Standardized front face pictures of the eyes region of 92 Caucasian women (50-67 years-old, 58.2±5.4) were evaluated by two experts using 7 6-points scales scoring: glabellar lines, horizontal frown lines, crows feet wrinkles, skin ptosis of the upper eyelid, malar region wrinkles, eyelid bags and lower eyelid festoons. Four naive evaluators (women's average age of the panel) also evaluated the pictures for perceived age.

Results
Multiple regression analysis shows that 5 attributes correlates with perceived age: eyelid bags (p=0.0002), crows feet wrinkles (p=0.0032), lower eyelid festoons (p=0.0061), horizontal frown lines (p=0.0065) and malar region wrinkles (p=0.0139). Using these signs, a second analysis enabled to established a predictive equation of perceived age that gives results in good agreement with perceived age estimated by naive evaluators.

Conclusions
Unexpectedly, glabellar lines and skin ptosis of the upper eyelid are not predictive of perceived age. In addition, the equation of perceived age reveals that, if eyelid bags is the factors that has the most weight, it is the four other factors, taken together, that have major impact on perceived age.

P37 Effect of weight loss on the dermal structure and oxidative stress of skin in overweight and obese Japanese males

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Purpose
Previous studies have reported decreased dermal echogenicity and increased oxidative stress in overweight Japanese males compared with normal healthy controls. The purpose of the present study was to clarify the effect of weight loss on dermal structure and oxidative stress of skin in overweight and obese Japanese males.

Methods
A longitudinal study of 1-year duration was conducted in Kanazawa, Japan. Japanese male volunteers aged between 20 and 64 years with a body mass index of ≥25 were recruited. A 20-MHz Dermascan C® ultrasound scanner was used to visualize dermal structure and measure echogenicity of the lower dermis. The mRNA expression level of *heme oxygenase-1* (*HO-1*) in hair follicles was quantitatively analysed by real-time reverse transcription polymerase chain reaction and was used as a marker of oxidative stress in skin tissues. Imaging and sampling of the thigh and abdomen were performed.

Results
Body weight decreases of 5% from baseline were observed in 8 of 16 participants (50%). Decreased expression of *HO-1* in the abdominal tissue was not observed in any participant. In thigh samples, decreased *HO-1* expression was observed in 1 participant (12.5%). Increased abdominal and thigh dermal echogenicity was observed in 2 (25%) and 4 participants (50%), respectively.

Discussion and Conclusion
A 5% decrease in total body weight may not affect dermal structure or oxidative stress in skin tissues. Future studies analysing basic patient characteristics in detail and comprising a higher number of weight loss participants should be conducted to validate the findings of the present study.

P38 Do different removal techniques of bed-bath cleaning agent affect skin physiological function and ceramide content? — comparing water-washing versus wiping

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Background: Bed-bath is one of the fundamental techniques to cleaning body, which is applicable not only to the patients in the hospital, but also in case of disaster where the water usage is restricted. Some bed-bath cleaning agents claim they can be removed by wiping, however, we don't know how the way of removal affect the skin condition.

Objectives: This study aimed to reveal the effect of different removal techniques of the cleaning agent against the skin physiological function and ceramide content.

Methods: Three kinds of bed-bath cleaning agents were chosen. We recruited 15 volunteers with informed consent, and they were randomly divided into three groups for allocation of each cleaning agent. One researcher applied a cleaning procedure on both forearms of the participant. After cleaning, the cleaning agent on the left or right arm was removed by water-washing or wiping, respectively. The cleaning procedure was done once a day for seven days. Before and after the cleaning, the measurement of skin physiological functions (pH, water content, transepidermal water loss), and the collection of the stratum corneum for ceramide quantification were performed. The ceramide quantification was done by the tape-stripping and dot-blot analysis.

Results: In all cleaning agents, there were no significant differences in the skin physiological functions and ceramide content between water-washing and wiping.
Conclusion: For the removal of the bed-bath cleaning agent, wiping and water-washing could equivalently keep the skin physiological function and ceramide content, which may allow the wider use of these agents.

P39 The influence of different shavers on the skin quantified by non-invasive reflectance confocal microscopy

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Background: The impact of personal care devices on skin is mainly assessed using subjective tools. However, new objective, accurate non-invasive *in vivo* imaging techniques have been developed. The aim of this study was to evaluate the ability of reflectance confocal microscopy (RCM) in quantifying morphological impact of shavers on skin. Furthermore, tape stripping (TS) as method to study morphological impact of shavers was evaluated.

Methods: In 12 healthy male subjects, for two consecutive days, a split-face test was performed in the neck; on one side a shaver was applied, while the other side was exposed to TS. The stratum corneum (SC) thickness was quantified using RCM and sensory observations were evaluated using questionnaires.

Results: For the development of personal care devices, consumer testing is an essential element. Usually, this relies on subjective tests as questionnaires. However, objective test methods, such as RCM, could help to identify potentially irritating and damaging materials. Shavers with different impact on skin, could be discriminated by RCM; shaver B removed more SC after application than the skin friendlier shaver A. Furthermore, changes in SC thickness induced by TS corresponded well to that of the shavers.

Conclusion: RCM is able to quantify the impact of different shavers on skin. Besides, TS appeared to be a suitable model mimicking the mechanical impact of shavers on skin. RCM in combination with the TS model appeared to be a suitable minimally invasive model to obtain morphological and cell biological data on skin-material interactions caused by different personal care devices.

P40 Interest of a shutter on solar simulator (V1.0) for determination of the MEDu

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Objective: Solar simulators are used for the determination of the Sun Protection Factor (SPF) *in vivo*. The first step of this evaluation is to determine the sensitivity to sunburn of each subjects. The Minimal Erythema Dose (MED) on unprotected skin (MEDu) is defined as the lowest dose of UV rays that produces a visible erythema 16-24 hours after exposure. UV dose at the output of the solar simulator is carefully calibrated and controlled before being applied on the subject's back. However, when switching off/on the simulator, energy variations can be observed with a higher power being delivered during the first seconds. This can have an impact on the MEDu determination.

The aim of this study was to evaluate the interest of a shutter for MEDu determination with a Solarlight Multiport 601-v1.0 (without the Enhanced optical system supplied with multiport 2.5).

Methods: A comparison of MEDu data collected at one year of interval, without (215 subjects) or with (306 subjects) the shutter, was first performed. An intra-individual study on 31 subjects was then performed to compare the MEDu obtained with or without the shutter.

Results: In the first study, the MEDu determined in the presence of the shutter was significantly higher (+19%) than in its absence ($p<0.001$). The second study, performed intra-individually, highlighted the same tendency: the MEDu in the presence of the shutter was significantly higher (+11%) ($p<0.0005$).

Considering higher MEDu leads to a lower SPF, this parameter must be, therefore, strictly controlled in the future.

P41 Human skin viscoelastic behavior identification using dynamic and quasi-static approaches

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The interest for the characterization of human skin viscoelastic behavior is continuously in progress, particularly for medical and clinical applications. Nowadays, the evaluation of these characteristics, using biophysical markers, has become a simple task by appropriated non-invasive methods.

They allow a quasi-static evaluation of the skin mechanical behavior. However, this evaluation does not mandatory reflect the contribution of its intrinsic mechanical properties.

In this study, an innovative contactless device ensures a controlled solicitation, using an air blast on the skin surface, has been developed. This device is equipped by a linear laser, of 800 sensors regularly distributed on 7mm length, which allows the estimation of normal displacement and vibrations.

With this instrument we are able to identify the viscoelastic behavior by two approaches: 1) A dynamic approach using an impact, to reveal the skin elastic character by the generation and the tracking of a surface wave propagation. This elastic character reflects the stored energy of the skin during the test. 2) A quasi-static approach using an adapted creep test with a controlled pressure. Based on a temporal tracking, the viscous character of skin is revealed.
To understand the age and gender effects, measurements have been realized on 60 healthy volunteers and classified into four age groups.

The obtained results confirm the efficiency of the developed instrumentation to estimate the contribution of the skin viscoelastic intrinsic properties, under the age and gender effect.

P42 Comparative metrology of devices for measuring skin moisturization based on electrical measurements

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It is generally appreciated that skin surface hydration levels can be monitored noninvasively based on changes in the electrical properties of the stratum corneum. Indeed the EEMCO guidelines for using either capacitance and/or conductance instruments for such purposes have existed since 1997. Since that time there have been a number of studies comparing how well various commercially available instruments measure the efficacy of moisturizing products. Typically, it was found that although there may be some confounding factors due to ingredients and probe geometry, measurements based on either capacitance or conductance were generally well correlated. Typically the percent improvement may have differed depending upon which set of measurements were used in the calculations but the rank order remained the same. In some of our more recent studies we have found exceptions to this general rule. In order to more fully understand these discrepancies, we have conducted a series of studies that allowed us to compare and contrast devices such as the Cornometer and Delfin Moisture Meter which are based on capacitance to the SkinCon and DermaLab Moisture Meter which are based on Conductance. Our findings with several of these formulations suggest that the use of such devices to measure the efficacy of moisturizing products may be much more complicated than generally assumed. Until these differences are better understood, we strongly recommend that in claim substantiation reports, the exact model of instrument and probe design must be clearly stated.

P43 Quantitative relationship between instrumental measures, image analysis, and clinical grading in the assessment of hyperpigmentation

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Finding the reliable technique for assessing efficacy in hyperpigmentation studies is important. It is of interest to compare the color values assessed by different methods, instrumental measures, image analysis and also compare those with the visual skin assessment. The skin lightening clinical study was conducted at Bejing Sino-German Union Cosmetic Institute Co., Ltd., Beijing, China. There were 180 female participants between the ages of 30-65 with hyper-pigmentation, melasma or solar lentigines. Upon recruitment, subjects were randomly assigned into one of four well known skin whitening treatments. The efficacy was assessed with color reader Konica Minolta CR-400 Chroma Meter by Konica Minolta Inc. (Tokyo, Japan), facial imaging VISIA-CR with Mirror software by Canfield Scientific Inc. (Fairfield, NJ, USA), and clinical grading by a dermatologist at baseline, and after 4, 8, and 12 weeks. The Pearson or Spearman (as appropriate) correlation coefficients were analyzed and visualized using scatterplots or heatmaps.

We found a strong positive, linear correlation between imaging analysis derived Individual Typology Angle (ITA°) and instrumentally derived ITA° values for all time points and both, pigmented and non-pigmented skin sites (Pearson $r > 0.8$, p-value < 0.0001). Moderate monotonic relationship was observed between instrumentally derived ITA° values and clinical grading of the skin tone and clarity (Spearman $\rho = 0.4 - 0.5$, p-value < 0.0001). Level of mottled hyperpigmentation, evenness and contrast showed a weak to moderate correlation with the ITA° values (Spearman $\rho = 0.2 - 0.4$, p-value < 0.001).

Both techniques, instrumental and image analysis represent a reliable assessment tool.

P44 Skin aging signs components evaluated by High Resolution and Reflectance Confocal Microscopy image analysis

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Skin Aging is a complex biologic process that can be influenced by the genetic background and environmental factors. Characteristic aging signs, such as the stratum corneum dryness, the loss of skin elasticity and firmness and pigmentation changes, are a consequence of an important epidermal and dermal structural modification.

The aim of the current work was to correlate structural modifications detected by Reflectance Confocal Microscopy, with melanin, casculature and photoinduced changes detected by High Resolution Image analysis.

After approval from the proper Ethical Committee, all subjects gave their informed written consent. A group of 80 women was enrolled with ages between 18-65 years old. Images were acquired using two different methods: Reflectance Confocal Microscopy (RCM, Vivascope 1500, Mavig) was performed in order to see structural changes related to aging process and at the same time a standard digital face photography (VISIA-CR, Canfield Scientific) was also performed in order to obtain data concerning Visible, Brown (hyperpigmentation), U.V. (photodamaged induced spots) and Red (vascular) spots of each subject. A region of interest in the malar area was defined and the analysis was performed on that area. Statistical correlations were performed using SPSS 21.

Results seems to indicate that melanin distribution, vascular changes and structural changes on fibers network have a strong and direct influence on the visible pigmentary aging signs.

P45 Evaluation of cellulitis with two different ultrasound probes: a comparative study

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Introduction
Aesthetic cellulite is a skin topography modification commonly localized on the pelvic region, lower limbs and abdomen, areas which present many receptors responsible for lipogenesis. The exact physiologic mechanisms underlying this skin condition are mainly the sexually dimorphic skin architecture, microcirculatory alterations, interstitial liquid infiltration (oedema) and localized hypertrophy of adipocytes.

The present work aims to compare two ultrasound's 20 Mhz probes with different focal lengths in order to evaluate the usual cellulite parameters.

Material and Methods
40 female subjects with aesthetic cellulite were included in the study after informed written consent and approval obtained from the competent Ethical Committee. The system, Two ultrasound probes, with different focus (13mm and 23mm) (connected to a Dermascan C unit, Cortex), were used to obtain images from the cellulite evaluation area (tights or legs) of all subjects. The Dermal-hypodermal junction length and the dermal thickness were calculated from the images obtained. A statistical comparison was performed using SPSS 21.

Results and Discussion
The calculated dermal-hypodermal junction length results show no differences between the two probes. Regarding the dermal thickness, results also didn't show any differences between the two probes. However, the definition of the ultrastructure is less visible with the probe with long focus length.

Conclusion
This results suggest that, although both probes can be used for quantification of cellulitis in the skin and general thickness studies, the probe with long focal length is less adequate for structural analysis due to the lack of definition of the image.

P46 Comparative study of two in vivo methods to evaluate anti-oxidant effect

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INTRODUCTION
Reactive Oxygen Species (ROS) are among the major factors that induce skin damage in vivo, mainly skin aging. To prevent the reactions of free radicals, the human body has developed defense mechanisms known as antioxidants. β-carotene is a Yellow chromophore molecule that when oxidized loses its chromophore capability and color.
The present work uses the this capacity to compare two invivo methods to evaluate anti-oxidant effects in the human skin.

METHODS
A group of 12 subjects was enrolled after informed written consent and approval of the competent Ethical Committee. All subjects used a cosmetic solution containing a mixture of known antioxidants on the forearm. Two protocols were defined: 1) A solution of β-carotene was applied on the forearm and in a control area for 15 min followed by a color evaluation. The areas were then irradiated with a UVA lamp and the color was measured again. 2) A solution of β-carotene was applied on the forearm and in a control during 48H, followed by a tape striping and color evaluation. A Chromameter CR-400 was used to obtain the b* color. Statistical comparison between the areas and protocols was performed using SPSS 21.

RESULTS and DISCUSSION
In protocol 1, the use of the cosmetic presented a 53.9% decrease in the oxidant activity of the UVA radiation. At the same time, the control presented a 37.7% increase in the oxidant activity. Similar results are obtained with the protocol 2, allowing a quantification of the anti-oxidant effects without the use of UV irradiation

P47 Is there an obesity paradox affecting skin physiology?

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The alarming prevalence numbers of obesity worldwide motivated the WHO to set the alarm for this modern days epidemic disease, affecting significant populations specially in the developed countries, in all ages. The relationship between obesity as a risk determinant of many diseases cardiovascular and non-cardiovascular, including aging, is known but, that is not the case regarding its impact over skin pathophysiology and skin disease. This study aims to study of the influence of excessive weight over major skin physiology variables specially focusing the epidermal water balance, includinh the barrier function, measure by TEWL, and the envelope function, focusing its biomechanical behavior. A convenience sample of 45 healthy female volunteers were selected and divided into four groups, according to its BMI (body mass index) in normal (control), overweight, obese (I and II) and morbid. Skin physiology was quantitatively assessed in all individuals, in four anatomical areas using non-invasive biometrical analysis by reference instrumentation. Descriptive and comparative statistics were applied adopting a confidence level of 95% . A non-linear alterations for both variables wad detected as a function of BMI, showing a graphic “U” a inverted “U” or a “ J “ shape tendency, suggesting that the weight gain might improve some properties while promoting a progressive adaptation of the skin to a new body contour.

P48 Application of TEWL in vitro for studying percutaneous absorption of various compounds through intact skin and skin representing diaper rash

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Trans-epidermal Water Loss (TEWL) is widely used as an indicator of barrier integrity *in vivo* for both normal and diseased skin. TEWL also increases in babies when the severity of diaper rash (based on the 0-3 visual scale developed in P&G) increases. To establish an *in vitro* model for diaper rash, the TEWL ratio of the site with rash to the control site (with no rash) determined in our clinical studies was used. Absorption of six model compounds was determined in intact skin and the skin representing a different degree of diaper rash based on TEWL increases following tape stripping of human skin. The six compounds (i.e., caffeine, benzoic acid, testosterone, salicylic acid, glyphosate, and taurocholic acid) cover different physicochemical properties. Results: 1) AquaFlux (used in the *in vitro* model) and Dermalab (used in our clinical studies) have a good correlation (r=0.97) ; 2) This *in vitro* model is able to detect the difference in percutaneous absorption between normal skin and skin representing mild or moderate to severe rash; 3) TEWL changes correlated well with changes of percutaneous absorption *in vitro*; and 4)The *in vitro* model with tape stripping produced consistent results of percutaneous absorption when compared with referenced models (skin treatment with sodium lauryl sulfate or fecal enzymes). Additionally, our results showed that impaired barrier has more impact on the absorption of hydrophilic than hydrophobic compounds and a greater effect on poorly absorbed than well absorbed compounds. However, studies with more compounds are needed to further demonstrate these observations.

P49 Local in vivo blood flow stress reciprocally influences TEWL

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Skin water content and flow among its different layers seems to be affected by the local perfusion conditions. However, only a few studies addressed the possible functional relationship between skin water balance and perfusion variables. Our study aims to look further into these mechanisms during stress tests used to explore the peripheral vascular function in vivo. A convenience sample of 56 subjects (both genders, 33.6 ± 14.9 years old) was studied after informed written consent. Variables measured at distal locations included local blood flow by laser Doppler flowmetry (LDF) and transepidermal water loss (TEWL) by evaporimetry. Provocation included a) a 3 min suprasystolic ankle occlusion and b) a 10 min saturated oxygen breathing. TEWL and local blood flow were studied during the provocation test and after that period, and compared with records obtained before the test (control). The Wilcoxon signed-rank test was used for variables changes between phases ($p<0.05$) and the Spearman coefficient to test correlation between variables. During both stress protocols blood flow decreased while TEWL increased, in both cases significantly. Furthermore, positive correlations between TEWL and blood flow were found during the provocation phase. These results clearly suggest a reciprocal relationship between TEWL and local blood flow, as previously published, although the mechanisms involved still need to be identified.

P50 Studying the impact of a 4 week-long use of an alcohol-based hand sanitizer on TEWL and epidermal hydration

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The stratum corneum protects the skin against physical, chemical, biological aggression and water loss. The repeated use of alcohol-based sanitizers is reported to cause skin dryness. This study evaluates the impact of a four week-long regular use of an alcohol-based gel hand sanitizer on skin hydration and on the epidermal 'barrier' function using a kinetic model to quantify TEWL desorption curves following a plastic occlusion stress test (POST). A group of 13 female subjects (19.7 ± 1.0 y.o.) was previously selected following specific inclusion criteria and informed written consent. These volunteers were asked to wash one of their hands, randomly chosen, for 29 consecutive days with a commercially available ethanol-based gel sanitizer. TEWL, superficial hydration (SH) and deep hydration (DH) were measured on days 1, 8, 15, 22 and 29, while a 30 min continuous TEWL recording following a 24 hour POST was made on days 1, 15 and 29 on a second skin area, from which relevant kinetic parameters were calculated. No differences were found for the control hand throughout the study. On the test hand TEWL increased transiently, between days 1 and 8, and then returned to baseline value, suggesting a functional recovery of the stratum corneum. The SH and the DH, improved between days 1 and 22. And the kinetic analysis revealed no significant differences. Under these conditions, results suggest that the continuous use of this alcohol-based sanitizer does not alter the epidermal barrier, on the contrary, it seems to reinforce its water balance while providing sanitation.

P51 Looking further into photoplethysmography components to study skin circulation

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Photoplethysmography (PPG) is a well-known technique used for a long time to monitor the individual's cardiovascular signals. In the last years laser Doppler flowmetry (LDF) and imaging (LDI) almost made us forget about PPG. However, it has the same oscillatory nature of LDF, also a very low cost and easiness to use. In this study, we compare the frequency ranges of cardiac and respiratory activities by a wavelet transform of ECG, PPG and LDF signals. A group of 10 young healthy subjects (both genders, 26.0 ± 5.0 years old) was selected after informed written consent. ECG, PPG and LDF signals were recorded for 30 min. The ECG (lead I) was recorded from both hands, while the PPG was recorded on the first toe and on the ear lobe, randomly chosen. A wavelet-derived periodogram was created for all signals, and their range and frequency of maximal amplitude compared ($p<0.05$). All periodograms have shown a well-defined cardiac band from 0.8 to 1.6Hz. No significant differences were found for the range or frequency of maximal amplitude between the signals. On the PPG-derived periodogram another high frequency band was visible from 0.4 to 0.2 Hz, which is consistent with the respiratory activity. No further differences were noted between toe and ear PPG signals. The respiratory activity band, however, was not clearly visible on the LDF-derived periodogram. These results confirm the potential interest of PPG to the in vivo monitoring of cardiac and respiratory performances.

P52 *In vitro* efficacy assessment of extracts and compounds from *Plectranthus* species for skin delivery

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Plectranthus spp. plants are widely chosen in many scientific investigations. Diterpenes and other metabolites isolated from these plants have been described regarding their antimicrobial, antioxidant, and anti-inflammatory activities, and preventing skin agieng.

In the cosmetic field, anti-ageing active agents can be uncovered from the *Plectranthus* plants, since they are rich in phenolic compounds with high antioxidant activity. Also, the *Plectranthus* extracts and isolated compounds could have potential for the enzymatic inhibition activity for two main enzymatic classes: Hydrolases and Oxidoreductase. Besides them, the level of acethycholine is also an important issue.

Therefore, this work is primarly focused in finding new bioactive agents for dermatological disorders concerning anti-epidermal pigmentation, anti-ageing and anti-sagging skin.

In this study, extracts were prepared by ultrassonic and by microwave extraction methods. Preliminary results revealed that *P. ecklonii* and *P. grandidentatus* organic extracts attained the most antioxidant activity, by the scavenging of DPPH method. The new enzymatic protocols provided reproducible and very promissing results, with inhibition values higher than 50%. The most efficient extracts for acetylcholinesterase, collagenase, elastase and tyrosinase inhibition were *P.ornatus* ethyl acetate, *P.ornatus* aqueous, *P.neochilus* methanolic, *P.grandidentatus* ethyl acetate, and *P.ecklonii* methanolic extracts, respectively. Parvifloron D was the compound with the highest inhibition activity in all of the studied enzymes.

Due to the last promising results, other bioactivities and the forthcoming work of IC_{50} values of the bioactive extracts are under study.

Unmet needs for cosmetics research

P53 The Relationship between the Three-Dimensional Face Shapes and Age Perceptions Received from Observation Angles

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Purpose
We previously confirmed that age is perceived differently depending on each face-observation angle. In this study, we examine the influence that the three-dimensional face shape has on the age perceptions received from various observation angles.

Method
Using a three-dimensional imaging system (VECTRA M3, Canfield Technology, USA), we obtained images of 280 Japanese women, aged 20 to 69. Using the shape data from each image, we created homology models with 11,255 peaks per person. We conducted principal component analyses for each ten-year age-group. We then performed a correlation analysis between the obtained principal component scores and the results from a previous study that obtained age perceptions when subjects observed faces from different angles.

Results and Discussion
A number of correlations were discovered between the principal component scores for the shapes in each age-group and the ages perceived by subjects observing the shapes from different angles.

The principal components such as cheek volume, jaw size, and cheekbone positions were not correlated with age perceptions obtained when the face was observed from the front. Instead, they were correlated with age perceptions obtained when the face was observed from specific angles.

Thus, the possible differences in age perceptions obtained when viewing the three-dimensional shape of the face at a particular angle can be described as shown.

P54 Chitosan encapsulated phytosomes of *Plectranthus madagascariensis*: an innovative antibacterial system for topical application

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The drug resistant bacteria have complicated the treatment of skin infections. As topical route is the most suitable for skin infections, numerous topical treatments have limited efficacy against resistant bacteria[1]. Some natural products as plants from *Plectranthus* genus have been used as skin conditioning and antibacterial in traditional medicines[2]. Extracts from P. madagascariensis (PM) have been investigated by our group due to their potent antibacterial activity namely against Staphylococcus aureus and S. epidermidis, including some methicillin resistant strains (MIC=1.95µg/mL)[3]. In this work an antibacterial PM extract was incorporated into phytosomes and then encapsulated with chitosan (Chi-PS-PM). The obtained particles were spherical and amorphous (SEM and AFM) with low polydispersity (0.2), an average size of 1082±363 nm and a favourable zeta potential (21±12mV). A sustained release over 10h of 82.9% (pH 5.5) or 100.8% (pH 7.4) was found for the most prevalent antibacterial compound in the extract (7α,6β-dihydroxyroyleanone). Also a reduction in the membrane flux (0.52±0.17µg/cm2/h) was verified when compared to the bulk extract (1.90±0.08 µg/cm2/h). The antibacterial activity against skin pathogens was maintained after encapsulation. Those results have showed that Chi-PS-PM is a promising system to be applied as antibacterial in topical formulations.

References:
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