



ISBS
INTERNATIONAL SOCIETY FOR
BIOPHYSICS AND IMAGING
OF THE SKIN

From surface to deepness

In memory of Pr P.G. AGACHE

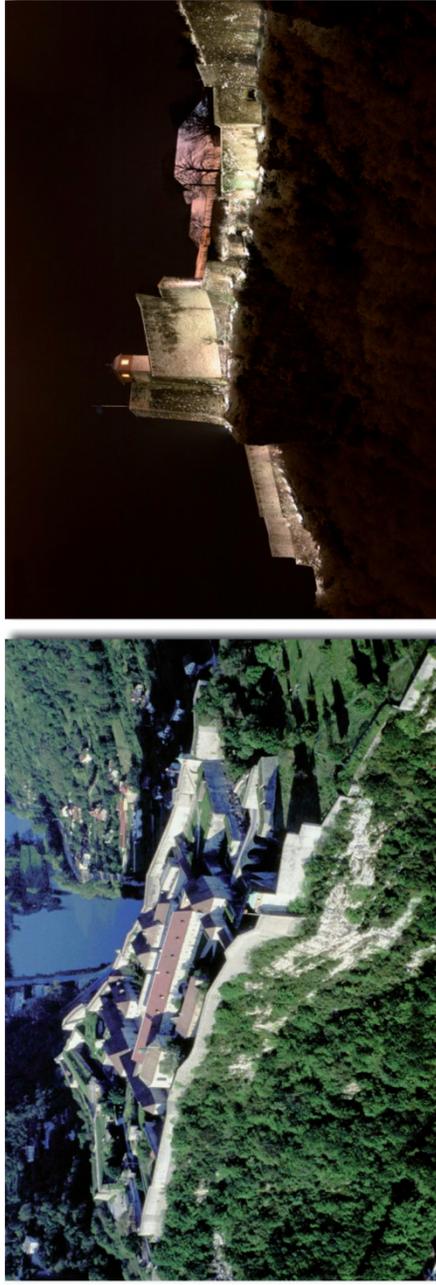
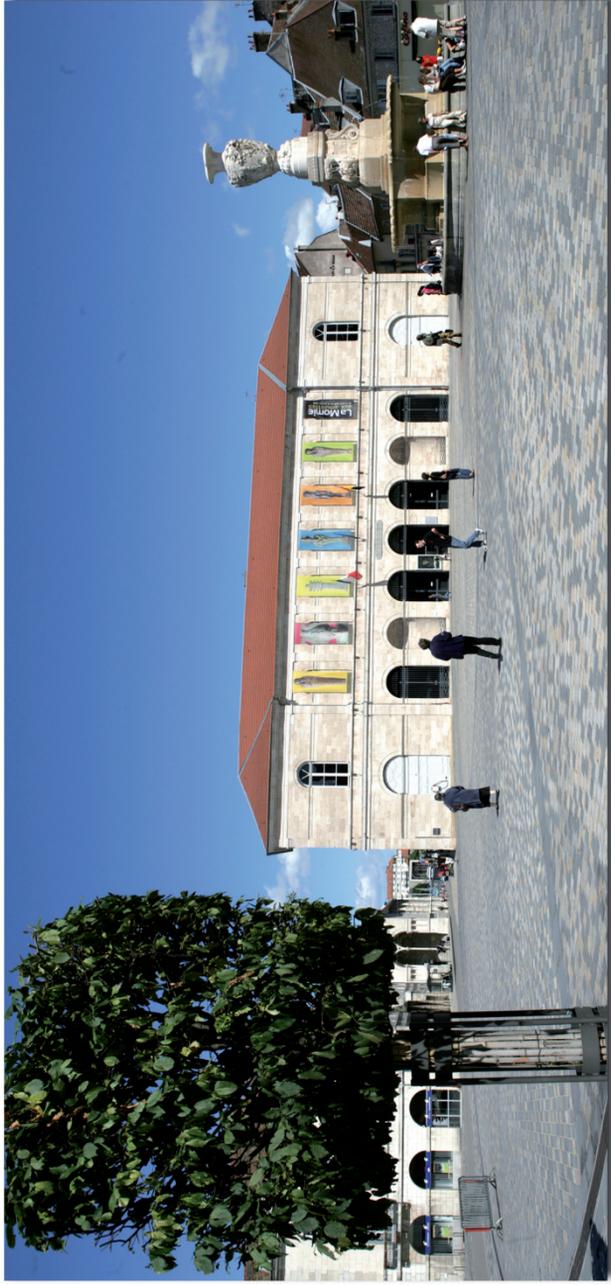
BESANÇON 2009 **ISBS International Meeting**

September 9-12, 2009
Besançon - France

CONGRESS BOOK

Under the Patronage of :





ISBS INTERNATIONAL SYMPOSIUM

BESANÇON (France) - September 9-12, 2009



250 ATTENDEES

29 COUNTRIES

3- ETHNIC SKINS

- **Skin colour diversity of Indian women**
J. Latreille, S. Gardinier, C. Guinot, E. Tschachler, F. Morizot 55
- **Factors influencing the severity of skin aging in Indian women**
E. Manger, J. Latreille, R. Jdid, C. Guinot, F. Morizot, E. Tschachler 56
- **Typology of maghreb skins**
N. Ismaili, Y. Afifi, B. Hassam, T. Lihoreau, A. Elkhyat, A. Jedy, P. Humbert..... 57
- **The correlation between Cutometer®'s parameters of skin elasticity and ages of Thai female volunteers (Poster)**
N. Waranuch, M. Sirada, K. Ingkaniman, W. Wisutitiprot..... 58
- **Grading of improvement and relapse in melasma of Thai females after 8 weeks-treatment with a combined cream of hydroquinone, steroid and tretinoin (Poster)**
W. Pratchyapruit..... 59

4- SKIN BIOMECHANICS

- **Analysis of the dynamics of femoral skin acoustic properties under the conditions of graduated traction for lower limb congenital shortening**
V. Shevtsov, L. Grebenyuk..... 61
- **Usage of mechanical acoustic parameters of investigated tissues for diagnosis of hyperextension and plasticity reserve in reconstructive restorative treatment in orthopaedics and plastic surgery**
V.I. Shevtsov, L.A. Grebenyuk, E.B. Grebenyuk, K.I. Noviko 62
- **Mechanical characterization of *in vivo* human skin using a 3D force-sensitive micro-robot and finite element analysis**
C. Flynn, P. Nielsen..... 63
- **Numerical identification of initial stress within *in vivo* human skin**
A. Chihouai, J. Chambert, L. Capek, F. Richard, L. Dzan, E. Jaquet..... 64
- **Evaluation of the elastic properties of the skin using the suction method (Cutometer®). Which parameters to use for claims in anti-aging treatments?**
A.O. Barel, R. Clysen, P. Clarys..... 65
- **Age-related changes in skin mechanical properties. Quantitative evaluation of 120 female subjects in a trial with a strict design (Poster)**
N. Krueger, S. Luebberding, M. Oltner, M. Streker, M. Krescher 66
- **History of high frequency ultrasound (Special lecture)**
K. Hoffmann..... 67

5- SKIN PHYSIOLOGY AND SKIN DISEASES

- **Decorative tattoos and motivation for their removal: a prospective study in 151 patients living in South of France**
J. Latreille, J.L. Levy, C. Guinot..... 69
- **The method of diagnosis of demodex**
N. V. Kaluga, A.A. Makarchuk 70
- **Effects of partial sleep deprivation on face appearance and skin properties**
F. Morizot, J. Latreille, S. Gardinier, L. Staner, C. Guinot, A. Porcheron, E. Tschachler..... 71
- **Objectivation of the individual sensory state by the assessment of specific biophysical properties of the skin in different climatic conditions**
A. Bigouret, F. de Oliveira, C. Gehin 72
- **Clinical visualization of photodamaged skin by facial averaging**
S. Hawkins, J. Carlson, J. O'Leary, B. Tiddeman, D. Perrett 74
- **Face morphing and age perception**
A. Porcheron, J. Latreille, C. Guinot, E. Tschachler, F. Morizot 75
- **Neonatal skin maturation – *vernix caseosa* and natural moisturizing factor**
R. Wickert, R. Utturkar, M. Vischer, W. Pickens, S. Hoath 76
- **Mannose-6-phosphate improves the colour of skin following damage**
S. Alam, J. Chantray, J. Bush, R. Hobson, S. O'Kane, M.W.J. Ferguson..... 77
- **TopO2 decay rates used as a metabolic indicator of the human skin *in vivo* (Poster)**
P. Contreiras Pinto, J.G. Morais, L. Monteiro Rodrigues ... 78
- **To understand skin circulatory physiology by low perfusion experiments with a monocompartmental model: the influence of age (Poster)**
P. Contreiras Pinto, J.G. Morais, L. Monteiro Rodrigues ... 79
- **Characterization of sensitive skin syndrome volunteer's barrier by dynamical analysis (Poster)**
P. Contreiras Pinto, C. Parreira, L. Monteiro Rodrigues ... 80
- **Approaching the topical formulation "heavy legs" claim (Poster)**
P. Contreiras Pinto, L. Monteiro Rodrigues 81
- **About the mechanisms involved in skin response to local temperature provocation (Poster)**
P. Contreiras Pinto, S. Moniz, L. Monteiro Rodrigues 82
- **Exploratory study of the typology of mature skin at different stages (Poster)**
J-M. Sainthillier, S. Mac-Mary, D. Monnier, P. Mermet, C. Tarrit, M. Mudry, C. Mudry, P. Humbert..... 83
- **Changes on body skin as a function of age (Poster)**
M. Lancin, A. Nkengne, G. Stamatas, F. Le Goff, A. Papillon, C. Bertin 84
- **Human skin wettability cartography (Poster)**
A. Elkhyat, Y. Afifi, B. Hassam, P. Humbert 85
- **Hair: hydrophobic/lipophilic balance - wettability and friction coefficient (Poster)**
A. Elkhyat, P. Humbert 86
- **Wettability and friction coefficient hydrophobic/hydrophilic balance influence (Poster)**
A. Elkhyat, P. Humbert 87

- **Nail: hydrophobic/lipophilic balance - wettability and friction coefficient (Poster)**
A. Elkhyat, T. Lihoreau, P. Humbert..... 88

- **Wettability of *Vernix Caseosa: ex vivo* studies (Poster)**
A. Elkhyat, T. Lihoreau, P. Humbert..... 89
- **Non-invasive methods of anatomico-structural skin changes evaluation in children with juvenile scleroderma (Poster)**
K. L. Vardanian, M. K. Osminina, N. A. Geppé, S. B. Tkachenko 90
- **Development of an optimized treatment regimen and method of forecasting wound healing outcome following cutaneous exposure to sulfur mustard in the weanling Yorkshire swine (Poster)**
J. Azeke, D. Usamiric..... 91

6- STRATUM CORNEUM AND BARRIER FUNCTION

- **The barrier function and water-holding capacity of the *stratum corneum* are not simply inter-related each other but are influenced by underlying pathological conditions as well as by body locations**
H. Tagami..... 93
- **Estimation of the *stratum corneum* diclofenac reservoir by chromametry**
R. Clijisen, P. Clarys, A.O. Barel 94
- **Non-invasive bioengineering assessment of the skin barrier function in patients with chronic venous insufficiency**
I. Angelova-Fisher, D. Wuthe, D. Zillikens, B. Kahlé..... 95
- **Measurement of inter- and intra-cellular water in *stratum corneum***
M. Misra, S. Shi, S. Yakovlev, M. Libera 96
- **Macro-scale mathematical model for hydration and TEWL in intact *stratum corneum***
R. Imhof..... 97
- **Experimental studies on the nature of *stratum corneum***
I. Sadiq 98
- **Assessing the effects of different semi-occlusive wound dressing over the epidermal barrier recovery (Poster)**
M.M. Pereira, L. Monteiro Rodrigues 99
- **Effect of sweating by exercise on *stratum corneum* hydration, skin surface sebum content and pH value (Poster)**
W. Siyu, L. Li..... 100
- **Side-by-side comparison of open chamber (TM 300) and closed chamber (Vapometer) TEWL (Poster)**
M. Steiner, S. Aikman-Greed, F.D. Dick 101
- **Can we really measure cutaneous water at different depths by bioimpedance? (Poster)**
C. Rosado, H. Raimundo, L. Monteiro Rodrigues 102
- **Influence of humidity and temperature on percutaneous absorption of VX through pig ear skin (Poster)**
D. Josse, C. Cruz, Y. Lhoutoune, P. Humbert..... 103

7- SKIN OPTICS AND DIGITAL IMAGING

- **Optical properties of the skin in the visible range Application to the measurement of skin complexion**
J. de Rigal, C. Yahi, A. Guiolet, C. Montastier, P. Humbert 105

- **Non-invasive optical methods for the study of infant skin**
G. Stamatas, J. Nikolovski 106

- **How to measure exactly the same location on the face as a function of time with digital photography?**
J. W. Wiechers, S. Mac-Mary, S. Vacheron, J-M. Sainthillier, E. Garcia, G. Khazaka, P. Humbert, B. Gabard..... 107
- **IOMA Sphere as a diagnosis tool in the evaluation of skin treatments**
E. Viviant 109
- **Innovative combination of *in vivo* methods to assess pores characteristics in surface and volume**
L. Colomb, G. François, C. Gevrey-Renaux, F. Flament, L. Bissey, J. Senée 110
- **Contactless apparatus with lateral and vertical micrometric resolution for real-time qualification of live tissue topography**
C. Millot, J.F. Quiniou, C. Roques-Carnes 111
- **Acquisition, visualization and exploration of stereoscopic images of skin with virtual reality**
K. Benzeroual, M. Haouach, G. Venturini, C. Guinot 112
- **Distant evaluation of arsenic-induced skin hyperpigmentation and hyperkeratosis using digital skin images**
N. Matveev, Th. Li, T. Islam, S.N. Kales, D.C. Christiani... 113
- **Advances in 3D Stereo Imaging**
J-P. Thirion..... 114
- **Relationship between aging patterns and facial sagging using a visual photographic scale (Poster)**
L. Vasquez-Pinto 115
- **Objective characterization of a face shape by image processing (Poster)**
F. Pons, S. Le Bruhac, L. Agopian 116
- **The role of color accuracy of digital images in distant evaluation of burns (Poster)**
N. Matveev, B. Kobrinsky, L. Budkevich, O. Starostin, A. Shinin, P. Tarasov 117
- **Three-dimensional facial simulations and measurements: changes associated with facial expression (Poster)**
E. Okada, Y. Maruyama, K. Onishi, A. Hayashi, M. Saze 118
- **A new light into skin whitening evaluation: development of an image analysis method and comparison with clinical trial approaching (Poster)**
L. Vasquez-Pinto 119
- **Intrinsic, solar and sunbed-induced skin aging measured *in vivo* by multiphoton laser tomography (Poster)**
M.J. Koehler, M. Kaatz..... 120
- ***In vivo* diagnosis of malignant melanoma by multiphoton laser tomography (Poster)**
M.J. Koehler, E. Dimitrov, J. Norgauer, K. König, M. Kaatz 121

8- IN VITRO BIOENGINEERING

- ***In vitro* skin surface titration: from macro to nano**
M. Wagner, V. Roucoules, M.F. Vallat, A. Mavon, H. Duplan .123
- **Tretinoin in polyethylene glycol-phospholipids micelle: Stability improvement and human fibroblast viability**
A. Wichit, A. Tangsumranjit, T. Pitaksuteepong, N. Waranuch .124

- **Three-dimensional imaging of collagen reorganization induced by mechanical stress in engineered skin tissue**

M. Huart, H. Pernot, N. Boyera, A.-M. Pena, A.-M. Minondo, A. Potter, C. Ebenhahn, F. Leroy, A. Black, E. Beaurepaire, J.L. Martin, M.-C. Schanne-Klein, A. Colonna 125

- **Cell imaging connected with protein mapping to investigate glyphosate-induced toxicity on human keratinocytes**

C. Heu, C. Elie-Caille, E. Lesniewska, M. Ewald, L. Nicod 126

Empetrum nigrum fruit extract: a multilevel skin protector

T. Saguet, L. Couturier, F. Yvergnaux 127

- **Exploration of anti-aging activity of Corsican «immortelle» Essential Oil. Technique and evaluation on human skin explants maintained in survival.**

P. Gasser, E. Lati, K. Fontes, Y. Millou, J.L. Pierrisnard, C. Toured, D. Davenne 128

- **Autologous cultured fibroblast injection for facial acne scar (Poster)**

H. Seunghian, L. Onsek, L. Gunwo, K. Jaeyoung, M. Seon-ghee, P. Gyuman, O. Chihwan 129

- **Cellomics® imaging technology reveals inhibition of fat accumulation in adipocytes by natural food constituents (Poster)**

I. Warnke, R. Goralczyk, J. Schwager 130

- **Stroma vascular-derived cells from human subcutaneous adipose tissue exhibit contractile properties stimulated by a selected hexapeptide (Poster)**

J. Franchi, C. Viennet, S. Robin, A. Zakaroff-Girard, F. Pellicier, S. Schnebert, M. Lafontan, J. Galitzky, M. Dumas, P. Humbert 131

- **A new lipooaminoacid able to restore aging-induced modifications in functional and biomechanical properties of human dermal fibroblasts (Poster)**

L. Cattuzato, N. Chevrot, S. Dumont 132

- **In vitro method measuring calcium percutaneous egression in normal and tape-stripped human skin (Poster)**

E. Jungman, H. Maibach 133

- **Potential of Thai breadfruit extract for application in anti-aging product (Poster)**

J. Viyoch, B. Supasiri, D. Binda, C. Viennet, P. Humbert 134

- **Comparison of in vitro - in vivo SPF correlation of 12 sunscreen commercial products using two sorts of skin substitutes (Viro-Skin x PMMA) (Poster)**

I. Salgado-Santos, C. Haroutounian 135

9- ENGINEERING & PROTEOMICS

- **Effect of flaggrin gene mutations on biophysical and biochemical skin properties in individuals with pathological dry skin**

S. Gardinier, J. Latreille, V. Mlitz, C. Guinot, E. Tschachler 137

- **Influence of TNF- α polymorphism -308 on irritant dermatitis in health care workers**

R. Wickert, J. Davis, M. Visscher 138

- **Quantitative in vivo imaging of tissue visco-elasticity using Supersonic Shear Imaging (Special lecture)**

M. Tanter, J.-L. Gennisson, M. Pernot, M. Fink 139

- **Functional food, food supplements and the skin (Special lecture)**

K. P. Wilhelm, G. Springmann, S. Biefeldt 141

10- CLAIMS

- **Developing a proper testing program for cosmetic advertising claim substantiation in the US**

S. Schwartz 143

- **New method of skin rejuvenation in aesthetic cosmetology and plastic surgery**

N.V. Kaluga 144

- **Corneometry in assessing health safety of skin purification products**

O.I. Voloschenko, O.V. Payetska, O.I. Yalovenko, T.G. Momot 145

- **Smile, beauty, self-perception and fulfillment in the society**

S. Zemmouri 146

- **Evaluation of bust morphological modifications by fringes projection by using a cosmetic product (Poster)**

D. Hottelart, M. Thomas, J.-C. Pittet, J.-J. Servant, K. Vie 147

- **Assessment of the nourishing effect of a lip balm exploratory study (Poster)**

J.-M. Sainthillier, S. Mac, C. Tarrit, P. Mermet, D. Mougin 148

- **Exploratory epidemiological study on caucasian women face skin depending on age (Poster)**

D. Hottelart, J.-C. Pittet, G. Simoneau, K. Vie 149

- **Evaluation of the cellulite through thermal camera measurement (Poster)**

A. Papillon, A. Nkengne, C. Bertin 150

- **Corsican « immortelle » Essential Oil is a real anti-aging ingredient (Poster)**

P. Gasser, E. Lati, K. Fontes, Y. Millou, J.L. Pierrisnard, C. Toured, D. Davenne 151

- **Acetyl hexapeptide-3 in a cosmetic formulation acts on skin anisotropy - clinical study (Poster)**

K.A. Tadini 152

- **Assessment of the effect of a topical treatment on early active stretch marks in post-partum women: a simple blind, randomized intra-individual study (Poster)**

N. Lachmann, J.-C. Pittet, M. Fischer, P. Msika 153

- **Contribution of instrumental methods for the assessment of hyaluronic acid fillers (Poster)**

C. Rouvrais, A. Degouy, A. Rouquier, D. Black, G. Josse, A.-M. Schmitt, V. Turlier 154

- **Comparative instrumental study of aesthetic dermatology acts and cosmetic protocols (Poster)**

J.-M. Sainthillier, S. Mac, M. Pfulg, V. Gribinski, V. Guillaou, J. de Rigal, C. Montastier, P. Humbert 155

INDEX

..... 156

ISBS APPLICATION FORM

..... 158

ISBS MEMBERS 2009

..... 159

TRIBUTE



PR PIERRE AGACHE †

Pierre AGACHE, who has died in September 2003, aged 76, led a professional life of incomparable intensity. Born in Roubaix in the north of France, he studied medicine in Lille in 1947, was intern at Lille hospital in 1951, then senior registrar in 1958. An accomplished specialist in Dermatology and Venereology, he became interested in fundamental research early in his professional life. He gained a hydrology certificate and a serology certificate at the Pasteur Institute of Lille, then became Professor "Agrégé" of Medicine in 1965, and in 1969 Professor of Dermatology and Head of the department of Dermatology in Besançon, succeeding Professor Laugier who had just been appointed in Geneva (Switzerland).

All his life, he developed new ideas and projects. Although he retired in 1993 from the Hospital and in 1996 from the University, he endlessly worked as a teaching specialist and researcher in our team where he always played an active part. In 1996 he was appointed Professor Emeritus by the University of Franche-Comté.

As far as 1969 when he arrived in Besançon, Professor Agache always aimed at maintaining the excellent qualities of the Dermatology Department regarding patients care as well as dermatopathology. He very quickly understood the importance of developing parallel specialities, such as Allergology, Angiology, Photobiology. Within a few years, the size of the Allergology Laboratory was increased tenfold and in 1974 he was the instigator of the Certificate of Allergology and Clinical Immunology which was under his responsibility.

With a particular interest in Angiology he set up new techniques such as photoplethysmography, laser-doppler, transcutaneous oxygen pressure measurement and he recruited an engineer for this unit. He also created a Phlebology outpatient unit. Today this unit of Angiology is integrated in the Dermatology Department.

Professor Agache was also the main initiator of an original field of research: skin measurements, which he used to describe as the practical application of knowledge in skin physiology. In 1980 he published in an international journal the results of his work on the measurement of sebum excretion, an innovative idea in France then. This was followed by investigations on sweat production, transepidermal water loss, mechanical properties, hair growth, scarring and photobiology.

Interested by anything that could benefit patients, in 1976 he set up a new treatment of skin diseases by UV originating from the United States which was a revolutionary method at the time: PUVAtherapy. Besançon became the second unit in France providing this therapy. Many remember this unit on the Faculty grounds with 4 doctors and 2 assistants helping patients coming from far afield and receiving treatment till 10 pm !

Never short of new ideas, Professor Agache could not conceive his career at the Hospital and University without working in research, and encouraging young dermatologists and researchers.

Less than 7 years after his arrival in Besançon he created the Laboratory of Cutaneous Biology and Biophysics in the Faculty of Medicine that he equipped with the help of the INSERM (National Health and Medical Research Institute), especially in ultramicrotomy. Two themes were developed: firstly research on verruca, then skin biomechanics. In 1969, very little was known on the virus of human verruca, apart from its morphological aspect in ultrastructure. With hard work, Professor Agache helped Dr Orth of the Pasteur Institute of Paris, a specialist of rabbit verruca, to isolate the genome of the virus of painful plantar verruca and show that this virus was different from the virus of mosaic verruca. These viruses were called type I and type II papilloma viruses. In Besançon, an internationally renowned team continues to work on this subject that he initiated.

Research in skin Biomechanics earned him worldwide recognition as one of the top international specialists in Biometrology. As a non conventional precursor, he quickly started to have regular contacts with foreign Universities. He also worked closely with the Laboratory of Applied Mechanics of the Faculty of Science and the Technology Institute of Besançon; this initiative was at the time very original and even subject to criticism.

Besançon became recognized internationally in this new field of research and Professor Agache organized in 1983 the 4th Intercontinental Congress of the International Society of Bioengineering and the Skin (ISBS) in Besançon, after Miami, Cardiff, Philadelphia and before San Francisco. Specialists from 20 countries, among them United States and Japan, participated in this meeting.

In 1980 Professor Agache contributed to the creation in Besançon of the INSERM unit of hormonal biochemistry. He also set up structures of biomedical engineering dedicated to all disciplines and contributed to the creation of a biomedical engineering structure, (a hospital unit) of GBM and finally a chair of professor. This structure, the 7th in France, was designed to develop medical equipment since this technology was lacking in France. He was founder President from 1980 to 1984.

It would be too long to detail all his work in terms of communication and Congress organization, but thanks to his efforts the Department of Dermatology of Besançon was recognized as one of the most important internationally. In 1991 he was elected President of the French Society of Dermatology and Venerology. This Society benefited from his numerous innovative and original ideas.

As a world known expert, he was a member of the Conseil Supérieur d'Hygiène Publique in France and a European expert whose opinion was highly regarded.

Professor Agache was undoubtedly a great researcher and a precursor, but also an excellent teacher who was able to explain complex physics or chemistry laws with simple words and images.

To his last day, he worked on a book to be published in English, which is the consecration of his career, about skin measurement methods, with contributions from international experts.

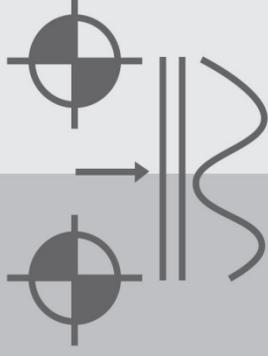
His human qualities, friendliness, consideration and relaxed approach were greatly appreciated and all his former colleagues and members of his team will remain grateful to him.

Pr Philippe HUMBERT

Head of Department of Dermatology

University Hospital

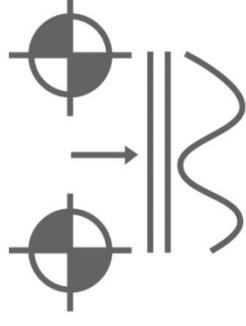
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ISBS
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ISBS International meetings

1976	Miami	USA
1979	Cardiff	Wales
1981	Philadelphia	USA
1983	Besançon	France
1985	San Francisco	USA
1986	Liège	Belgium
1988	Milwaukee	USA
1990	Stresa	Italy
1992	Sendai	Japan
1993	Lübeck	Germany
1994	Cincinnati	USA
1996	Zurich	Switzerland
1998	Boston	USA
2000	Jerusalem	Israel
2002	Paris	France
2002	Edinburgh	UK
2002	Baltimore	USA
2003	Hamburg	Germany
2004	Orlando	USA
2005	Philadelphia	USA
2006	Atlanta	USA
2007	Cardiff	Wales
2008	Seoul	Korea
2009	Dallas	USA
2009	Besançon	France
2010	Buenos Aires	Argentina



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Editor in chief of Skin Research & Technology

- Jørgen SERUP

The President of the Franche-Comté University

*is pleased to invite you to an award ceremony in honour of
Professors Albert KLLIGMAN, Howard MAIBACH, Ronald MARKS,
Gérald PIÉRARD, Hachiro TAGAMI and Mr. Gabriel KHAZAKA
during which they will be distinguished by the Franche-Comté University
as Honorary Professors*

*Wednesday, September 9th, 2009, 15:00
Salle de parlement du Palais de Justice - Besançon (France)*



ALBERT KLIGMAN

Albert M. Kligman studied first Psychiatry and Mycology at Penn. University in Philadelphia. His first papers concerned some techniques of staining in mycology. He was then invited in Paris in 1945 for consulting about rebuilding the mushrooms plants around Paris. After the mushrooms in the forests he went to skin and took his medical degrees at Penn during the fifties. From 1955 up to the nineties he has been considered as the leader (or better the pope) of Dermatology. He is a 180° open minded scientist expert in biology, clinics, creating methods, using new instruments, for skin research.

Among the numerous scientific discoveries in Dermatology, the following advances made by Albert Kligman can be quoted: the allergy test (with Magnusson), the irritation test (with Frosch), Biology of sebum (with Shelley), skin microbiology (with Williamson), Poison ivy (identification of urushiol), All-trans retinoic acid for acne (in the sixties) and treatment of photoaging (1985), skin whitening (Kligman's trio), Effect of UVa and UVb on skin (in collaboration with his wife Lorry Kligman), Regression test for moisturizers, etc.

A lot doctors today are indebted to him because he has been great Professor who created a sponsorship (Simon Greenberg) for helping students

It is quite impossible to summarize Albert's activity and personality in so few lines. His personality as a Scientist but also as a man overwhelms most of his fellows: He is without any doubt the Master of Dermatology of the end of the XX° century.



Regression test for moisturizers, etc.

My life in dermatology

«I have lived through the most exciting, thrilling, spectacular molecular biology, pharmacology, biochemistry, etc. time in all of dermatologic history.

I finished my residency at the University of Pennsylvania in and topical drugs which enable them to treat effectively the 1951, which marked the end of a period which I have called multifarious, diverse diseases that comprise dermatologic practices.

paleodermatology, which I characterized as empirical, morphology, Dermatology is no longer simply a skin game.

didactic, authoritarian, descriptive dermatology, the legacy of the Many disorders formerly thought to be limited to the skin, European masters.

Mid-century also marked the beginning of a new movement which involvement of internal organs. Then too systemic diseases I called neodermatology, based on experimental, innovative, frequently have cutaneous manifestations that require the research-oriented basic investigations, culminating in what we expertise of dermatologists for diagnosis and treatment.

now call evidence-based medicine. To be a competent dermatologist today one has to be an internist as well as an externist.

At the birth of neodermatology there were only four M.D., Ph.D. Dermatologists are avid scholars. 90% leave their practices Lerner, and Walter Shelley. Sadly, I am the sole survivor of this to attend the national meeting of the American Academy of

pioneer group of researchers. Dermatology!

At that time dermatologists were not considered real doctors and No medical specialty spends as much effort and money in were mocked by the establishment for their primitive methods of nurturing the careers of young investigators. The Dermatology

treating skin lesions, using noxious topicals that soiled, stank, Foundation in the USA furnishes 3 million dollars annually in and stained. This contrasts stunningly today when one-third of awarding scholarships and fellowships, much of which comes from

applicants for residencies are M.D., Ph.D.'s. Half of the staffs the membership.

of the leading departments are M.D., Ph.D.'s. Applicants come At age 93, I am happy to have lived long enough to watch this from the top of the graduating class, the best and the brightest. incredible transformation of dermatology from a backwater

Competition for a residency position is furious. It seems that clan of clowns to the eminent position that dermatology now everyone wants to become a dermatologist. holds in the hierarchy of medical sciences.

To become a full professor in our leading departments of dermatology requires knowledge of immunology, genetics,

A. Kligman

HOWARD MAIBACH



Howard Maibach is Professor in the Department of Dermatology University of California, San Francisco. He became Dermatology Professor in 1973 and Chief of the Occupational Dermatology Clinic in 1984.

He received many awards and is an honorary member of different societies over the world.

His present researches are dedicated to dermatopharmacology, dermatotoxicology, psoriasis and exogenous dermatoses.

He published more than 2000 papers, and was Editor of an impressive number of medical books. He is a recognized specialist in skin toxicology and percutaneous absorption, as well as *stratum corneum*, among many others topics

«Students of skin bioengineering development need only examine Taken together, with the collaboration of physicists, the lectures/posters/commercial exhibits of the ISBS meetings to mathematicians, chemists, biologists, and physicians, the ISBS embryo (to-days bioengineering) will reach maturity – and solve clinical questions that plague us today».

As a decade's long (and continued) student of Albert Kligman, I share his vision statement of the future (see his statement).

H. Maibach

RONALD MARKS

Ronald Marks trained in Medicine at Guy's Hospital, London 1953 – 59. He trained in Dermatology in the army 1960 – 65 and at St George's Hospital, London, St John's Hospital and the Institute of Dermatology, London and the University of Miami, Miami 1965 – 72. RM became Senior Lecturer and Consultant at St John's Hospital for diseases of the skin 1972 – 73 and then in the University Hospital of Wales 1973 – 1982 when he was promoted to Professor of Dermatology in the University of Wales. He was appointed Clinical Professor of Dermatology in Miami in 1993. He retired from the University Of Wales in 1998 and became Professor Emeritus. He continues to practice, teach and research in this subject. RM is current chairman of the International Society of Stratum Corneum Research, founder member of the Aging Skin Group of the British Association of Dermatologists and Life Present of the ISBS. He is currently Medical Director of Cutest Systems Ltd in Cardiff and is a member of the London Expert Witness Institute. He has published more than 400 papers and written or edited and contributed to more than 40 books. His main research interests are, non invasive instrumentation, the stratum corneum aging and photodamage.



He has published more than 400 papers

Dermatology Counts

«Of course it does! You don't need me to tell you that. Just ask the average patient with severe generalized atopic dermatitis or with widespread lesions of senile pemphigoid whether they prefer an up to date dermatologist or an inexperienced non specialist to treat them – you will soon find out that dermatology does indeed count. But dermatology counts in another sense as well. How many of you were in Besancon in 1984 at one of our very first congresses? It is with pleasure and pride that I remember that meeting. I was Chairman of our Society at that time and the Mayor of the City spoke to us, made me a Freeman of the City and awarded me a medal! At that time we were an excited, keen and innovative bunch of scientists with the common obsession to measure and count the characteristics of skin in health and when diseased. Have we achieved our aims? In some ways we have. Look around you in the exhibition halls of the major meetings of the AAD or the EADV. There is now a profusion of instruments of all sorts – admittedly many of them, such as lasers and phototherapy machines for treatment but also sophisticated ultrasound, Confocal microscopy and laser Doppler imaging devices. It seems that at last our clinical colleagues can hear the skin call out, "Measure me"! "Count me"! But we cannot relax, there is a long way to go. We still do not have techniques or instruments for assessing the extent and severity of disease. When we do have these our patients will profit and we will have made a major step forward».

R. Marks

GÉRALD PIÉRARD

Gérald E. Piérard was born in 1946. He is Professor at the University of Liège, Belgium and Chairman of the department of Dermatopathology, CHU Sart Tilman, Liège, Belgium.



Gérald Piérard is a creative researcher and dream up innovative ways of skin analysis, *in vitro* and histologically. He interested in many fields of Dermatology and Medicine, such as skin fungal infections, sebum, skin aging, etc.

He was past president of the International Society of Dermatopathology, the International Society for Bioengineering and the Skin, the European Group for Efficacy Measurement of Cosmetics and Other Topical Products (EEMCO) and the European Society for Cosmetic and Aesthetic Dermatology (ESCAD).

He is President of the Mosan Study Group of Pigmented Tumors, and a member of the board of 10 dermatological societies. He is Associate editor of 14 scientific journals, founding member of 6 scientific societies, and member of 28 international societies. He is Honorary member of the Tunisian Society of Dermatology, Doctor Socium Honoris Causa of the Polish Society of Dermatology. He received 13 national and international awards.

He published 189 books, monographies and chapters of books and more than 1500 publications in scientific journals.

The Liège analytical vision of skin conditions

« Man just landed on the moon. At the same time, a young MD Other friends were also of importance in my scientific life. To become resident in Dermatology. What a contrast for him ! On cite only a few, I would like to mention the late Pierre Agache the one hand, a fantastic adventure supported by a technological and Bernard Ackerman, and the ever active colleagues Philippe Humbert, Enzo Bernardesca and Jean-Luc Lévêque. feat. On the other hand, a "wonderful" world merely based on descriptive aspects, dogmatism and subjectivity. With my team of researchers, all of them being dual MD's and PhD's, it was our contention that the future of dermatology resided In the early '70, I followed a leitmotiv: measure when you can, and if there is no measurement, invent one". At that time, I in seeing with the eyes and looking with the brain. We created received the support of Charles Lapière who encouraged me to in 1988 the département of dermatopathology with a section of explore this way of thinking. Soon, I had the chance to meet three analytical microscopy and a laboratory of skin bioengineering mentors. First, Ronald Marks was fascinating with his many and imaging. We are grateful to Gabriel Khazaka for a fruitful inventions of hand-made bioengineering devices. The concepts cooperation with his company pushing onward and upward the he derived from his findings were so clever! He deserves the progresses in skin bioengineering. honor to be designed as one of the most brilliant experimentalist Have we reached our initial goal? Surely not, a small step was in dermatology. However, as a French-speaking dermatologist, made forward but much effort remains to be made. Hopefully, I experienced from time to time some difficulties to "translate" the story is not over and I foresee a brilliant future for the next what appeared to me as a Welsh accent altered by a pipe in the generation in Liège and elsewhere. Bioengineering will help mouth. Albert Kligman was my second mentor. I was fascinated streamlining the endeavour of the future dermatology. However, by the flood of new ideas, always stimulating the brain. And last we must be aware that in spite of all technologies it all comes down but not least, I met Howard Maibach who impressed me with to shaping one individual at a time in the field of dermatology».

G. Piérard

HACHIRO TAGAMI

Professor Hachiro TAGAMI was born in Tokyo in 1939. He is now emeritus Professor of Tohoku University. He was Professor and Chairman at the Department of Dermatology, Tohoku University School of Medicine. He is member of many scientific societies, and was Vice President of the Japanese Cosmetic Science Society, belonged to Board of Director of the Japanese Dermatological Association, the Japanese Society for Investigative Dermatology, the Japanese Society for Inflammation, and the Japanese Society for Psoriasis Research.

He received different awards such as Minami Memorial Award from the Japanese Dermatological Association for the study on the leukotactic factor derived from psoriatic scale, a Scientific Award from the Japan Foundation for Skin Health and Industry for the development of the method to evaluate the skin surface hydration, the Yasuda-Sakamoto Award from the Japan Lydia O'Leary Foundation for the original investigations performed in the field of dermatology in Japan.

He was especially interested in research in Inflammation and immunity of skin diseases, particularly psoriasis, atopic dermatitis, contact dermatitis, warts and superficial fungal infections. He is also particularly recognized for his knowledge and researches in instrumental analysis of skin functions, particularly that of the stratum corneum under various conditions. So far 541 papers in English have been published in international journals, together with 32 papers in English as chapters for different books.



Serendipity in my young days

«Born 1939 in Tokyo, I luckily survived not only those destructive air raids during the World War II but also tuberculosis and glomerulonephritis.

I chose Kyoto University to study medicine, because there are many original old temples and palaces in Kyoto. However, I was fascinated by the attractive personality of Professor Ofuji and his lecture. At that time, most Japanese professors published their papers in Japanese journals just as a mere echo of the authorities in the Western countries. However, Dr. Ofuji was full of original ideas, trying to publish his papers in international journals, e.g., eosinophilic pustular folliculitis (Ofuji).

In my first year of residency, he suggested me to join the team of Dr. Kligman at the University of Pennsylvania for research training. In fact, Dr. Kligman was the best mentor for young dermatologists. In the subsequent 2.5 years, I enjoyed numerous in vivo studies on skin functions such as the barrier function of the stratum corneum in various experimentally produced skin changes. I remember that it was such a relief for me to see him every Friday evening when I presented all the findings obtained in the past one week, because I could find his enjoying face and expect his comfortable encouragement.

These experiences in US motivated me a lot. After my return to Kyoto University, I started my own research on several projects, this time assisted by Dr. Ofuji's pertinent comments. One of such projects is about psoriasis, which had been recognized as a unique metabolic dermatosis showing greatly enhanced epidermal proliferation. From the production of intra-corneal microabscesses, he suggested the presence of chemotactic factors in the scales. Thus, I

H. Tagami

GABRIEL KHAZAKA



Gabriel Khazaka was born in 1943 in Damascus / Syria. After his baccalauréat, he performed studies of law at the University of Damascus, then he studied Economy and Marketing at Cologne University in Germany and obtained in 1971 his Academic degree – Diploma of trader

He practiced Trainee at pharmaceutical company Nattermann, Cologne, and was an expert and marketing Manager at company Nattermann.

In 1980 he became General Manager at company Schwarzhaupt Medizintechnik in Cologne and began to develop medical engineering devices for diagnosis, e.g. for eyesight testing, hearing tests, reaction tests and for blood pressure

In 1986 he established the Courage + Khazaka electronic GmbH Company in Köln (Cologne –Germany) dedicated to the development, manufacturing and distribution of dermatological testing devices. He is owner of numerous inventions in the field of skin diagnosis as well as several patent applications in this field.

Since 1986 to the present day he is the General Manager of the company Courage + Khazaka electronic GmbH, a world-wide leader of skin testing devices.

«It is a great joy for me to be honoured for my work of the last 25 years, at the ISBS meeting. It is not only the honour that is so important to me but also the very location of this meeting. When I left my hometown of Damascus in 1964 and came to Europe for the first time as a young man of 20 years of age, I would never have imagined my life would turn out this way. As a child I was raised by the Christian Sisters of Besancon in Syria and as a boy I kept on dreaming of this city, so far away. In my dreams I walked the streets of Besancon many times and imagined myself in the middle of this city. Now my childhood dreams have come true.

From the beginning, after founding Courage + Khazaka, the members of the ISBS society have always supported me in every possible way. Especially in the beginning, I could count on Prof. Pierre Agache's input into my work. Since I come from the business side of this field I am always grateful for the ideas and opinions of the scientists. This does not only stimulate our work but it also puts our developments to the test critically thus helping us to constantly improve our products, which have found worldwide recognition. In the ideas and the work of Prof. Philippe Humbert this collaboration lives on and also allows me to visit Besancon from time to time, the city of my childhood dreams.

In this context I would like to take the opportunity to thank also Dr. Karl-Heinz Schrader, Prof. Hagen Tronnier, Prof. Ulrike Heinrich, Prof. Vera Rogiers, Prof. Ronald Marks, Prof. Howard

Maibach, Prof. Gerald Piérard, Prof. Enzo Berardesca, Prof. Noel Kim, Prof. Peter Elsner, Prof. Klaus Wilhelm, Prof. André Barel, Prof. Hristo Dobrev, Dr. Joachim Fluhr, Dr. Hans-Peter Nissen and the many other scientists who have always evaluated my works critically and objectively.

Skin has always been extremely exciting to me and from the beginning I have loved the idea of inventing instruments to investigate its different parameters. I hope that for the future we will be able to contribute to exploring the secrets of the skin. We are especially proud that our devices are used around the world in beautiful locations like Besancon, but recently have also been used in space. After my studies of Business Economics in Cologne, Germany I have always work in the field of medical technical systems. When founding Courage + Khazaka I broke new ground in the field of objectively measuring the skin. For all new technologies and ideas which may come up in the future I will always have a sympathetic ear. One of my dreams for the future is to supply simple and inexpensive technology to third world countries in order to help diagnose skin diseases in an early state and thus treat them accordingly and prevent them worsening. I hope that this area will receive more attention from all of us in the near future».

G. Khazaka



Gladimir V. G. BARANOSKI

Gladimir V. G. Baranoski received a Ph.D. in Computer Science from the University of Calgary (Canada) in 1998. Currently, he is an Associate Professor at the School of Computer Science and the leader of the Natural Phenomena Simulation Group at the University of Waterloo (Canada). He is also an elected member of Executive Committee of the European Association for Computer Graphics and associate editor of Elsevier journal Computers & Graphics. The results of his research on the modeling of light interactions with natural materials (e.g., plants, soil and human tissues) have been made available to the scientific community through the publication of articles in remote sensing, applied optics and computer graphics journals and conference proceedings.

**Modeling light and skin interactions:
pitfalls and perspectives**

- **G. Baranoski**

Computer simulations are regularly being used by researchers to understand and predict the responses of skin tissues. In this lecture, we will discuss theoretical and practical challenges that should be tackled to improve the efficacy and accuracy of current simulation frameworks. Furthermore, since similar modeling approaches are also used in scientific fields, this lecture also aims to foster cross-fertilization with these fields by identifying common needs and complementary resources. The lecture begins by providing a concise review of relevant biophysical background, followed by a discussion on the fundamental concept of predictability. It continues by examining the specific constraints and pitfalls found



Stephen BOPPRT

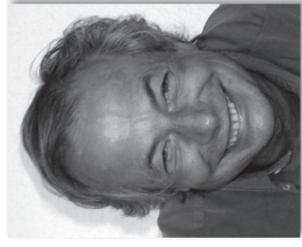
Prof. Stephen Boppert received his PhD in Medical and Electrical Engineering from MIT, and his MD from Harvard Medical School. At the University of Illinois at Urbana-Champaign, he is Professor of Electrical and Computer Engineering, Bioengineering, and Medicine, and Head of the Biophotonics Imaging Laboratory at the Beckman Institute for Advanced Science and Technology, one of the first and largest interdisciplinary research institutes in the United States. His research interests include the development and translation of novel optical imaging and diagnosis technologies for medical and biological applications. He is currently Leader of an Illinois Imaging Initiative focused on developing imaging science, technology, and applications across multiple disciplines.

Innovations in skin imaging

- **S. Boppert***

Recent advances in optical imaging and diagnosis technologies now enable high-resolution, non-invasive, label-free imaging and characterization of in vivo skin, as well as in vitro and grafted engineered skin. This keynote presentation will review several state-of-the-art skin imaging techniques including optical coherence and multiphoton microscopy, second harmonic generation microscopy, and coherent anti-Stokes Raman microscopy. In addition, optical coherence elastography and magnetomotive techniques using magnetic nanoparticles enable quantification of skin biomechanical properties. The ability of optical imaging techniques to track cellular and molecular changes *in vivo* and longitudinally over time offers the potential to investigate a wide range of processes. Combined, these new technologies can provide a wealth of new anatomical, molecular, and biomechanical information about healthy, diseased, damaged, aging, rejuvenated, and repaired human skin.

* BOPPRT SA, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois USA



Michel MANFAIT

*Engineer in Physics, PhD in Physics, Fellow in Pharmaceutical Sciences
Professor (Faculty of Pharmacy, University of Reims Champagne-Ardenne, France)
Head of MEDIAN Unit, CNRS UMR 6237, University of Reims Champagne-
Ardenne
Field of research
Molecular Biophysics. Vibrational microspectroscopies. Spectral imaging. Optical
(Raman and FTIR) characterization of cells and tissues.*

**Skin and vibrational microspectroscopies
(Raman and FT-IR)**

- **M. Manfait***

Vibrational microspectroscopies (FTIR and Raman) are highly potential tools to investigate biological samples at various scales from cell analysis to *in vivo* development. This presentation will consider biomedical applications of spectral micro-imaging (hyper spectral, multimodal), from frozen and paraffin embedded (without chemical

dewaxing) skin tissue sections. Examples will focus on characterisation of cancer lesions of skin. Perspectives of an *in vivo* Raman analysis with an up to date dedicated instrumentation will be shown.

* Unité MEDIAN CNRS UMR 6237, UPR Pharmacie, Université de Reims Champagne-Ardenne,
51 rue Cognacq Jay, 51096 Reims Cedex, France



Laurent MISERY

Pr Laurent Misery is professor of dermatology at the University of Brest, France, head of the department of dermatology at the university hospital and director of the laboratory of skin neurobiology. He performed numerous studies about interactions between skin and the nervous system. He has recently published a book about pruritus.

**The skin: A sensory organ. From neuronal mechanisms
to self-perceived sensitive skin**

- **L. Misery***

Skin is one of the five sensory organs. Its nervous network is dense and fine, allowing numerous different perceptions. Nerve endings are coursing up to the upper layers of epidermis (but not *stratum corneum*). Synapses between nerve endings and all cells from the skin can be observed, with a high neuronal plasticity: obviously with Merkel cells but also with immune cells (Langerhans cells, dermal dendrocytes, mast cells...) and epidermal (keratinocytes, melanocytes) or dermal cells (fibroblasts, endothelial cells). Neurones produce neurotransmitters that could bind to receptors which are expressed by all skin cells. Hence, skin and the nervous system are able to communicate, defining the neuro-immuno-cutaneous system (NICS): data can be transmitted from skin to the nervous system since skin is a sensory organ and nerve cells can modulate all properties of the skin: immunity, vasomotricity, hair growth, keratinocyte activation, collagen production, etc.

Very interesting new data have been recently provided about the expression of sensory proteins belonging to the TRP (transient receptor channel) family and about Merkel cells. TRP are expressed not only on neurones but also on keratinocytes and other cells. This suggests

that keratinocytes are able to act as sensory cells then to transmit sensory data to neurones. Merkel cells are cells which remained mysterious. We could provide new techniques for long-term cultures, allowing us to demonstrate that they can act as mechanoreceptors by themselves. More interestingly, we showed that they can modulate the epidermal cells through the release of neurotransmitters by their dendrites.

Clinical consequences of the sensory properties of the skin are numerous. It allows to better understand pathophysiology of sensitive skins. Skin sensitivity is defined by the occurrence of erythema and/or sensations like burnings, prickings, itch or pain after exposure to various factors: UV, heat, cold, wind, water, cosmetics, stress, etc... The presence of both vascular and sensory abnormalities indicates the intervention of the skin nervous system. The triggering by factors that could be physical, chemical or psychological is only possible if TRP and similar sensory proteins are activated. Hence, skin sensitivity is due to abnormalities on keratinocytes. We can suppose that the activation of TRPs is enhanced, with a lower threshold, in people presenting with sensitive skins.

* Laboratory of Skin Neurobiology, University Hospital Morvan, Brest, France



Lars NORLÉN

Lars Norlén, Assoc. professor, Karolinska Institute, Stockholm, Sweden

Current position:

Group Leader in Electron Tomography/Biophysical Dermatology, Medical Nobel Institute, Department of Molecular Cell Biology, Karolinska Institutet, Stockholm, Sweden
Specialist in Clinical Dermatology, Dermatology Clinic, Karolinska University Hospital, Stockholm, Sweden

Previous position:

Group Leader in Biophysical Dermatology, Dept. of Physics, Group of Applied Biomedical Physics, University of Geneva, Geneva, Switzerland, 1999-2004

Awards: 1999 Wenner-Gren Fellow, Wenner-Gren Foundations, Stockholm, Sweden

2008 LEO Goldprize in medical research

Conference organization:

Chairman of the Wenner-Gren Symposium - «The Human Skin Barrier As A Biomembrane Model», June 28-30, 2007, Wenner-Gren Center, Stockholm.

Chairman of the Tomography Symposium - «Progress in Cryo-electron Tomography of Vitreous Sections», Karolinska Institutet, November 21-23, 2008.

INVITED SPEAKER



Cees W.J. OOMIENS

Cees Oomens is associate professor in Biomechanics and Continuum mechanics. He studied physics at Eindhoven University of Technology. He obtained his PhD at Twente University (1985) on a study on the mechanical behavior of skin.

At the present time his research is focused on damage and adaptation of soft biological tissues, with emphasis on skeletal muscle tissue and skin.

A major application field is a study on the etiology of pressure ulcers. The goals are to develop an objective method to identify patients at risk of developing a pressure ulcer and to develop techniques for early detection of deep tissue injury.

A multi-scale approach is adopted, ranging from studies on cultured cells, tissue engineered constructs of muscle and skin, in-vivo animal studies and human studies. Theoretical models include convective and diffusive transport processes in deforming structures and interaction with growth, synthesis and damage development.

Mechanical properties of human skin layers

- C.W.J. Oomens*

Cryo-electron microscopy and tomography of vitreous skin sections

- L. Norlén*

Presently, in the post-genomic era, major efforts are directed towards unravelling biological mechanisms at the molecular level.

Until now these efforts have been hampered by the difficulty in obtaining near-native in-situ data with molecular resolution.

Tomography of vitreous tissue sections (tissue TOVIS) has the potential to obtain three-dimensional structural data of biomolecules in their native cellular context with a resolution of a few nanometers.

The talk will outline some major challenges as experienced from the application of TOVIS to human skin.

The mechanical properties of human skin have been studied seriously since the beginning of the seventies of the last century. Although the *stratum corneum* and the role of temperature and humidity on its properties, had the interest of researchers between 1970 and 1985, most older studies on mechanical properties ignored the top layers and were focussed on the dermis. Many of these studies were performed in-vivo, using tensile, torsion, suction and indentation testing. Since the late nineties, however, new imaging techniques and inverse methods became available, allowing a separation of the properties of the different layers of the skin ranging from the subcutaneous adipose tissue, via dermis to epidermis and *stratum corneum*. At the same time clinical developments (f.e. trans-epidermal drug delivery) and new developments in cosmetic products and personal care fed the need to acquire this knowledge.

In the second part of the presentation some in-vitro work will be presented from ex-vivo studies on adipose tissue, epidermis and *stratum corneum*. Preparation techniques will be discussed. The presented testing methods are shear tests on a rheometer and micro-indentation testing.

Apart from the stiffness and the strength of the individual top layers it is extremely important in many clinical or cosmetic applications to understand and quantify transport of small, large, neutral and ionised molecules through all layers of the skin. This is part of biomechanical research too and a few examples of recent work on this subject will be discussed briefly.

In the presentation some of the recent developments of this multi-scale approach to the mechanics of skin will be discussed. Part of the discussion will be based on in-vivo studies to find mechanical properties of *stratum*

* Karolinska Institute, Stockholm, Sweden

* Eindhoven University of Technology, Biomedical Engineering Department, P/O Box 513 5600 MB Eindhoven, The Netherlands



Vera ROGIERS

Prof. Vera Rogiers is Head of the Department of Toxicology, Dermato-Cosmetology and Pharmacognosy at the Vrije Universiteit Brussel (VUB). She is professor of toxicology and organizes postgraduate courses on "risk assessment of cosmetics in the EU" (<http://safetycourse.vub.ac.be>).

Her main research activity is situated in *in vitro* experimental toxicology. Actual focus is on (i) epigenetic modification of primary hepatocytes to stabilize their specific phenotype in culture and (ii) production of functional human hepatocytes from mesenchymal progenitor cells of different adult human tissues. She is author of more than 220 publications in international peer-reviewed journals and is editor of several scientific books. She has been invited speaker (>130 times) in and organized herself (>40) international congresses. She has obtained several international scientific awards and is reviewer of a number of international scientific journals in toxicology, pharmacology and dermatology. She is, at the EU level, co-chair of the SCCS and member of ESAC. She is chairperson of ecopa, bringing together Academia, Industry, Animal welfare and Governmental Institutes in the different EU Countries.

Safety and efficacy: cosmetic dossier and the EU regulation

- **V. Rogiers***

Key points in the actual EU cosmetics legislation can be summarized as follows:

- Cosmetic products must be safe for the consumer
 - The responsibility lies with the manufacturer, first EU importer or marketer
 - The safety of the end product is based upon safe ingredients (toxicological profile, chemical structure, exposure)
 - Safety must be guaranteed without animal testing (strict deadlines apply, namely 11 March 2009 and 11 March 2013)
- Risk assessment lies at the basis of safe cosmetics and is carried out in the EU via 2 different channels, namely at the Commission level (DG SANCO) and within the industry (safety assessor). Ingredients for which some concern exists with respect to human health are evaluated by the SCCS (Scientific Committee on Consumer Safety, former SCC(NF)P) and concern annexes II, III, IV, VI and VII of Dir 76/768/EEC. All ingredients (actives and non-actives) of cosmetics brought on the EU market also must undergo a safety evaluation by an independent safety assessor.
- The reflection of this exercise is present in a so-called
- "dossier" (PIR=Product Information Requirement; TIF=Technical Information File; PIF=Product Information File) that must be kept available for inspection by the appropriate authorities.
 - A list of general toxicological requirements for cosmetic ingredients can be found in the "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" (SCCP/1005/06). Although most of the hazard tests are traditionally based upon the use of experimental animals, the actual EU cosmetics legislation imposes the use of the 3R's concept (Refinement, Reduction and Replacement) and focuses in particular on validated "Replacement" methodology.
 - Validated alternatives have been developed over the last 25 years and were in particular successful in addressing hazard for acute and local toxicity.
 - A number of these validated tests are refinement/reduction methods and for these strict deadlines exist in the EU with respect to testing and marketing:
 - Acute oral toxicity: fixed dose procedure, acute toxic class, up-and-down method
 - Skin sensitisation: local lymph node assay
 - Eye irritation: several screenings for severe and strong

* Department of Toxicology, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium

irritants

Validated replacement methods are available for the endpoints:

- Skin corrosivity: Transcutaneous Electrical Resistance, EpiSkin™, EpiDerm™
- Skin irritation: EpiSkin™, modified EpiDerm™, Skin Ethic™ RHE
- Phototoxicity test: 3T3 Neutral Red Uptake Phototoxicity test
- Dermal absorption: *in vitro* test with human or pig skin

- Embryotoxicity: Whole Embryo Cytotoxicity test, MicroMass test, Embryonal Stem cell Test

Validated alternative tests, however, are lacking for quantitative risk assessment and especially for systemic and long-term toxicity testing (sub acute & sub chronic toxicity, chronic toxicity and carcinogenicity, developmental toxicity, toxicokinetics), representing a major obstacle for the further implementation of *in vitro* testing in regulatory toxicology.

Some changes are expected in the near future, not in relation to animal testing, but in the form of the actual legislation. Indeed a "Directive" implies that every change made must be first implemented in the national legislation of each Member State before it is

available and applicable all over Europe. This is very time-consuming and leads to different interpretation of the same piece of legislation. Simplification of the procedure became a necessity and therefore a Cosmetic Regulation will come into force, applying the same market control rules at the same time in all EU Member States. In this so-called RECAST, apart from some limited changes in content e.g. one single EU notification; some new provisions for CMRs (present in food and for which no alternatives exist); an extension of the qualifications of the safety assessor and in general better harmonized standards, no substantial changes will occur. The cosmetic "dossier" will become a "Cosmetic Safety Report" with two parts (A= cosmetic product safety information and B = cosmetic product safety assessment). This report still will contain the same risk assessment procedures as before. No changes in the animal testing decisions are foreseen within this RECAST scenario.

More information with respect to cosmetics safety can be found in the book: "Safety assessment of cosmetics in Europe", eds. Vera Rogiers and Marleen Pauwels, in book series 'Current Problems in Dermatology', vol 36, Karger (Basel, Switzerland)

In memory of Pr. P.G. Agache



From surface to deepness JSBS International Symposium

In association with ESCAD
European Society for Cosmetic & Aesthetic Dermatology

Besançon

From 9th to 12th of September 2009

PROGRAMME

President : Pr. Philippe HUMBERT



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Durations (including questions) :
Invited speakers : 45'
Special lectures : 30'
Communications : 15'



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Institut national
de la santé et de la recherche médicale



In honor of Professors A. Kligman, H. Maibach, R. Marks, G. Pierard, H. Tagami and Mr. G. Khazaka

In memory of Pr. P.G. Agache



From surface to deepness JSBS International Symposium

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European Society for Cosmetic & Aesthetic Dermatology

Besançon

From 9th to 12th of September 2009

PROGRAMME

15h 00		AMPHI B	
SESSION 3 : ETHNIC SKIN	Chairwomen	C. Guinot	
■ Skin colour diversity of Indian women		J. Latreille	
■ Factors influencing the severity of skin aging in Indian women		E. Mauger	
■ Typology of Maghreb skins		N. Ismaili	
SESSION 4 : SKIN BIOMECHANICS	Chairmen :	A. Barel	
		C. Flynn	
		S. El Gammal	
■ Analysis of the dynamics of femoral skin acoustic properties under the conditions of graduated traction for lower limb congenital shortening		L. Grebenyuk	
16h 00 - 16h 30 Break			
■ Usage of mechanical acoustic parameters of investing tissues for diagnosis of hyperextension condition and plasticity reserve in reconstructive restorative treatment in orthopaedics and plastic surgery		L. Grebenyuk	
■ Mechanical characterization of <i>in vivo</i> human skin using a 3D force-sensitive micro-robot and finite element analysis		C. Flynn	
■ Numerical identification of initial stress within <i>in vivo</i> human skin		J. Chambart	
■ Evaluation of the elastic properties of the skin using the suction method (Cutometer®)		A. Barel	
17h 30 End of the day			
20h 00 GALA DINNER: "Grange Grillard" typical dinner in ARBOIS (Jura)			



Victor Hugo



Victor Hugo

Friday 11 September

8h30	<i>Invited speaker</i> : Lars Larién	Molecular cryo-electron tomography of skin
9h15	<i>Special lecture</i> : Klaus Hoffmann	History of high frequency ultrasound
9h45 - 10h15 Break		
10h15	SESSION 5 : SKIN PHYSIOLOGY and SKIN DISEASES	Chairpersons :
	■ Decorative tattoos and motivation for their removal : a prospective study in 151 patients living in South of France	S. Hawkins R. Wickett
	■ The method of diagnosis of demodex	C. Guinot
	■ Effects of partial sleep deprivation on face appearance and skin properties	N. Kaluga
	■ Objectivation of the individual sensory state by the assessment of specific biophysical properties of the skin in different climatic conditions	F. Morizat
	■ Clinical visualization of photodamaged skin by facial averaging	A. Bigouret
	■ Face morphing and age perception	S. Hawkins
	■ Neomatal Skin Maturation - <i>Verrix Cassaze</i> and Natural Moisturizing Factor	A. Porcheron
	■ Mannose-6-phosphate improves the colour of skin following damage	R. Wickett
		S. Alam

In honor of Professors A. Kligman, H. Maibach, R. Marks, G. Pierard, H. Tagami and Mr. G. Khazaka

In memory of Pr.P.G. Agache



From surface to deepness JSBS International Symposium

In association with ESCAD
European Society For Cosmetic & Aesthetic Dermatology

Besançon

From 9th to 12th of September 2009

PROGRAMME

AMPHIB		
10h30	SESSION 6 : STRATUM CORNEUM and BARRIER FUNCTION	Chairmen : H. Tagami H. Maibach
	<ul style="list-style-type: none"> The barrier function and water-holding capacity of the <i>stratum corneum</i> are not simply inter-related each other but are influenced by underlying pathological conditions as well as by body locations Estimation of the <i>stratum corneum</i> diclofenac reservoir by chromametry Non-invasive bioengineering assessment of the skin barrier function in patients with chronic venous insufficiency Measurement of inter- and intra-cellular water in <i>stratum corneum</i> Macro-scale mathematical model for hydration and TEWL in intact <i>stratum corneum</i> Experimental studies on the nature of <i>stratum corneum</i> 	R. Cljssen I. Angelova - Fischer M. Misra B. Imhof I. Sadiq
12h45	Lunch	
13h30	Invited speaker : Michel Manfait	Skin and vibrational microspectroscopies (Raman and FT-IR)
14h15-14h30	Break	



Louis Pasteur



Hôpital Saint-Jacques - Besançon

AMPHI A		
14h30	SESSION 7 : SKIN OPTICS and DIGITAL IMAGING	Chairmen : B. Gahard J.W. Wiechers
	<ul style="list-style-type: none"> Optical properties of the skin in the visible range: Application to the measurement of skin complexion Non-invasive optical methods for the study of infant skin How to measure exactly the same location on the face as a function of time with digital photography? IDMA Sphere as a diagnostic tool in the evaluation of cosmetic skin treatments Innovative combination of <i>in vivo</i> methods to assess pores characteristics in surface and volume 	J. de Rigal G. Stamatias J.W. Wiechers E. Viviant L. Colomb
15h45-16h15	Break	
	<ul style="list-style-type: none"> Contactless apparatus with lateral and vertical micrometric resolution for real-time qualification of live tissue topography Acquisition, visualization and exploration of stereoscopic images of skin with virtual reality Distant evaluation of arsenic-induced skin hyperpigmentation and hyperkeratosis using digital skin images Advances in 3D stereo imaging 	C. Roques - Carmes C. Guinot T. Islam J.P. Thirion

In honor of Professors A. Kligman, H. Maibach, R. Marks, G. Pierard, H. Tagami and Mr. G. Khazaka

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AMPHIB		
14h30	SESSION 8 : IN VITRO BIODENGINEERING	Chairwomen : A. Colonna D. Maugin
	<ul style="list-style-type: none"> <i>In vitro</i> skin surface titration : from macro to nano Tretinoin in polyethylene glycol-phospholipids micelle : stability improvement and human fibroblasts viability Three-dimensional imaging of collagen reorganization induced by mechanical stress in engineered skin tissue Cell imaging connected with protein mapping to investigate glyphosate-induced toxicity on human keratinocytes 	M. Wagner A. Wicht A. Colonna C. Heu
15h30-16h00	Break	
	<ul style="list-style-type: none"> Empetrum nigrum fruit extract a multilevel skin protector Exploration of anti-ageing activity of Corsican « immortelle » Essential Oil. Technic and calculation of various markers on human skin explants maintained in survival 	T. Saguet D. Davenne
16h30	SESSION 9 : ENGINEERING and PROTEOMICS	Chairwomen : A. Colonna D. Maugin
	<ul style="list-style-type: none"> Effect of Flaggrin gene mutations on biophysical and biochemical skin properties in individuals with pathological dry skin Influence of TNF-α polymorphism-308 on irritant dermatitis in health care workers 	J. Latreille R. Wickett
17h15	End of the day	



Citadel of Besançon



Salines Royales - Arc 6 Semains

CITADEL PARTY		
Saturday 12 September		
8 h 30	Invited speaker : Cees W.J. Oomens	Mechanical properties of human skin layers
9 h 15	Special lecture : Mickaël Tanter	Quantitative in vivo imaging of tissues visco-elasticity using Supersonic Shear Imaging
9h45-10h15	Break	
10h15	Invited speaker : Vera Rogiers	Safety and efficacy: cosmetic dossier and the EU regulation
11h00	Special lecture : Klaus P. Wilhelm	Functional food, food supplements and the skin
11h30	SESSION 10 : CLAIMS	Chairpersons : V. Rogiers R. Bazin
	<ul style="list-style-type: none"> Developing a proper testing program for cosmetic advertising claim substantiation in the US New method of skin rejuvenation in aesthetic cosmetology and plastic surgery Corneometry in assessing health safety of skin purification products Smile, beauty, self-perception and fulfillment in the society 	S.R. Schwartz N.V. Kaluga O.I. Voioschenko S. Zemmouri
12h30	CLOSING CEREMONY	
14h00	Conducted tour : Salines Royales and Pasteur Route	

In honor of Professors A. Kligman, H. Maibach, R. Marks, G. Pierard, H. Tagami and Mr. G. Khazaka

Capacitance imaging of skin lesions

- J-L. Lévêque*

Very few information are available concerning possible changes in the skin surface water content of lesional skin compared to normal, not involved, skin. Capacitance imaging is a relatively new technique allowing differences in the skin surface hydration to be routinely imaged and quantified with a lateral resolution of 50 micrometer. Moreover this method is very convenient for observing and quantifying the micro-relief of the skin surface (1)(2).

By using this technique, various skin lesions were observed and characterized in terms of surface hydration and surface micro-relief: acne, seborrheic keratoses, warts, scars, angiomas, naevi, etc.

Compared to the surrounding not involved skin, some of these lesions appear more hydrated (warts, some lentigos,

seborrheic keratoses, rubis,..) although some others appear drier (achromia, parakeratosis, kerato-acanthomas, some scars..) (3)(4) Acne lesions would appear more or less hydrated according to their degree of inflammation. Surface hydration of lentigos is variable, probably related to their degree of evolution. Photoaged skin of exposed skin is characterized by unevenness in skin hydration, some diffuse pigmented areas being more hydrated than surrounding skin although some other are drier (4).

General results obtained on pigmented lesions suggest that synthesis and diffusion of melanin and flaggrin are closely related.

Chairmen: Jean-Luc LÉVÊQUE - Gérard PIÉRARD

- (1) Lévêque JL, Querleux B: SkinChip a new tool for investigating skin surface in vivo. *Skin Res Technol*, 2003 Nov, 9(4), 343-7.
- (2) Batisse D, Giron F, Lévêque JL: Capacitance imaging of the skin surface. *Skin Res Technol*, 2006 May, 12(2), 99-104.
- (3) Xhauf-laire-Uhoda E, Piérard GE: Contrast skin capacitance imaging of seborrheic keratoses and melanocytic naevi. *Dermatology*, 2006, 212, 394-397.
- (4) Laquitze S, Bazin R, Rosillo A, Lévêque JL: Dermatoheliosis of the chest: Characterization by capacitance imaging. *Int J Cosmet Sci*, 2009, 31, 225

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The influence of *stratum corneum* hydration on body fat determination by bioelectrical impedance analysis

- P. Clarys*
- P. Dericmaecker*
- R. Clijjsen**
- J. Taeymans**
- A.O. Barel*

Introduction: The use of bioelectrical impedance analysis (BIA) of the body (or parts of the body) is a modern method for the estimation of the amount of body fat. Depending on the used instrument contact between the instrument and the body is made at specific skin sites (e.g. hands or feet). It was the aim of the present study to evaluate the influence of the *stratum corneum* hydration at the contact points used for BIA on the body fat estimation

Methods: Subjects were students of the Faculty of Physical Education and Physiotherapy of our university (128 females and 126 males, mean age respectively 20.8 ± 1.2 years and 21.4 ± 1.6 years, BMI respectively 21.3 ± 2.0 kg/m² and 22.6 ± 1.9 kg/m².)

Using the Corneometer CM820 *stratum corneum* hydration was measured at the volar part of the forearms (reference value) and the sole of the right foot (metatarsal and heel region) and the palm of the right hand (metacarpophalangeal and carpal region) before and after contact with respectively the Tanita and the Omron body fat analyzer. Changes in *stratum corneum* hydration during the contact time were calculated (ΔH). As gold standard for body fat estimation the under water weighing method (UWW) was used and procentual deviation of the gold standard was calculated for the Tanita instrument (PDT) and the Omron (PDO).

Results: *stratum corneum* hydration at the volar part of the forearm was respectively 55 ± 10 a.u. for the females and

58 ± 13 a.u. for the males. During contact with the Tanita instrument *stratum corneum* hydration increased significantly at both measuring sites at the foot for both sexes. Changes in *stratum corneum* hydration after contact with the Omron were less consistent (see table).

Females	Before BIA	After BIA	p
Foot MT	61 ± 26	71 ± 26	<0.0001
Foot C	61 ± 27	70 ± 27	<0.0001
Hand ACM	93 ± 23	93 ± 22	0.608
Hand POC	91 ± 23	88 ± 24	0.001

The estimate of body fat differed significantly as a function of the used method and instrument for the females (OWW: $25.2 \pm 5.3\%$; Tanita: $22.0 \pm 4.9\%$; Omron: $18.0 \pm 3.8\%$ ($p < 0.0001$)) and the males (OWW: $12.9 \pm 5.8\%$; Tanita: $10.3 \pm 5.1\%$; Omron: $9.6 \pm 5.1\%$ ($p < 0.0001$)). No relation was found between *stratum corneum* hydration measured at the reference skin sites and PDT and PDO. *stratum corneum* hydration values measured at the respective contact sites before BIA, nor ΔH at the respective skin sites were related with PDT or with PDO.

Conclusion: The BIA measuring procedure with the Tanita instrument provokes an occlusive effect at the skin contact site. However, our results indicate that BIA for the determination of body composition is not influenced by *stratum corneum* hydration.

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New techniques for occupational skin health surveillance

- H. Taylor*
- P. Xiao*

Ill health due to skin exposure remains a considerable problem, particularly in the workplace. In our aim to reduce the incidence of occupational skin disease and ill health due to skin exposure we need to understand how exposure to substances and physical factors is affecting the skin and how best to identify early signs or pre-clinical signs of skin disease. This project investigated possible new techniques for occupational skin health surveillance. The project focused on techniques that would identify sub-clinical damage that could lead to irritant contact dermatitis.

A number of techniques were investigated. Of those investigated those that showed potential were the use of *stratum corneum* hydration gradients (calculated using Optothermal Transient Emission Radiometry hydration measurements), occlusion with a Corneometer probe and tape stripping. The hydration gradient studies showed that there

are differences between normal and sub-clinically damaged skin with the means of normal and damaged skin being significantly different. Occlusion with the Corneometer probe allows measurement of the rise in hydration during occlusion. The gradient of the initial rise in hydration differs between normal and sub-clinically damaged skin. The tape stripping studies used transepidermal water loss measurement to monitor the removal of the *stratum corneum*. This study highlighted a difference in the number of tape strips required to remove the *stratum corneum* of normal and sub-clinically damaged skin.

This presentation will outline and discuss the findings of these initial studies. It will also discuss the future possibilities of these techniques for use in skin health surveillance and in increasing our understanding of irritant skin damage.

* London South Bank University, 103 Borough Road, London, UK

Corneoxenometry: a *stratum corneum* toxicity model

- G. Piérard*
- P. Quatresooz*

Predicting the damaging effects of peeling agents on human *stratum corneum* is hazardous in conditions close to the *in vivo* situation. Comparisons between the effects of different compounds or different concentrations of a given compound may prove to be difficult to perform. By contrast, the *in vitro* corneoxenometry bioassay is safe, quite easy to handle and serve to predict some specific effects occurring in clinical practice.

The effects of different concentrations of glycolic acid (GA) and those of problematic commercially available phenol formulations were tested using the corneoxenometry bioassay. In short, cyanoacrylate skin surface strippings were harvested from healthy volunteers. They were dipped in the test products for a controlled time and stained for 30 s with toluidine blue and basic fuchsin. After rinsing, the color of the samples were measured in the L* and Chroma C* system from which the colorimetric index of mildness (CIM) is derived following CIM= L* - Chroma C*. When needed a gas chromatography was used for dosage purposes.

Ga at 5, 10, 20, 30, 50 and 70% concentrations were tested

as well as phenic acid present in commercially-available formulations at 4.4 and 10% claimed concentrations.

The bioassay using GA showed consistent reactivities with a dose-effect relationship when using a 3 min exposure time. The corneoxenometry CIM values progressively decreased with increasing GA concentrations. Significance was reached beginning with the 20% concentration. No statistical differences were obtained between any of two consecutive levels of the tested GA concentrations. Contrasting with these findings, data appeared unreliable and not informative for a 10-min exposure time.

The data about the commercially-available phenol formulations were astonishing and suggested a mislabelling of the claimed concentrations. The control by gas chromatography confirmed our concerns. One of the test products contained phenol at a concentration more than 1,000 folds higher than the claimed concentration.

In conclusion, corneoxenometry is an easy bioassay easy to perform. It is relevant for predicting *in vivo* adverse effects.

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SkinGlossMeter – a novel device for the measurement of skin gloss

- M. Niinimäki*
- K. Myller**
- J. Nuutinen*
- T. Lahtinen**

Background: Easily applicable instrument to measure gloss of skin has not been available. Concepts of skin shine, radiance, etc has been used but they are usually poorly defined.

Purpose: To develop a hand-held device for easy determination of local gloss of skin and to relate the measured skin gloss values with industrial gloss standard.

Methods: A small hand-held and battery-operated device with a patented diffractive optical element and laser light source (635 nm) was constructed. The specular reflectance later called gloss was defined as part of the incident light reflecting back at a small angle (≤ 5 degrees). The specular reflectance was measured from a gloss standard. Furthermore, the gloss of volar and dorsal forearm, cheek and forehead was measured with six healthy Caucasian volunteers.

Results: There was a significant correlation between the SkinGlossMeter reading and gloss standard. The coefficient of variation for ten repeated measurements was less than 1% on gloss standard. Respectively, on human skin the coefficient of variation was typically between 2 and 5 %. Moreover differences between glosses of anatomical sites were found. The time for single measurement was 2 sec.

Conclusions: A novel glossmeter for easy assessment of gloss was developed. Preliminary measurements indicate that in addition to skin the instrument can be applicable also with lip and nail. The SkinGlossMeter allows rapid and accurate measurement of gloss with applicants and formulations affecting human skin appearance.

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** Department of Physics, University of Kuopio; Finland



Jean-Claude GUIMBERTEAU

Jean-Claude Guimberteau was born in 1947. He is co-founder and scientific director of the « Institut Aquitain de la Main », and Director of Research at the « Société Française de Chirurgie Plastique Reconstructrice et Esthétique.

He is member of the French Hand Society (GEM) and of the French Plastic and Reconstructive surgery society (SOFCPRE). He was trained in the Hand and Plastic department of the Bordeaux University (Dr AJM. Goumain and Pr J. Baudet) During this surgical trainings he was one of the pioneers in microsurgery and transplantations. He spent few months (1976) as visiting fellow with Pr J-M. Converse in New York (USA) and Pr R. Millard in Miami (USA). Introducing innovative concepts in hand anatomy, physiology and secondary flexor tendon repair, he has been supported many years by Pr Cl Verdan and Pr H.E. Kleinert.

He is member of the French Academy of surgery, of the Haute Autorité de la Santé and General Secretary of the European federation of hand surgery units .

The skin excursion

- J-C Guimberteau*

This film is the continuation of the previous one « Strolling under the skin » which has shown a subcutaneous multifibrillar architecture enclosing microvolumes called microvolumes and which as they are piled up not only ensured the shape but also the mobility, the adaptability and resistance to the gravitational force.

The skin, boundary of this optimal efficiency world sine it allows life through this fractal and chaotic structure, has not been really studied in its morphodynamic aspect and chiefly in its continuity with the rest of the body. The aim of this film and always thanks to endoscopic observations carried on the living matter, during surgeries to show the existence of mobility mechanism,

the architectural organisation in the derm, the epiderm and hypoderm.

It enables us to prove that the tissue continuity is complete, always organised in a multi fibrillar and vacuolar system, enveloping either lots of cell as far as the epidermis concerned or few cells for the derm but always with a fractal chaotic organisation with a tension – compression forces balance, close to the subcutaneous structures already observed particularly at the level of tendons .

A vision of the body organisation in complete continuity through this inner architectural, will lead to new reflexions .

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***In vivo* reflectance confocal microscopy: a new non-invasive tool for microscopical skin inflammation evaluation and management**

• **M. Ardigo***

Reflectance Confocal microscopy of human skin is a new technique for in-vivo investigation of microscopical features of the skin with cellular resolution by the effect of wavelength on imaging and the role of specific reflectance indexes as a contrast agent. This technique allows optical sectioning of turbid objects. A thin plane or section can be optically or non-invasively imaged within a scattering medium with high resolution and contrast close to optical histology. With confocal microscopy is possible to show thin section of human skin *in vivo* and nuclear and cellular detail can be visualized without biopsy and histological processing. Dynamic process can be also observed non-invasively as the capillary blood flow.

Reflectance Confocal Microscopy have been used in medical application for the evaluation of neoplastic and inflammatory

melanocytic disorders due to the high reflectance index of melanin, but also keratinocytes, inflammatory cells, adnexal and dermal structures can be easily evaluated giving morphological and dynamic information. For that reason, also several inflammatory skin diseases (i.e. psoriasis, contact and irritant dermatitis, vitiligo, ect.) have been also evaluated disclosing the possibility to manage skin inflammation in term of diagnosis and follow-up.

Moreover, biological effects of drugs (i.e. hydroquinone), physical therapies (i.e. laser) and “cosmetics” (i.e. peelings) on the different skin layers and structures have been also evaluated giving interesting and useful information obtained using this instant and entirely non-invasive and infinitely repeatable technique for microscopical analysis.

Chairmen: Bernard QUERLEUX - Klaus P. WILHELM

References:

- Ardigo M, Cota C, Berardesca E, González S. *Concordance between in vivo reflectance confocal microscopy and histology in the evaluation of plaque psoriasis. J Eur Acad Dermatol Venerol.* 2009 Feb 19.
- Ardigo M, Maliszewsky J, Dell'anna ML, Berardesca E, Picardo M. *Preliminary evaluation of vitiligo using in vivo reflectance confocal microscopy. J Eur Acad Dermatol Venerol.* 2007 Nov;21(10):1344-50.
- Ardigo M, Maliszewski J, Cota C, Scope A, Sacerdoti G, González S, Berardesca E. *Preliminary evaluation of in vivo reflectance confocal microscopy features of Discoid lupus erythematosus. Br J Dermatol.* 2007 Jun;156(6):1196-203.
- Pellacani G, Gutera R, Longo C, Averamidis M, Seidenari S, Menzies S. *The impact of in vivo reflectance confocal microscopy for the diagnosis accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol.* 2007 Dec;127(12):2759-65.
- Yamashita T, Akita H, Asiner S, Miyakawa M, Lerner EA, González S. *In vivo assessment of pigmentation and vascular compartments changes in UVA exposed skin by reflectance-mode confocal microscopy. Exp Dermatol.* 2007 Nov;16(11):905-11.

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Melanocytes and skin pigmentation: *in vivo* visualization and characterization by Reflectance Confocal Microscopy

- **G. Pellacani***

Reflectance-mode confocal scanning laser microscopy is a new tool for the non invasive study of the skin at quasi-histopathologic resolution, giving rise to images corresponding to horizontal sections of the epidermis and superficial dermis. Since the contrast in confocal microscopy images is mostly provided by the differences in reflectivity of tissue organelles and cytoplasmatic melanin, pigment disorders and melanocytic lesions represent the best suitable candidates for confocal microscopy examination. Providing high-resolution and instantaneous imaging of cellular and architectural details in human skin, it is able to distinguish different amount of melanin within keratinocytes, as proven in different skin phototypes. Moreover, hypopigmentation disorders, such as vitiligo, and hyperpigmentation ones, such as melasma, have been explored, providing precious information on histopathologic characteristics without a biopsy and offering the possibility to evaluate pigment dynamics at cellular level

References:

- Pellacani G, Cesinaro AM, Seidenari S. Reflectance-mode confocal microscopy for the *in vivo* characterization of pagetoid melanocytosis in melanomas and nevi. *J Invest Dermatol.* 2005 Sep;125(3):532-7.
- Pellacani G, Cesinaro AM, Seidenari S. *In vivo* assessment of melanocytic nests in nevi and melanomas by reflectance confocal microscopy. *Mod Pathol.* 2005 Apr;18(4):469-74.
- Ardigo M, Matizewsky J, Dell'anna ML, Berardesa E, Picardo M. Preliminary evaluation of vitiligo using *in vivo* reflectance confocal microscopy. *J Eur Acad Dermatol Venerol.* 2007 Nov;21(10):1344-50.
- Pellacani G, Guitera P, Longo C, Avramidis M, Seidenari S, Menzies S. The impact of *in vivo* reflectance confocal microscopy for the diagnosis accuracy of melanoma and equivocal melanocytic lesions. *J Invest Dermatol.* 2007 Dec;127(12):2759-65.

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Comparison of the *stratum corneum* thickness on forearm, leg, face and palmar measured by confocal Raman spectroscopy and confocal microscopy

- **A. Böhling***
- **S. Bielfeldt***
- **P. Buttgererit***
- **U. Ely***
- **K.P. Wilhelm***

Environmental conditions i.e. UV-light, mechanical or chemical stress and external treatments effect the outer skin layers most. To ensure homeostasis the *stratum corneum* (SC) reacts very dynamic to such effects. One of the most prominent of its changes is the adaptation of its thickness. High precision *in vivo* measurement of SC thickness therefore is deemed to be a fruitful parameter in skin research. Confocal techniques in general can be used for the determination of several physical parameters at a defined depth below the skin surface. An important approach of these techniques is that measurements can be performed *in-vivo* without disrupting the skin of volunteers. In this study we used confocal Raman spectroscopy and confocal laser scanning microscopy for the assessment of SC thickness. The determination of the thickness was performed on the volar forearm, lower leg, palm and cheek. Both techniques can not measure SC thickness directly. Therefore the development of effective and precise methods for analysis is necessary. In case of confocal laser scanning

microscopy the boundary surface between SC and stratum granulosum can be detected by the change of the lateral structure within the images. This border can either be detected by eye of an independent observer or by a specialized pattern matching algorithm. In this study we compared the results of both methods. In case of confocal Raman spectroscopy the determination of SC thickness was based on water content measurements. The water concentration in the SC is low whereas the water content in the viable epidermis is distinctly higher. For the calculation of SC thickness the change of the water content at the layer between SC and viable epidermis was assessed. Based on Fick's 1st law of diffusion a linear modelling leads to the precise determination of SC thickness.

This study leads to the conclusion that both confocal techniques can be used for precise, reproducible and effective *in vivo* assessment of SC thickness. The results reveal a validation of both methods with a good correlation of all measured values.

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Skin hydration as measured by *in vivo* Raman spectroscopy: dry skin characterization and effect of body wash cleansers

- S. Zhang*
- C. Xiao*
- T. Hancewicz*
- M. Misra*
- D. Drutis*
- V. Foy*
- M. Edmunds*
- A. Johnson*
- K. Ananth*

in vivo confocal Raman spectroscopy has become more and more widely used as a clinical tool in basic skin understanding and efficacy evaluation of skin products. Efforts have been made and will continually be required in interpreting the profiles of important skin components. Skin hydration from the water profiles in the *stratum corneum* (SC) is particularly interesting and controversial, although the profiles themselves can be easily determined.

In this presentation, we will critically review the methods recently reported (and our own unreported) in the calculation of *stratum corneum* thickness from the water profiles determined by confocal Raman spectroscopy. There has been no convincing comparison of the Raman method with any other methods in the literature as it is applied to the skin at the body sites that are most relevant to skin care. The hydration effect of skin products can be examined in two different ways: comparison of the water levels at a given depth or total water for a specified depth range. When the depth range is the entire SC "thickness" as calculated with one of the methods above, the water content becomes the total water in SC.

We have conducted a clinical study to examine the above approaches with various dry skin conditions that range from

moisturized skin to dry skin (dryness grades from 0 to 3.5 in the 0-4 scale). The results show that better skin always has higher water levels at all depths throughout the SC. However, the total water within the calculated SC boundary as defined above is lower for the moisturized skin due to a thinner SC.

We have also conducted a clinical trial to study the effect of body wash cleansers on skin hydration. Clinically controlled washes were applied to the inner forearms of two test panels of more than 20 subjects each. In one panel, a small but statistically significant difference in the surface skin hydration was detected, as expected, between the cleansers containing different surfactant systems. In the other panel, however, one body wash with known harsher surfactants unexpectedly caused an increased water level in the deep layer of the skin, which is likely due to a preclinical condition of edema as supported by other evaluations in the study.

These studies show that although its interpretation may not be straightforward, the water distribution in SC is now readily measurable with *in vivo* Raman spectroscopy and provides an extra dimension of data in understanding the skin and product effects on skin.

High spatial resolution *in vivo* Raman analysis of human skin

- S. Naito*
- S. Tokuhara*
- M. Igaki*
- Y. Masukawa*

Introduction: Characterization of chemical components in depth profile, such as water, of living human skin is essential to understand the moisturizing mechanism and barrier function of skin from the view point of material dynamics. However, we could not obtain enough information with conventional methods, such as tape-stripping, IR-ATR spectroscopy and photo-acoustic spectroscopy. Recently, Caspers developed a confocal Raman spectroscopy specially designed for chemical component depth profile analysis of human skin, which immediately became one of the most important devices for non-invasive skin measurement.

However, the spatial resolution of the commercially available Raman spectroscopy is relatively large, about 5 μm in depth. Since the typical *stratum corneum* thickness is approximately 15 μm , we can obtain only an ambiguous component depth profile of *stratum corneum* with this device. Better spatial resolution is necessary for the detailed study of dynamics of chemicals in the *stratum corneum*.

In order to solve this problem, we developed a higher spatial resolution Raman system for skin components depth profile analysis.

Methods: The new Raman probe was equipped with an oil immersion objective lens of N.A.1.3. We chose borosilicate glass as a window material in order to minimize the refractive index mismatch between the optical window and the skin surface. We took a commercial confocal Raman spectroscopy (Nanofinder30, Tokyo Instruments), and adapted it to perform skin measurements. The focal position in the depth direction was controlled by a piezo stage system, and the focal position

in the lateral direction was controlled by a galvano scanner system. The excitation laser wavelength was 632.8 nm.

The spatial resolution of the Raman system was evaluated with the FWHM method. We first measured water content depth profiles of untreated forearm skin with the Raman system. We then measured changes to the water content depth profile after the application of water vapor to the forearm skin.

Results and Discussion: The depth spatial resolution of our Raman system was 1.8 μm . This resolution is much better than the commercial skin Raman system, 5 μm .

The water content of the forearm skin measured with our system increased in accordance with the depth from the skin surface, and the water content value reaching a plateau at the depth of about 15 μm . While this water profile was similar to the general water profile measured with the commercial skin Raman system, the water profile measured with our system showed a non-continuous point, one that has not been ever found with the commercial system, at the depth of about 8 μm . This data indicates that dynamics of water of *stratum corneum* is not homogeneous in the depth direction.

After the application of water vapour, water content of the skin at the depth of 0 to 3 μm showed an immediate increase within one minute. This indicates that water vapor is easily absorbed into the skin surface.

Conclusion: We developed a high spatial resolution skin Raman system, which is very valuable for studying the dynamics of chemicals in the *stratum corneum*.

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In vivo measurement of the human epidermal thickness in different locations by multiphoton laser tomography

- M.J. Koehler*
- P. Elsner*
- K. König*
- M. Kaatz*

Background: The *in vivo* measurement of epidermal thickness is still challenging. While ultrasound, optical coherence tomography and confocal laser microscopy are employed with moderate success, this issue has not been addressed by multiphoton laser tomography.

Objectives: In the present study, *in vivo* measurement of four different morphometric epidermal parameters is performed.

Methods: 30 healthy volunteers aged 21-82 years were included in the study after informed consent and approval of the local ethics committee. At the dorsal forearm and the dorsum of the hand, thicknesses of total epidermis, viable epidermis and *stratum corneum* and the depth of the papillary dermis were calculated from depth-resolved intensity curves

after correlation with multiphoton images.

Results: We have shown consistently that in all age groups the four morphometric parameters are significantly higher at the hand compared to the forearm, while there were no differences between age groups. This is consistent with most previous findings.

Conclusions: The method presented here provides a novel *in vivo* investigation tool for the measurement of epidermal morphometric parameters that may be useful for the observation of epidermal changes over time in skin disorders, of therapy side effects or in cosmological science.

In vivo Raman investigation of *stratum corneum* lipids: effect of natural aging

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The outermost layer of the skin, *stratum corneum* (SC), provides an outstanding barrier against the external environment and is also responsible for skin impermeability towards most solutes. The barrier function depends upon the presence of a unique mixture of lipids in the intercellular spaces of the SC. The major SC lipids components are long chain free fatty acids, cholesterol and ceramides. Ceramides that comprise nine subclasses play a crucial role in maintaining the barrier function of the skin. Previous studies showed that the barrier function is related to the ceramide organization order: orthorhombic (highly organized), hexagonal (intermediate) and disorganized order.

Vibrational spectroscopies, Raman and FTIR, are rapid techniques that permit to have conformational and molecular information of biologic structures. Several studies have reported the application of infrared spectroscopy and Raman spectroscopy for revealing the organization of the lipids of SC

and their barrier function. Raman spectroscopy is a non-destructive analytical method for determining the structure and conformation of molecular compounds and studying the *in vivo* organization of SC lipids.

Previous studies showed that there are several bands or bands ratio in the Raman spectra of SC lipid constituents which directly monitor lipid conformational order and lipid structure.

In this work, we try to highlight, using Raman spectroscopy, the changes in the organization and the structure of the *in vivo* SC lipids due to the age of volunteers.

Using statistical data processing we are able to separate the Raman spectra in function of the volunteer ages. Raman spectral signatures show small variation indicating a slight increase in the lateral packing along aging of the skin.

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Non-invasive multiphoton tomography of human skin with DermaInspect™

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Clinical non-invasive multiphoton tomography provides clinicians and researchers with in-vivo optical biopsies with subcellular resolution based on two-photon autofluorescence, second-harmonic generation, and fluorescence-lifetime imaging. The multiphoton tomograph DermaInspect™ (JenLab) is a novel 1M class medical system using a femtosecond-laser source which operates in the near-infrared (NIR) spectral range between 710-920 nm. Non-linear excitation in perfect spectral overlap with the optical window of tissue allows to follow natural endogenous fluorophores inside human skin such as NAD(P)H, flavins, elastin, collagen, melanin, keratin, and porphyrins down to a tissue depth of 200 μm without applying any contrast agents. The DermaInspect™ clearly visualizes individual intratissue cells, intracellular mitochondria, melanosomes, and the morphology of the nuclei as well as extracellular matrix elements. Employing optional spectral filters and using the possibility of measuring the signal decay (FLIM) pixelwise the DermaInspect™ provides up to 5D imaging. Its distinct functionality enables the diagnosis of dermatological disorders such as psoriasis, fungal infections, nevi, and in particular, early stage melanoma. Furthermore, the novel imaging technique offers the possibility of tissue engineering, determination of skin-aging effects as well as in situ screening of the diffusion and intradermal accumulation of topically applied cosmetic

Background/Purpose: The ASCLEPIOS system developed by the M2D+ team of the Le2i laboratory (Université de Bourgogne, France) allows determination of a skin reflectance spectrum over the visible wavelength range in each pixel of a 2D image, thereby generating a hyperspectral (3D) cube. Reflectance spectra mainly result from the reflectance of two skin chromophores, epidermal melanin and dermal haemoglobin. A source separation method was applied on the mixed reflectance spectra, resulting in two component spectra for melanin and haemoglobin, respectively. We also obtained through this process quantification of each chromophore in each pixel of a 2D skin image. The accuracy of the pure source spectra obtained was validated by comparison with the theoretical spectra of each chromophore [1]. *In vivo* assessment of the 2D quantification of chromophores was performed on an image of a café-au-lait macule where only melanin accounts for the difference in pigmentation from normal skin.

Method: Independent component analysis [2] was used as a source separation method. Melanin is mostly found in the epidermis and haemoglobin in the dermis. Thus, melanin and haemoglobin reflectance spectra are assumed to be completely independent from each other. As the known source spectra are non-Gaussian, this criterion was exploited in the separation process. In order to handle the noise in the obtained melanin spectrum, a polynomial fit method has been established to obtain a source spectrum close to the theoretical one. Consequently, melanin quantification was re-estimated using

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a linear mathematical model. As 'café-au-lait' macules result from increased melanin production from a normal number of melanocytes in the basal epidermal layer, the proposed method was tested on a skin image of a 'café-au-lait' spot on lightly pigmented skin

Results: Source spectra obtained for melanin and haemoglobin were similar to their calculated theoretical spectra. The amount of melanin calculated from the 2D quantification process was significantly increased in the pigmented area as compared with normal skin. In contrast, haemoglobin quantification was almost uniform, irrespective of visible pigmentation.

Conclusions: We have developed a quantification method for skin chromophores such as melanin and haemoglobin using an algorithm for blind source separation from hyperspectral data. A café-au-lait macule could be clearly differentiated from normal skin based on its melanin content. Likewise, erythema intensity could also be quantified from haemoglobin content. Therefore, ASCLEPIOS device combined with our blind source separation method could allow non-invasive monitoring of pigmentation and erythema in a number of skin diseases.

References:

- [1] Data by S. Prabl and S. Jacques from website <http://omlc.ogi.edu/spectral/>.
 [2] A. Hyvärinen, J. Karhunen, E. Oja, *Independent Component Analysis*, John Wiley, New York, 2001.

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An open system to generate hyperspectral cubes for skin optical reflectance analysis

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Optical visible light analysis of the skin mainly relies on RGB colour imaging. Multispectral imaging of human skin represents a more accurate approach for *in vivo* analysis of its optical properties. Few dermatology-oriented multispectral devices have been hitherto developed. Their diagnosis abilities for pigimentary tumors or other clinical applications are still being evaluated [1].

We have developed a multispectral imaging system for *in-vivo* skin optical analysis. It is based on a charge-coupled device camera, a rotating wheel with a set of seven interference filters and a computer. All interference filters were chosen specifically to cover the visible spectrum range from 400 to 800 nm. Light transmitted by an optical fibre is filtered in each waveband to illuminate the area of skin under investigation. A dedicated software drives the rotating wheel and yields a set of seven monochannel images resulting in one multispectral image.

In the next step, our system generates a hyperspectral cube from this multispectral image. The reconstruction process uses a light propagation model and a robust method based on an artificial neural network [2]. The robustness of neural network associative memories against noise allows generation of a hyperspectral cube integrating reflectance spectra for a high number of single pixels. The advantages of this reconstructed hyperspectral cube are both its independence towards the illuminating conditions allowed by the artificial neural network learning phase and the large amount of data generated.

The hyperspectral cube obtained with our multispectral imaging system is amenable to further processing such as classical 2D segmentation algorithms on each reflectance plane from the cube, reconstruction of reflectance spectral curves in each pixel, using various signal processing methods to extract information. In clinical practice, this system can be used for objective monitoring of skin diseases, independently from the light source, or quantification of skin chromophores. A 2D analysis of pigmented skin tumours in a wide range of spectral bands can also be performed. Hopefully, this approach might improve the accuracy of their diagnosis.

Acknowledgments. This research project has been funded by a joint grant from the Conseil Régional de Bourgogne and the Laboratoire Dermatologique Bioderma company.

- [1] Elbaum M, Kopf AW, Rabinovitz HS, Langley RG, Kamino H, Mihm MC Jr, Sober AJ, Peck GL, Bogdan A, Gulkowicz-Krusin D, Greenebaum M, Keem S, Oliviero M, Wang S. *Automatic differentiation of melanoma from melanocytic nevi with multispectral digital dermoscopy: a feasibility study.* *J Am Acad Dermatol* 2001;44:207-18.
- [2] Mansouri A, Sanchez M, Marzani F, Gouton P. *Spectral reflectance estimation by neural networks from a multispectral set.* *IEEE Conference on Physics in Signal and Image Processing, Toulouse, January 31st-February 2nd, 2005.*

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Analysis of wrinkle morphology using vertical scanning interference microscope

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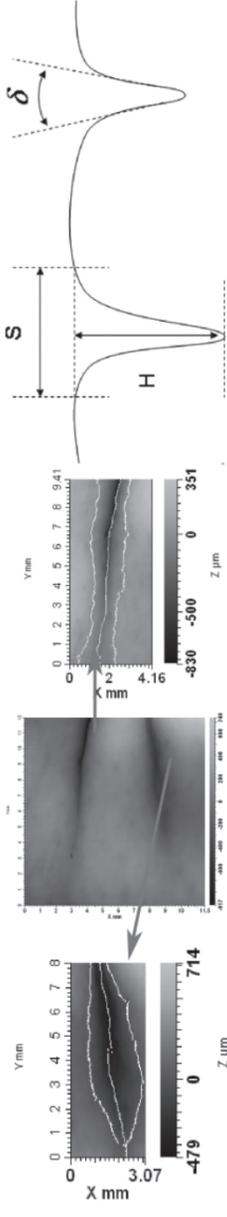
Objective: To evaluate the *in vivo* efficacy of a cosmetic product based on cross-linked hyaluronic acid microspheres at improving wrinkle morphology, silicone replicas of crow's feet were analyzed using a white light interference microscope in vertical scanning mode associated to a new procedure of wrinkle detection.

Materials and methods: 20 female volunteers (mean age 61.5±4 y.o) participated in a randomized double-blind, placebo-controlled, half-face clinical study. Each volunteer applied a W/O formula containing 2% (w/v) of cross-linked hyaluronic acid microspheres to one side of the face and the same formula containing no product (placebo formula) to the other side. A silicone replica of each crow's foot was taken before and 6 hours after application of the products. With the help of Pr. H. Zahouani and his team from the Laboratory of Tribology and Systems Dynamics (Ecole Centrale de Lyon, France), all the replicas were then analyzed using a vertical scanning interference microscope and the local geometrical parameters were detected using an innovative procedure. The objective of the methodology is to locate the edge of the wrinkle automatically by assessing the brutal variation of the gradient of the relief. For this, a threshold is fixed for all of the images. Each 512x512-pixel image is then scanned line by line and column by column in order to identify the edge of the wrinkle point by point. When the edge is identified, a

root mean square plan is established, that goes through all the points outside the wrinkle and will be the reference for the calculation of wrinkle width, depth and angle (1).

Results and discussion: 6 hours after application of the products, wrinkle angle was significantly reduced with both products but a higher reduction was obtained with the product containing cross-linked hyaluronic acid microspheres than with the placebo (7.2% and 6.7% respectively, p<0.05). The product containing the cross-linked hyaluronic acid microspheres reduced wrinkle depth by 9.4% (significant vs. placebo, p<0.001) and wrinkle width by 5.1% (significant vs. placebo, p<0.001). In contrast, the placebo appeared to worsen wrinkle depth and width (+30%, p<0.01 and +26%, p<0.001 respectively) probably due to the oily base induced skin softening.

Conclusion: Silicone-based replicas of crow's feet wrinkles are usually analyzed using profilometry techniques. Here we present a new method for analyzing silicone replicas by detecting wrinkle edge which allows precisely detecting individual wrinkle parameters and their evolution over time. Using this method, we demonstrated the efficacy of cross-linked hyaluronic acid microspheres at improving the appearance of crow's feet wrinkles 6 hours only after a single application.



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MR of the skin at 3 Tesla: a quality and feasibility study on healthy volunteers

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Purpose:

To assess skin MR quality at 3 Tesla. To describe skin anatomy at 3 Tesla. To discuss advantages of 3 Tesla MR imaging of the skin.

Materials and methods:

A 3 inch customized surface coil was specially designed and elaborated for our 3 Tesla MR system. Thin sections were obtained with a three dimensional Fiesta acquisition sequence and a two dimensional spin echo T1 sequence. Prospective analysis was performed twice by two radiologists independently. 36 healthy volunteers were included and underwent MR examinations on two skin areas: the face and the calf. Image quality was assessed on visibility of skin layers and artifacts quantification.

Results:

An intense magnetic field, thanks to its high signal-to-noise ratio, enables to realize images of the skin with a high spatial resolution (Pixel size=58 μ), in a satisfactory acquisition time. Image quality was excellent for the calf with a high intra and inter-observer correlation (Pearson coefficient). Motion artifacts resulted in a small loss in quality and reproducibility for the face.

Conclusion:

Skin MR is a recent imaging modality still in evaluation. Imaging at 3T magnetic field allows high quality imaging of the skin. It has the potential to become in the near future the best non invasive technique to analyse skin characteristics and its diseases.

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Skin colour diversity of Indian women

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The India is currently the 2nd most populated country in the world after China and demonstrates a wide geographical and cultural diversity. The aims of our study were: 1) to investigate skin colour variation in a large sample of Indian women originated from the North, the West, the South and the East, 2) to investigate age-related changes in skin pigmentation according to phototype and 3) to study the increase of facultative pigmentation above the constitutive pigmentation "Sun Exposure Index" according to age and phototype.

The study was conducted on 804 Indian women, aged between 20 and 70 years old from Phototype II to V. The women must be residing in Mumbai for not less than 5 years. Skin color was measured on four skin sites: - Forehead (FH), Cheek (C), Forearm (FA) and Inner upper arm (I), using a spectroradiometer CM 2600d (Minolta, Japan), under controlled environmental conditions (T: $22.4 \pm 0.8^{\circ}\text{C}$, RH: $38.2 \pm 4.9\%$) during the dry season (mid December to mid April). The women did not apply any skin care product on the test sites at least 12 hours before measurements. The spectrophotometer provides chromametric parameters: Lightness (L^*), Red intensity (a^*) and Yellow intensity (b^*), as well as, the spectral reflectance of the skin at each wavelength across the visible waveband: 400-700 nm, with a 10 nm spectral interval. The Individual Typological Angle (ITA) was also calculated.

Chairwoman: Christiane GUINOT

The range of ITA on each skin site was very large. On the cheek, the minimum value was equal to -54.3° (Dark skin category) and the maximum to 51.1° (Light skin category) which highlights the diversity of skin lightness of the Indian women faces. The comparison of the investigated sites skin colour indicated also that in all phototypes, the forehead was found to be the darkest area, which may be attributed to various factors such as chronic sun-exposure, hyperpigmentary disorders (melasma, mechanical-induced hyperpigmentation...). In all phototypes, skin pigmentation was found to increase with age on sun-exposed areas (C, FH and FA), whereas, as expected, it was found to decrease with age on unexposed area (I). Moreover, the age-related increase in facultative pigmentation seems to occur earlier in phototypes II to IV than in phototype V (20-30 vs 50-59), whereas the decrease in constitutive pigmentation (i.e. melanogenesis) seems to occur at the same age in all phototypes (i.e. 40-49). Finally, the Sun Exposure Index (SEI) was found to increase significantly with age in all phototypes. However, this phenomenon was significantly more pronounced in phototypes V than in phototypes II-IV.

In our study, we have confirmed that Indian skin demonstrates wide variation in skin colour ranging from fair to dark skin.

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Factors influencing the severity of skin aging in Indian women

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The aim of this study was to investigate the potential effect of lifestyle factors on skin aging in Indian women. A "Skin Age Score" (SAS) was specifically developed based on an individual evaluation of various skin aging features in various areas of the face.

The study was conducted on 804 Indian women living in Mumbai, aged between 20 and 70 years old, from skin phototype II to V. A total of 12 age-related features (coarse wrinkles on forehead, furrows between eyebrows, wrinkles on the nose, crows' feet wrinkles, wrinkles under the eyes, wrinkles above the upper lips, slackening tissue, drooping eyelid, bags under the eyes, naso-labials folds and pigmented irregularities on the cheek and the forehead) were investigated by a trained dermatologist using photographic reference scales. Self-assessed questionnaires were used to ask the Body Mass Index (BMI: underweight/normal/overweight/obese from the WHO classification), menopausal status (yes/no), vegetarian (yes/no), tobacco chewer (yes/no), smoker status (yes/no) and cumulative lifetime sun exposure (severe / moderate / none or mild). Skin colour was measured using a spectrophotometer CM 2600d (Minolta, Japan) and a Sun Exposure Index (SEI) was calculated from these parameters.

The SAS was developed using a second-order confirmatory

factorial analysis. The skin aging features were first grouped according to 3 categories (wrinkles, tissue slackening and pigmented irregularities), and partial scores were obtained for each category. A global score was built using the partial scores. To express the resulting SAS in years, the distribution of the score was modified so that the average and distribution of individual score values were identical to those of the chronological age of the total sample. Multiple regression analyses were performed on women older than 30 years to determine whether phototype and certain lifestyle traits were associated to the scores values.

After adjustment on age, the SAS is higher for menopausal women and fair phototypes, and tend to be associated with SEI and tobacco chewer. In addition, the corpulence was found to be associated with wrinkles and sagging scores. The wrinkles score decreases when BMI increases, and on the contrary, the sagging score increases with BMI.

In this study we confirmed the importance of menopausal status and skin colour in global skin aging. "Skin age" indicators, constructed with this approach based on visible signs of aging, could be useful for longitudinal application, especially to test the efficacy of anti-aging interventions and therapies for skin.

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Typology of maghreb skins

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Objective: To study the biometric characteristics of maghreb skin using common cutaneous exploration techniques and by comparing the results by age bracket and by sex.

Materials and Methods: This prospective, randomised monocentre study was carried out on the forehead, the cheeks and the forearm of healthy volunteers giving informed written consent.

Healthy volunteers were included of both sexes and of maghreb origin who agreed to apply nothing to the face and arms 24 hours before the study and not to participate in any other test during the study period.

Study procedure: after 15 minutes rest in the laboratory (forearm exposed to air) and after photographs were taken of the front and side (cross polarisation, UV filter) the following measurements were taken:

- Epidermal thickness with echography
- Cutaneous relief (surface, volume, roughness) using visioScan
- Lipid indices on the forehead and cheeks with the sebumeter (Courage and Khazaka)
- Hydration indices on the forehead, cheeks and forearm using the Corneometer CM825 (Courage and Khazaka)
- The colour (L*, a*, b*) using a chromameter

Statistical study: a descriptive analysis was postponed for each parameter. The bilateral significance threshold was 5% and the tendency was between 5 and 10%.

Results: Thirty five volunteers were grouped together for three months. The results were analysed and compared to those found in Asian and African skin.

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The correlation between Cutometer®'s parameters of skin elasticity and ages of Thai female volunteers

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Skin elasticity is one of aging signs that can be measured using several equipments. Cutometer® is one among those used to quantify skin elasticity. Skin property can be demonstrated in term of Cutometer®'s parameters; U_a/U_f : gross elasticity, U_r/U_e : net elasticity, U_v/U_e : ratio of viscoelastic to elastic distension and U_r/U_f : relative elastic parameters. However, the correlations of its parameters with skin properties reported in many publications are often in disagreement. Besides, a report on Asian population is limited.

Seventy-four Thai females volunteers of ages 30-60 year old were measured their skin properties using Cutometer®.

The values of 4 parameters, U_a/U_f , U_r/U_e , U_v/U_e and U_r/U_f were analyzed for their correlation with volunteers' ages. The results indicated that these parameters had correlation with age in different degree. Only U_v/U_e indicated positive correlation while other parameters presented reverse correlation. Among these, U_a/U_f showed the highest correlation ($p < 0.001$). Moreover, the correlations were sensitive to age of volunteers. The older the volunteer group the lesser the correlation was presented, especially U_a/U_f . However, U_v/U_e was the most sensitive to volunteers' age than other parameters.

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Grading of improvement and relapse in melasma of Thai females after 8 weeks-treatment with a combined cream of hydroquinone, steroid and tretinoin

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Background: Melasma is a common skin problem in any races including Asians. It commonly occurs in Thai females, age 30-40 years and females outnumber males about 13:1. In addition to multiple etiologic factors, the environmental factor of Thailand as a tropical and sunny climate country constitutes a definite factor responsible for improvement and relapse of pigmentation after any treatments. At present, the topical treatment consisting of hydroquinone (HQ), steroid and tretinoin together with sunlight protection is a standard treatment for melasma.

Objective: To investigate the clinical outcomes induced by a bleaching cream consisting of 4% HQ in 0.02% triamcinolone together with an adjunct 0.05% tretinoin cream in Thai females.

Materials and Methods: Forty Thai females with melasma on both cheeks participated in the study. The applied side was randomly selected between the right and left cheeks. The subjects were instructed to properly apply the cream to the assigned cheek before bed time for 8 weeks and were followed up them at 2-week intervals during the study. The clinical outcomes were evaluated by maximeter®, transepidermal water loss (TEWL), and corneometer®. The subjects' satisfaction was evaluated by a physician and patient static global assessment. During the study the subjects were advised to avoid sunlight and to use the sunscreen SPF-60 provided by us in the morning and at noon. After 2 months of treatment, the subjects stopped the use of the test cream and continue only the sunscreen use for the subsequent 10 months.

Results: Thirty-four subjects completed the first phase of the clinical trial. The average age of them was 46 years old, 28 (82%) of them had skin type IV and 21(62%) had a family history of melasma. They have developed melasma for 5-10 years. Improvement of pigmentation at the 2nd month was found in 32 (94%) of them. As compared to the pretreatment value of pigment (mean 362+ 62 a.u.), the treated melasma lesions showed a decline in pigmentation as the following mean difference values; 41, 63, 74, and 77 a.u. at the 2nd, 4th, 6th, and 8th week of treatment, respectively. The cream induced a significant increase in TEWL, which reached almost 2-fold values, being associated with erythema. However, all the subjects tolerated well the treatment. After stopping the treatment, 22 subjects further completed the regular 2-month-follow-ups for another 10 months. As compared to the pigmentation value (mean 283 + 51a.u) which obtained at the 8th week, these subjects demonstrated a gradual increase in pigmentation, i.e., 42, 46, 44, 41, and 32 a.u of mean difference values, which, however, was still significantly lower than the pretreatment values even at the 4th, 6th, 8th, 10th, and 12th month of the study, respectively.

Conclusion: We found that the melasma of Thai females showed a moderate relapse after stopping the cream treatment within 2 months. This may be mainly due to the strong sunlight in Thailand, which is noted almost all year round. A sunscreen is required for either short-or long-term treatment of melasma but it shows only a little efficacy in preventing the relapse of melasma pigmentation.

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Analysis of the dynamics of femoral skin acoustic properties under the conditions of graduated traction for lower limb congenital shortening

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Introduction: The necessity of the objective evaluation of skin integument acoustic properties has occurred in connection with the active development of new technologies, allowing to hinder the processes of aging by improvement of approaches while selecting an optimal technique of treatment in plastic and esthetic surgery, orthopaedics. **OBJECT** of the work was to analyze the dynamics of skin acoustic properties in patients with congenital abnormalities of growth and development of the lower limb for surgical lengthening by the method of transosseous distraction osteosynthesis. **METHODS.** Femoral skin acoustic testing was performed in 21 subjects at the age of 16–21 years (18.7±0.7) with congenital shortening of the lower limb. Femoral lengthening was performed monofocally with the Ilizarov fixator and accounted for 3–7 cm (4.2±0.4 cm). Normal subjects at the same age (n=10) were included in the control group. Acoustic analyzer ASA (Russia and Yugoslavia) was used, measurements were made in the directions with respect to limb anatomical axis («angular orientation») – in parallel with [C(z)], transversely to [C(x)], at angle 45 degrees with respect to [C(45)] and at that 135 degrees – to [C(135)] of Cartesian coordinate system. The subject examined was in supine position, the knee angle was 180 degrees. **RESULTS.** It has been established that the value of sound velocity in different angular directions in the skin of the femur shortened in the preoperative period doesn't differ reliably from the parameters in the intact segment. Significant (according to

Student t-test) increase of velocity parameters was observed under the conditions of graduated traction for all four orientations of the sensor. Thus, the sound velocity in the skin of the femur being lengthened longitudinally C(z) amounted for 89.8±3.40 m/s (versus 63.1±3.7 initially, p<0.01), for C(x) orientation – 64.9±2.9 m/s (before treatment – 54.9±3.3 m/s, p<0.01), in C(45) oblique diagonal directions – 84.2±31 m/s (versus 62.5±3.7, p<0.05) and C(135) – 77.4±3.50 m/s (versus initial values 58.6±2.90 m/s, p<0.01). The reliable excess of velocity parameters in the skin of the femur lengthened remained throughout fixation, and in the immediate period after treatment completion their decrease was observed up to 56.4±3.4 m/s for C(x), 60.5±2.9 m/s – for C(z), 62.3±2.5 – for C(45) and 60.1±2.9 m/s (p>0.05).

Conclusion: Under the influences of graduated traction in «fixator–limb» the skin integument turns into stressed-and-deformed condition, remaining in the nearest 2–3 months (fixation period). The restoration of skin mechano-acoustic condition takes place after 6–11 months. Apart from dominant traction influences, hyperhydration effects also influence the velocity of surface acoustic wave propagation in the skin of the femur being lengthened. The applied importance of the studies made consists in the necessity of predicting the overtraction signs of skin integument for limb shape and size modeling.

Chairmen: André Barel, Cormac Flynn, Stephan El Gammal

Usage of mechanical acoustic parameters of investigated tissues for diagnoses of hyperextension and plasticity reserve in reconstructive restorative treatment in orthopaedics and plastic surgery

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Introduction: Diagnosis meaning of mechano- acoustic testing of the skin has been studied by researchers mostly in clinical settings (Thacker J.G. et.al., 1977; Shevtsov V.I., Grebenyuk L.A., 1998). Particularly, it was performed by creation of tissues reserve for separation of hand web spaces in patients with syndactylia by graduated stretching, and the analysis of objective signs of biomechanical skin condition allowed us foresee possible development of its irreversible hyperextension.

The goal of the study is development of quantitative criteria of mechano-biological skin condition in the limbs in order to determine the reserve of its plasticity and also to foresee the possible development of skin hyperextension.

Results: To measure the speed of surface acoustic wave at different detector orientations, we used an acoustic analyzer ASA (Russia/Yugoslavia). It was established that in 9 patients (aged 3-16 y.o.) with congenital forearm stumps, who underwent surgical lengthening by 4-5 cm, the skin plasticity index of lengthened stump in the nearest follow-up after lengthening was 0.68 – 0.9 compared with the intact segment t index of 1.11-1.52. In case of tissue deficit in patients with congenital anomalies of development (congenital segment stumps) the amount of stump lengthening that can be achieved is limited by soft tissues component conditions. The peculiarity of reconstructive management of such patients is the development of stress deforming condition in tissues of the lengthened segment.

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Mechanical characterization of *in vivo* human skin using a 3D force-sensitive micro-robot and finite element analysis

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Human skin is a complex multi-layer material, which exhibits non-linear stress-strain, orthotropic and viscoelastic characteristics [1]. The measurement of its mechanical properties is important in many diverse areas such as the design of artificial skin [2], wound healing [3] and surgery simulation [4]. Several experimental methods have been developed to measure the mechanical properties of skin. These methods include applying suction, torsion, normal indentation or extensions to the skin surface [5-8]. Some techniques such as suction and torsion are unable to measure the skin's anisotropy. Extensometry tests typically apply only in-plane deformations in a limited number of directions. In order to characterise the complex mechanical properties of skin, it is necessary to apply a rich set of deformations to the region of interest.

The goal of this study is to measure the force response of *in vivo* human skin under a rich set of deformations imposed by a novel force-sensitive micro-robot [9]. The micro-robot is capable of inducing controlled three-dimensional deformations on the surface of skin *in vivo*. The three-dimensional reaction force vector at the probe tip can also be measured.

Skin on the back of the upper arm and the upper and lower parts of the volar forearm were studied. The arm rested on a support with the area of interest facing down. The cylindrical probe was glued to the surface of the skin and was moved in a cyclical motion such that the surface of the skin was subject to an in-plane deformation of about 1.5 mm. The deformation

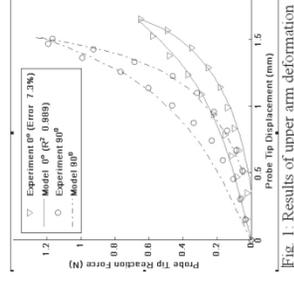


Fig. 1. Results of upper arm deformation

was repeated along five different directions – 0, 30, 90, 120 and 150o relative to the longitudinal axis of the arm. The probe tip force was measured during each cycle of deformation. A finite element model was developed to determine material parameters and the *in vivo* tension of the skin. An Ogden strain energy function was used along with a two-term Prony series to model viscoelasticity. An orthogonal prestress was applied representing the tension in living skin.

The force response for all three areas of the arm was highly non-linear and contained significant hysteresis. The upper arm and lower volar forearm exhibited significant anisotropy – the force-displacement curve was stiffer in a direction 90o to the longitudinal axis of the arm. The upper part of the volar forearm did not exhibit significant anisotropy. The finite element model accurately simulated the anisotropy, non-linearity and hysteresis observed in the experiments. The error of fit ranged from 5 to 10%. The Ogden model parameters for the upper arm were $\mu=0.014$ MPa and $\alpha=32.89$; for the upper volar forearm $\mu=0.025$ MPa and $\alpha=33.23$; and for the lower volar forearm $\mu=0.005$ MPa and $\alpha=32.05$. The *in vivo* skin tension calculated by the model ranged from 1 to 6 kPa on the upper arm, 5 to 6 kPa on the upper forearm and to 1 to 5 kPa on the lower forearm. The simulations demonstrate the importance of prestress in a model when characterising *in vivo* human skin.

References:

1. Wilkes, G.L., et al., *CRC Crit Rev Biotechnol.* 1:453-495, 1973.
2. Bellamy, K., et al., *Biomat.* 24:5061-5066, 2003.
3. Cerda, E. *J Biomech.* 38:1598-1603, 2005.
4. Mabrouk, M., et al., *Haptics-E.* 2:1-1, 2001.
5. Delalleau, A., et al., *Skin Res Technol.* 14:152-164, 2008.
6. Batisse D., et al., *Skin Res Technol.* 8:148-154, 2002.
7. Zahouani, H., et al., *Skin Res Technol.* 15:68-76, 2009.
8. Delalleau, A., et al., *Wear.* 264:405-410, 2008.
9. Chen, A. *A three-axis parallel drive force-sensitive micro-robot probe*, University of Auckland, 2007

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Numerical identification of initial stress within *in vivo* human skin

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It is well-known that the mechanical behaviour of *in vivo* human skin is viscoelastic, non linear and anisotropic. Many experimental researches have been undertaken to evaluate the mechanical properties of this biological tissue and have led to propose reliable mechanical modelling of *in vivo* skin. Nevertheless, it is necessary to take the natural stress of the skin into account in order to improve predictive models. Several authors have developed experimental devices to determine this natural tension; on the other hand other authors have considered it as an intrinsic parameter of the global behaviour.

The present study aims at determining the field of natural tension stresses in human skin (*in vivo*) by comparing the experimental opening displacement of an incision performed during a neck surgery with the numerical one obtained by finite element method. During the surgery, pictures are taken before and after the phase of incision. Elementary numerical analysis of these pictures allows us to evaluate the shape and size of the incision.

The framework of numerical analysis by finite element method is assumed to obey a 2D modelling under plane stress conditions. The considered skin domain is square-shaped and large compared to the size of the wound. The skin material is homogeneous and the mechanical behaviour of the skin is restricted to linear elasticity. The human skin is considered as isotropic at first and then as orthotropic.

In order to assess the initial stress field, two numerical

strategies are performed.

First, the numerical simulation of wound closure is carried out by suturing the incision which is defined by geometrical dimensions coming from the experimental procedure. Since the skin domain is large compared to the size of the incision, the displacements at the external boundary of the domain are assumed to be zero. A prescribed displacement field is applied on the edges of incision to close the wound. As a result of the suturing, the skin domain deforms and creates the so-called initial stress field.

Second, the phase of incision is simulated by combining finite element computations with an optimization method. The lines where the incision is to be performed are assumed to follow free boundary conditions. The external boundaries of the considered domain are subject to an unknown initial biaxial stress state. During the phase of incision, the stress releases in the vicinity of the wound which is in the process of opening. The unknown biaxial stress field is estimated by solving a least-squares method through the minimization of an objective function that depends on the residuals between the experimental data and the numerical simulations. The nonlinear minimization based on the evaluation of the opening displacement at the mid-point of the incision is solved by using the Levenberg-Marquardt method.

These two methodologies provide data stating as reference for the functional state and allowing the interpretation of the experimental tests of *in vivo* mechanical investigations.

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Evaluation of the elastic properties of the skin using the suction method (Cutometer®). Which parameters to use for claims in anti-aging treatments?

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In cosmetic claims concerning the efficacy of anti-aging products and treatments, general terms such as elasticity, firmness, tonus, etc. are used. Based on the suction method, the Cutometer (Courage-Khazaka, Germany) evaluates quantitatively the elastic and viscoelastic properties of the skin. In the Strain versus Time mode (which is mostly used) the vertical deformation of the skin due to vacuum, is measured in function of time and various linear skin deformation parameters are recorded (Ue, Uv, Ur, Uf and Ua); these parameters are dependant to skin thickness. From these linear parameters ratio parameters can be computed (general elasticity, elastic recovery and viscoelastic ratios (Ua/Uf, Ur/Ue, Ur/Uf and Uv/Ue). Furthermore the Cutometer software has been extended by area parameters (deformation x time) in a single (one cycle, Fo and F1) or repetitive (10 cycles, F2, F3 and F4) deformation /recovery curve.

The aim of this study was to investigate in a comparative way the use of all proposed software parameters (linear deformation and ratio parameters, and surface parameters) for the characterization of the mechanical properties of the dermis. The different classical linear, linear ratios, surface and surface ratios parameters were computed “*in vitro*” using silicone sheets and rubber balloons as simple reference systems for evaluating the calibration, accuracy, range,

linearity and repeatability in order to validate the Cutometer. The influence of various parameters such as aperture of the probe, applied load (negative pressure), duration of suction and relaxation and combination of both pressure and time on the linear and surface parameters were investigated. Accuracy is measured by calibration with a standard device furnished by Courage-Khazaka. The repeatability (SD in %) is very good (less than 1 %) for Uf, Ur/Ue, Ue/Uf, 2-5 % for Uv/Ue and 5-11% for F0, F1 and F1/F0. The increase of suction (from 50 to 500 mbar) at respectively 1, 3 and 10 s suction/relaxation provokes an increase of Uf, Ur/Ue, Uv/Ue, F0 and F1 and a decrease of Ur/Uf and F1/F0. The increase of the suction/relaxation time (1, 3 and 10 s) at respectively 300 and 500 mbar is characterized by an increase of Uf, Ur/Ue, Uv/Ue, F0 and F1 and a decrease of Ur/Uf and F1/F0. All linear, linear ratios, surface and surface ratios are load (suction pressure) and time of suction/relaxation dependant. As a conclusion we recommend the following *in vivo* experimental conditions for the use of the Cutometer when assessing the efficacy of anti-aging treatments: 6 mm aperture, 300-500 mbar suction, 1 or 3s suction/relaxation time and 2 cycles of suction/relaxation. Preference to the classical linear ratio parameters instead the surface and surface ratio parameters which show a less good repeatability (SD (5-11%)).

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Age-related changes in skin mechanical properties. Quantitative evaluation of 120 female subjects in a trial with a strict design

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- M. Kerscher*

Background: The most commonly used method to determine the mechanical ability of skin is the creep test using suction chamber devices. Until now there is no scientific consensus upon which skin deformation parameters are particularly suitable to describe age related changes in human skin mechanics. The aim of this study was to examine common mechanical skin parameters to find those best representing the influence of aging.

Material / methods: 120 healthy women aged 18-65 were distributed evenly to 4 age groups (18-29 y, 30-39 y, 40-49 y, 50-65 y). Women in group 1 to 3 had to be in the follicular phase of their menstrual cycle. Subjects in group 4 had to be post-menopausal. Beside hormone substituted or aesthetic treated subjects also subjects with a history of strong smoking habits or extensive use of tanning beds were excluded from the trial.

The biomechanical properties of the skin were measured using the Cutometer® MPA 580 on four body sites: cheek, neck, volar forearm and back of the hand. In order to analyze parameters referred to 1 mm skin thickness we also used 20 MHz sonography (DUB 20, Taberna Pro Medicum, Lüneburg, Germany).

Results: High correlation ($r > 0.5$) with age was found for

the parameters gross elasticity (U_a/U_f), immediate recovery (U_r), ratio of elastic (U_r/U_f) and delayed (U_d/U_f) recovery to skin distensibility as well as for the maximum recovery of the first (U_a) and the last curve (U_{a5}). Only low correlation ($r < 0.3$) with age was found for the ratio of delayed to immediate distension (U_v/U_e), skin fatigue (U_{f5-U_f}) and the residual deformation (U_f-U_a).

Discussion: In summary, especially parameters of the recovery phase are suitable to evaluate aging and anti-aging effects on skin mechanics. While the ratio of delayed to immediate distension (U_v/U_e) did not change with aging, the reverse parameter which represents the ratio of delayed recovery to immediate recovery (U_d/U_r) increases with aging. With a higher correlation rate of the immediate recovery (U_r) compared to the delayed recovery (U_d) it is obvious, that aging primarily influences the ability of the skin to react elastically after an elongation.

Conclusion: According to the results of our study we recommend to evaluate aging effects on the mechanical properties of skin by using the parameter immediate recovery (U_r). If there is no possibility to use sonography for analyzing absolute parameters referred to 1 mm skin thickness, the ratio of elastic recovery to distensibility (U_r/U_f) should be used.



Klaus HOFFMANN

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History of high frequency ultrasound

**Decorative tattoos and motivation for their removal:
a prospective study in 151 patients living in South of France**

- J. Latreille*
- J-L. Levy**
- C. Guinot***

In last years, there has been a dramatic increase in body tattooing in western countries, and in parallel an increase demand for tattoo removal. The purpose of our study was to describe a large sample of French patients asking for tattoo laser removal, to identify potential groups of patients sharing similar characteristics and to investigate the patients' motivations for being tattooed and for tattoo laser removal.

To reach these objectives, a prospective exploratory study was performed in a Private laser clinic in France. One hundred and fifty one consecutive patients asking for tattoo removal were included prospectively from November 2006 to July 2007. Patients' characteristics, descriptors of the tattoos concerned and motivations for tattoo removal were collected during a medical interview.

The population was composed of 65 women and 86 men, aged between 17 and 60 years. The women were on average younger than the men, 51% had less than 30 years versus 29% for the men. Sixty-four patients (42%) declared having only one tattoo and six patients (7%) at least ten. The majority of the tattoos that the patients want to remove were located on a visible skin site: the arm (43%), the shoulder/the back (26%) or the hand (9%). Tattoo could be either recent or old. Indeed, 11% of the patient had the tattoo since less than one year and 24% for more than 20 years. Fifty-eight percent of

the tattoos were small (defined as less than 30 cm²), 77% were monochrome, 72% were customized and 39% amateur. Seventy-six percent of the patients declared that they have been satisfied or very satisfied after the realization of their tattoo.

The motivations mentioned for tattoo removal were: aesthetic, social discredit, employment, family or partner pressure, change of life, change of partner and "no more in the frame of mind". The motivations were found associated with the degree of satisfaction after realization of the tattoo, but also with sex and age. Four profiles of patients were identified. Types 1 and 3 is composed of patients with amateur tattoos stating more frequently "social discredit" for tattoo removal, whereas types 2 and 4 is composed of patients with professional tattoos referring more frequently "aesthetic motivation". Types 1 and 4 are mainly composed of men (over forty and under forty, respectively), type 2 of women under thirty and type 3 of patients of both sex over forty.

Our findings showed that decorative tattoos can become a social problem and can create psychological burdens. Therefore, it seems important to have information campaigns about tattooing and tattoo removal targeted according to the different profiles of patients that we determined, in order to avoid unwanted tattoos.

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The method of diagnosis of demodex

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The problem calling demodex is old like the world but is still actual. The Demodex mite brings many problems and discomfort for people whose skin is affected by this mite. The theme of elaboration of the new methods of treatment and diagnoses of parasite mite is still actual for doctor-cosmetologists. The Demodex mite parasites into sebaceous glands, hair follicles and glands of eyelid cartilages of man and mammalia. The typical localization of mites is so called «the triangle of demodex»: nasal fold, chin and also lashes, eyebrows, more rarely in the area of back, ears, hair area of head, neck. There is an inflammatory process in reply of the mechanical irritation and onset of products of mite vitality. We have worked out and patented a new method of diagnoses of demodex which increases the precision of diagnoses thanks to complex approach. This problem is decided by next way: skin material is taken from 5 sites. The fence of skin material from 5 sites provides the necessary representation of skin state in the area of mite localization. In case of the demodex revelation during the stage of eggs, larvae or the revelation of only empty egg membranes, the procedure of fence of skin material and microscopy is repeated every 7 days between analysis, because during this time (approximately third of vital cycle of demodex) the stage of larva turns into the stage of mature mite. Moreover, the revelation of minimum 1 specimen of demodex during any stage of development is diagnosed as an invasion. In case of the repeated revelation of only empty egg membranes it's diagnosed the absence of invasion. This hard approach of the definition of invasion (the revelation of minimum 1 specimen of demodex during

any stage of development) is correct because of the biological programmed vitality of this mite, especially during the conditions of systematic disorders in the organism of patient which is revealed into the skin state of patient in sites of mite localization. Then it's necessary to determine long or short demodex mite. The determination of long or short demodex is allowed to put the term of treatment (120 days and 180 days). In addition the reason of skin imbalance which is necessary to treat is determined. The general examination of organism of the patient on the revelation of immune, hormonal and metabolic disorders is performed. The complex approach in diagnoses, including not only the presence of parasites but also the state of organism of the patient, which was not able to provide the defense of skin from the mite infection more than correct. In this case the subjective complaints of patients and objective analyses of laboratory are used according to known methods. Thus the complex approach in diagnoses of demodex is allowed not only to heal patients from the pathology of skin but also to find the reason of change of the reactivity man organism as a result of disorders of nervous, endocrine, vascular and metabolic processes. The complex diagnosis is allowed to compose the medical program which to lead not only to treatment of demodex but also to improve immunity, the creation of medium is not favourable for parasite mite, normalization of vascular state of skin, the removal of accompanied diseases making worse immunity, disturbing metabolic processes into the organism of patient. The execution of medical program with complex and whole approach is allowed to have good results.

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Effects of partial sleep deprivation on face appearance and skin properties

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A reduction of sleep time on a chronic basis is a hallmark of life in modern society ("modern 24h-society"). Sleep has important homeostatic functions and sleep deprivation has effects on brain plasticity, energy conservation, tissue restoration, immune response and thermoregulatory function.

Our objective was to investigate the effect of partial sleep deprivation on facial appearance and on skin functions (skin barrier, skin hydration, skin temperature, sebaceous secretions and skin sensitivity).

Fifteen healthy Caucasian women, aged from 30 to 40 years, have been admitted to the study centre for 12 days. The experimental period was divided into 3 sections: a baseline section of 2 days (D1, D2) with women sleeping for 8 hours (BL, from 11:00 pm to 7:00 am); a chronic partial sleep deprivation section of 6 days (D3 to D8) where they rested for only 4 hours in bed (PSD, from 3:00 am to 7:00 am) and a sleep recovery section of 4 days (D9 to D12) consisting in 3 nights of 10 hours in bed (RP, from 9:00 pm to 7:00 am).

Skin color (Spectrocolorimeter® CM-2600d from Minolta), skin barrier function (Tewameter® TM300, Courage and Khazaka), skin hydration (Corneometer® CM825, Courage and Khazaka), sebaceous secretion (Sebumeter® SM810PC, Courage and Khazaka) and Current Perception Thresholds of skin nerve fibers (Neurometer®, Neurotron Inc) were assessed on facial and forearm skin in controlled environmental conditions at D1, D3, D6, D8, D9 and D11. For each time point, measurements were taken at 8:00 am, 2:00 pm and

8:00 pm. In addition, skin barrier recovery kinetic was assessed after skin barrier disruption by tape stripping at D3, D8 and D12. Facial skin images were taken in standardized conditions at D1, D3, D6, D8, D9 and D11.

On the forearm and on the cheek, a significant decrease in skin hydration was found at the end of the PSD period followed by a return to values comparable to baseline ones during the recovery period. Diurnal variations of skin capacitance with a peak at 8:00 pm were observed throughout the entire experiment. A tendency to a decrease in sebum

secretion during PSD followed by a rebound during RP was observed. In this study, no daily variation in sebum secretion was found. TEWL measurements were found to decrease during PSD with a tendency to augment from D11 without reaching baseline values. In parallel, no change in skin temperature was found on the cheek whereas a slight decrease during PSD was found on the forearm for this parameter. No modification of facial skin color parameters was observed throughout the experiment whereas a decrease in a* values paralleled by an increase in L* values was found on the forearm. In this study, Partial Sleep Deprivation was not found to alter skin barrier recovery capacity. Fatigue-related features (paleness, dark circles and tissue slackening) were found to be more pronounced at the end of PSD.

Our results suggest that sleep restriction results in skin modifications which differ from those induced at the epidermal level by psychological stress reported previously for acute sleep deprivation or periods of marital dissolution.

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SKIN PHYSIOLOGY AND SKIN DISEASES

Objectivation of the individual sensory state by the assessment of specific biophysical properties of the skin in different climatic conditions

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The CSTB in Nantes is specialized in the study on the climate effects on buildings and on human comfort. To improve human comfort in different climatic conditions, the CSTB must understand the interactions between the environment, the human body and individual perception. As the skin is a sensory organ and the first barrier between the environment and the human interior, some CSTB researchers have chosen to study the biophysical properties of the skin to objectify human perception. Moreover, some authors (1) have already established correlations between some skin parameters (as the skin temperature) and thermal sensation. So it seems to be judicious to use this approach with more skin parameters to assess not only the thermal sensation but also the overall perception. Hence, the CSTB develops protocols to measure some parameters on human skin in environments with controlled temperature (as in the Jules Verne climatic wind tunnel).

The aim of this work is to validate a non-invasive method in order to assess the external environment influence on the biophysical properties of the facial skin and on individual sensation. We could also identify specific biophysical parameters that give objective information about individual well-being.

The approach is multidisciplinary: it brings together expertise in skin biophysics, in thermal science, in neuroscience, in physiology, in psychophysics (...). The idea is to combine some measurements of biophysical skin parameters with the

that consistently elicits a nerve response and A β , A δ and C fiber groups were selectively stimulated. Both methods are complementary and it allows more reliable results on the activity of nerve fibers.

During all experiments, the volunteers declared their individual thermal and well-being feeling through a questionnaire. And for some subjects, the activity of their autonomic nervous system has been recorded with the measurements of some physiological parameters: the heart rate, the respiratory rate and the electro-dermal resistance. With these measurements, we could have objective information about the emotional reactivity and we could differentiate the stress phenomenon and the thermoregulation phenomenon. So, we could identify if a parameter changes with the characteristics of the environment or with individual stress.

This study is being analyzed. After a statistical analysis, we could highlight different correlations between some biophysical parameters and individual perception and the sensitivity thresholds. Maybe we could identify different classes of individuals with particular behaviours with the environment.

Some previous studies showed a significant positive correlation between the skin temperature and individual perception in various environments (cold, neutral and warm). Now we must work on other parameters as the hydration, the sebum excretion, the perception thresholds (...). With this experimental data, we could improve a model to evaluate thermal and global perception and we could determine the efficiency of a cosmetic product that should, for example, improve human comfort in non-standard environments.

References:

(1) Danni Wang, H. Zhang Ph.D, Edward A. Arens, and C. Huizenga, «Observations of upper-extremity skin temperature and corresponding overall-body thermal sensations and comforts» (May 31, 2007). Center for the Built Environment. Indoor Environmental Quality (IEQ) TSA-2001 Neurosensory Analyzer Model TSA II User and service guide - Medoc

(2) Dr Oliveira F., Thèse: « confort thermique ressenti par l'homme dans son environnement climatique », INL 2008

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Clinical visualization of photodamaged skin by facial averaging

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Perceived facial health, age and attractiveness has been shown to correspond a variety of factors including facial symmetry, lines and wrinkles, facial shape, color, evenness of skin tone, mottled hyperpigmentation, and sallowness. Traditional approaches for fully understanding differences between ethnic, aging and photodamaged subject groups, therefore, has necessitated the incorporation of different visual, subjective and objective instrumental methods to characterize all attributes of interest. More recently, multidimensional approaches have been proposed as a way to holistically characterize improvement from skin care products.

Facial averaging, originally proposed by Perrett et al. in the early 1990s, has evolved as a powerful tool for characterizing all aspects of photodamaged skin, utilized in many ways including understanding the drivers of attractive and healthy skin appearance. One key advantage to facial averaging is that the technique captures changes across all appearance attributes with a single method, and therefore can be a very powerful tool for characterizing clinical benefits with skin care formulations. Recently, we have demonstrated significant improvement in the visual modeling of texture

in facial averages, resulting in the ability to more accurately model highly nonlinear features – for example, a very coarse wrinkle that is location-dependent, discrete pigmentation, etc. The objective of this research was to develop accurate and sensitive predictive tools and models for visualization of the multidimensional clinical signs of aging, with emphasis on very realistic appearance of facial skin in both averaged and transformed images.

Healthy female subjects ages 20-70 provided informed consent to participate in IRB-approved facial studies. Two ethnicities were evaluated – Caucasian subjects with Fitzpatrick skin types I-III and Asian subjects with Fitzpatrick skin types IV/V. Facial average examples will be shown that represent a range of photodamage attributes, and characterize improvement with cosmetics over time. Visual evaluation of photodamage of computed facial averages showed good correlation to mean severity of photodamage, calculated from individual subjects within the averaged group. Increasing the realism and accuracy of facial averages presents a significant breakthrough, and has wide ranging applications in the cosmetic industry.

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Face morphing and age perception

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In our modern society, looking younger is becoming an important concern for most of the women. The main objective of the present study was to determine the contribution of each type of age-related features to age perception.

Seventeen pictures of Caucasian women faces, selected from the database of the CE.R.I.E.S, were morphed in order to individually reduce the wrinkles ("wrinkles procedure"), the tissue slackening ("lifting procedure") and the aging spots ("spots procedure"). The whole set of images (original faces and the morphed faces) was presented to a group of female judges (45-55 years old) using an eye tracker. They were asked to evaluate a possible age difference between the original picture and the 3 transformed pictures (1/ without wrinkles, 2/ without slackening, 3/ without spots). The gaze pathway of the judges was recorded during the evaluation in order to document the visual processing of faces when estimating age. The number of fixations and the length of fixations in 6 Areas Of Interest (AOI) covering the whole face (eyes, mouth&nose, forehead, right cheek, left cheek and oval) were analysed.

Compare to the original faces, « lifting procedure » was

found to make the faces significantly younger than « spots procedure» and « wrinkles procedure ». The age difference reached on average -4,3 years after the "lifting", whereas it reached -3,6 years after the two other procedures. Interestingly, the decrease in severity of the nasolabial fold was the most contributive effect of the rejuvenating morphing procedure. Regarding the face processing, the determination of an age difference was faster when aging spots were erased than when skin slackening was reduced. Older judges (menopausal women) were more focusing on the mouth area than younger judges (non-menopausal women). Younger judges would be less sensitive to aging of this area, as wrinkles of the upper lips have been shown to occur later than the other age features.

The present study confirms that changes of face appearance by virtual transformations can explain the individual contribution of the different categories of age-related features to age perception. In addition, virtual rejuvenation of faces has been shown to alter eye gaze behaviour in relation to the age of the observer.

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Neonatal skin maturation –
vernix caseosa and natural moisturizing factor

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Background: Full term neonatal skin hydration decreases rapidly and then increases during the first two postnatal weeks, indicating adaptive changes in the water handling properties of the upper *stratum corneum* (SC). Transition from high to low humidity at birth may initiate epidermal changes such as the proteolysis of filaggrin to natural moisturizing factor (NMF). Newborn skin with *vernix caseosa* left intact at birth is more hydrated, less scaly, and undergoes a more rapid decrease in pH than with skin with *vernix* removed. *Vernix* retention facilitates postnatal hydration and influences acid mantle formation.

Purpose: To examine the ontogeny of NMF generation in neonatal SC and the potential roles of NMF and *vernix caseosa* (VC) in postnatal adaptation and to determine the implications for premature epidermal barrier maturation.

Methods: NMF was quantified from the upper SC (1) in full term infants at birth and one month and (2) at birth and 24 hours in parallel cohorts of full term neonates randomly assigned to have VC retained or removed at delivery. The infant values were compared to NMF collected from the surface of neonatal foreskin and adult volar SC and from samples of *vernix caseosa*. Samples collected 24 hrs post birth with D'squame tapes were analyzed using reverse-phase high performance liquid chromatography and fluorescence

detection. Statistical evaluations were made with Student's t-tests and ANOVA.

Results: NMF levels were extremely low at birth. They were significantly higher one month later but lower than adult values. Retention of VC resulted in significantly higher NMF levels 24 hours after birth compared to infants with *vernix* removed at delivery. NMF levels paralleled the higher SC hydration and lower skin pH observed for VC retention. Relative to infant and adult SC, *vernix* had higher levels of glutamic acid and histidine. NMF increased as a function of SC depth in adults but decreased with depth in neonatal foreskin SC.

Conclusions: NMF levels increase significantly after one month and production may be initiated with the transition from high to low humidity in the neonatal period. Retention of NMF-containing *vernix* may facilitate increased hydration and skin pH reduction at birth. The NMF in the skin surface samples of infants with *vernix* retained appears to come from the *vernix* rather than the SC. The results corroborate reports on the influence of humidity on filaggrin proteolysis and NMF formation in animal models. The findings are relevant for the skin care of premature infants for whom SC barrier maturation and acidification continue long after birth.

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Mannose-6-phosphate improves
the colour of skin following damage

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Background: The ability of mannose-6-phosphate (M6P) to improve the colour of skin following epidermal and dermal damage is reported. A single-centre, double-blind, within-subject, placebo-controlled, randomised trial investigated the efficacy and safety of M6P to improve the colour of skin at split thickness skin graft donor sites.

Methods: Two 1.5cm x 2 cm split-thickness skin grafts were harvested from the lower back of subjects and then treated with topical M6P (300mM or 600mM) or placebo within 30 mins and again 24 hours later. Subjects attended daily follow-up visits until Day 14 and then on Days 16, 18, 21, 24 and 28 during which dressings were removed and the sites photographed. Quantitative measures of skin colour (ΔE), redness (Δa), and lightness (ΔL) compared with surrounding untreated skin were derived from the analysis of standardized, colour calibrated photographs using CIElab (D50, 20). Differences were compared between M6P- and placebo-treated sites using the Wilcoxon signed-rank test.

Results: Seventy eight healthy subjects (51% male; 18-

70 years) were randomised to receive 300mM (N=39) or 600mM (N=39) topical M6P. Over the 28 days following treatment, significant improvements ($p=0.0045$ and $p=0.0003$, respectively) were seen in the colour (AUC ΔE) of M6P-treated skin compared with placebo-treated skin. Treatment with M6P 600mM significantly lightened the skin (median ΔL) compared with placebo over the first 2 weeks post surgery ($p<0.05$). During the first week skin redness (Δa) was reduced compared with placebo in 74.4% of subjects treated with M6P 300mM and 84.6% of subjects treated with M6P 600mM. Both doses of M6P had a favourable safety profile, comparable with placebo.

Conclusions: Topical administration of M6P to split thickness skin graft donor sites significantly improves the colour of treated skin compared with placebo. The reduction in redness and darkness of skin following treatment with M6P results in skin that blends better with the surrounding area compared with placebo.

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TcpO₂ decay rates used as a metabolic indicator of the human skin *in vivo*

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Introduction: Transcutaneous variables such as TcpO₂ and skin's microcirculation (LDF) had been used to approach skin metabolic activity, particularly in conditions that are related with the normal physiologic state. The sensibility of these variables to changes increase with the use of dynamical protocols that overstimulates skin and permits the study in extreme conditions. The 100% Oxygen ventilation atmosphere used as a challenge test evokes the capacity to understand and quantify the maximum Oxygen disposition in the skin, which ultimately may be related with several skin conditions involving cutaneous perfusion. The objective of this work was to develop an experimental analytical model to study oxygen disposition in the skin following a 100% Oxygen ventilation from which an *in vivo* indicator of skin's «vitality» e.g. of skin's homeostasis may be defined.

Methods: 54 volunteers, both gender, without dermatological or circulatory pathology, non smokers, were included, following informed written consent. Two groups were defined (Group I 18-35 y.o; Group II: 40-65 y.o). A dynamical protocol was defined with basal measurements (10 min) followed by 100% O₂ ventilation (10 min) and recovery (10 min). Trans Epidermal Water Loss (TEWL, Tewameter TM300), Microcirculation blood flow (LDF,

Periflux PF5010) and transcutaneous gases (TcpO₂ and CO₂, Periflux 5040) were obtained in one foot randomly chosen. A mono-compartmental model was developed to analyze the data of TcpO₂ evolution. The most relevant parameter was calculated (t_{1/2}elimination). Non linear regression was used with special software developed for MS Excel. Statistical comparisons between groups were performed using SPSS 16.0 The confidence level was 95%

Results: During provocation there is an increase of TcpO₂ values that saturates in about 10 min. In the same period the blood flow drops by a local vasoconstrictor effect. Modeled data shows that older volunteers present a statistical significant slower elimination rate (p=0.031). These data suggests that there is a decrease in the metabolic capacity of these tissues even in non pathologic volunteers, which, due to aging process, can present some degree of metabolic impairment.

Conclusion: The proposed experimental and analytical methodology allows the quantification of dynamical parameters such as t_{1/2} elimination that can be sensitive to early metabolic changes and therefore can be used as a skin «vitality» predictor.

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To understand skin circulatory physiology by low perfusion experiments with a monocompartmental model: the influence of age

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Introduction: Monocompartmental evaluation of Laser Doppler Flowmetry (LDF) and transcutaneous oxygen (TcpO₂) data has been applied to low perfusion experiments, accepting that oxygen disposition rates may be reliable predictors of vascular impairment. After defining a new compartmental model to analyze TcpO₂ and LDF data from dynamical maneuvers, the authors applied this model to a group of normal individuals (young versus old) to evaluate the applicability of the model and the influence of age over those parameters.

Methods: Following informed written consent, 54 volunteers, both gender, without dermatological or circulatory pathology, non smokers, were included. Two groups were defined (Group I 18-35 y.o; Group II: 40-65 y.o). All volunteers were submitted to two dynamical low perfusion protocols that involved a postural change (leg elevation during 10 min) from decubitus (Protocol 1) and an induction of a reactive hyperemia following a suprasystolic occlusion during 10 minutes (Protocol 2). Transepidermal Water Loss (TEWL, Tewameter TM300), Microcirculation blood flow (LDF, Periflux PF5010) and transcutaneous gases (TcpO₂ and CO₂, Periflux 5040) were obtained in one foot randomly chosen. A monocompartmental model was developed to analyze the data of TcpO₂ evolution. The most relevant parameter

was calculated (t_{1/2}elimination half-time life). Non linear regression was used with special software developed for MS Excel. Statistical comparisons between groups were performed using SPSS 16.0 The confidence level was 95%

Results: Results obtained from non linear regression suggests that compartmental model correctly describes the data from TcpO₂ values in both protocols. Since all volunteers are healthy with no clinical evidence of vascular impairment, data obtained from the model should be similar in the two groups. Results show that in both protocols the elimination rates of oxygen are slower in Group II than in Group I. However these differences are not statistically significant (p=0.557 and p=0.435), probably due to high variability in the data. Nevertheless, results indicate a data trend suggestive of some degree of vascular impairment, not obvious in the raw data.

Conclusions: The proposed compartmental model clearly fits to experimental data and can produce reliable parameters to characterize low perfusion experiments. Slower TcpO₂ elimination rates observed with both protocols in Group II, although not expressed in the raw data, may reflect or «be related with» the initial stages of the aging process.

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Characterization of sensitive skin syndrome volunteer's barrier by dynamical analysis

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Introduction: Several studies suggest that 50% of the population considers to suffer from some cutaneous sensibility. Some of these individuals do not show any objective skin sign and therefore his characterization is often difficult or even impossible. The auto-perception of these symptoms is the only way to diagnose the condition. The use of dynamical measurements such as the Plastic Occlusion Stress Test (POST) combined with compartmental analysis had been suggested to be a more sensitive method to discriminate small differences in the skin barrier function. So, the present presentation tries to illustrate this condition by compartmental analysis in individuals with the auto perception of sensitive skin.

Methods: Following informed written consent, 33 female volunteers were selected and divided in three groups (Group I n=15 – auto perception of sensitive skin; Group II n=12 control group with ages below 23 y.o; Group III n=6 control group with ages above 45 y.o). All of the volunteers were submitted to a POST protocol with 24H of occlusion in the hand, following a continuous measurement of Trans

Epidermal Water Loss (TEWL, Tewameter TM300, C+K, Germany) during 30 min. A bi-compartmental model previously developed and validated was used to analyze TEWL decay curves and t1/2evap was calculated. Descriptive statistics (MS Excel) and non parametric comparative tests for related data were obtained by the SPSS 16.0 software, and a confidence level of 95% adopted.

Results: Several studies failed to present differences between sensitive skin and normal skin. Our results show that t1/2evap was significantly higher in sensitive volunteers that in the control group no matter the inexistence of TEWL differences between the 2 groups.

Conclusion: Results confirm that objective indicators of the Sensitive Skin Syndrome may exist although very discrete, highlighting the need to use, for this purpose, methods and techniques with appropriate sensitivity. These findings, revealing some dynamical modifications in the volunteer's skin water dynamics, may explain the reported dryness commonly associated to the syndrome.

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Approaching the topical formulation “heavy legs” claim

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Introduction: The “heavy legs” symptom seems to be related with an early expression of chronic venous failure, estimated to affect 40% of the population in developed countries. This high prevalence justifies the increase of medicinal (OTC) and health care products (cosmetics included), mostly topical cutaneous formulations, used to improve this condition. However the claim substantiation of these topical formulations is not established. Most of the times the “application” commonly involves a mechanical (massage) effect that could be associated to an eventual reduction of the symptom expression, independently from any other activity. The objective of the present work was to develop a sensitive methodology allowing to characterize the “heavy legs” condition and the related topical formulation's claim.

Methods: Following informed written consent, 7 female volunteers with complains of “tired legs”, confirmed by clinical evaluation, were included in the study. A reference gel was applied during 28 days in one leg of each volunteer. The other leg serves as control. At Day 0 (D0), Day 14 (D14) and Day 28 (D28) all the volunteers were submitted to instantaneous

and dynamical measurements of microcirculation blood flow (Periflux 5010, Perimed, Sweden), sonographic (Dermascan C®, Cortex Technology, Denmark) and centimetric measurements. A data normalization procedure was adopted. Descriptive statistics (MS Excel) and non parametric comparative tests for related data (Wilcoxon Signed Ranks Test) were obtained by the SPSS 16.0 software, and a confidence level of 95% adopted.

Results: When compared with D0, LDF values present a significant decrease of both basal and dynamical values after Day 14 and Day 28 suggesting that the observed effect may result from a combination between the massage and the formulation. Centimetric data show an increase in control leg diameter. In the application leg the differences are less evident, suggesting some protective action of the formulation.

Conclusions: The presented methodology seems to be useful to assess the present condition and the related claim support.

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About the mechanisms involved in skin response to local temperature provocation

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Introduction: Laser Doppler Flowmetry (LDF) and TcpO₂ measurements may be used to assess pathologies with cutaneous expression such as peripheral vascular disease. Sometimes these techniques are combined with dynamical approaches such as postural changes, reactive hyperaemia and local temperature changes to increase sensibility of the applied methodologies, allowing to look deeper into the (regulatory) mechanisms involved.

Skin temperature adaptations following local heating hasn't be fully understood. 42°C produces a biphasic response with an early peak of LDF dependent from an axonal reflex followed by a nadir and a second peak related with the release of Nitric Oxide (NO). At higher temperatures (44°C) this typical behaviour is modified and a maximal response may be obtained from the beginning of the heating.

The present work tries to understand the contribution of the axonal reflex to the response at 44°C by the application of a local anesthetic that blocks the afferent sensory pathway.

Methods: Following informed written consent, 11 female volunteers, without dermatological or circulatory pathology, non smokers, were included. A local anesthetic patch (EMLA®

Lidocaine+Prilocaine, AstraZeneca, Portugal) was applied to all volunteers 1H30m before application of the protocol in one forearm randomly selected. The other forearm served as control. Laser Doppler Flow measurements (LDF, Periflux PF5010) were performed during 42°C (control), and 44°C local heating. Statistical comparisons between groups were performed using SPSS 16.0. The confidence level was 95%

Results: Results from 42°C heating have shown a normal biphasic response. With EMLA results are significantly different since there is a decrease in the first part of the response due to the reduction of the axonal reflex. At 44°C the response is not biphasic. With EMLA there is no reduction in the response suggesting that the axonal reflex is not involved in this response.

Conclusion: The physiological mechanism of the response to the 44°C local heating does not involve the axonal reflex. The heating provocation seems to involve several regulatory mechanisms that can be used in vascular medicine and experimental dermatology to understand and follow up several skin conditions related with microcirculation changes.

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Exploratory study of the typology of mature skin at different stages

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Introduction: Post-menopausal skin aging has intrinsic and extrinsic origins that induce considerable appearance and feeling disparities within a class of age.

Objective: The aim of this study was to try and identify different stages of maturity (3) of the skin of the face of menopausal women.

Material and Methods: 150 women, aged between 50 and 80 (63 ± 7 years), were enrolled. Investigations combined clinical scoring, biometrical assessments (corneometry, cutometry, digital photography and 3D analysis by fringe projection) and questionnaires. Images created from the photographs and the 3D images were scored by several investigators to rank the subjects according to 3 stages of skin maturity.

Results: The most pertinent variables to differentiate these maturity stages (elastosis, wrinkles on the cheeks, on the upper lip, microrelief (St), spots, elasticity...) were identified by factorial modelling. Among all these variables, the actual age was not selected by the statistical model, contrarily to the apparent age ("the age women think they look"). From these classifications and the subjects' photographs, a typical face at each maturity stage was constructed.

Conclusion: This study reveals the clinical and physiological characteristics of the different stages of facial aging. It is thus possible to imagine cosmetic treatments specifically adapted to the needs of menopausal women

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Changes on body skin as a function of age

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Facial skin aging has been a great concern in cosmetology and dermatology and many publications have documented the age-related transformations of skin. However to our knowledge, few studies have been conducted to systematically investigate the changes of skin attributes in different body sites. This study was designed to assess the link between age and skin body attributes such as hydration, firmness, color, stretch marks and cellulite. The study involved 150 healthy women Caucasian volunteers aged between 18 and 70 years of age and with a Body Mass Index (BMI) between 20 and 26 kg/m². The skin hydration (corneometer), firmness (cutometer) and chromophores distribution (DRS and spectral imaging) were evaluated on different anatomical sites. Several measurements where also done to characterize the level of cellulite (clinical grading, profilometry, 3D, centimetric measurements). Clinical grading was performed to measure the skin dryness,

sagging, brown spots, spider veins and stretch marks. Finally, the whole body shape was evaluated on digital pictures. The skin firmness on all body sites (waist, neck opening, buttock, abdomen, hip, thigh, upper arm) significantly decreases with age. The dryness of the lower leg shows a trend to increase with age. The orange peel aspect (on buttock, abdomen, hip and waist), the stubborn cellulite (on abdomen, buttock and contracted buttock, thigh, hip and waist), the quantity and the length of stretch, the centimetric values (of the abdomen, the hip, the waist and the arm) and the brown spots number on the neck opening significantly increase with the age. In conclusion, this study has allowed to demonstrate that skin firmness, brown spot on neck opening, quantity and length of the stretch marks, the dryness of the lower leg correlate with age. In addition, the circumference (in cm) of certain areas (abdomen, hip, waist and arm) also correlates.

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Human skin wettability cartography

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Introduction: For decades the surface hydrophobicity has been reported to play an important role in many biological processes, such as cellular adhesion, contact inhibition, elasticity, functionality of tissue membranes, functioning of intracellular structures, and adhesion of infectious microorganisms. The skin affinity with water is estimated by measuring of its water contact angle (θ_w).

Cutaneous pH. Hydration index HI (arbitrary unit: au). Lipidic index LI ($\mu\text{g}/\text{cm}^2$).

- Water contact angle (θ_w): measured by a tool specifically designed for *in vivo* and *in vitro* measurement

Results and discussion: In the seborrhea sites, the HI and skin wettability are higher. The cutaneous pH was not significantly different from one area to the other

Objective: To establish a cartography of skin's wettability by θ_w measuring at nine sites. The hydration and lipidic index (HI, LI) and the skin pH are measured.

Materials and Methods:

- Volunteers: Ten females volunteers took part in this study. Their average age was 23.2±2.5 years (extreme: 20 and 27 years).

- Study sites: see table

- Biometric Measurements: Performed by the combined Courage et Khazaka GmbH-Köln, Germany which to measure:

	1	2	3	4	5	6	7	8	9
pH	5.0±0.5	4.9±0.4	4.5±0.5	4.7±0.5	4.1±0.4	4.8±0.4	4.6±0.4	4.1±0.5	4.5±0.5
HI	76±5	58±4	50±6	53±5	45±7	47±8	42±7	59±5	30±5
LI	126±27	55±11	28±8	22±8	-	-	-	-	-
±w	60±7	68±5	80±10	84.7±5	91±6	98±6	100±6	94±12	101±4

1: forehead; 2: cheek; 3: back; 4: thorax; 5: volar forearm; 6: Thigh earlier(cuisse antérieure); 7: Posterior thigh (cuisse postérieure); 8: Abdomen; 9: leg (Jambe)

Conclusion: The sebum makes the skin more hydrophilic

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Hair: hydrophobic/lipophilic balance - wettability and friction coefficient

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Introduction: The human hair is by nature a fibrous material with a negatively charged hydrophobic surface. It is naturally coated with an oily secretion (sebum). The hair is particularly sensitive to hyper-seborrhea which modifies its physical properties. Contrary to greasy hair, dry hair is not well lubricated and becomes breakable. Furthermore, hygiene practices and exposure to physical, thermal and chemical constraints for cosmetic reasons mainly, are likely to modify the aspect of the hair and its biometrological characteristics significantly. It seems obvious therefore that the parameters of wettability and the friction coefficient should be measured.

Material and method: The wettability and the friction coefficient were assessed by tools designed specifically for measurements *in vivo* and/or *ex vivo*[1,2]. The hair tested was washed and degreased first, before being rinsed with water again, then left to dry in the ambient air. The hair was then coated with sebum by rubbing it on the forehead, an area of the face where the skin is oily.

Results: The strongly hydrophobic tendency of the hair is characterized by a very high contact angle with water $\theta_w = 110^\circ \pm 5$. The impregnation of the hair by natural sebum decreases its hydrophobia ($\theta_w = 75^\circ \pm 4$). The friction coefficient of the hair differs according to several factors (see table): flat, curly, dry, greasy hair but also according to the direction of friction

Friction direction	Hair		
	flat	curly	flat + sebum curly + sebum
Near the ends	0.24±0.01	0.39±0.02	0.14±0.01 0.25±0.03
Near the roots	0.37±0.02	0.51±0.03	0.20±0.02 0.34±0.04

Friction coefficient of the hair; sliding distance: 10 mm; velocity = 0,5 mm/sec; normal load = 0,1N; number of friction = 10; friction with a sphere of diameter = 10 mm. T°C = 21°C - 23°C, %RH = 60-70%.

Conclusion: The hair is a hydrophobic/lipophilic surface. Similarly to the skin, the sebum decreases the friction coefficient and the hydrophobia. Treatment by hydrogen peroxide decreases the high hydrophobia of the hair (103° to 90°) The strong variations of the friction coefficient μ show the influence of the rubbed site (roots, centre, ends) and of the direction of friction (towards the ends or the roots).

Prospect: The hair presents specific characteristics of wettability and friction depending on whether they are coated or not with sebum. The variations are sufficiently discriminative to study the effect of cosmetic products applied to the hair in order to modify its aspect. The analysis of the properties of surface provides a better understanding of the mechanisms involved in the more or less flexible nature of the hair.

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Wettability and friction coefficient hydrophobic/hydrophilic balance influence

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Introduction: Friction coefficient depends on several parameters: types of probe motions (rotational vs. linear), surface roughness and physicochemical parameters of surfaces in contact).

Objective: Was to investigate whether hydrophobic/hydrophilic balance (Ho/Hi) of the skin surface influence the friction coefficient μ .

Method: The Ho/Hi balance is determined using the relationship between the critical surface tension γ_c (Zisman's principle: which delimits the wetting capacity) and the surface tension of water γ_{H_2O} (water: reference element of Ho/Hi balance).

The Ho/Hi balance for six surfaces (human skin forearm, Teflon®, silicone impression material 'Silflo®', vinyl polysiloxane impression material 'resin' steel and glass) were measured and their influences were compared to friction coefficient μ .

Results: This study shows that the higher hydrophobia tendency of the surfaces, the lower friction coefficient. The use of three sliding materials (Teflon®, steel and glass) of different Hi/Ho balance confirms the importance of these physicochemical parameters in μ . For example, Teflon® with high hydrophobia has a low μ . Friction coefficient increased when hydrophobia of sliding and slider surfaces decreased.

Conclusion: Friction coefficient value depends on the type of slider surface and its physicochemical properties. *In vivo*, the friction coefficient may quantify the influence of lubricant/emollients/moisturizers. For example, the friction coefficient of hydrated skin (through the action of moisturizing products) is higher than the friction coefficient of dry skin. The relationship between the friction coefficient and the Hi/Ho balance can be reversed in the presence of water and sebum on forehead, for example.

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Nail: hydrophobic/lipophilic balance - wettability and friction coefficient

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Introduction: Hardness of the nail is also based on its affinity with water. A too dry nail becomes breakable and fissures more easily, and a too wet nail weakens and loses a part of its impermeability, opening the way to contamination. The main constituent of nail is keratin; nail is also composed by lipids and minerals. The keratin - which is among the elements that made up the skin and hair - is a fibrous material insoluble in water, in order to ensure impermeability and protection against external agents. The degree of spreading water on the nail is an indicator of its hydrophobic or hydrophilic tendency. The objective of this work was to measure the water contact angle of the nail (water being the reference element of hydrophobic/hydrophilic balance). The effects of ethnicity, sex, washing and degreasing are studied.

Material and method: 36 volunteers of four different countries (China, France, Iran, Morocco) and both sexes (21 women, 15 men) participated in this study. The water contact

angle θ_w is measured by a system developed for *in vivo* and *in vitro* measurements.

Results: The surface of the nail is a hydrophilic surface with $\theta_w=68^\circ$. No significant difference function of ethnicity and sex were observed. The washing with soap and water, and the degreasing (acetone) increase its hydrophobicity. θ_w goes up respectively to 81° and 89° .

Discussion: Today, the nail wetting is very little studied and therefore comparisons of our results are limited. The value of nail wettability is located between that of the forearm ($\theta_w=90^\circ$) and the forehead ($\theta_w=55^\circ$).

Conclusion: The washing or the degreasing of the nail increases its hydrophobicity; therefore it becomes more dry, fragile and brittle.

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Wettability of *Vernix Caseosa*: *ex vivo* studies

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Introduction: Well before birth, the fetal skin is protected from the external environment (amniotic liquid) by a specific layer called *Vernix Caseosa* (VC). This protection begins towards the 24th gestation

n week and is made of a layer containing up of 80% water, 10% lipid and 10% protein.

The water contact angle θ_w is a indicator of the surface hydrophobic/hydrophilic tendency.

Objective: The objective of this work was to quantify the hydrophilic/hydrophobic tendency of VC in order to compare it with *in vivo* human skin wettability.

Materials and methods: The water contact angle θ_w was measured on the fleshy VC by a tool specially conceived for *in vivo* and/or *in vitro* wettability studies.

Results: The water contact angle θ_w was about 88° . This value classed VC within hydrophobic tendency surfaces.

Discussion: In this work, our results were closed to those in the literature. The values classed VC as a surface of hydrophobic tendency compared to that measured on the volar forearm (poor in sebum). In the forehead (rich in sebum) the $\theta_w = 55^\circ$.

Conclusion and Perspectives: The physicochemical parameters of VC is very recent (2001) and less published. However, in order to best understand the exact nature, role and for future exploitation of this material, both faces not yet studied till today, should be tested.

The bibliographic data show that VC material may be used *in vivo* for dry skin hydration and could help in anti microbial and innate immunity defense.

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Non-invasive methods of anatomico-structural skin changes evaluation in children with juvenile scleroderma

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Background: Development of non-invasive methods of anatomico-structural skin changes evaluation is one of the priority aims nowadays. Data obtained with the help of these technologies allows evaluation of pathological skin changes *in vivo*, which is particularly important in pediatric practice. One of actual problems has till now definition specific anatomico-structural criteria of activity sclerodermic inflammation.

Aim of our research was to study anatomico-structural skin changes in children with juvenile scleroderma by means of non-invasive methods and to compare measures of a thickness children's healthy skin and juvenile sclerodermic lesions.

Patients and Methods: With the help of ultrasound dermatoscanning (DermaScan C Ver.3) and confocal microscopy (VivaScope1500®) we examined 107 children with juvenile scleroderma from 3 to 16 years old with localized skin syndrome, among them 36 patients in the oedema stage, 34 in induration and 37 in fibrosis and atrophy stage of the disease.

Results: In sclerodermic lesions in oedema stage epidermis is thickened 1,78±0,12mm in comparison with children's healthy skin 1,50±0,52mm, epidermic-dermic joint is thinned, marked dermal oedema, hyperchogenic zone's extension, homoigenizing and fibrinoid degeneration of connective tissue structures are observed.

In the induration stage epidermis is thinned 1,06±0,9mm, border of subepidermal layer is uneven; decrease of hypochogenic zones in derma, collagen fibers disorganization, deformation of stratum papillare, marked inflammatory infiltration and perivascular fibrosis are observed.

In the atrophy and fibrosis stage epidermis as well as border between epidermis-dermis joint is not visible, marked heterogeneous hyperchogenic zones, progressive collagen fibers disorganization are observed. Stratum papillare is smoothed, walls of papillary vessels are thickened due to fibrosis while the blood flow is decreased.

Conclusion: Thus non-invasive methods allow investigation of anatomico-structural skin changes without tissue damaging, helps evaluating pathological process's dynamics in different stages of the sclerodermic inflammation.

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Development of an optimized treatment regimen and method of forecasting wound healing outcome following cutaneous exposure to sulfur mustard in the weanling Yorkshire swine

- J. Azeke*
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A modified 2³ factorial study was performed to develop an optimized treatment regimen for superficial dermal sulfur mustard casualties. The proposed treatment program would comprise debridement of the HD-induced lesion at 2 days post-exposure followed by the application of autologously transplanted skin cells harvested at the time of surgery. In addition, the benefit of post-exposure/pre-surgical topical anti-inflammatory creams was also investigated. Three superficial dermal wounds were created on each of 24 weanling Yorkshire pigs alongside a sham-exposed negative control site. While the positive control wound received no intervention following exposure, both optimization sites were either left untreated or treated four times daily with Clobetasol propionate and diclofenac sodium gel topical anti-inflammatory agents (TAIAs). Both optimization sites were debrided to 100um or 200um at 48hrs post-exposure using a dermatome or surgical laser. Concurrently, autologous skin cells were harvested from a naïve donor site on each animal. Sham and positive control sites were given no interventional treatment following agent exposure.

Non-invasive bioengineering was performed 7 and 14 days post-surgery (PS07 and PS14) to assess the mechanical, colorimetric, barrier function, and morphological properties of the experimental sites. De novo re-epithelialization was monitored using a subjective visual scoring protocol. Ultimate wound healing outcome was assessed by immunohistochemistry (IHC) and histopathology (HP)

on excised lesions. Partial least squares regression was used to quantify the effects of each treatment variable (TAIA, debridement method, and depth) on wound healing outcome. Correlation analysis was also performed to ascertain the suitability of using non-invasive methods to characterize and/or predict end-point histological and visual outcomes.

Statistical analysis (p<0.05) indicated that the application of TAIAs prior to surgery was the most significant contributor to desirable wound healing, having a cumulative effect of 1.52 versus 0.89 and 0.84 for debridement method and depth, respectively. Significant correlation was observed between HP, IHC, and the visually assessed wound healing scores at PS14. Colorimetry and ultrasonography were highly correlated with IHC, HP, and visual wound healing scores. Colorimetric data at PS07 were also correlated with IHC, HP, and visual assessments acquired at PS14. These findings suggest a potential means of forecasting final wound healing outcomes from early non-invasive bioengineering assessments.

This study demonstrates the importance of pre-surgical TAIAs treatment on healing of superficial dermal HD injuries. The use of several non-invasive bioengineering metrics as wound healing prognostic indicators was also validated. Using such prognostic tools, caregivers would be better able to provide patients better personalized care and thus ensure the most desirable wound healing outcomes.

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STRATUM CORNEUM AND BARRIER FUNCTION

The barrier function and water-holding capacity of the *stratum corneum* are not simply inter-related each other but are influenced by underlying pathological conditions as well as by body locations

- H. Tagami*

The barrier function and water-holding capacity constitute the indispensable functional properties of the *stratum corneum* (SC). Both can be instrumentally evaluated *in vivo* as transepidermal water loss (TEWL) or as high frequency impedance, i.e., conductance and capacitance. From the observation of their behaviors in commonly observed skin changes, it is generally thought that they are correlated each other. Recently, it is reported that the the flaggrin gene mutations that causes dry, scaly skin changes of ichthyosis vulgaris based on the deficiency in flaggrin-derived amino acids, i.e., the natural moisturizing factor, in the SC may also induce SC barrier impairment, leading to the development of atopic dermatitis by facilitating the penetration of various environmental antigens. However, the elevated TEWL values recorded instrumentally in patients with ichthyosis vulgaris is rather mild to allow the permeation of those large molecular environmental antigens, although the skin surface hydration state is extremely low even compared with sénile xérosis, another well known dry skin condition.

Moderate impairment in the SC barrier function such as noted in the dry limbs of ichthyosis vulgaris patients is also noted in fresh scars and retinoid-treated skin or even in normal facial skin, in which the skin surface is rather well hydrated associated with a smooth skin surface, exhibiting,

moreover, a moderate elevation in TEWL. In fact, the facial skin or genital skin in normal healthy individuals shows much higher TEWL than that of other bodily locations, although they are covered by very soft and hydrated SC. It is because the functional properties of the SC are not only under the influence of the epidermis but also are influenced by the underlying dermal conditions.

In the case of atopic dermatitis, we should think the possibility of the development of fine fissures in the dry skin, which induces focal structural disruption in the SC. Although we may not be able to detect any measurable TEWL increase, these defects definitely enable the permeation of large molecular environmental allergens to induce allergic skin reactions. Thus, it appears rather difficult to simply correlate the values of those instrumentally measured functional parameters each other to explain the pathogenesis of atopic dermatitis. Most of all, the primary pathogenetic mechanisms underlying the development of atopic dermatitis is not the SC barrier disruption but it is the extremely poor water-holding capacity of the SC, which leads to the total disruption of the SC barrier function in a dry environmental condition. To prevent the development of atopic dermatitis in such individuals, skin care to improve the skin condition is important, especially in winter season.

- 6 -

STRATUM CORNEUM AND BARRIER FUNCTION

Chairmen: Hachiro TAGAMI - Howard MAIBACH

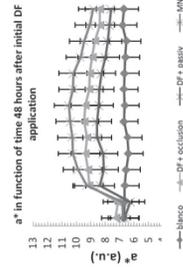
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Estimation of the *stratum corneum* diclofenac reservoir by chromametry

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Introduction: The *stratum corneum* (SC) is known to form a reservoir for topically applied substances [1]. Investigations on the reservoir function of SC are important in order to understand the pharmacokinetics of topically applied substances, and to elaborate the optimal treatment procedure [2]. The aim of our study was to estimate the reservoir properties of Diclofenac (DF) after a single topical application with and without occlusion.

Methods: A group of 14 healthy volunteers (females and males, aged 20-25) free of local or systemic treatment with any drugs participated in this study. During the duration of the experiments the volunteers were asked to maintain their daily activities but to abstain swimming and extensive showering. A 1% DF (Voltaren Emulgel®, Novartis) formulation (12mg) was applied on the volar forearms on randomized defined circular skin areas of 7cm². The product was applied for 20 minutes under two different conditions at the same time; passive diffusion under a semi occlusive sponge and a passive (open) application without occlusion. Bioavailability of DF in the SC under the two conditions was assessed by quantification of a methyl nicotinate (MN) induced erythema at respectively 48 and 72 hours post DF application. When DF is present in the *stratum corneum* the nicotinate response is depressed in a concentration dependent way. The response of the MN induced erythema was quantified with the Minolta Chromameter operating in the L*a*b* mode. Measurements were carried out before DF



Conclusion: Up to 72 hours post an initial open or semi occlusive DF application we found an inhibition of the MN response. This can be used as an indication for the presence of active DF in the *stratum corneum*. The reservoir formation seems to be independent of the application modality. Evaluation of the MN response at longer time intervals post DF seems necessary to estimate the duration of the DF reservoir.

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Non-invasive bioengineering assessment of the skin barrier function in patients with chronic venous insufficiency

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Background: Chronic venous insufficiency (CVI) comprises all symptoms caused by permanent venous and capillary hypertension. While the clinical manifestations of the disease have been well characterized, there is little knowledge on the skin barrier function in the affected individuals.

Aim: The aim of the study was to assess non-invasively the epidermal barrier function in patients with CVI stage C2 and C4 according to the CEAP classification and compare the findings to a group of healthy controls (stage C0).

Patients and methods: 30 patients with CVI without concomitant diseases and 15 healthy, aged-matched volunteers were included in the study following photoplethysmography and duplex sonography examination of the lower extremities. The skin barrier function was assessed by measurement of transepidermal water loss (TEWL), capacitance and skin colour symmetrically on five different fields as follows: flexor surface of the shin, medial and lateral malleolus, dorsum of the foot (arcus venosus) and forearm (control site).

Results: Compared to the volar forearm, there was a tendency for increased TEWL as well as significant reduction of *stratum corneum* hydration on all measurement sites on the lower extremity. Furthermore, compared to the control group, the patients with CVI had significantly higher TEWL values on all measurement sites on the lower extremities while no differences in capacitance between the patient and control group was observed.

Conclusion: Changes in the epidermal barrier function in CVI patients are detectable by bioengineering methods in early stage of disease and manifested by significantly increased TEWL. Our results suggest that CVI is not a contributing factor to the reduced *stratum corneum* hydration in the patients' group. These findings could help understand the factors facilitating the development of a venous leg ulcer and contributing to disease progression and its complications.

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STRATUM CORNEUM AND BARRIER FUNCTION

Measurement of inter- and intra-cellular water in *stratum corneum*

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Spatially resolved low-loss electron energy-loss spectroscopy (EELS) is a powerful method to quantitatively determine the water distribution in frozen-hydrated biological materials at high spatial resolution. However, hydrated tissue, particularly its hydrophilic protein-rich component, is sensitive to electron radiation induced changes. This sensitivity has traditionally limited the achievable spatial resolution because of the relatively high noise associated with low-dose data acquisition. We show that the damage caused by high-dose data acquisition affects the accuracy of a multiple-least-squares (MLS) compositional analysis because of inaccuracies in the reference spectrum used to represent the protein. Higher spatial resolution combined with more accurate compositional analysis could be achieved

if a reference spectrum was used that better represents the electron-beam-damaged protein component under frozen-hydrated conditions rather than one separately collected from dry protein under low-dose conditions.

By employing changes in the data analysis methods we have been able to measure concentration of water at spatial resolution of 10 nm, which is 5-10 times better than that in previous studies of frozen-hydrated tissue. Application of these methods to frozen-hydrated sections of porcine skin is sufficient to resolve intra-cellular water fluctuations as well as the water concentration in the inter-cellular lipid-rich regions of skin where water-mediated activities are believed to play a significant role in the degradation of corneodesmosomes.

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STRATUM CORNEUM AND BARRIER FUNCTION

Macro-scale mathematical model for hydration and TEWL in intact *stratum corneum*

- R. Imhof*

The aim of this work is to develop an improved understanding of how *stratum corneum* (SC) properties of hydration, TEWL and swelling are affected by ambient conditions of temperature and humidity. The method used is to calculate the steady-state flux of water diffusing from the viable epidermis, through the SC, into the ambient air. The SC is modelled on a macro-scale, where the bricks & mortar structure, tortuosity and other micro-scale properties are subsumed into an effective diffusion coefficient. The effect of water binding in the SC is represented by a hydration-dependent effective diffusion coefficient. The interaction between the SC surface and the adjacent air is described by a sorption isotherm. SC swelling is assumed to be isotropic and additive, where the volume of hydrated SC is given by the sum of the volumes of dry SC and water of hydration.

We will present results of calculations using both constant and hydration-dependent diffusion coefficients, with and without swelling. Calculated hydration depth profiles will be compared with those measured using confocal Raman spectroscopy. Also presented will be calculations of the dependence of TEWL and SC thickness on ambient temperature and RH, again comparing these with published measurements.

Work is in progress to extend the model to include calculations of changes of TEWL with thickness of SC removed in tape stripping experiments and time-dependent changes of TEWL and hydration when steady-state conditions are perturbed.

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STRATUM CORNEUM AND BARRIER FUNCTION

Experimental studies on the nature of *stratum corneum*

- I. Sadiq*

The *stratum corneum*, the outer-most layer of human body, perform important functions of protecting the body from the external dangers, controlling the egress and ingress of water and other chemicals in skin, being a biosensor of external stimuli and acting as a guardian of cutaneous homeostasis. A number of studies were designed to investigate various aspects of structure and function of *stratum corneum*. A variety of techniques and procedures were used to explore various properties of the *stratum corneum*. Its thickness *in vivo* was estimated using confocal microscopy and optical coherence tomography. The changes in thickness after application of a variety of chemicals were studied. The precipitation of certain chemicals were observed as crystalline deposits by confocal microscope. Hydration was studied using a number of instrumental modalities. The time-course of enhanced hydration after a variety of treatments were recorded.

Purpose: To evaluate the impact of different wound dressings in the recovery of the skin "barrier" function.

Methods: 30 healthy women, ages ranging 19 - 49 y.o. (26,7±10,5) were selected after informed written consent. A Sodium Lauril Sulfate (SLS) solution (5%) was applied under occlusion (24h) in predefined sites of both forearms (volar). This induction phase was followed by the repairing phase with the application of different wound dressings: hidroxipoliuretán (PermaFoam®), Hialuronic acid (Hyalofill®), polyurethane film (Opsite Flexigrid®) and gauze soaked in saline. Site distribution was previously randomized (Latin square). The biological impact of this experimental procedure was evaluated by Trans Epidermal Water Loss (TEWL) (Tewameter® TM 300, Courage - Khazaka Electronic GmbH, Koln, Germany), Eritema (Chromameter® CR 300) and Blood Perfusion (Periflux® PF 5010). The variables were measured in basal conditions and in 30 minutes, 4, 8, 11, 13, 15 and 20 days after the patch removal. Wilcoxon Rank and Friedman signs tests

Assessing the effects of different semi-occlusive wound dressing over the epidermal barrier recovery

- M.M. Pereira*
- L. Monteiro Rodrigues* **

(non parametric) were applied and a significance level of 95% adopted.

Results: TEWL signal can be used to follow up the barrier recovery process even under subclinical conditions. Eritema and blood perfusion can also be used to complement these results. Data shows that, under the present experimental conditions, the recovery of the skin "barrier" was faster in the sites covered with hidroxipoliuretán and hyaluronic acid dressings.

Conclusions: The present study objectively illustrates the importance of the occlusion over the cutaneous barrier recovery. Nevertheless more experimental approaches are needed to better understand the mechanisms involved, specially those involving the impact of different wound dressings in wound healing.

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Effect of sweating by exercise on *stratum corneum* hydration, skin surface sebum content and pH value

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Background: The physiological indexes of skin include *stratum corneum* hydration, skin surface sebum content and pH value, which could reflect physiological state of the local and systemic organism, and also could be affected by many factors from internal or external changes. Many studies have been put on these physiological indexes, but there is no report of studying on effect of sweating by exercise on sebum, hydration and pH value of face skin.

Objective: To observe the effect of sweating by exercise on *stratum corneum* hydration, skin surface sebum content and pH value of forehead and pars zygomatica of healthy individuals of different ages in order to collect the numerical data as the reference for exterior use drugs and before / after sports' cosmetics.

Method: *Stratum corneum* hydration, skin surface sebum content and pH value were measured on both forehead and pars zygomatica of 102 healthy Chinese individuals (5~60y) in summer at quiescent condition, beginning sweating, profuse sweating, and 1 hour after sweating with COMBINATION DEVICE (Sebumeter®SM810/Corneometer®CM825/Skin-pH-Meter®pH900). To compare the three indexes at different states (quiescent condition, beginning sweating, profuse sweating, and 1 hour after sweating) in general group and different subgroups (age 5~12y, age13~17y, age18~35y, age36~50y, age51~60y).

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Side-by-side comparison of open chamber (TM 300) and closed chamber (Vapometer) TEWL

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Introduction: We compared a closed-chamber TEWL meter (transepidermal water loss, Delfin Vapometer [DV]) against an open-chamber TEWL meter, which is viewed as the reference standard for TEWL measurements (Courage & Khazaka TM 300). The TM 300 was used in two modes, the standard open chamber method [CKO] and a closed mode (CKC) with a semi-permeable membrane chamber cover.

Methods: 540 TEWL measurements were taken in 17 participants with sessions of three and six sets of measurements on different days, measuring the TEWL on the dorsum and palm of both hands on each occasion. Four participants took part on either day one or day two only. The order of TEWL measurements was randomised to exclude confounding by interference when taking repeated measures. On the first day three sets of measurements were done; in the morning; around lunchtime; and late afternoon. On day two one baseline measurement before application of a barrier cream or emollient and then five measurements at specified time intervals thereafter were performed. The measurements

were done under standardised climate conditions in an air-conditioned room: the air-conditioning was switched off during measurements to prevent air movement interfering with open chamber measurements.

Results: 540 measurements were done with each TEWL meter respectively (DV, CKO, and CKC) at the same area of the skin of the hands of the 17 participants. The mean room temperature was 22.5°C and mean relative humidity was 56%. The range of the TEWL values were 2.3 to 84.7 g/m²h for the open-chamber TM 300, 2.6 to 72.4 g/m²h for the closed-chamber TM 300 and 5.1 to 214.1 g/m²h for the Delfin Vapometer.

Conclusion: There was acceptable agreement between the TM 300 and Delfin Vapometer in the lower and mid ranges but for higher TEWL values the devices showed increased variability with higher measurement values for the Vapometer.

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Can we really measure cutaneous water at different depths by bioimpedance?

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Introduction: The MoistureMeter-D® is a bioimpedance measurement device supplied with four probes of increasing sizes, to enable measurements at consecutively deeper depths up to a maximum of 8 mm. The aim of this work was to conduct a systematic study to assess the discriminative capacity of the device, after oedema induced by application of a mild irritant. The performance of the device was compared with results provided by ultrasonography, and a visual scoring was used to confirm induction of irritant contact dermatitis.

Methods: A total of ten healthy volunteers participated in this investigation. The study was conducted in either the right or left volar forearm, where three sites were marked. On site a) a 3.5% aqueous solution of sodium lauryl sulphate (SLS) was applied using a large Finn® chamber, b) was used as a control of the possible effects of the occlusion caused by the Finn chamber and c) was the untreated control. The location of a), b) and c) were randomized. The chambers were applied for 24h and one hour after removal of the adhesives the same measurements were performed in all sites. Erythema was assessed by a visual scoring system. Water content was systematically sampled using the probes supplied with the MoistureMeter-D® (Delfin Technologies, Finland)- XS5, S15, M25 and L50. Finally, skin thickness and segmentation were measured using an ultrasound device (Dermascan, Cortex Technology, Denmark).

Results: All the volunteers exhibited an irritant skin

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Influence of humidity and temperature on percutaneous absorption of VX through pig ear skin

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Background: S-(2-diisopropylaminoethyl) o-ethyl methylphosphonothioate (VX) is an anticholinesterase liquid of low volatility that penetrates the organism mainly through the skin. Environmental parameters play a crucial role for barrier function and permeation process of the skin. To estimate the influence of different climatic conditions on percutaneous absorption of VX, an ex-vivo technique with control of the surrounding conditions may make possible to replicate the microclimate above the skin during its exposure to VX.

Purpose: The aim of this study was to evaluate the influence of humidity and temperature on percutaneous absorption of neat VX by using a new apparatus and method to fix and control environmental parameters.

Methods: The apparatus used in this study is constituted of two parts: (1) An INOX chamber with six holes. Each hole has a diameter equals to Franz cell opening receiver compartment. The chamber is thermostated by a water bath. Reservoirs is integrated in the chamber and filled with saturated salt solutions to create a precise relative humidity. (2) Five Franz cell receiver compartments are fixed to the chamber. Each cell receiver compartment is fastened opposite to each chamber

hole. Each pig ear skin fragment (3.14 Cm2) is clamped between Franz cell receiver compartment and the chamber's hole. Each piece of skin is in contact with the interior side of the chamber across each hole. Before application of 15.7 µl of neat VX, the skin was let to equilibrate with the conditions created in the chamber. A sensor introduced in the chamber let to control temperature, humidity.

Results: An increase of temperature from 21°C to 35°C increased significantly the permeation rate of VX. At 21°C an increase of humidity from 41% to 71% increase slightly the permeation rate. At 35°C an increase of humidity from 41% to 71% decreased slightly the permeation rate. At given humidity, an increase of temperature led to an increase of total skin absorption (skin and receptor fluid) A decrease of humidity slightly increased the total skin absorption.

Conclusion: Variations in temperature and humidity have different degrees of effects on VX absorption. Since these parameters could change under field exposure conditions, the device used in this study is useful for creating a number of realistic situations encountered at different scenarios of skin exposure to chemicals.

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**Optical properties of the skin in the visible range
Application to the measurement of skin complexion**

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- C. Yarhi*
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- C. Montastier*
- P. Humbert**

Radiant skin, dull skin. Are these often-heard expressions a subjective notion or do they denote an optical reality? The first visible physical characteristics of the skin are its color and its glow. The term glow covers two more complex notions – specular reflection (the mirror effect on the surface), which is variously diffuse according to the grain, and the actual diffusion itself. Specular reflection is modified by the presence of sebum, sweat or the application of cosmetics. By diffusion is meant the light that has penetrated into skin and which, reflected by its internal structures, reemerges to transmit the color information for the components of the dermis. The spatial combination of these two kinds of light depends on the ratio between their respective intensities and the grain. To provide an answer to this question a study was carried out on a population of 30 women from 25 to 35 years of age, using a colorimetric method, a polarized light spectroscopic method, and measurements of microcirculation and surface roughness, supplemented by an assessment of skin condition by an esthetician.

Methods: An esthetician assessed the skin condition of the face with a standardized method under diffuse lighting. A 25-criteria assessment grid, scored from 0 for most favorable to 9 for very unfavorable, broken up into 4 thematic blocks (color, skin tone, grain, and general state of the *stratum corneum*), was used. From these criteria, the esthetician was able to determine whether the skin was “radiant” or “dull”. Skin color was measured on the forehead and the cheek with a Minolta CR 300 colorimeter, according to the protocol recommended by the EMMCO.

Capillaroscopy measurements were performed using the video-capillaroscopic device of the dermatology ward at the Besançon hospital. The parameters chosen were length, surface area, and quantity of capillaries visible under the skin, as well as the

number of capillary loops.

An apparatus based on the properties of totally polarized light (400 nm – 800 nm) was used. The polarization plane of the incident light was maintained; according to the dimensions of the skin contours the reflected light was not depolarized. The light that penetrated into the skin and was diffused by its internal structures was depolarized, partially absorbed and reemitted. The geometric configuration of the probe yields two spectra, one for the reflected light and, simultaneously, one for the strongly colored diffused light. From these two spectra were calculated the color parameters, indexed 0 for retrodiffused light and 35 for reflected light. Un-indexed contrast parameters combine both spectra.

Results and conclusion:

All the clinical evaluation data were subjected to a P.C.A., which clearly separated the population under study into two groups, one with radiant skin and one with dull skin. From the clinical point of view dull skin is somewhat red, thick and opaque with a tendency to be oily and a dilated grain, whereas radiant skin is pinker, clearer, finer and with fewer imperfections. The capillaroscopy data showed that dull skin is significantly more vascularized, with no increase in blood flow. The color measurements derived from polarized light spectroscopy were in line with the observed microcirculation – redder with less specular brightness for dull-skinned individuals. On the contrary, the contrast between reflected light and diffused light is a parameter directly linked to radiant skin. The surface roughness of the skin was also seen to be greater for individuals with dull skin.

The analysis of these results gives us better insight into the differences between these two skin conditions and underscores the preponderant role of the condition of the *stratum corneum* in the notion of skin radiance.

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Non-invasive optical methods for the study of infant skin

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Until recently, the study of infant skin *in vivo* has been limited to simple non-invasive techniques focusing on skin surface properties such as *stratum corneum* (SC) hydration, trans-epidermal water loss, and SC pH. With this work we demonstrate the development of non-invasive optical methods adapted for measurements on infant skin and the use of such methods to document skin maturation changes during the first years of life. Optical methods can be classified into methods relating to spectroscopy, microscopy, macro-imaging, or a combination of the above. Skin spectroscopy can be achieved *in vivo* with the use of fiber optic probes that can come in contact with the skin site of interest. Diffuse reflectance spectroscopy was used for the evaluation of the concentration of skin chromophores such as oxy- and deoxy-

hemoglobin and melanin. Epidermal cell proliferation rate was assessed through the use of fluorescence spectroscopy. Fourier-transform infra red spectroscopy was used for the determination of the amount and organization of skin surface lipids. Using *in vivo* laser scanning reflectance confocal microscopy we discovered differences between infant and adult skin microstructure. Finally, using multi-modal macro imaging we documented early signs of sun-induced damage on infant skin. The use of all these methods was well tolerated by infants and their parents. In conclusion, non-invasive optical measurement techniques are powerful and useful tools in the study of infant skin properties within a clinical setting. Their use allows for novel insights on infant skin and the skin maturation process.

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How to measure exactly the same location on the face as a function of time with digital photography?

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The fight against skin aging is truly international, although the symptoms may differ throughout the world. Whereas Caucasians notice wrinkles as one of the first signs of their passing years, Asians observe skin discolourations. All of us want to have a skin with a perfect colour (a uniform complexion without any discolouration) and a perfect smooth surface (without any wrinkles or other signs of roughness). Hence, there must be products to achieve these effects as well as ways to measure whether these products are successful.

Anti-aging skin measurements pose two different problems: time and location. On the one hand, the anti-aging effect of treatment will usually not be immediate. Photography is normally employed to capture the so-called "before treatment" situation because people simply cannot remember exactly how they looked like 4 weeks or 4 years ago. Whereas skin colour can be measured from photographs, parameters like roughness and wrinkles are more difficult to assess. After all, skin colour is a two-dimensional parameter, whereas skin roughness or a skin wrinkle is a three-dimensional phenomenon. In addition, for skin colour measurements it is important to study a larger skin surface area whereas for skin roughness and wrinkle measurements, it is extremely important to measure exactly the same small skin surface in the "before treatment" and "after treatment" pictures. The dilemma is to ensure that you measure exactly the same location as a function of time.

The work described here set out to solve these two issues simultaneously, i.e., how to measure skin surface parameters

from digital photographs (pseudo-three-dimensional measurements from a two-dimensional photograph) on exactly the same location at multiple time-points during skin treatment. The solution was found in software that can ensure exact repositioning of the face of the subject as well as assessment of skin surface parameters.

One of the first things we discovered was the importance of equal illumination in conjunction with the phototype of the subject being studied. A 10 million pixel Canon camera was combined with LED's and mirrors to ensure that this was achieved, of which the settings differ from phototype to phototype. A removable chin support ensured the same distance between the subject and the camera.

When working with the software of this equipment, a subject file is first created for each person that is measured, allowing treatment details and follow-up measurements to be stored systematically. When an anti-aging product is applied and the same subject is measured, for instance, every four weeks, a series of sequential photographs is generated that visualises the benefit of using this product. Automatically, the same lighting conditions are chosen that were used with the first photograph and that are based on the subject's skin phototype and zoom. These constant lighting conditions yield consistent pictures and solve the time issue as described above. But when the subject positions his or her face on the chin support at a follow-up visit, the operator can superimpose the face of the

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subject of this visit (the so-called dynamic picture) on top of a semi-transparent image taken during the first visit (the so-called static picture) and readjust the face to ensure that the positioning of the face is almost exactly the same as during the first measurement.

The importance of this becomes obvious when performing the skin analyses. First, the operator selects up to four pictures (s) he would like to compare. When (s)he zooms in one picture, the other picture(s) is/are also automatically zoomed in. In these enlarged pictures, it is still possible to further reposition one picture relative to another picture, so that exact positioning on multiple pictures is achieved.

A series of different analyses can now be performed on the pictures: spots (colour gap), skin colour (luminance, erythema, pigmentation) and homogeneity of complexion (average colour gap and maximum colour gap) can be measured, based on colour and contrast differences in the digital photographs.

But a three-dimensional phenomenon such as wrinkles should be also available from such kind of system, as soon as they are visible in two-dimensions. By translating contrast in pictures into numbers, pseudo-volume, surface, depth, visibility index (wrinkles) and roughness index calculation can be calculated and have thus been incorporated in the software. Moreover, because the system operates via the internet, new measuring opportunities that are being constantly developed can even be investigated on photos taken previously.

This novel equipment will greatly facilitate facial skin measurements. Without the need for expensive equipment accurate measurements of skin colour and pseudo 3-dimensional wrinkles and roughness can now be taken from 2-dimensional pictures.

IOMA Sphere as a diagnosis tool in the evaluation of skin treatments

- **E. Viviant***

The clinical evaluation of aesthetic results due to our treatments in the cosmetic facility is difficult and subjected to the eye of the beholder. Unless the effects are so obvious and beyond discussion, doctor and patient are often in a delicate position whether to end or continue the treatment. With new tools to evaluate sub-clinical changes, the effect of the treatment can be monitored and acted upon in the very early stages of change. Non responders are directed towards other treatment modalities, while the slow responders or the disbelievers are urged to continue.

Until now the science to measure skin functions, better known as skin biometry, required a variety of expensive and delicate instruments in need of proper calibration protocols and complicated handling. These conditions could only be met in a research setting such as big cosmetic labs and university facilities.

Recently a practical tool for biometry, named the "IOMA Sphere", brought this science within the reach of a standard clinical setting.

The IOMA Sphere is an innovative patented facial skin imaging system that accurately provides revealing photos and objective measures of sub-dermal conditions and the ability to track improvements in consumers' skin.

By employing advanced lighting and filtering techniques, IOMA Sphere™ captures high-resolution images of clients' facial skin and enables the doctors, in less than 15 seconds, to accurately reveal specific skin conditions.

The imaging part contains Sagging (Natural light), Fine lines (Parallel polarized light), Forehead furrows (Parallel polarized light), Redness, vessel analysis (Cross polarized light), UV damage (UV fluo light), Clogged pores (Blue fluo light) and Bacterial activity (Blue fluo light)

IOMA Sphere integrates the: analysis of Nasal Labial Folder (Neutral light) Lips shape and volume (Neutral light), 3 side views features, reference image for each view, Automatic face position recognition, reference images updatable for each view, 1:1 magnification, side by side single and 3V comparison, product and treatment recommendation, report generation, self-diagnosis for image quality for each single image, customer history, business tracking, Autofocus, Mixed light temperatures flashes, calibrated camera colorimetry, fully integrated, touchscreen technology, networking capability.

The IOMA Sphere reveals the artifacts before they become visible with the naked eye.

The acquired data can be subjected to a statistical and audit report for further analysis.

Among the most useful clinical applications we selected the skin surface analysis after several types of injectable, Lasers and peelings. Even the anti-aging effect of creams can be objectively evaluated.

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Innovative combination of *in vivo* methods to assess pores characteristics in surface and volume

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Introduction: Sebaceous activity, through the number of active sebaceous glands (Sebutape®) or sebum excretion (Sebumeter®) is known to be highly dependant from age, gender, hormonal status, diet and many other parameters. Nevertheless, pores features, which could be also linked to sebaceous activity, was not often studied.

This paper attempts to characterize age differences in skin pores features (visible size, density and volume estimation) using two *in vivo* systems. The efficacy of a cosmetic product on pore characteristics will also be presented.

Materials & Methods: Two *in vivo* imaging systems were used to detect and characterize skin pores.

The first one was a polarized lighting dermatoscope (called the Dermascor®) allowing a 4 magnification skin images with a circular 35 mm-diameter field. Using polarisers in parallel position associated with in-house software, we characterized the density of the pores (number of pores / cm²) and their visible size (area in mm²).

The second one was a fringes projection system (DermaTOP®) associated to an in-house advanced algorithm allowing the measurement of the pores volume estimation.

Using these methods 52 volunteers were investigated in this study. They were distributed in two equal age groups: [18-25] and [50-60] years old. An one month daily topical application was done on the whole population.

Results: We have revealed significant age effect on pores characteristics. Visible pores surface as well as pores volume estimation were higher for the older panel than for the young one, but pores density was lower. Significant change on pores characteristics after one month of treatment has been observed. Visible pores surface and pores volume estimation decreased with a cosmetic product application, and this for the 2 age groups.

Conclusion: The association of two evaluation systems and in-house developed algorithms allows us to perform an *in vivo* objective evaluation of pores characteristics either in surface and volume. We have revealed pores change with age as well as with a cosmetic product. Using these methods, we are planning to carry out another study investigating pores features and sebaceous activity.

Contactless apparatus with lateral and vertical micrometric resolution for real-time qualification of live tissue topography

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The methodologies classically selected to characterize the topography of living tissues which intrinsically have a limited reflecting power, are based on fringe projection [1-5]. This technique, combined with a data analysis by means of the Fourier-transform method or phase measurement delay, shows limitations in lateral and vertical resolution. Moreover, it is not compatible with the values involved in the elementary mechanisms of human skin healing for example.

To avoid such experimental limitations, new equipment of 3D topography contactless measurement, working in real time, and based on the analysis of wave front, has been designed and developed.

The basic concept is built on the principle of the PHASEVIEW device [6], for which the system of data acquisition has been adapted to the metrology of live tissues, in particular in terms of observing a large area, thus limiting

optical aberrations.

Moreover, analyzing experimental data required an important electronic adaptation to enable the presentation of results via a proprietary software. The analysis mainly concerns representations (3D map, contour map), histograms (height or slope distribution), surface or volume criteria (developed area, retention volume), statistical criteria (moment of n order of height distribution), parameters from signal processing (Fourier transform), and fractal data (perturbation indexes).

At first, the performances of the apparatus labelled «z live» were tested on grained surfaces, either flat or curved, and compared with accuracy data obtained by a Scanning Mechanical Microscope (SMM) type [7]. Subsequently, the *in vivo* study of the skin was compared with a metrology of replicas. Finally, some leg wounds were studied, enabling a kinetic approach of healing phases.

- [1] SURREL J., SURREL Y., «La technique de projection de franges pour la saisie des formes d'objets biologiques vivants», *J. Opt.* 29, 6 - 13, 1998.
- [2] MARSAILL D., GHARBIT, MEAUME S., GENTILHOMME E., ZAHOUANI H., HUMBERT E., «Mesures volumétriques des plaies. Technique de la profilométrie par projection de franges», *Journal des plaies et cicatrisation, Spécial tome VI*, n°27, mai 2001.
- [3] MARSAILL D., «Ingénierie optique et microsystèmes silicium. Développement d'une instrumentation dédiée à la biologie cutanée», *Thèse de Doctorat d'Université, UFR-ST Université de Franche-Comté*, 2004.
- [4] FRANKOWSKY G., BERLIN Y., «Fast and contactless optical measurement of 3D shape and the surface roughness by projected microfringes», *Conf. Proc. of the Int. Oberflächentekologium, Chemnitz, Feb 2/3, 2004*.
- [5] CREATH K., «Phase measurement algorithms», *Progression in Optics XVII*, 343-393, 1988.
- [6] www.phaseview.fr
- [7] WEHBI D., QUINIOU J-F., ROQUES-CARMES C., «Rôle de la rugosité des surfaces en science des matériaux», *Traitement thermique*, n°222, pp. 63-73, 1988.

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Acquisition, visualization and exploration of stereoscopic images of skin with virtual reality

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Recurrently, new imaging techniques are considered in dermatology, but they are rarely based on stereoscopic skin photographs. Our system deals with three-dimensional (3D) interactive analysis of the skin, and more precisely with stereoscopic images acquisition, system calibration, 3D visualization using virtual reality, skin interactive exploration and knowledge discovery.

The aim of our project was to conceive a global system that allows dermatologists 1) to acquire 3D skin photographs with no constraint for the patient, i.e. without contacts, lasers and in a very short acquisition time, 2) to explore the images in 3D with the highest fidelity to the original set of photographs, 3) to perform 3D measures (distances, surfaces, volumes), 4) to discover and share knowledge with other experts.

The acquisition system is compound of a mechanical support, two cameras and two calibration targets. First, the mechanical parameters of the system are calibrated with a new method that increases the accuracy of parameters estimation. Second, a set of two photographs are taken under standardized lighting conditions and the colour calibrated using a digital colorchecker chart. Third, the acquired images are explored using a virtual reality equipment compound of a large

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Distant evaluation of arsenic-induced skin hyperpigmentation and hyperkeratosis using digital skin images

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A number of developing countries (including Bangladesh, India and China) experiences large-scale contamination of potable water with arsenic, a potent carcinogen. The first symptoms of arsenic poisoning are specific ("rain-drop") skin pigmentation, or hyperkeratosis, or both (Hossain MK, Khan MM, Alam MG, et al, 2005). Therefore, large-scale surveillance of arsenic-induced skin changes might be important both for assessment of the scope of arsenic poisoning and for early recognition of precancerous skin lesions.

Presently, in many regions of Bangladesh systematic surveillance of patients with such lesions is not feasible, due to lack of dermatologists. One of the solutions could be using digital skin images, taken by a local aide with a simple digital camera. Afterwards, the skin images could be the evaluated by an experienced dermatologist (in Bangladesh, or abroad). Such an approach resembles "store-and-forward", or "asynchronous" mode in teledermatology.

The aim of our investigation was to assess the feasibility of distant evaluation of skin hyperpigmentation and hyperkeratosis at digital skin images of Bangladeshi patients drinking As-contaminated water. We used 193 digital skin images of the patients, obtained during the study organized by Harvard School of Public Health in Bangladesh in 2003-2004 (1280x960 pixels, obtained using digital camera Nikon Coolpix E5000). These images were independently reviewed by two dermatologists; no additional data were provided to the physicians.

Thus, it was demonstrated that distant evaluation of arsenic-induced skin lesions is generally possible, but special measures should be taken to provide proper color reproduction of digital skin images (e.g. use of standard color charts or special software for color correction) to secure high diagnosis accuracy.

We calculated interobserver agreement rate for skin pigmentation and hyperkeratosis and tried to define which factors might influence the agreement rate. Results: The interobserver agreement rate was found to be 52% for hyperpigmentation and 77% for hyperkeratosis. That was close to the reported level of interobserver agreement for the cases when no additional data were provided to consultant - 46.4-57.2% (Hermann FE, Sonnichsen K, Blum A, 2005). One of the factors which might impede the agreement rate could be improper color reproduction of obtained digital skin images. We defined color characteristics of lesion-free skin areas at available digital images. Mean H (hue) of such areas was found to be 6.7, SD=12.2. Approximately 70% of the images (136 of 193) had H within mean H + 1 SD; such images were considered to have small range of color distortions. Other 57 images represented greater range of color distortions. Interobserver agreement rate for hyperpigmentation was higher at images with small range of color distortions compared to images with greater distortions (57% vs. 40% respectively, p <0.05). No significant difference was found in evaluation of hyperkeratosis (79% vs. 72%, p >0.05).

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Advances in 3D Stereo Imaging

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3D is a current revolution in the imaging of the skin, skin structure and skin pathologies. 3D can serve multiple purposes ranging from photo-documentation to quantitative measurement with a quality and an accuracy far better than what can be achieved with simple 2D pictures.

Outside from the classical but quite expensive tomography imaging systems (CT, MRI, Ultrasound, Confocal microscopy...), 3D surface of the skin can be obtained via two broad classes of technologies. "Active vision", by which strips or patterns are projected onto the surface and then measured via a (generally single) image – and "passive vision", which is pure observation of the subject, generally based on Stereovision. Stereovision has the advantage to mimic human vision, therefore enabling 3D stereo-visualization as well as 3D surface reconstruction. It is generally more compact and easier to manipulate than active vision tools that need power supply and camera video mode to record the results of projected strips.

Between 3D stereovision technologies, auto-calibration must be distinguished from absolute calibration. With auto-calibration techniques, one can reconstruct a surface, generally with less details than absolute calibration system. Absolute quantitative measurements of 3D surfaces and 3D volumes are only possible using absolute calibration. Although cheaper than active vision systems, stereovision systems are still one

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order of magnitude more expensive than 2D photography, due to the high level of accuracy and calibration process in their design.

QuantifiCare has developed 3D LifeViz, an absolute calibration stereovision technology, with field of view intended for face and breast imaging. It is now issuing its second generation of systems with its LifeViz "Micro", designed for a 5x4cm field. LifeViz Micro is very compact and easy to use, it enables stereo-visualisation and quantitative measurements of fine wrinkles, crows feet, acne lesions, acne scars, BCC lesions, small ulcers and scars, striae, varicose... In some conditions, it is accurate enough to access to skin texture information.

We are presenting the result of extensive validation experiments of the 3D LifeViz micro system, demonstrating an in-depth accuracy of down to 8 microns in some conditions. Developing further the accuracy of the system will be limited by factors like skin transparency which would make difficult the assessment of accuracy and ground truth.

Finally, we are presenting a variety of clinical applications including, acne, scars, fine wrinkles and basal cell carcinoma, and including also a presentation of the different quantitative tools available in the 3D quantification package. This makes the 3D stereovision technology an ideal tool for research labs working on skin.

Relationship between aging patterns and facial sagging using a visual photographic scale

- L. Vasquez-Pinto*

Besides chronological age, life style and habits contribute to the generally agreed perception that some individuals may look "old for their age". One of the visual clues underlying the perception of aging is facial sagging. Therefore, it can be used to establish visual methods of evaluating skin aging.

This study developed a photo standard scale for evaluating facial sagging and correlated it with several well known habits that improve the extrinsic aging.

A four-point photo scale was prepared using 128 photos of women aged 18-59 years. Photographs were taken under standardized conditions (Visia Complexion Analysis – Canfield Scientific, Inc) and were divided into 4 categories by 11 dermatologists according to their perception of increased facial skin sagging. Dermatologists were blinded to information regarding volunteers' age, habits and life style. The correlation between the dermatologists answers were not statistically different ($p=0,05$). Kappa value was $0,57-0,87$, indicating a strong agreement between dermatologists.

Cluster analysis was performed and the four-grade sagging scale was determined (I: $1,35\pm0,64$; II: $2,58\pm0,20$; III: $3,55\pm0,29$; IV: $4,52\pm0,18$). The Euclidian distance of each volunteer was settled relatively to the cluster centre and photographs were then distributed in the four statistically identified clusters. The nearest volunteer relatively to cluster centre was considered representative of her group.

Information regarding volunteers' age, phototype, smoking habits, sun exposure due to work demands or leisure, hormonal replacement and cosmetic habits were collected at the moment they were photographed. Normality tests, equality tests for standard deviations and distributions and non-association

test were performed to identify which variable predisposed skin sagging. As expected, skin sagging increased linearly with age. The average age was 25, 41, 48 and 54 years for grades I, II, III and IV respectively. Interestingly we observed a difference of approximately 10 years between each grade. Hormonal replacement also showed a significant correlation with skin sagging, although it was a weak one ($\emptyset=0,279$). Phototype and cosmetic habits did not showed any influence in skin sagging in this study.

Curiously, we did not observe statistical differences between smoker and non-smoker volunteers nor any impact of the amount of cigarettes/day. Nevertheless, we did find a positive correlation between skin sagging and smoking time ($p=0,0001$). Volunteers in grade I have been smoking for 7 years, on average, while for other grades they have, on average, 22-36 years of smoking habits.

Sun exposure for leisure varied between 3-6 hours/day only in vacation periods. This was not enough to establish a significant correlation with the increase of skin sagging. However, those volunteers who were sun exposed for working demands, and therefore had longer and continuous periods of exposure, showed significant and strong association with skin sagging ($\emptyset=0,591$).

In summary, this work validated a visual photographic scale to assess facial skin sagging. Phototype, smoking, sunbathing and cosmetic habits did not influence skin sagging. On the other hand, significant correlation was observed with age, hormonal replacement, smoking span and sun exposure for working reasons.

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Objective characterization of a face shape by image processing

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The study of face shape is useful not only in Cosmetology, but also in Medicine and Aesthetic surgery. The characterization of a face's general shape (oval, oblong, elongated, etc) corresponds to a ratio between various representative segments: width and height ratio, eyes gap, forehead height, etc. Face is also characterized by more or less pronounced angles which break the global shape, most often at the cheekbones, jaw and chin's levels.

When aging, elasticity loss and skin relaxation contribute to modifying the face's overall shape, which is not determined only by bone structure anymore: jowls appear, neck skin shows deep folds called dewlaps. These changes are visible through the appearance or modification of the angles characterizing face shape.

Objective measuring methods exist regarding face oval and its modifications; they are based on several techniques:

- Measuring distances between the face's representative points: at the levels of chin, cheekbones, forehead and face overall height. The ratios between these measurements are done to characterize the observed modifications.

- Jaw's angles measuring after tracing two representative lines of the face determining a specific angle.

We propose an original method using image processing that measures all the angles on the lower part of the face's shape. Standardized photographs of the face are taken full-face and sideways with a cross-polarized light enabling to avoid artefacts due to lights reflected by flashes. The control of parameters (distance between sensor and volunteer, shooting angle), enables the exact positioning of the volunteer.

The image obtained is then processed so that the only remaining element is the overall shape of the face (detached

from the background). A software elaborated especially for this use analyses the face's curve and extracts the coordinates of the points constituting it. An oriented list of points is obtained and serves as a basis for the calculation enabling shape analysis. For each point of this list, a couple of points are associated, distant from n pixels on both sides. This enables to work with two vectors associated within one point and following face shape in this very point. The angle between these two vectors is determined by scalar product and the result is associated with the current study point. The calculation is then repeated for the overall points of the face's curve, providing for each point of this curve an angle value. The analysis of this series of values enables to isolate the salient angles of the face and quantify its overall shape.

This technique presents the possibility to perform measurements on different scales. The size of the vectors of analysis is indeed variable. An analysis with small-sized vectors will be influenced by curve's weak angles variations such as skin sagging. On the contrary an analysis with bigger-sized vectors will put forward global information about the face's oval shape. The study of face symmetry is possible by comparing the curves obtained between the left and right parts of the face. Different studies are possible: full-face study, sideways study, studies focused on the neck and chin zones.

This system of objective measurements of face oval and neck shape permits to classify a face according to its shape or following age bracket. Moreover, comparisons are possible on the effects of cosmetic products claiming acting against oval relaxation, or on the effects of techniques in Medicine and Aesthetic surgery.

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The role of color accuracy of digital images in distant evaluation of burns

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Burns are very common type of injury. In Russia, only 1/4 of all patients with burns are treated in combustiologie departments, specialized in burns treatment, while the majority of the injured have to be treated in non-specialized surgical departments. Non-specialized departments are characterized with higher mortality rate (6.5% vs. 4.3% in combustiologie departments); it demonstrates the role of physicians' expertise in the treatment of burns.

One of the ways to share the expertise of combustiologists with surgeons working in non-specialized departments could be use of telemedicine. One of the aims of telemedical consultations could be diagnosing the grade of the burn using digital images of the injured area, as the grade of burn is very important for definition of correct approach to treatment. Unfortunately, few surgeons in non-specialized departments can perform the correct assessment of burns degree in all cases, which often leads to improper treatment of the patients.

To date, no studies have been performed in Russia to evaluate feasibility and diagnosis accuracy of distant evaluation of burns basing on digital images.

To solve the problem, we have conducted our study within The Burns Department of Moscow Research Institute for Paediatrics in Children's Surgery.

The aim of the investigation was to access the feasibility and diagnosis accuracy of digital images of burns areas in children. The same set of 30 burns images was demonstrated to 8 experienced combustiologists. To evaluate if improper color reproduction of the images might impede diagnosis accuracy,

special software was developed, able to change the colors of an image in a random way. The observers were not aware of the rate of color change, but the figures characterizing the color distortion were recorded by the software and subsequently were used for statistic analysis. An observer had to define the grade of burn at the demonstrated burn image. All the images were of the same resolution, 3 megapixels, acquired with the same digital SLR camera. The images were presented to physicians at the same calibrated monitor.

Average diagnosis accuracy (the rate of correct definition of the grade of burn) was found to be 68%. The accuracy of diagnosis significantly depended on the level of color distortion of the images: for the group of images whose color distortions (measured as difference of H [hue] of lesion-free skin areas) did not exceed 28, diagnosis accuracy was of 73%, while the images with greater color distortions provided diagnosis accuracy of only 58% ($p=0.024$). Difference of S (saturation) and V (value, or brightness) did not influence the accuracy of diagnosis.

Therefore, it is generally possible to use digital burns images for their distant evaluation in the specialized combustiologie center. Meanwhile, special measures should be undertaken to secure maximum color accuracy of the images, as this might increase the rate of diagnosis accuracy.

Further studies will be devoted to use of computerized image analysis for assessment of burns degree and calculation of burn area at digital images of the lesions.

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Three-dimensional facial simulations and measurements: changes associated with facial expression

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Introduction: Recent innovations in laser scanning technology provide a potentially useful technique for accurate three-dimensional documentation of the face. In this study, linear and area measurements of the facial contour and facial units have been recorded in a variety of chosen facial postures using surface laser scanning combined with three-dimensional lighting techniques on seven healthy volunteers and three patients with facial nerve paralysis.

Methods: Three-dimensional surface measurement of the face was taken using a laser light scanner (Cyberware Laboratory 3030/SP), which projects a low-power laser beam onto the face of the subject. Computer graphics lighting techniques were used to produce facial images constituted by highlights and shadows, which emboss facial contour and units. The

quantitative measurement of changes in facial angles and areas were made to analyze morphological changes of the face accompanying facial expression.

Results: Changes of angles and widths of the cheek units were found to be associated with dimensional changes imposed by the action of the underlying mimetic muscles.

Discussion:

One of the characteristic advantages in our study is that the facial images, observed from any angle with the light projected from any location, can be obtained with only one scanning, and this technique allows simulation of the facial contour and units that we would actually encounter in daily life.

A new light into skin whitening evaluation: development of an image analysis method and comparison with clinical trial approaching

- L. Vasquez-Pinto*

The efficacy of skin lightening products containing multiple pigmentation inhibitory mechanisms was examined by both *in vivo* and *in vitro* studies. *in vivo* studies usually comprise double-blind random clinical trials. In these studies, uniformity of skin tone and reduction of age spots due to solar melanosis are evaluated with subjective tools such as efficacy perception questionnaires addressed to both dermatologists and volunteers. Alternative methodologies, like colorimetric methods and pantone based colour charts, were developed in order to reduce this bias. This study developed two image analysis routines to evaluate objectively the efficacy of a cosmetic product in reducing age spot size and improving skin tone evenness. Subjective efficacy perception of both dermatologists and volunteers was compared to objective image analysis results.

A 4 week clinical trial was performed with 40 women, aged 40-59 years, with solar melanosis spots clinically diagnosed. Volunteers used a 5% vitamin C formulation every night and a SPF15 cosmetic cream at daytime. Photographs were taken under standardized conditions (Visia Complexion Analysis – Canfield Scientific, Inc) at days 0, 7, 14 and 28, after beginning the described treatment. At each time point, both the volunteer and the dermatologist answered efficacy perception questionnaires. Photographs were analysed using two image analysis routines developed to evaluate the size of age spot relatively to total face size

and the degree of unevenness of skin tone. Statistical analysis were performed to validate the image analysis routines as an objective tool to assess product's efficacy. Objective results were then compared to clinical results.

After 4 weeks of continuous product use, a significant improvement in skin tone unevenness was observed in 78% of the volunteers ($p=0,001$). These results were more precise than the subjective efficacy perception evaluation performed. Dermatologists observed an improvement of volunteers' skin tone unevenness in 56% of trial group. Similarly, 53% of volunteers declared that they perceived an improvement in their skin tone unevenness.

Age spot image analysis showed a significant decrease in spot size after 4 weeks of treatment ($p=0,006$). This reduction was observed in 80% of the volunteers and showed a strong correspondence with volunteers' perception.

In summary, this work validated two image analysis routine developed to objectively assess a cosmetic product efficacy in increasing the skin tone unevenness and reducing age spots size. Image analysis results were similar to volunteers' perceived decrease in age spot size. However image analysis of skin tone unevenness was more effective than subjective clinical trial results. These objective image analysis routines should be used in cooperation with clinical trials to support skin lightening product claims.

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Intrinsic, solar and sunbed-induced skin aging measured *in vivo* by multiphoton laser tomography

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- **M. Kaatz***

Skin aging is accelerated by extrinsic factors, particularly actinic damage. Over the last decades, both clinical and pathological differences between intrinsic and actinic aging have been characterized. In one work, we aimed at quantifying skin aging by non-invasive *in vivo* methods. Young healthy volunteers using indoor tanning facilities and aged people were compared with appropriate controls by measurements of skin elasticity with the Cutometer and the Reviscometer and by semi-quantitative evaluation of the dermal matrix composition by the multiphoton laser tomograph DermalInspect. We found differences between the sun-protected volar forearm and the dorsal side as well as between young and old test persons with all three methods. No significant

differences were found between the skin of indoor-tanned

test persons and control. Also, gender had no influence on the severity of skin aging. The most consistent results were obtained with the DermalInspect. The considerable inter-individual variation due to the cross-sectional design of the study may have disguised the factual skin damage caused by tanning beds.

In another work, the focus was on age-dependent morphological changes of the dermal fibre network. With a filter system, the fibres of collagen and elastin were optically separated. We defined several parameters to describe the fibre network and found the parameters to be different when comparing young and old volunteers.

A score system for the evaluation of the fibre morphology was proposed and the score was shown to correlate well with age.

In vivo diagnosis of malignant melanoma by multiphoton laser tomography

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- **E. Dimitrow***
- **J. Norgauer***
- **K. König***
- **M. Kaatz***

The incidence of malignant melanoma has shown a dramatic increase over the past three decades. On the one hand, patient outcome and curability depend on early diagnosis, on the other hand, only few excised melanocytic skin lesions turn out to be malignant at histopathological examination. Therefore, non-invasive diagnosis of pigmented skin lesions is of outstanding interest. *In vivo* multiphoton laser tomography (MLT) represents a recently developed diagnosis tool that allows non-invasive tissue imaging.

In the investigation of eighty-three melanocytic skin lesions by MLT we identified distinct morphological differences in melanoma compared with melanocytic nevi. In particular, six characteristic features of malignant melanoma were specified and statistically evaluated. Furthermore, we investigated fluorescence intensity and lifetime in order to yield additional information for diagnoses of suspicious pigmented skin lesions.

Sensitivity values up to 95% and specificity values up to

97% were achieved for diagnosis classification. The most significant diagnosis criteria include architectural disarray of the epidermis, poorly defined keratinocyte cell borders as well as the presence of pleomorphic or dendritic cells. Remarkable differences in lifetime behaviour of keratinocytes in contrast to melanocytes were detected. Fluorescence lifetime distribution was found to correlate to the intracellular amount of melanin. Spectral analysis of melanoma revealed a main fluorescence peak around 470 nm in combination with an additional peak close to 550 nm throughout all epidermal layers. Excitation at 800 nm shows a selectively observable fluorescence of melanin containing cells and offers the possibility of cell classification.

Procedures of selective imaging as well as spectral fluorescence lifetime imaging by means of multiphoton laser tomography support diagnosis decisions and may improve the process of non-invasive early detection of melanoma.

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***In vitro* skin surface titration: from macro to nano**

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- M.F. Vallat*
- A. Mavon*
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Stratum corneum is a heterogeneous tissue composed of lipid-depleted corneocytes embedded in a lipid-enriched extracellular matrix. It comes from the epidermal differentiation of the skin. The wetting properties of this upper layer are of major interest in the understanding of interfacial phenomena, such as adhesion of microorganisms or proliferation of resident flora. Until now, the wettability has been characterized through different parameters such as surface energy, critical surface tension, or hydrophilia, via macroscopic contact angle measurements. But this method does not allow to discriminate the effect of the corneocytes with the one of the extracellular matrix on the final surface properties, because of the size of the liquid drop.

This study, performed *in vitro* on human skin explants provided by PFDC, consists in understanding the wetting properties of the *stratum corneum* from macroscopic and nanoscopic points of view.

1°) Knowing that macroscopic contact angles are sensitive to the pH of the liquid probe, the first aim is to determine the “macroscopic pKa values” of the *stratum corneum*. Consequently, dynamic contact angles are measured between

test-liquid drops (aqueous solutions ranging from pH 1 to pH 12) and the *stratum corneum* in order to obtain the contact angle titration curve of the *stratum corneum*.

2°) The second purpose of this study is to reach the pKa values of the different functional groups located on the complex-cornified envelope. This consists in measuring adhesion forces between an AFM (Atomic Force Microscopy) tip (functionalized with specific groups, such as amine, carboxylic, hydroxyl or methyl groups) and single-isolated corneocytes through buffered liquid media (ranging from pH 1 to pH 12).

The variations observed in the contact angle titration and chemical force titration curves will be discussed in terms of acid-base or electrostatic interactions and hydrogen bonding.

The importance of the liquid medium pH, which governs the driving force between molecules and skin, will be demonstrated.

The comprehension of the pH-dependent properties of the *stratum corneum* shall provide a better understanding of the role of individual corneocytes in the final surface properties of the *stratum corneum*.

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Tretinoin in polyethylene glycol-phospholipids micelle: Stability improvement and human fibroblast viability

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All-trans retinoic acid (tretinoin) is an effective substance for dermatological treatment of wrinkle skin. It is responsible for regulating proliferation and differentiation of skin cells. Unfortunately, its' susceptibility to reactive oxygen is one limitation factor for the development of cosmeceutical products. This study aimed to explore PEG-PE micelles in their ability to stabilize all-trans retinoic acid (tretinoin) and reduce its' toxicity to skin fibroblasts. The results indicated that tretinoin in PEG-PE micelles was more stable than

free tretinoin. Tretinoin concentration remained more than 80% after storage at room temperature for 28 days. The data suggested that PEG-PE micelles could retard chemical degradation of tretinoin. Moreover, tretinoin in PEG-PE micelles at 10 nM significantly increased cell viability of human fibroblasts (1.8 fold) comparing with free tretinoin. Therefore, micelles of PEG-PE copolymer could possible are useful as a novel carrier for the development of tretinoin topical formulation.

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Three-dimensional imaging of collagen reorganization induced by mechanical stress in engineered skin tissue

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We study the response of engineered dermal tissues to changes in their mechanical environment. We characterize two kinds of Episkin™ reconstructed skin models which consist of human keratinocytes cultured atop a dermal equivalent made of fibroblasts-populated collagen gel (lattice model). In the first model (type BA prototype), no stress is induced during dermis growth and cells are allowed to contract freely the gel matrix. In the second model (RealSkin model), mechanical stress is generated by interfering with gel contraction thanks to specific, proprietary methods.

equivalent of the reconstructed skin. We observe that the reconstructed human epidermis is a multilayered stratified epithelium which is morphologically similar to the native epidermis (basal, spinous, granular and *stratum corneum* layers). Most importantly, we show that mechanical strains induce orientation and reorganization of the collagen fibers; these morphological alterations are confirmed by scanning electron microscopy studies.

Moreover, this morphological reorganization induces a modification of the biomechanical properties of the lattice. To correlate these observations with increases in fibroblasts activity, we carry out ultrastructural and biochemical analyses on our samples and characterize the regulatory role of strains on the expression of main proteins of the extracellular matrix.

Moreover, this morphological reorganization induces a modification of the biomechanical properties of the lattice. To correlate these observations with increases in fibroblasts activity, we carry out ultrastructural and biochemical analyses on our samples and characterize the regulatory role of strains on the expression of main proteins of the extracellular matrix.

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Cell imaging connected with protein mapping to investigate glyphosate-induced toxicity on human keratinocytes

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During the skin aging which is accelerated by environmental factors, the generated reactive oxygen species make unstable the «proliferation/differentiation/apoptosis» coupling of epidermal cells. A biochemical study, previously realized on human keratinocyte cultures treated by a pesticide, glyphosate, allowed the validation of an *in vitro* model of skin aging.

In this work, we propose to study the loss of HaCaT cell integrity appearing after this chemical induced-oxidative stress. An original approach, combining a micro- to nanoscale cell characterization and analysis of fluctuating protein expression, is proposed: a) the exploration of cell growth and morphology through fluorescence and AFM imaging, b) a functional investigation using force spectroscopy for detection of oxidative stress biomarkers in membranes and c) the establishment of proteomic comparative profiles between safe and glyphosate-treated cells.

Our results revealed up to now that a glyphosate treatment induced cell impairment in an acute dose-dependency.

Indeed, we observed a breakage of plasma membrane and a release of cytosol, microtubules disorganization and fragmentation, and finally aggregation of chromatin. These observations characterized apoptotic key events. Our first force spectroscopy results revealed that a glyphosate coated tip interacted specifically with keratinocyte membrane structures. This nanobiotechnological tool will allow cell mapping establishment and screening original anti-oxidant molecules.

The proteomic profiling would highlight cell oxidative stress cascade and identify corresponding major biomarkers in our skin aging model. Original molecules with cytoprotective potentials will be tested and compared to well-known antioxidants, in order to correct pesticide induced cutaneous disorders.

These data all together will contribute to better encircle the mechanisms of chemical and environmental hazards acting on the human skin.

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Empetrum nigrum fruit extract: a multilevel skin protector

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Various external aggressions are responsible of skin aging, as oxidative stress, inflammatory disorder, mechanical stress. These effects are amplified in extreme environment. In this area, vegetables could supply animal defences when consumed.

The aim of this work was to select a plant coming from extreme environment and to test its capacity to act as a biological and structural skin protector. We focused our work on *Empetrum nigrum* fruit, which grows in subarctic region. Fruit juice is rich in polyphenol and concentration of anthocyanes is very high. From *Empetrum nigrum* fruit an extract (ENF) has been realised.

After demonstrating very good antioxidant activity due to the anthocyanes, biological activities have been studied on ENF. In a first step, this extract has been considered for its potential in modulation of extracellular matrix degradation. The second step was focused on contractile forces generated by fibroblasts. The third study consisted in tested the same extract, *in vivo*, in order to evaluate mechanical properties and microcirculation of human skin.

In a first time, elastase activity inhibition has been studied. The model used was polymorphonuclear (PMN) culture. PMN liberates constitutive elastase and this enzymatic secretion is increased when PMN cells are activated. In the presence of 2% of ENF, the elastase activity was greatly inhibited for 81% of constitutive and 71% of inductive elastase.

In a second time, MMP-1 liberation has been studied. Human

normal keratinocytes secretion of MMP-1 after stimulation is accentuated by a proinflammatory substance, phorbol myristate acetate. 2% of ENF reduces significantly MMP-1 secretion (0,7 pg.ml⁻¹.µg⁻¹ vs 6.1. pg.ml⁻¹.µg⁻¹ for control)

Moreover, the effect on contractile forces generated by striae distensae fibroblasts embedded in collagen lattices, with GlasBox® technology has been studied. Tensile forced developed by fibroblasts from red striae distensae are higher than force developed by fibroblast from adjacent normal skin. This increasing force kept striae distensae development. ENF in culture medium restore biomechanical properties of striae distensae fibroblasts to normal level.

After *in vitro* tests, *in vivo* study has been realised on volunteers.

The ability of the active substance, 3% ENF, to improve the biomechanical properties of the skin has been measured by cutometry. In comparison with his placebo, the product induced significant amelioration of tonicity parameter (17%) and firmness (7%), after 56 day of product uses. Moreover skin microcirculation has been tested by laser Doppler technology. ENF increased significantly skin microcirculation (6%).

In conclusion, ENF extract demonstrates high activity in antioxidant response, but although in protecting MEC and microcirculation. Moreover, ENF extract improves skin mechanical properties and protects against red striae distensae.

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IN VITRO BIOENGINEERING

Exploration of anti-aging activity of Corsican « immortelle » Essential Oil. Technique and evaluation on human skin explants maintained in survival.

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- K. Fontes**
- Y. Millou**
- J.L. Pierrisnard**
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Essential oils are not so easy to investigate as cosmetic active ingredients.

Two key points: they are hydrophobic and have only low molecular weight components.

Skin explants maintained in survival can be a good model to investigate their potentialities.

A large study about Everlasting essential oil (Immortelle – Helichrysum italicum from Corsica) was conducted this year.

We describe in this presentation the protocol, the histological studies, antibodies used. Histology is shown, software LEICA QWIN utilisation and transfer of results to Excel for quantification are described.

The following markers were investigated:

Glycosaminoglycans
Collagen Type I, Collagen Type III, Collagen Type IV, Collagen Type VII
Ki 67 mitotic index
Laminin -5
Sirtuin -1
Fibrillin -1
Connexin 43
Caveolin -1

The results of this study about Helichrysum italicum Essential oil are provided in a poster of this symposium

Autologous cultured fibroblast injection for facial acne scar

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- K. Jaeyoung*
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Background: Dermal fillers for the correction of facial wrinkle and acne scar. Injected bovine collagen was used as dermal filler. But is could be induced the hypersensitivity reaction (up to 6%) such as granulomatous reaction, necrosis and abscess.

Objective: The purpose of this study was to determine efficacy of autologous living fibroblast injection in phase II clinical trial for treatment of depressed acne scar. Previous studies that injection of autologous fibroblast increase collagen formation, accompanied by a concomitant increase in thickness and density of dermal collagen.

Method: This was comparison study of injectable living autologous fibroblast for treatment of facial depressed acne scar. Living fibroblast This IRB-approved study enrolled 24 patients with depressed acne scar. Skin sample were taken from retroauricular area (3mm punch biopsy) and send to S-biomedics Inc. Laboratory. Fibroblasts were selected culture, and multiplied over 7 week period using a proprietary process. Injection with Living fibroblasts (2×10^7 cell/ml) were given

as three dose administration at two week intervals. Efficacy evaluation was performed 4 month after the fast injection by visual grading and stereoiimage optical topometer (SOT, 3D parameter; Sq and Sa).

Results: Living fibroblast produced statistical significantly greater improved in acne scar. Difference between pre-treatment and 4 month after archived statistical significant increase Sq and Sa in SOT and visual grading. No serious adverse effects were seen.

Conclusion: Our results indicate that autologous fibroblast injection can safe and effectively produce improvement in depressed acne scar.

Acknowledgement: This study was founded by S-biomedics (www.sbiomedics.com) Inc. Seoul, Korea). Also, it was supported by Seoul Research and Business Development Program (grant number 10574) and Medium-term Strategic Technology Development Program (The Ministry of Knowledge Economy, Korea).

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Cellomics® imaging technology reveals inhibition of fat accumulation in adipocytes by natural food constituents

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Adipocytes are specialized cells in the white adipose that function as energy storage for triacylglycerides (TGs). An increase of adipocyte volume (fat accumulation) and cell number (adipogenesis) is characteristic in cellulite, an alteration of the gluteal-femoral adipose in women, resulting in orange peel-like skin and dimpling at various grades. There is a high need for actives to alleviate this unfavorable appearance by both cosmetic and dietary interventions. Valid *in vitro* test systems are required to allow for a larger screening of higher number of compounds and rapid quantitative detection of relevant fat cell differentiation markers.

An *in vitro* adipose cell system in murine C3H10 T1/2 cells was established to investigate the inhibition of intracellular fat droplet accumulation and cell differentiation by active food constituents. Differentiation was induced by rosiglitazone in the presence of different doses of test compounds for 7 days. Accumulation of intracellular lipid droplets was measured with a newly developed morphological assay that uses the Cellomics® HCS reader and SpotDetector Bioapplication software from Thermofisher. This advanced technology

allows for the capture and analysis of fluorescent images in a fast and automated manner. The fluorescent dye, Bodipy 493/503 was employed to visualize and quantify fat droplets in differentiated adipocytes, while nuclei were localized with the Hoechst 33342 dye. Furthermore, the expression levels of adipogenic genes like aP2, Acrp 30, LPL, CPT-1 β , Glut-4 and FAS were examined by RT-PCR.

Representative compounds of some 'families' of food chain ingredients such as polyphenols, catechins, carotenoids and PUFAs were tested. The ω -3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and the carotenoids (all-E)-lycopene and β -carotene exhibited the strongest inhibitory effects on the rosiglitazone stimulated lipid formation. 2-Hydroxy-tyrosol (HT) and epigallocatechine gallate (EGCG) showed a moderate inhibition. The results demonstrate the suitability of this system, and suggest that the application of supplements fortified with naturally occurring active compounds could be an approach to diminishing orange-peel skin.

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Stroma vascular-derived cells from human subcutaneous adipose tissue exhibit contractile properties stimulated by a selected hexapeptide

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- **S. Robin******
- **A. Zakaroff-Girard****
- **F. Pellicier***
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- **M. Dumas***
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Adipose tissue development is the result of adipocyte hypertrophy and the recruitment and differentiation of regenerative precursors located in the stromal-vascular fraction. This fraction represents a heterogeneous cell population surrounding mature adipocytes. Several groups have demonstrated that mesenchymal cells within the stromal-vascular fraction of the subcutaneous adipose tissue display multilineage developmental plasticity *in vitro* and *in vivo*.

For illustrating this plasticity, we previously demonstrated that these adipose tissue stroma vascular-derived cells (SVC) treated *in vitro* with TGF beta were able to express as fibroblast do, the phenotypic smooth muscular cell marker alpha smooth muscle actin (alpha SMA). This study investigates the contractile properties of SVC compared to

dermal fibroblasts (DF) obtained from the same individuals, skin locations, and culture conditions. When placed in floating collagen lattices (8.105cells/mL type I collagen), SVC and DF contraction kinetics (up to 10 days) were identical. When immobilized in the GlaSbox® device for a 48h measurement of strengths during isometric dermal remodeling, the SVC embedded in collagen lattices exhibit equivalent or higher tensile strengths than DF. Moreover, a treatment with 3% of the selected hexapeptide (Phe-Val-Ala-Pro-Phe-Pro) significantly stimulated SVC and DF isometric forces. We conclude that the adipose tissue stroma vascular fraction is a reservoir of fibroblast-like cells able to reorganize and tense in depth the dermal collagen matrix. This illustrates the ability of this particular hexapeptide to modulate the SVC and DF plasticity and their possible role in controlling the biomechanical properties of the dermis and hypodermis.

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A new lipinoacid able to restore aging-induced modifications in functional and biomechanical properties of human dermal fibroblasts

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In addition to disturbance in the production and organization of extracellular matrix (ECM) components, skin aging seems to be able to modify the biomechanical strengths developed by dermal fibroblasts *in vivo*. Many studies on the contraction activity of fibroblasts have been performed on the well known model of free retraction-collagen gels (i.e. lattices). However, restrained lattices are more similar to the human dermis than the previous ones. The aim of the study was, on the one hand, to compare the genetic profile and biomechanical properties of young and elderly dermal fibroblasts, and, on the other hand, to evaluate the effect of a new cosmetic active ingredient (a lipinoacid, LAA, previously described as an antiradical and proteasome activity-promoting product) on functions and biomechanical properties of elderly fibroblasts. For this purpose, molecular experiments were performed on replicative-senescent or not human dermal fibroblast monolayer cultures, both by cDNA arrays and by qPCR, to evaluate LAA ability to restore a younger genetic profile (BIOalternatives, France). Contractile forces developed by normal fibroblasts extracted from either a young (30 y-old) or an aged (65y-old) skin were measured in the LAA-treated, TGF- β 1-treated (reference molecule), or untreated GlaSbox® lattices (i.e. a device enabling the measure of the contractile forces developed by fibroblasts within a restrained lattice, Bioexigence, France). Immunostaining experiments were also performed to evaluate the distribution of proteins involved in focal adhesions (paxillin and vinculin) and of a cytoskeleton component (vimentin). Finally, clinical trials (vs. placebo) were conducted on Caucasian female volunteers to confirm

LAA anti-age ability (Dermscan, France). In the first trial (42d-treatment, mean age: 55y-old), measure of wrinkles was assessed by skin printing and image analysis. In the second one (56d-treatment, mean age: 58y-old), measure of skin biomechanical properties was assessed using Ballistometer®. At the molecular level, the variations observed in cDNA experiments showed that LAA induced a restoration effect on the genetic profile of senescent fibroblasts, which could be confirmed and quantified by qPCR for the following selected genes: type I collagen α 1 subunit and biglycan, two ECM components; Serpin 1H, involved in collagen fiber organization, and HSP90, involved in the regulation of proteasome activity. In the GlaSbox® model, a decrease in elderly fibroblast contractile forces in comparison with those of younger fibroblasts was observed (\approx -20%). Treatment of lattices with either TGF- β 1 or LAA totally restored the level of contractile forces. Similarly, while paxillin, vinculin and vimentin showed a disorganized pattern in elderly fibroblasts in comparison with younger ones, treatment of lattices with either TGF- β 1 or LAA partially restored the normal pattern. Finally, anti-wrinkle efficacy and improvement of skin elasticity could be demonstrated on Caucasian women. Taken together, these results confirm the anti-age efficacy of LAA and suggest that this ability is strongly correlated to its ability to act on ECM production and organization but also on fibroblast/ECM interactions. In the future, signalling pathways regulating such aging-induced and LAA-restored biological processes could be investigated.

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In vitro method measuring calcium percutaneous egression in normal and tape-stripped human skin

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Ions play a crucial role in skin homeostasis. Calcium, a participant in wound healing, is localized at an increasing gradient from the stratum basale to the stratum granulosum in normal skin. *In vivo* and *in vitro* studies showed disturbances in this gradient when the skin is damaged. We measured here, with a calcium specific electrode, *in vitro* calcium percutaneous egression from dermis to epidermis in normal and tape-stripped skin. Tape stripped skin released a greater

concentration of calcium ions over time than normal skin. Also calcium uptake from the dermis is greater in tape stripped skin. This method reproduced the increased of calcium skin permeability when the cutaneous barrier is disrupted. This project aims to develop a non-invasive method to measure calcium flux concentration in normal and tape stripped skin that might be reproduced and validated *in vivo*.

and tape-stripped skin. Tape stripped skin released a greater

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Potential of Thai breadfruit extract for application in anti-aging product

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In cosmetic industry, the demand for multi-functional products and their efficiency are the keys of trend on innovations and cosmetic market. Recently, we found that extract of heartwood of plant in Moraceae family, namely *Artocarpus incisus* (breadfruit or Sa-kae in Thai) exhibited tyrosinase inhibitory activity in the dose-dependent manner. The obtained IC50 value was $10.26 \pm 3.04 \mu\text{g/mL}$ while kojic acid, a well-known lightening agent, provided IC50 of $7.89 \pm 0.18 \mu\text{g/mL}$. In addition, the extract at the concentration of 2 to 25 $\mu\text{g/mL}$ was able to decrease the melanin production of melanocyte B16F1 cells. In this study, therefore, was aimed to evaluate if the extract has also a potential on anti-aging property, particularly on antioxidation activity and human keratinocyte and fibroblast proliferation. To obtain yellow powder of the extract, heartwood of *A. incisus* grown in Phitsanulok Province, Thailand was firstly macerated by using diethyl ether, and the organic solvent was then evaporated under vacuum. Artocarpin, a flavonoid component generally found in the extract, was determined by HPLC method to quality control of the extract from each batch. The content of artocarpin found averaged $45.2 \pm 0.5 \%$ w/w. DPPH assay was used to determine antioxidative activity of the extract.

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Comparison of *in vitro* - *in vivo* SPF correlation of 12 sunscreen commercial products using two sorts of skin substitutes (Vitro-Skin x PMMA)

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Background: The *in vivo* Sun Protection Factor (SPF) test is the world wide most suitable assay for efficacy evaluation of sunscreen products. Considering the premise that SPF test is performed in human volunteers and that this assay is not risk-free, some safety measures must be taken prior to *in vivo* assessment. Take into account that most of Sunscreens products act as chemical UV absorbers and or physical barriers that reflect the incident radiation an *in vitro* spectrophotometric technique could be useful to predict the *in vivo* behavior of the sunscreen product.

Purpose: The scope of this work was to compare the *In vitro* - *In vivo* SPF Correlation of 12 sunscreen commercial products using two of the most used commercially available substrates for SPF *in vitro* evaluation. These substrates are Vitro-Skin from IMS and Helioplate HD6 from Helioscience.

Methods: The *in vitro* spectrophotometric readings were carried out by Labsphere UV 1000-S. For the *in vitro* evaluation 3 substrates for both of the two types were tested

per product and the results were the mean of those 3 substrates, a 10% coefficient of variation was considered as adequate. *In vitro* assessments were based on the latest publication of the International Sun Protection Factor Test Method. Results of a minimum of 10 subjects and a maximum of 20 shall be used until the fulfillment of the methodology statistical criteria.

Results: The mean of all *in vitro-in vivo* obtained data were plotted in a linear regression graphic in order to calculate the linear correlation coefficient (R2) and to obtain the Straight line equation. A R2 greater than 0.8 is generally described as strong, whereas a correlation less than 0.5 is generally described as weak.

Conclusions: Both substrates (Vitro Skin and Helioplate HD6) showed strong *In vivo* - *In Vitro* correlation (R2 greater than 0.8) and could be used to forecast the SPF prior to *in vivo* assessment, in order to minimize the risks to human subjects.

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Effect of filaggrin gene mutations on biophysical and biochemical skin properties in individuals with pathological dry skin

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Two prevalent FLG null alleles in the European population (R501X and 2282del4) and two less common variants (R2447X and 3702delG) have been reported to be linked to ichthyosis vulgaris, and to be strong susceptibility factor to atopic dermatitis (AD or eczema), eczema-associated asthma and AD with allergic sensitizations. The aim of the study was to examine the effect of these FLG gene mutations on biophysical properties and *stratum corneum* (SC) composition in subjects with atopic dermatitis (AD).

To reach this objective, a study was conducted on 196 French adults Caucasian subjects, 18 to 46 years old, classified in two groups: atopic dermatitis subjects (AD group, n=97) and healthy skin subjects as controls (control group, n=99). The genotypes of four FLG variants (R501X, 2282del4, R2447X et 3702delG) were analysed. The severity of AD was determined using the OSCORAD index. Raman measurements were performed on the forearm of each subject to assess in the SC the amount of lipids, lactate, NMF components (amino acids and derivatives), relative amount of water as well as the thickness of the SC. Moreover, capacitance, trans epidermal water loss (TEWL), temperature and pH parameters were also measured on the forearm.

Fourteen subjects (AD: n=10; Control: n=4) were carriers of at least one mutation of the three FLG variants: R501X, 2282del4 and R2447X. The 3702delG variant was not detected in our sample. The allelic frequencies of FLG mutations tended to be higher in the AD group than in the control group ($p < 0.0993$). The AD carriers of at least one mutation presented significantly lower values of NMF components (amino acids and derivatives) than the AD subjects without mutations and than the control subjects. They also presented significantly higher values of TEWL, lower total amount of water and a thinner SC than the control subjects. Besides, a significant negative correlation was found between OSCORAD and the Ceramide 3, the cholesterol and NMF components, even if no significant difference of Ceramide 3 and of cholesterol was found with the mutation. The AD severity was also negatively correlated with the capacitance parameter.

Our findings support the role of filaggrin protein in the maintenance of skin barrier function and suggest a diminution of lipids (Ceramide 3 and cholesterol) when the severity of AD increases.

Chairwomen: Anne COLONNA - Danielle MOUGIN

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Influence of TNF- α polymorphism -308 on irritant dermatitis in health care workers

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Background: Consistent use of proper hand hygiene by health care workers (HCW) reduces the transmission of hospital acquired infection. A major reason for lack of compliance is the skin irritation produced by repeated hand hygiene. There is significant variation between individuals in susceptibility to skin irritation. Understanding the sources of this variation might allow prediction of susceptibility and intervention prior to the development of skin problems that lead to lack of compliance with hygiene protocols. It has been reported that the presence of an A allele (GA/AA genotypes) at position -308, on the TNF- α gene, is associated with more severe response to SLS in a patch test compared to the GG genotype.

Purpose: The purpose of this study was to evaluate the relationship between TNF- α polymorphism -308 and the irritant response in HCWs comparing both hand irritation in work place and response to patch testing with SLS between subjects with and without the A allele.

Methods: DNA genotyping was done by quantitative polymerase chain reaction (Q-PCR) on blood samples to determine presence of a TNF- α polymorphism at locus -308 on 74 HCW in a neonatal intensive care unit. Hand dermatitis was evaluated by expert visual grading and digital imaging of photographs. A subset of HCW was selected for

patch testing. Patches containing 0.05% SLS, 0.1% SLS, or distilled water were applied to the outer upper arm on days 1, 2, 8, and 9 and removed after 6 hours. Visual grading of dryness and erythema, TEWL (Vapometer), and digital photography were used to quantify the effects of the patch treatments. We also measured *stratum corneum* cohesion by tape stripping an untreated site to approximately 3 times baseline TEWL with D-Squares and quantifying the amount of SC removed by each D-Square®

Results: The correlation between knuckle dryness and TNF- α polymorphism -308 tended toward significance ($p = 0.1$, chi-squared test). No correlation was found between TNF- α polymorphism -308 and visual erythema or excess erythema from image analysis. Repeated measures analysis of the patch test data showed significantly higher response to SLS in the AA/GA group compared to GG in both TEWL and visual erythema grades in agreement with literature. Analysis of the SC cohesion and digital image analysis from the patch tests is ongoing.

Conclusions: It is clear that TNF- α polymorphism -308 has an influence on response to irritant but the hand data show that other factors must also play significant roles in the variation in the levels of chronic dermatitis in HCW.

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Mickaël Tanter, Ph.D., is a research professor of the French Institute for Medical Research (INSERM). He is currently heading the team Inserm ERL U979 "Wave Physics for Medicine" at Institut Langevin (CNRS UMR 7587), ESPCI, Paris, France. He received a Diplôme d'Ingénieur (High Engineering Degree) from Ecole Supérieure d'Electricité in 1994 and was awarded in 1999 a Ph.D. degree from Paris VIII University in Applied Physics.

His main activities are centered around the development of new medical imaging and therapy techniques. His current research interests a wide range of topics: ultrasonic therapy and echographic imaging of the brain, elasticity imaging of organs using ultrafast scanners. Mickaël Tanter is the recipient of 16 patents in the field of ultrasound imaging. He is the author of more than 70 technical peer reviewed papers. He is a member of the scientific boards of the Focused Ultrasound Surgery Foundation and IEEE Ultrasonics. Finally, he is also co-founder of Supersonic Imagine, a MedTech company in the field of ultrasonic medical imaging and therapy.

Quantitative *in vivo* imaging of tissue visco-elasticity using Supersonic Shear Imaging

- M. Tanter*
- J-L. Gennisson*
- M. Pernot*
- M. Fink*

This lecture presents a review of the applications of Supersonic Shear Imaging (SSI) modality. This technique is based on the combination of a radiation force induced in tissue by an ultrasonic beam and an ultrafast imaging sequence capable of catching in real time the propagation of the resulting shear waves. The local shear wave velocity is recovered using a time of flight technique and enables the two dimensional (2D) mapping of shear elasticity. This imaging modality is implemented on conventional probes driven by dedicated ultrafast echographic devices. Consequently, it can be performed during a standard echographic exam. Clinical results demonstrate the clinical feasibility of this

new elastography technique in providing quantitative assessment of stiffness of breast tissues. Experimental results will emphasize the potential of this elastography technique for many other potential applications such as liver, cardiovascular, ophthalmologic and muscular applications. Beyond elasticity imaging, a complete *in vivo* assessment of tissue rheology can be performed using this approach. Dispersion effects affecting the propagation of visco-elastic waves in soft tissues are a key to understanding the rheological behavior of human tissues. The potential of this new technology in providing quantitative and *in vivo* skin mechanical properties will be emphasized.

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Klaus-Peter Wilhelm

Klaus-Peter Wilhelm, M.D., Professor, born on March 3rd 1960, is President and Medical Director of proDERM Institute for Applied Dermatological Research, Schenefeld/Hamburg, Germany, and extraordinary Professor of Dermatology, Medical University of Lübeck, Germany.

Dr. Wilhelm received his M. D. degree in 1986 from Medical University of Lübeck and was awarded the title extraordinary Professor in 2002. From 1988 to 1990 he was a Visiting Scientist at the Department of Dermatology, University of California, San Francisco, Medical School in Dr. Howard Maibach's laboratory. He completed his residency at the Department of Dermatology, Medical University of Lübeck, Germany, from 1990 to 1993. In 1994 he founded the contract research institute proDERM in Schenefeld/Hamburg. Since then he has been President and Medical Director of proDERM.

Functional food, food supplements and the skin

- **K. P. Wilhelm***
- **G. Springmann***
- **S. Bielfeldt***

Functional food and food supplements are foods or dietary products that should provide a health benefit beyond basic nutrition. The worldwide market for these products is estimated to be in excess of \$ 100 billion.

Such products are regulated in Europe under the food (supplement) legislation. However, there is a potential distinction and separation between food supplements and drugs. While initially functional food and food supplements were mainly provided as a «soft alternative» to pharmaceutical drugs to improve health parameters that could be early linked to nutrition i.e. lower cholesterol levels, prevent osteoporosis, induce natural sleep etc.

In recent years the idea that food supplement could also benefit the skin, i.e. improve its cosmetic appearance, strength it against environmental pollution, reduce wrinkles aging parameters etc has gotten more widespread attention.

Such «nutraceuticals» are used to supplement the diet by increasing the total dietary intake of specifically defined important nutrients, targeted a particular skin benefit. This definition includes nutritional supplements, such

as vitamins, minerals, herbal extracts, antioxidants, amino acids, protein etc. Since this growing market is significant, and because it addresses skin health care, assumption of skin benefits must be substantiated with scientific evidence.

Regulation requires scientific proof of any health claims made with food supplements and even provides significant protection comparable to patent laws for the innovator.

Since systemically administered compound with mostly have a lower concentration in the skin as compared with topically applied compounds the effect of food supplements is usually smaller on skin parameters than that of topically applied cosmetics. Therefore, the planning and execution of claims support study require extra care and sophistication to detect those skin benefits.

This presentation will critically review the concept of «nutraceuticals» with an emphasis of regulatory requirements and designing appropriate study protocols to verify claims.

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**Developing a proper testing program for
cosmetic advertising claim substantiation in the US**

- **S. Schwartz***

Advertising Claim Substantiation varies from country to country and is under the general oversight of various government and non-government agencies. However, the concept of good clinical design is universal. To get a better understanding of the requirements it is important to understand the agencies under which "regulations" would apply as well as the country definition of a drug and cosmetic. One critical key to a successful program is the ability to write a concise protocol, keeping in mind that it should be able to be repeated when used by those of reasonable scientific background to show the same or similar results as obtained to support the claims. It is most important to know how and

when to use technology and how much weight it carries in the support of the claims and the proper use of trained observers and consumer data, remembering that in the US the gold standard is a "reasonable basis" for supporting the claims and that a very high level of proof is based on "consumer perceivable" whose data is derived from the use of trained observers and the consumer. The overall scope will scope will be on the US and its "regulation" of the industry and focus on the use of Triangle Testing which comprises the use of all three key support elements, trained observer, technology and the end user, consumer.

Chairpersons: Vera ROGIERS, Roland BAZIN

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New method of skin rejuvenation in aesthetic cosmetology and plastic surgery

- **N.V. Kaluga***

The skin disorders, which are appeared as wrinkles, folds, are related with the loss of collagen and elastin in considerable degree as well as deterioration of blood microcirculation, the lowering of tonus of mimetic muscles. It's known the plenty of methods of rejuvenation which are founded on the introduction of biological active medicines, providing the aesthetic effect, in the face zones (Botox, Disport, hyaluronic acid, «Alloplant» and others). The introduction of these medicines is related with the risk of overdosage which can lead to the deterioration of neurotrophic control of some skin sites. However, it's known that the skin aging is not local but systemic process.

The purpose of our study is the application of blood and salivary cytokinos of patient for his rejuvenation. It's generally known that cytokinos are special substances with high concentration of factor of skin growth. This task is decided by the method of skin rejuvenation, composing the course of introduction of medicines in dermal and subdermal layers, the complex of autocytoquinas of patients is used which is introduced by the method of electrophoresis, ultrasound and subcutaneous injections 5-10 times every 1-5 days at 50-100 mkg/ml. If the rates of collagen and elastin are lower then the standard, in addition the course is performed 5-10 times every 1-5 days 1 dose autocytoquinas (50-1000 mkg/ml) in the forearm during the procedure. In this case 1 dose of autocytoquinas which is introduced in forearm or skin of face is divided on some micro-injections.

The introduction of medicine without injection by the method electrophoresis, ultrasound excludes the problems of skin trauma and pain of this procedure. The injection is less painful into the forearm than the complex local introduction of medicine in plenty of sites along the wrinkle as it's performed in analogy. The introduction of autocytoquinas absolutely excludes the allergic reaction because the medicine is performed from the blood of patient or salivary of patient (patent UA N° 29936). The rejuvenation of autocytoquinas was performed in patients of different ages. The results of rejuvenation are fixed by the measurement of collagen and elastin in skin of patient, simultaneously the immunological and biochemical analysis were performed.

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Corneometry in assessing health safety of skin purification products

- **O.I. Voloschenko***
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- **O.I. Yalovenko***
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The research was done into the ability of skin to keep and restore moisture after using cosmetic products for skin purifying. The level of volunteers' skin hydration was measured by the indicator Corneometer CM 825 made by «Courage+Khazaka electronic GmbH» under the constant values of moisture and temperature during the whole experiment. The care product subject to testing was: Shampoo and Shower gel 2 in 1, which contains aqua, sodium laureth sulfate, sodium chloride, cocamidopropyl betaine, parfum, polyquaternium-7, sodium citrate, benzofphenone-4, propylene glycol, 2-bromo-2nitropropane-1,-3-diol, citrus aurantifolia, zingiber officinale extract, sorbitol, CI 19140, CI 42090 and moisturizing creme. 15 volunteers took part in the experiment. Their left forearm were treated with the Shampoo and Shower gel 2 in 1 and 24 hours later with the moisturizing creme, while the right forearm, the control one, was treated with water only. The marked area of the forearm was washed by the above care product according to the use instruction and the moisture level was measured by Corneometer CM 825 before washing, 30 min. after washing, 2 hours after washing, 4 hours after washing, 24 hours after washing, and then a moisturizing creme was applied. After that the moisture level was measured 30 - 60 - 90 min later.

The research showed the following results: average in the group values of the moisture coefficient - background - 33,56 ± 1,00; 30 min after washing - 22,53 ± 0,68, t = 9,1235; 2 hours after washing - 22,46 ± 0,76, t = 8,8491; 4 hours after washing - 21,53 ± 0,82, t = 9,2751; 24 hours after washing - 22,46 ± 0,94, t = 8,0635. The obtained data show that after washing of the tested area of the forearm with the

Shampoo and Shower gel 2 in 1 there appeared statistically reliable reduction of the skin moisture level on the given area by 33 - 36 %, which is not restored naturally even 24 hours later. The control area, washed with water, showed stable results without reliable changes: background - 34,02 ± 0,87; 30 min after washing with water - 32,54 ± 0,74, t = 1,3005; 2 hours after washing - 33,07 ± 0,90, t = 0,7606; 4 hours after washing - 34,22 ± 0,69, t = 0,1802; 24 hours after washing - 33,17 ± 0,96, t = 0,6577. After the application of the moisturizing creme on the tested area the full and stable hydration restoration was observed - in the test group the value of the hydration coefficient: 30 min after application - 35,25 ± 0,70, t = 1,3810; 60 min after application - 31,91 ± 0,85, t = 1,2535; 90 min after application - 31,37 ± 0,107 t = 1,4946. Under this single washing the volunteers did not notice any significant adverse feelings. However, after the cream application all the volunteers stated that felt more comfortable than before the application of the moisturizing creme. Consequently, it is possible to make a conclusion that Corneometer CM 825 is able to determine negative tendency for the human body, which cannot be registered while assessing the effect only by visual monitoring and subjective feelings.

The conducted experiment demonstrates an opportunity to develop the direction of using corneometry in assessing health safety of skin purification products, provided the development of the criterion significant range of fluctuations of the moisture level coefficient, characterized by the absence of tendency to develop skin irritation.

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Smile, beauty, self-perception and fulfillment in the society

- S. Zemmouri*

Materials and methods:

The study has been done in a private periodontal clinic center in casablanca from August 1992 to Dec 2008, englobed more than 2500 cases, aged between 14 and 42 years, and repartited between female and masculine. Criteria of inclusion were: age, presence of inflammation symptomatology, periodontal pockets, no treatment before, bone less.

Clinical exams were done by probing of deep pockets, Xrays images; for some patients biological analysis glycemia was explored (no microbiological exams).

Clinical types of peridontitis: PPP pre pubertal, PAJ, PPR, chonrical periodontitis.

Results: through our investigations based on the observations of 17 years on a population of 2500 cases, around 30% presented PAJ, the female representing more than 60%.

Conclusion: the rate of prevalence of the acute juvenile periodontitis showed around 30 to 35% the importance of this kind of disease in the oral and buccal pathology to the teenagers and the young persons.

The fonctionnal and aesthetic handicap is important that's why it is important to develop some program of detection and prevention in the target populations of school college and university.

This study is retrospectival; we can adopt the approach below for obtaining benefit of this results:

-starting a microbiological, genetical and immunological study by a recall policy of those patients
-starting paralely a prospectival clinical study in the paediatric population and also schools and universities, with the support of great international laboratories.

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Evaluation of bust morphological modifications by fringes projection by using a cosmetic product

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- J-C. Pittet**
- J-J. Servant***
- K. Vie*

Background: Age induces breasts shape change: dermal fiber production slows down and the support tissue slackens. It is difficult to put cosmetic product effectiveness forward on breast shape and requires a very precise device to see the product effect.

Purpose: The aim of the controlled and open-label study is to evaluate the shaping effect of the Clarins Gel Buste. The product is daily applied on breast from the base up to the chin with massages up and down until complete absorption during 28 days.

Method: The device used is the 2 sensors FaceSCAN-EO (EOTech) with the OptoCAT 2007R2 software, (version 1.0.05). The measurement principle is based on the projection of structured light and fringes of each sensor positioned at 60°. Any surface formed in each sensor's volume will be measured and reconstructed in 3D. After realignment of each subject's

acquisitions before and after treatment, an area is defined beneath each breast to quantify the breast uplift under the effect of the treatment. The quantification thus consisted in a measurement of the distance between the position of the lower part of each breast, before and after treatment.

Results: On the 32 volunteers, the daily application product induced a significant mean difference of 0.5mm ($p=0.0476$, Student's t-test) observed in the position of the breasts after treatment, and may reach -4.80mm, translating into an uplift of the bust. This effect is observed in 65.6% of the volunteers.

Conclusions: The device FaceSCAN-EO appears to be very sensitive to detect a small uplift on bust. This device could be used for other parts of body and prove cosmetic products effects on body shape.

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Assessment of the nourishing effect of a lip balm exploratory study

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- D. Mouglin**

Background: The main characteristics of the lips are their fragility and sensitivity to dryness and exposure to UV. This phenomenon is an issue for many people, more specifically with the presence of chapped lips in winter.

Objective: The aim of this study was to objectivate and illustrate the nourishing effect of a lip balm in the winter season (November to December 2008) after repeated applications during 28 days.

Material and Method: 30 women (mean age 31 ± 8.9) with moderately dry lips were enrolled (dryness score between 1 and 2.5 at inclusion). The assessments at D0 and D28 combined clinical scoring of dryness (9-grade scale, from 0 to 4, with 0.5 increment), standardized photographs taken in normal and UV light (Faraghan system), corneofix (measurement of the desquamation index and analysis of fluorescence by microscopy), and self-assessments.

Results: A very significant improvement was observed regarding the main criterion: the clinical score decreased by approximately half a unit after one month of treatment (for 80% of the subjects, and without any case of aggravation).

Both the fluorescence assessed from digital photographs and the desquamation index decreased. In some cases, the analysis carried out from UV photographs revealed a phenomenon of agglutination of the corneocytes generating a fluorescing hyperkeratosis. Finally, 80% of the subjects declared that they perceived a positive effect on tightness, dryness, chapped lips, softness and smoothness of the lips.

Conclusion: This study has shown the efficacy of the balm after only one month of treatment. Furthermore, the complementarity of various techniques to objectivate the mechanism of action of the product and the interest presented by these new techniques (UV photographs/microscopy) in the assessment of skin dryness have been demonstrated.

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Exploratory epidemiological study on caucasian women face skin depending on age

- D. Hottelart*
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- K. Vie*

Background: Age and photo-aging modifies the cutaneous physiology: aging of the capillary network, pigmentation disorders, modification of network lines and wrinkles appearance.

Purpose: The aim of the study is to evaluate the evolution and the impact of several parameters on skin: clinical parameters, instrumental measurements from photographs analysis and filled in questionnaire. These evaluated parameters are characteristic of skin state and colour, depending on age, and attitudes toward sun and stress exposure. 150 Caucasian women, with phototype II or III, without rosacea, are included and divided according to 5 age brackets (20-30, 31-40, 41-50, 51-60 and over 61).

Method: Realisation of standardised photographs with cross and parallel polarised light and natural light of the entire face. 4 investigators realised clinical grading from these photographs: wrinkles (glabellar, nasolabial fold and underneath eye), skin texture, complexion homogeneity (pigmentary and vascular component) and skin coloration around eyes with photogrades. A questionnaire, about attitudes toward sun and stress exposure, was filled in by volunteers. Finally, quantification of vascular and pigmentary homogeneity (indexes defined by Haralick) was realised from photographs: about 6 x 6 cm area on cheek.

Results: On the 146 volunteers included for the analysis, a good correlation existed between the criteria from clinical grading and the age: glabellar wrinkles ($R^2 = 0.67$) and underneath eye wrinkles ($R^2 = 0.66$). But it is not the case for skin texture ($R^2 = 0.002$) and skin coloration around eyes ($R^2 = 0.01$). These results are authenticated with the good concordance between the 4 investigators (between 48.7% and 63.3%). These results should be balanced with the stress exposure that significantly increases the glabellar wrinkles grade ($p = 0.013$) and the sun exposure has a significant influence on the skin texture grade ($p = 0.028$). As regards the complexion homogeneity, there is no concordance with age ($R^2 = 0.36$) according to the clinical grading. This result is the same for the pigmentary homogeneity ($p = 0.66$) obtained from photographs analysis. On the other hand, it appears that vascular homogeneity is significantly linked to the age ($p = 0.046$). All these results should be balanced with freckle presence and tendency to rosacea which can develop with age.

Conclusions: This exploratory study shows the interest to establish connections between some parameters linked to the age and observe factors which can modify them. It would be interesting to carry on the study and compare clinical grading with some other instrumental measurements.

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Evaluation of the cellulite through thermal camera measurement

- **A. Papillon***
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Cellulite (local lipodystrophy) represents very common skin condition among women. The main clinical feature of this process is the appearance of "orange peel" skin in the affected areas principally the lateral thighs and buttocks. The "orange peel" skin aspect is related to the uneven distribution of adipocytes. The skin temperature is also affected by this uneven distribution.

The contact thermography has been widely used to assess the severity of cellulite, however the methods present drawbacks related to its sensitivity and its precision (Contact method). The objective of our study is to develop a thermal method for evaluation of the cellulite; avoiding contact with the skin.

A high sensitive infrared camera is used to acquire the images under well controlled environmental conditions. The acquisitions are made on the middle of the thigh of each volunteer for whose positioning, well being and emotional state are properly handle to avoid temperature variations. The acquired images are subsequently automatically analyzed and several

parameters describing the thermal homogeneity are extracted. The method was evaluated for repeatability, reproducibility and accuracy. The repeatability and reproducibility were assessed on 5 healthy volunteers by 4 different manipulators and the data were analyzed with ANOVA. The accuracy was estimated from a panel of 45 healthy volunteers. The correlation between the thermal homogeneity parameters and a clinical grading of the orange peel appearance done by an expert grader was performed.

The results show no significant difference between the trials and the manipulators and the percentage of variation from one measurement to another was lower than 5%. Moreover, the thermal homogeneity was significantly correlated with the clinical grading of the orange peel.

In conclusion, the proposed method was found repeatable, reproducible, and accurate as the results obtained correlated with the clinical grading of the orange peel aspect.

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Corsican « immortelle » Essential Oil is a real anti-aging ingredient

- **P. Gasser***
- **E. Lati***
- **K. Fontes****
- **Y. Millou****
- **J.L. Pierrisnard****
- **C. Toured****
- **D. Davenne*****

A large study about Everlasting essential oil (Immortelle - Helichrysum italicum from Corsica) was conducted this year. Photos are provided
Variability of results according skin donors and oily vehicle is discussed

Botany of Helichrysum species

Chemical analysis of Helichrysum italicum Essential Oil and quality markers.

Conclusion: Helichrysum italicum (Immortelle) demonstrates through this studies very original and powerful anti-aging properties.

Results of the following markers

Neutral Glycosaminoglycans: Very strong increase

Collagen Type I: dermal increase

Collagen Type III dermoepidermal junction improvement

Sirtuin-1: extremely strong epidermal increase

Fibrillin-1, Connexin 43 are also improved

* Bio-Eke, Longjumeau, France

** L'Occitane, Manasquan, France

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Acetyl hexapeptide-3 in a cosmetic formulation acts on skin anisotropy - clinical study

- K.A. Tadini*

Acetyl hexapeptide-3 has been used in anti-aging topical formulations since it has demonstrated effects in improving the skin appearance. However, there are few scientific studies about its effects on epidermis and dermis, when vehiculated in topical formulations, mainly using objective measurements, which are an important tool in clinical efficacy studies. Thus the aim of this study was to determine the clinical efficacy of the acetyl hexapeptide-3 using biophysical techniques.

Formulations with and without acetyl hexapeptide-3 were applied to the ventral forearm and the face area of human volunteers. Skin conditions were evaluated after 2 and 4-week period daily applications, by analyzing the *stratum corneum* water content (Corneometer® SEM 575) and the skin mechanical properties, using two instruments, the Cutometer® SEM 575 and Reviscometer® RV600 to identify

skin changes after the use of the formulations under study.

Formulations studied increased water content of the *stratum corneum* in the face region, which remained constant until the end of the study. In contrast, only formulation with acetyl hexapeptide-3 exhibits a significant effect on mechanical properties, by decreasing the anisotropy of the face skin. No significant effects were observed in cutometer parameters.

Considering the acetyl hexapeptide-3 effects on the anisotropy face skin, it can be considered an effective ingredient for improving conditions of the cutaneous tissue, which can be used in anti-aging cosmetic formulations. Moreover, the Reviscometer® RV600 showed as a sensitive instrument able to detect skin effects of cosmetic products.

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Assessment of the effect of a topical treatment on early active stretch marks in post-partum women: a simple blind, randomized intra-individual study

- N. Lachmann*
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- P. Msika*

Background: **Stretch marks are a well-recognized, common** skin condition that rarely causes any significant medical problems but are often a significant source of distress to those affected. The origins of stretch marks are poorly understood but these disfiguring lesions are usually caused by excessive stretching of skin, especially observed during pregnancy.

Objective: We investigated the response of early, clinically active stretch marks (stage 1 of the Deprez-Adatto classification) to topical application of a cream specifically formulated to reduce stretch marks. This cream contains patented ingredients: lupcol, natural biopeptides and arabinogalactane which counteract tissue inflammation and stimulate extracellular matrix (ECM) remodelling.

Materials and methods: In a simple-blind, randomized, intra-individual comparative study, 16 post-partum women presenting symmetrical and comparable stretch marks on each of their thighs (or hips) at the baseline, applied the cream twice daily on one of their thighs during 2 months. The other thigh served as the untreated control. Each thigh was evaluated monthly by clinical examinations and by analysis of skin section of stretch marks obtained by ultrasound imaging (35MHz) before and at the end of the study. Viscoelastic properties and skin hydration were also measured at each

kinetic time. Cross polarized photographs were taken to assess the inflammatory component of stretch marks.

Results: After 1 and 2 months, we observed significant improvements on treated stretch marks compared to the control. After 2 months, we observed a decrease in mean length and width of 10 % ($p < 0.01$) and 20% ($p < 0.01$), respectively, compared to the untreated stretch marks. We also observed significant improvement on the global quality of the skin around the stretch marks on the treated area compared to the control: the relief of the skin and its roughness were significantly decreased, respectively - 11% ($p = 0.019$) and -18% ($P = 0.038$), the firmness and hydration were significantly improved after 2 months. Ultrasound images and cross-polarized photographs confirmed these clinical improvements.

Conclusion: These results demonstrate the efficacy of a topical cream on clinical appearance of early, active stretch marks in post-partum women. We observed a significant global improvement of the severity of stretch marks after 2 months. The action of the cream was not limited to stretch marks. The cream proved to be effective in improving global skin quality.

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Contribution of instrumental methods for the assessment of hyaluronic acid fillers

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- D. Black*
- G. Josse*
- A-M. Schmitt*
- V. Turlier*

Context: In the literature: assessment of filler performance has only been made by qualitative or semi-quantitative methods.

Objective: The aim of this study was to measure the performance of a new hyaluronic acid (HA) based filler product using semi-qualitative and original quantitative methods.

Study: During 12 months after injection: Follow up of cutaneous modifications induced by injection of HA-based filler product (Glytone® 3) in the nasolabial folds (NLF) on volunteers having had no previous filler injection and who presented moderate to average NLF scores. Measurement time-points: before (0) and after 1, 3, 6, 9 & 12 months.

Semi-qualitative methods:

- Blind (hidden time-point) clinical scoring of the depth of the nasolabial folds (NLF) from standardised photographs using the Lempere scale (0 to 5) by 2 trained investigators who did not participate in the injections (principal efficacy criterion)

- Self evaluation of satisfaction

Quantitative Instrumental measurements:

Volume of NLF from skin replicas and *in vivo* 3D fringe projection images (Dermapop, Eotech, France)

Tissue characterisation and thickness of NLF by RF ultrasonography (Dermcup 2000, ATYS)

Discussion: Clinical scoring, self assessment of satisfaction, measurement of the NLF volume by skin replicas and 3D fringe projection imaging are well correlated, and show the same effect: highly significant improvement in reducing NLF depth during the 12 months following injection.

Ultrasound images showed hypodermic localisation of HA implants. Another method, Magnetic Resonance Imaging, has already shown dermo- hypodermic localisation of HA implants. The slight decrease in dermal thickness is due to compression by the underlying HA filler. Thus, the filler effect is not due to an increase in dermal thickness

Conclusion: The results of the subjective and objective measurements are correlated and demonstrate significant product efficacy in treating nasolabial folds of moderate to intense severity for up to 12 months.

The instrumental data confirm the clinical results observed, and help to understand the mechanism of action of the injected reticulate HA. These objective measurements can only be carried out by trained staff in specially equipped centres.

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Comparative instrumental study of aesthetic dermatology acts and cosmetic protocols

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- J. de Rigal****
- C. Montastier***
- P. Humbert*****

Background: Mesotherapy and chemical peels are commonly performed in dermatological practice. However few data are available to compare their cutaneous effects on the face with those resulting from the repeated application of cosmetic products.

Objective: A mesotherapy study (meso study) and a chemical peel study (peel study) devised according to criteria defined by LaClinic of Montreux were compared with two different cosmetic protocols combining a serum, a day cream and an eye contour cream for the meso study and a cream for the peel study.

Material and Method: In the meso study, two groups of 15 subjects were followed during 8 weeks: one group had 4 sessions of mesotherapy and the second group was enrolled in a cosmetic protocol using a 10% vitamin C serum. In the peel study, two groups of 10 subjects were followed during 2 weeks: one group had one chemical peel and the second group was enrolled in a protocol involving a 0.1% retinol serum.

The selection of the assessment tools was based on the similarity of their approach with the faculties of the human eye: imaging by Chromasphere® and by Skinevidence for the meso study, 3D analysis by fringe projection and imaging by SSA (Skin Surface Analyser) for the peel study.

Results: In the meso study, the increase of luminosity (L*), pink component (a*) and heterogeneity, on high resolution images of the colour of the cheeks was demonstrated, as well as the modification of the isotropy of the skin surface. In the peel study, a diminution of the microrelief was observed, associated with the increase of the heterogeneity measured on the cheekbone.

Conclusion: Both preliminary studies have contributed to the direct review and the objective comparison of dermatological acts and specifically adapted cosmetic protocols. The instrumentation based mainly on 2D and 3D imaging has shown its sensitivity and interest.

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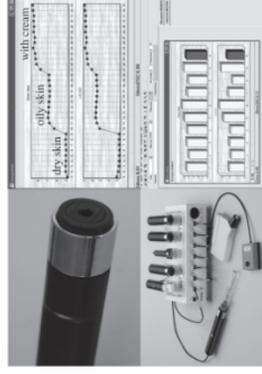
INDEX

- 3D fringe projection 154
3D stereovision imaging 114
3 Tesla MR 52
- A**
Acetylhexapeptide 152
Acne scar 129
Acoustic properties 61
Adipose tissue stroma 131
Advertising claim substantiation 143
Aesthetic dermatology 144
Age 58, 84
Age effect 110
Aging 74, 79, 149
Aging patterns 115
Alpha SMA 131
Anti-aging 65
Anti-aging evaluation 128
Antioxidation 134
Arsenic 113
Atopic Dermatitis 137
- B**
Barrier function 93
“Barrier” recovery 99
Bioelectrical impedance 34
Biometry 57
Biometry 109
Biophysical parameters 72
Body fat 34
Body skin 84
Breasts 147
Burns 117
- C**
Calcium flux 133
Capacitance 33
Cellomics® Imaging Technology 130
Cell proliferation 134
Cellulite 84, 150
Chemical force titration 123
Chromametry 94
Chronic venous insufficiency 95
Claims 26
Claim support 81
Climatic conditions 72
Clinical grading 149
Clinical trial 119
Collagen 151
Collagen reorganization 125
Color 105
Color accuracy 117
- Colour 77
Confocal 106
Confocal microscopy 43
Constitutive modelling 63
Contact angle titration 123
Contract Dermatitis 138
Contractile forces 132
Corneodesmosomes 96
Corneometry 145
Corneoxenometry 36
Cosmetic evaluation 109
Cosmetic study 83
Cutaneous ionic transport 133
Cutaneous pH 85
Cutometer® 58
Cutometry 127
Cytokines 144
- D**
Degreasing 88
Demodex 70
Dermal fibroblasts 132
Dermis 38, 65
Dermocosmetic 152
Diagnosis 70
Diclofenac 94
Diffraction optical element 37
Digital images 113, 117
- E**
Early stretch marks 153
Echography 139
EELS 96
Efficacy evaluation 129
Elasticity 65
EMLA 82
Empetrum nigrum 127
Epidermis 38
Ethnic differences 74
Ethnic skin 57
Exploratory study 83, 148
- F**
Faces age perception 75
Face shape 116
Facial Averaging/Morphing 74
Facial sagging 115
Facial simulation 118
Facial unit 118
Femosecond laser 48
Filaggrin 137
Finite element method 64
- Firmness 84
FLIM 48
Food supplements 141
FPS in vitro 135
Friction coefficient 86, 87
Fringes projection 147
Function 98
- G**
Glow 105
Graduated traction 61
- H**
Hair 86
Healing phases 111
Health safety 145
Heavy legs 81
High spatial resolution 45
Human keratinocytes 126
Human skin 85, 87, 89
Hyaluronic Acid Filler 154
Hydration 35, 84, 85, 97
Hydrophobic/hydrophilic balance 85, 87, 88, 89
Hydroquinone 21
Hyperspectral cube 50, 51
- I**
Image analysis 119
Image processing 116
Imaging 33, 106
Imaging system 50, 51, 109
Immortelle 151
Indian women 55, 56
Infant 76
Inflammation 41, 42
- J**
Juvenile scleroderma 90
- L**
Lip balm 148
Lipidic index 85
Lipids 47
- M**
Mannose-6-phosphate 77
Mathematical modelling 78, 79, 80
Mechanical properties 25, 26, 66
Mechanical stress 125
Melanoma 121
Melasma in Thai 59
- Metabolic index 78
Microcirculation 127
Mite 70
Modeling light 19
MoistureMeter-D 102
Molecular resolution 23
Multiphoton 125
Multiphoton laser tomography 46, 120, 121
Multiphoton tomography 48
Multi-scale 24
Multispectral image 50, 51
- N**
Nail 88
Nano-imaging 126
Natural food ingredients 130
New technic 62
NMF 76
- O**
Oedema assessment 102
Open / closed chamber 101
Optical diagnosis 21
Optical properties 19
- P**
PEG-PE micelle 124
Perception 72
Periodontitis 146
Photographs 107, 149
Pigmentation 9
Post partum 153
Primary fibroblast 134
Primary keratinocyte 134
Prospective study 69
Proteomics 126
- R**
Raman spectroscopy 21, 43, 45, 47, 137
Reflectance Confocal Microscopy 41, 42
Retinoic acid 124
- S**
Sclerodermic 90
Sebum 85, 86, 100
Sensitive hands 80
Skin aging 56, 66, 83, 107, 115, 116, 144
Skin barrier 95
Skin colour 55
- Skincare 119
Skin complexion 105
Skin elasticity 58
Skin explants 128
Skin gloss 37
Skin Health Assessment 35
Skin hydration 44, 96
Skin microcirculation 82
Skin pores features 110
Skin properties 62
Skin relief 112
Skin surface pH 100
Skin temperature 150
Sleep deprivation 71
Soap 88
Spectrophotometer 55
Spectroscopy 106
Stratum corneum reservoir 94
Stratum corneum 36, 93, 97, 98, 123
Stratum corneum hydration 34, 100
Stratum corneum thickness 43, 44
Stretch marks 84
Subcutaneous adipocytes 130
Suction method 66
Sulfur mustard 91
Supersonic Shear Imaging 139
- T**
Tattoo removal 69
TepO₂ 78
Tensile strength 131
TEWL 35, 80, 95, 97, 101, 138
Thai breadfruit 134
Thermal IR camera 150
Three-dimensional surface measurement 118
TNF- α 138
Tomography 23
Typology 69
- U**
Ultrasonography 154
Ultrasound imaging 153
- V**
Vascular-derived cells 131
Vascular disease 81
Vernix Caseosa 76, 89
Visco-elasticity 139
- W**
Water-holding capacity 93
Wettability 85, 86, 87, 88, 89

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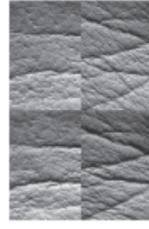
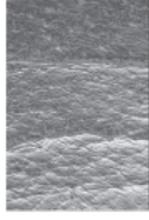
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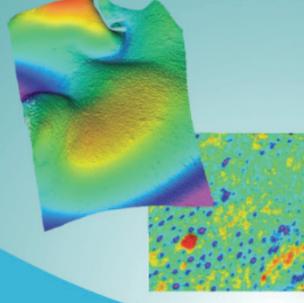
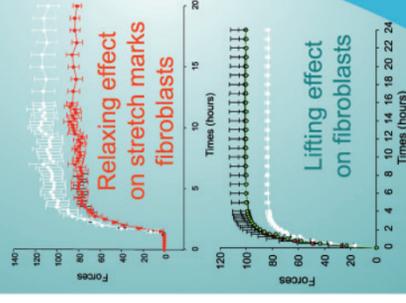


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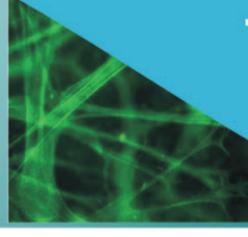
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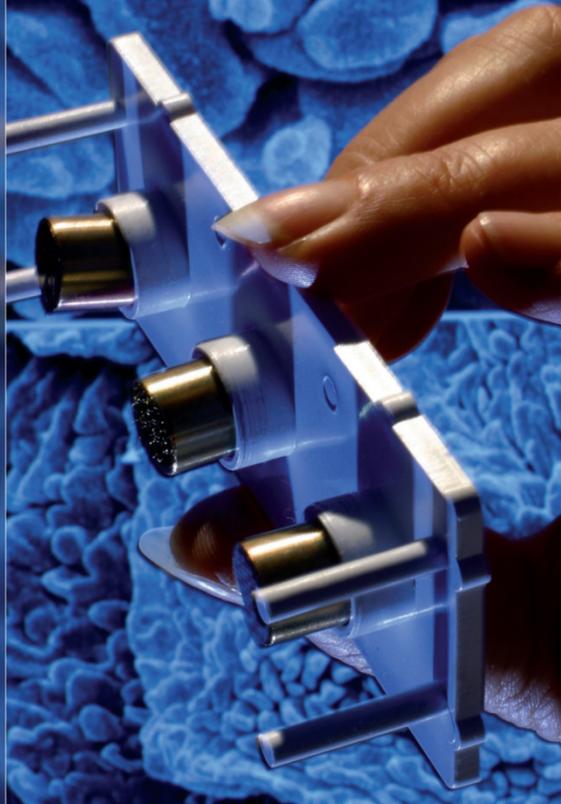
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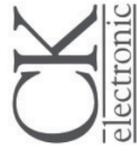
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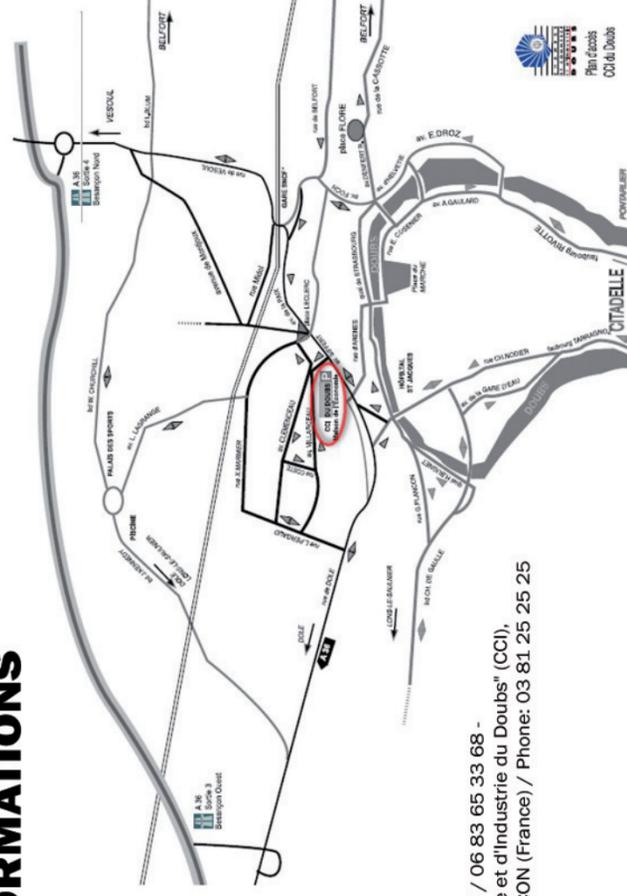


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