CONFERENCES

VIRTUAL MEETING | 2022



7-8 April 2022 Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies - 2nd meeting



LOCATION

VIRTUAL MEETING

CONVENORS

Dr Franciska Vidáné Erdő

LE STUDIUM VISITING RESEARCHER

FROM Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, HU

RESIDENCE AT Nanomedicines and Nanoprobes (NMNS), University of Tours - FR

Prof. Emilie Munnier

Nanomedicines and Nanoprobes (NMNS), University of Tours - FR

Dr Franck Bonnier

LVMH Recherche - FR







CONVENORS

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FROM: Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, HU **IN RESIDENCE AT:** Nanomedicines and Nanoprobes (NMNS), University of Tours - FR

Prof. Emilie Munnier Nanomedicines and Nanoprobes (NMNS), University of Tours - FR

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ORGANIZING COMMITTEE

Sophie Gabillet, General Secretary

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LE STUDIUM Loire Valley Institute for Advanced Studies • Région Centre-Val de Loire • FR



ABSTRACTS

Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies 2nd meeting



Created in 1996 on the CNRS campus in Orleans La Source, LE STUDIUM has evolved to become the multidisciplinary Loire Valley Institute for Advanced Studies (IAS), operating in the Centre-Val de Loire region of France. LE STUDIUM has its headquarters in the city centre of Orleans in a newly renovated 17th century building. The amazing facilities are shared with the University of Orleans. In 2014 new developments and programmes linked to the smart specialisation of the Centre-Val de Loire region came to strengthen existing IAS collaborative relationships with the local and the international community of researchers, developers and innovators.

LE STUDIUM IAS offers to internationally competitive senior research scientists the opportunity to discover and work in one of the IAS's affiliate laboratories from the University of Tours, the University of Orleans, National Institute of Applied Sciences (INSA) Centre Val de Loire and ESAD Orléans, as well as of nationally accredited research institutions located in the region Centre-Val de Loire (BRGM, CEA, CNRS, INSERM, INRAE). Our goal is to develop and nurture trans-disciplinary approaches as innovative tools for addressing some of the key scientific, socio-economic and cultural questions of the 21st century. We also encourage researchers' interactions with industry via the IAS's links with Poles of Competitiveness, Clusters, Technopoles, and Chambers of Commerce etc.

LE STUDIUM has attracted two hundred and thirty experienced researchers coming from 47 countries for long-term residencies. In addition to their contribution in their host laboratories, researchers participate in the scientific life of the IAS through attendance at monthly interdisciplinary meetings called LE STUDIUM THURSDAYS. Their presentations and debates enrich the regional scientific community at large (researchers of public and private laboratories, PhD students, research stakeholders' representatives, etc...)

For the period 2015-2021, LE STUDIUM operates with an additional award from the European Commission in the framework of the Marie Skłodowska-Curie Actions (MSCA)-COFUND programme for the mobility of researchers. Since 2013, LE STUDIUM is also an official partner of the Ambition Research and Development 2020 programmes initiated by the Centre-Val de Loire Regional Council to support the smart specialisation strategy (S3) around 5 main axes: biopharmaceuticals, renewable energies, cosmetics, environmental metrology and natural and cultural heritage. New programmes are currently designed to include all major societal challenges.Researchers are also invited and supported by the IAS to organise, during their residency and in collaboration with their host laboratory, a two-day LE STUDIUM CONFERENCE. It provides them with the opportunity to invite internationally renowned researchers to a cross-disciplinary conference, on a topical issue, to examine progress, discuss future studies and strategies to stimulate advances and practical applications in the chosen field. The invited participants are expected to attend for the duration of the conference and contribute to the intellectual exchange. Past experience has shown that these conditions facilitate the development or extension of existing collaborations and enable the creation of productive new research networks.

The present LE STUDIUM CONFERENCE named "Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies 2nd meeting" is the 117th in a series started at the end of 2010 listed at the end of this booklet.

We thank you for your participation and wish you an interesting and intellectually stimulating conference. Also, we hope that scientific exchanges and interactions taking place during this conference will bring opportunities to start a productive professional relationship with presenting research laboratories and LE STUDIUM Loire Valley Institute for Advanced Studies.

> Yves-Michel GINOT Chairman LE STUDIUM

INTRODUCTION

Following the successful 2019 edition of the International Interdisciplinary Conference titled "Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies" held in Tours (France), the event is back for a 2nd edition. This event will take place as an online conference.

The conference aims to gather industry scientists and academics to discuss the state-of-the-art in the research and development of skin models and novel analytical techniques for efficacy and safety testing of cosmetic products. Cosmetic science is a buzzing field driven by constant innovation therefore testing, measuring, evaluating are essential to demonstrate innocuity and/or effectiveness of skincare products while durability and personalisation have become key market trends.

Talks and discussions will contrast various industrial and academic viewpoints and visions regarding the characterisation and evaluation of cosmetic products, particularly as these relate to the use of in vitro, ex vivo and in vivo models of human skin and recent progress in methodologies, technologies and instrumentation. Multi-techniques, multi-scales approaches, non-invasive and non-destructive analysis, hold promises for better characterisation of reconstructed skin models, studying physiological processes and visualise the effects of skincare products from bench top experiments to volunteers.

Sharing among researchers, suppliers, manufacturers, and other actors of the cosmetic industry is sought to provide a snapshot of latest scientific results and achievements but also to discuss current challenges to be addressed.

The region Centre-Val de Loire Regional Council actively supports the research and development in the field of cosmetic sciences. Held in the heart of the Cosmetic Valley technopole, the Conference is meant to promote national and international collaborations across industry and academia. Regional, national and international scientists in the field are strongly encouraged to attend and present their research. While a panel of renowned speakers will be invited, slots for oral presentations will be dedicated to rising scientists in the field to offer them a chance to present their work and to encourage their participation in the discussions.

The organising committee is very much looking forward to welcoming you.

This international conference is organised in the framework of the COSMETOSCIENCES ARD CVL Programme.

LE STUDIUM **CONFERENCES** Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies | 7-8 April 2022

PROGRAMME

THURSDAY APRIL 7TH (13:45 - 17:40 ; GMT+2:00 - PARIS)

13:45 Official Opening by **Prof. Emilie Munnier** (NMNS) & **Sophie Gabillet** (General Secretary of LE STUDIUM Loire Valley Institute for Advanced Studies)

SESSION 1: IMAGING AND DIAGNOSTIC TECHNIQUES FOR SKIN RESEARCH

14:15 Dr Stephan Bielfeldt - Confocal Raman spectroscopy: Noninvasive *in vivo* investigation of human skin on a molecular level

14:35 Dr Maxim Darvin - Two-photon excited fluorescence lifetime imaging for non-invasive visualization of mast cells and macrophages in human skin in vivo

14:55 Dr Miklós Gyöngy - Monitoring of skin using an optical-ultrasound imager for potential cosmetic applications

15:15 Dr Mélanie Pedrazzani - Line-field optical coherence tomography (LC-OCT) assisted by artificial intelligence for three-dimensional microscopic quantification of *in vivo* healthy epidermis

15:35 Lena Waszczuk - Morpho-molecular characterization of tattooed skin biopsies with adverse reactions using co-localized line-field confocal optical coherence tomography (LC-OCT) and confocal Raman microspectroscopy (CRM)

15:55 Coffee break

SESSION 2: PENETRATION ENHANCERS AND ADDITIVES FOR TOPICAL FORMULATIONS

16:10 Prof. Dominique J. Lunter - Studying the impact of emulsifiers on SC lipids by confocal Raman microspectroscopy

16:30 Dr Nikolett Kis - Investigation of the effect of chemical permeation enhancer glycols on the skin measuring by NMR and Raman spectroscop

17:00 Michal Szczepanczyk - Catalase activity in keratinocytes, stratum corneum, and defatted algae biomass as a potential skin care ingredient

17:20 Dr Mais Saleh - Nanostructured Vitamin E Phosphate: Characterizing its percutaneous Penetration into Excised Human Skin and release from cosmetic formulations

17:40 End of the day

FRIDAY APRIL 8TH (09:00 - 12:40 ; GMT+2:00 - PARIS)

SESSION 3: SKIN MODELS IN COSMETIC AND DERMATOLOGICAL RESEARCH

09:00 Dr Catherine Grillon - New reconstructed epidermis models closer to skin physiology

09:25 Marek Puskar - Evaluation of serum growth factors in wound healing using a full-thickness in vitro human skin model

09:45 Dr Silvia Letasiova - In vitro skin irritation tests using reconctructed epidermis tissue model, EpiDerm, for evalution of safety and efficacy testing of cosmetics.

10:05 Hichem Kichou - Evaluating Strat-M® synthetic membrane as substitute to in vitro and ex vivo skin models for permeation studies

10:25 Dr Jean-André Lapart - How's AFM a fantastic tool in cosmetic research?

10:45 Coffee break

SESSION 4: SKIN-ON-A-CHIP - DIFFUSION STUDIES AND OTHER APPLICATIONS COMPARATIVE EVALUATIONS OF SKIN MODELS

11:00 Dr Franciska Erdo - Studying topical drug delivery in Skin-on-a-chip & by Confocal RAMAN spectroscopy

11:20 Dr Jeyaraj Ponmozhi - Realtime analysis of drug (diffusion, toxicity, would healing, repair, inflammation, aging with different shear stress studies) on skin cells in a microfluidic skin-on-a-chip device.

11:40 Dr Rime Michael-Jubeli - Multi-scale analytical characterization of the skin barrier from *in vitro* to *in vivo*

12:00 Dr Gerwin Puppels - Comparing cultured skin, *ex vivo* skin, and *in vivo* skin by near-infrared confocal Raman spectroscopy

12:20 Dr Ana-Maria Pena - *In vivo* melanin 3D quantification and z-epidermal distribution by multiphoton FLIM, phasor and Pseudo-FLIM analyses

12:40 End of the conference

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ARD CVL COSMETOSCIENCES PROGRAMME



In an international environment characterised by changing regulatory regimes and increasing harsh competition, research and innovation are key factors to ensure smart specialisation and sustainable economic development of territories and stakeholders. In the very well established perfume and cosmetic industry of region Centre-Val de Loire, the COSMETOSCIENCES programme aims at giving a significant impetus to research projects with a strong character of innovation to unlock industrial development blockages by opening the door to new concepts and enable new startups. It fosters French leadership in the sector and the leadership of the region Centre-Val de Loire, particularly with regard to sustainable cosmetics.

Anchored in the region Centre-Val de Loire, this project articulates around the structuring of research at the national level on this cosmetic theme, including through the research group (GDR) Cosmactifs, created by CNRS in January 2015. It brings 48 laboratories together and is driven by the University of Orléans. Focused on economic development, this project shares in the international influence of the French cosmetics industry across the region Centre-Val de Loire.

Together with the Cosmetic Valley competitiveness cluster and in conjunction with the cosmetic industry the programme creates the Centre of Expertise for the Cosmetics Industry. Located at the very heart of the territory covered by Cosmetic Valley, the centre's mission is to support business growth in the perfume and cosmetics sector with research, training and development activities and services specifically targeting very small and medium sized enterprises (VSEs and SMEs). The centre focuses on three complementary developmental axes:

- 1. Cosmétopée and Sustainable Cosmetics,
- 2. Glycochemistry and Glycobiology
- 3. Innovation in Formulation, Cellular Tools and Technologies.



Recherche & Industrie, innovons ensemble

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Dr Ponmozhi Jeyaraj
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Hichem Kichou
$\ensuremath{Evaluating Strat-M}\xspace$ synthetic membrane as substitute to in vitro and ex vivo skin models for permeation studies
Dr Nikolett Kis
Investigation of the effect of chemical permeation enhancer glycols on the skin measuring by NMR and Raman spectroscopy

CONVENORS



Dr Franciska Vidáné Erdő

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Dr. Franciska Erdő has a background in pharmacology. She worked for research institutes in Hungary (BIOREX Ltd, IVAX Drug Research Institute) and Germany (Max Planck Institute for Neurological Research, Cologne and Charité University, Berlin) She has experience from pharmaceutical industry too (Sanofi-Synthelabo-Chinoin and SOLVO Biotechnology). Her main research interest was the analysis of pathophysiology of stroke, and development new therapeutic strategies. Since 2014 she has been working for Pázmány Péter Chatholic University, Budapest as an associate professor and as a labhead. Her main expertise is drug delivery accross the physiological barriers. Currently she is involved in a collaborative research on skin analysis and RAMAN spectroscopy at University of Tours in France.

Studying topical drug delivery in Skin-on-a-chip & by Confocal RAMAN spectroscopy

Studying skin composition and interaction with topical substances is important both in dermatology and cosmetoscience. Several techniques are utilized and are under development for these purposes. Skin models are in use with different complexity from 3D bioprinted skins through human reconstructed skin substituents to excised tissues. These models are successfully applied in diffusion cells and skin-on-a-chip devices. In the current study two model drugs (caffeine and guinidine) were investigated as a cream formulation in microfluidic skin-on-a-chip device and by confocal RAMAN spectroscopy. Excised skins and skin substituents were compared in regard of their chemical composition, barrier function and permeability. First the measuring system was optimized and then the properties of the skin models were characterized. Caffeine, as a hydrophilic drug easily penetrates through the skin, the more lipophilic guinidine reached a much lower concentration in the perfusion fluid. The accumulation of the drugs in the upper layers of the epidermis was similar showing that quinidine can well-penetrate to the lipophilic matrix of stratum corneum, but does not cross the full thickness skin barrier as easily. In the penetration of caffeine the transappendageal route has a crucial role, this can explain the two order of magnitude difference in absorption compared to quinidine. Furthermore, quinidine interacts with several transporters in the skin which influences its penetration profile.



Prof. Emilie Munnier

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Emilie Munnier is a full professor at the Faculty of Pharmacy of Tours where she has been teaching pharmaceutical technology and cosmetology for more than ten years. She is also a researcher in the EA6295 Nanomedicines and nanoprobes research team. After her degree of pharmacy, she obtained a PhD in Life and Health Sciences at the University of Tours dealing with nanotechnologies for health and finally her authorization to direct research in 2016.

Using her skills in formulation and analytical chemistry, she is currently dedicated to the encapsulation of active molecules to improve their penetration into the skin, but also to control their interaction with the ingredients of a finished pharmaceutical or cosmetic product. She has been the coordinator of several research projects involving players in the cosmetics industry, and is currently participating in the PIERIC and MINIONs projects, which are part of the ARD CVL Cosmetosciences regional research program. The objective is first to develop innovative formulations, then to improve the analysis of the tissues exposed by various methods, in order to elucidate the mechanism of action and to measure the effectiveness of these formulations.



Dr Franck Bonnier

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Franck Bonnier obtained his PhD from the Department of bio-spectroscopy for life sciences as a member of the Médian CNRS group, Université de Reims Champagne-Ardenne, France. Following his PhD, he joined the FOCAS Research Institute, DIT in 2008 as a post-doctoral research engineer under the National BioPhotonics and Imaging Platform, Ireland, and as a member of the Biophotonics and Imaging group that specialised in the application of biophotonics, especially Raman and Infrared spectroscopy, in the biomedical field. In September 2014, Franck Bonnier has joined the EA 6295 Nano-Medicine and Nano-Probes (NMNS) research group at Université de Tours (France) where he pursued his scientific work as associate professor in analytical chemistry. The focus remained the development of methodologies towards implementation of spectroscopic techniques coupled to chemometrics approaches as clinical and industrial tools. His work addressed concerns about diagnostic, therapeutic and cosmetic applicationa. Franck Bonnier is strongly involved in the ARD 2020 Cosmetosciences program, exploring translational research to position infrared and Raman spectroscopy for quality control and for testing efficacy of cosmetic products on skin models, in vitro to in vivo. Since January 2022, Franck Bonnier has joined LVMH research (Saint Jean de Braye, France) as project leader in the in vivo innovation group.

SPEAKERS

Dr Stephan Bielfeldt

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Stephan Bielfeldt has a degree in Bioengineering. He is Vice President Science & Consulting at proderm GmbH in Hamburg / Germany. proderm is a contract research organization specialized in clinical dermatological studies. Stephan is responsible for the development of new skin research methods, as well as the consultancy of customers in the field of clinical studies. He has been working in clinical research for more than 30 years. Special fields of his expertise are photobiology / sun protection, in vivo confocal. Raman spectroscopy and clinical studies of skin microbiome. His list of publications contains more than 100 scientific publications.

Confocal Raman spectroscopy: Noninvasive in vivo investigation of human skin on a molecular level

For more than 20 years now confocal Raman spectroscopy is used to measure molecular concentration profiles in vivo in the human skin. With a depth resolution down to 3 μ m the epidermis and upper dermis can be directly assessed and the molecular composition of a wide range of skin components and externally applied molecules be quantified.

Measurements can be performed on almost every region of the body surface, as on the extremities, the trunk, but also on areas more difficult to reach, as scalp, axillae, lips and inside of the mouth.

In the first decade of the 21st century the research focused on the chemical composition of the epidermis. Water distribution in the stratum corneum, the content and composition of natural moisturization factors (NMF) and skin lipids, where important topics.

In the last decade a new important field of application occurred, the quantitative penetration and permeation of externally applied components and actives.

In this presentation an overview on the methodology is given, with examples of water and NMF measurements. To explain the approach of quantitative measurement of skin penetration and permeation in vivo, results of caffeine penetration are presented. Simple solutions in water with and without a penetration enhancer were investigated. With the Raman technology the permeation rate of caffeine into the stratum corneum as well as the depletion rate into the living epidermis could be quantitatively measured.



Dr Maxim Darvin

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Maxim E. Darvin studied at the Moscow State Engineering Physics Institute, the Faculty of Experimental and Theoretical Physics, Department of Medical Physics, where he completed his master's degree in physics. After a postgraduate study, he was awarded the titles Dr. rer. med. in 2007 at the Humboldt University of Berlin, Germany and PhD in 2010 at the Saratov State University, Russia. He is the author and co-author of more than 200 scientific papers. His main interests are medical physics, laser techniques, quantum electronics, spectroscopy, biophotonics and skin physiology.

Two-photon excited fluorescence lifetime imaging for non-invasive visualization of mast cells and macrophages in human skin *in vivo*

Mast cells (MCs) and macrophages (M Φ s) are important multifunctional immune cells found in all tissues of the body. In the skin, resting and activated MC populations and M1- and M2polarized M Φ s are located in the dermis. Histomorphometric analysis of skin biopsies is used to determine the quantity of these cells and their activation and polarisation state. Non-invasive in vivo visualization of MCs and M Φ s in the skin is currently not possible.

We show for the first time that two-photon excited fluorescence lifetime imaging (TPE-FLIM) can be used for label-free non-invasive visualization of resting and activated MCs and M1- and M2-polarized MΦs in human skin in vivo with high sensitivity and specificity. First, we recorded TPE-FLIM parameters (lifetime and intensity) of human MCs and MΦs in vitro and demonstrate that they have specific TPE-FLIM parameters that are distinct from major components of the extracellular matrix and other dermal cells. Second, we confirmed the visualization of MCs and MΦs in the skin biopsies ex vivo based on their known TPE-FLIM parameters and subsequent cell-specific immune staining. Finally, we found cells with previously determined TPE-FLIM parameters for resting and activated MC populations [1] and M1- and M2-polarized MΦs [2] in the human skin in vivo.

The developed non-invasive method can advance the understanding of the role of MCs and $M\Phi s$ in health and disease diagnostics and therapy monitoring in dermatology and immunology.

References:

[1] Kröger, Scheffel, Nikolaev, Shirshin, Siebenhaar, Schleusener, Lademann, Maurer, Darvin. In vivo non-invasive staining-free visualization of dermal mast cells in healthy, allergy and mastocytosis humans using two-photon fluorescence lifetime imaging. Sci Rep. 2020;10:14930.

[2] Kröger, Scheffel, Shirshin, Schleusener, Meinke, Lademann, Maurer, Darvin. Label-free imaging of macrophage phenotypes and phagocytic activity in the human dermis in vivo using two-photon excited FLIM. bioRxiv. 2021;2021.11.29.470361.



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C. Grillon obtained her PhD in 1989 (University of Orléans), discovering specific lectins on immune and skin cells. After a postdoctoral position in the Chemistry Institute for Natural Substances (ICSN UPR2301) in Gif sur Yvette, she joined the CNRS as research scientist. She moved to Center of Molecular Biophysics in Orléans where she is co-responsible of the group "Cellular microenvironment and receptors pharmacology". She has a long-standing expertise in cell interactions, microenvironment and tumor angiogenesis. Her current research focuses on skin microenvironment (oxygenation), specific in vitro models, and aims to prevent skin aging and associated pathologies. She participates to the GDR Cosmactifs network and has coordinated the book "Models to evaluate cosmetic actives".

New reconstructed epidermis models closer to skin physiology

Co-authors : Nadira Hammas-Chettouh, Loick Ridou, Fabienne Fasani, Giovanni Busco

Among skin models, reconstructed human epidermis are largely used in cosmetic domain either to evaluate compounds activity or for regulatory tests such as toxicity. They represent a good alternative to animal testing. These 3D model of skin epidermis are simpler than full reconstituted skin, are easier to prepare and can also be purchased from various companies. But are they representative of physiological skin? For which applications?

We investigated one aspect of this question basing our research on knowledges of skin microenvironment. Inside the skin, cells are submitted to a low oxygen level varying for one layer to another. In the basal part of the epidermis, oxygen partial pressure decreases to 1.5-3%, a condition called physioxia. Moreover, skin aging is associated with oxygen level decrease. These levels are very far from the classical conditions used to maintain cells in culture. In fact the oxygen partial pressure in an incubator is around 18-19%, what is called normoxia.

In the current study, we present the comparison of the preparation of reconstructed epidermis in physioxia and in normoxia and their characterization in terms of morphology, proliferative potential, and differentiation but also their capacity to answer to oxidative stress, a crucial activity for skin health preservation.

This presentation shows the importance of taking into account the oxygen parameter in reconstructed epidermis model and particularly in the case of biological activity studies.



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Miklós Gyöngy was born in Budapest, Hungary, in 1983. He received the M.Eng. degree in Engineering and Computing science from the University of Oxford in 2005, and the D.Phil. degree from the same institution in 2010, after gaining acceptance to the Life Sciences Interface Doctoral Training Centre Programme. In 2010, he joined the Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, where he founded the faculty's ultrasound laboratory and where he is currently an Associate Professor. In 2018 he founded a university spin-off, Dermus, which develops software and hardware solutions for multimodal optical-ultrasound imaging of the skin.

Monitoring of skin using an optical-ultrasound imager for potential cosmetic applications

Co-authors : Gergely Csány¹, László Hunor Gergely², Norbert Kiss², Klára Szalai², Kende Lőrincz²

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Optical-ultrasound imaging is a cost-effective method of simultaneously imaging the skin surface and the region under the surface. To the best of our knowledge, the Dermus SkinScanner is the first device capable of multimodal optical-ultrasound imaging, extending traditional dermoscopy with a window into the depths of the skin. Among other clinical applications, it can image chronic skin inflammation diseases such as psoriasis and atopic dermatitis, with the potential to provide more objective metrics of inflammation than are currently possible. In the current talk the latest results regarding skin imaging are presented, especially regarding the monitoring of skin inflammation. The talk concludes with a discussion of possible forays into cosmetic applications based on the existing experience with the device, including testing of cosmetic products, monitoring of skin hydration, and monitoring of cosmetic interventions such as use of fillers.



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J.Ponmozhi is an active researcher who works in biomicrofluidics - in interdisciplinary research areasin IPS Academy, Institute of Engineering & Science. She did her UG in Mechanical engineering from Madras University, India, M.E by research in the area of nanofluids from Universidade de Aveiro, Portugal and PhD from Faculdade de Engenharia da Universidade do Porto, Portugal in biomicrofluids belonging to chemical and biological engineering. She has established 3 labs (nanofluids lab, microfluidics lab and microbiology lab) to execute her three-government funded project:

1.) India and Japan bilateral cooperation for the work on Bacterial vaginosis in collaboration with radiology people from QST, Takasaki, Japan.

2.) India and Sri Lanka for the work on early detection of Oral cancer in collaboration with Oral medicine & periodontology doctors from University of Peradeniya, Sri Lanka

3.) Skin-on-a-chip work for developing drug diffusion studies in collaboration with Prof. Franciska

4.) Institute funded root-on-chip for rice plant in collaboration with Prof. Gamini Seneviratne from National Institute of Fundamental Studies, Kandy.

Realtime analysis of drug (diffusion, toxicity, would healing, repair, inflammation, aging with different shear stress studies) on skin cells in a microfluidic skin-ona-chip device.

Advantages of microfluidics could outnumber the advantages in reconstructed and excised skin samples in certain cases for the study on permeability, toxicity, irritation, corrosion, disease models (eg. oral cancer, etc.), pharmacology, therapeutic approaches, pharmacokinetics and formulation optimization with skin cells deposited in the microchannel and could mimic the exact in vivo conditions. Rather constructing the skin models with full-thickness skin, just the skin cells of different layers namely stratum corneum, epidermis (HaCaT), dermis (Fb) & epithelium (EC), etc. can be cultured and deposited or adhered on the microfluidic channels with thickness of about less than 10µm. The deposition of the skin cells can be made by passing the cultured assay at different shear stress as the shear stress controls the porosity of the skin layer thickness we plan to develop for each lavers. The drug diffusion can be studied at varied concentration and varied shear stress on single skin cell layers developed with our desired thickness and also overlapping skin cell layers just as the same to mimic the human skin. The skin cells viability can be maintained at our required level for the study (disease models)by providing the nutrients at our required flowrate. The shear stress in the microchannel will affect many factors such as cell-cell communications, extracellular gradients, local concentrations of compounds secreted due to drug reaction with skin cells, the drugs penetration into the skin cells. The results obtained for our skin layer thickness for each type can be extrapolated to the actual thickness of the skin layers for epidermis (32-42μm); dermis (949-1350 μm); hypodermis (847 – 2979 μm).

It was reported by different research groups that this microfluidic models has better sensitivity, specificity and accuracy. To highlight the use of microfluidics, the research work by a German group has to be looked through for their work of developing native skin with hair follicles.



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Hichem KICHOU is currently PhD student at the EA 6295 NanoMedicine & NanoProbes, Faculty of Pharmacy, University of Tours, France. His research aims to develop a multi-methodological analytical approach providing a comprehensive evaluation of interaction and diffusion of actives ingredients (cosmetic, pharmaceutical) in human skin. Chromatography techniques are preferred to establish kinetics of penetration of molecules trough the different layers of the skin while Raman confocal imaging (RCI) coupled to multivariate analysis is proposed to investigate penetration profiles in stratum corneum and epidermis.

Evaluating Strat-M® synthetic membrane as substitute to in vitro and ex vivo skin models for permeation studies

Strat-M® is a multi-layered polymer membrane designed for transdermal diffusion testing without the need to account for human skin variability, safety and storage limitations. The aim of the study is to determine the permeation kinetics of the model Active Cosmetic Ingredient (ACI) resorcinol in Strat-M®, EpiSkin® reconstructed human skin and excised human skin and to correlate these results with the characteristics of applied formulations (PBS, hydrogel, Oil/Water emulsion). While high performance liquid chromatography (HPLC) is used to assess rates of diffusion, Confocal Raman Imaging (CRI) and Normal Phase - High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (NP-HPLC/HR-MSn) were applied to characterise the 3 models at the molecular level to reach a better understanding of formulation-skin model interactions. HPLC data from emulsions suggest permeability of Strat-MTM is ~3-fold higher compared to excised human skin but ~3-fold lower compared to EpiSkin® RHE. Concerning PBS solution and hydrogel, the permeability through Strat-M® was ~5-fold higher compared to excised human skin and ~3-fold lower compared to EpiSkin® RHE. CRI coupled to K-means clustering analysis shows that Strat-M® are composed of three layers characterised by spectral features from polyether sulfone and polyolefin, mimicking the skin's multi-layered morphology. However, lipid analysis using NP-HPLC/HR-MSn confirm Strat-M® do not exhibit any lipids found in EpiSkin® and excised human skin, hence its barrier function to active compounds is solely due to the organisation and nature of polymers. Results obtained with resorcinol suggest Strat-MTM could be a more suitable model than reconstructed human skin for the upstream screening of hydrophilic ACI penetration.



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I graduated as a pharmacist in 2018 with a summa cum laude qualification at the University of Szeged. Already during my studies, I was interested in pharmaceutical technology, so I have started working as an undergraduate student researcher at the Institute of Pharmaceutical Technology and Regulatory Affairs. I studied the effect of different dermal formulations and the electroporation treatment on the physiological parameters of the skin. Now, I am a Ph.D. candidate at the University of Szeged, and my Ph.D. research work examines skin permeation enhancement using passive and active methods. Part of this work is the study of electroporation, film-forming systems, and the study of chemical permeation enhancers in Lund University, Sweden.

Investigation of the effect of chemical permeation enhancer glycols on the skin measuring by NMR and Raman spectroscopy

Dermal drug delivery is an attractive alternative to conventional drug administration due to its advantages. However, the outermost layer of the skin, the stratum corneum (SC), provides an effective barrier against dermal permeation. Permeation enhancement techniques, such as chemical permeation enhancer glycols, are widely used to overcome the barrier function of the skin.

The aim of this research work was to examine three well-known glycols to define their effects on the SC structure and define the correlation with their permeation enhancer properties. We used NMR spectroscopy to characterize the molecular structure of SC, and Raman spectroscopy to examine the permeation of a model drug into the different skin layers.

The results of NMR measurements reviled that the glycols increase the mobility of SC components, furthermore, they affect the mobility of keratin filaments. A saturation level for all glycols was detected, after which the addition of chemicals did not increase the mobility. The results of Raman spectroscopy correlated well with the results of NMR measurements because a significant permeation enhancer effect of glycols was observed.



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As a very curious scientist, Jean-André LAPART, PhD in biology, is passionate about technological innovation and has over 8 years of experience in cellular and molecular biology with a deep expertise in microscopy (confocal, expansion ExM, 3D-SIM and STED). At BioMeca, he is in charge of running the laboratories safely and efficiently and he participates actively in developing innovative solutions within the R&D department.

About the company, BioMeca helps biotech, cosmetic and pharma industries to understand effects and mechanisms of action of active ingredients, formulas, and drugs through the structural and mechanical characterization of biological samples.

BioMeca offers a cutting-edge technological platform and tailor-made solutions to meet issues and challenges of health and well-being.

How's AFM a fantastic tool in cosmetic research?

Atomic force microscopy (AFM) is a tool for nanoscale analysis-based approach allowing to obtain mechanical information about structures. Although it is basically used for the characterization of stiff materials, AFM can be used to extract mechanical and structural properties of biological samples. Indeed, in addition to biologically answer to a stimulation, biological samples also respond to a biomechanically manner as for example in the case of the hydration process. At BioMeca ®, we used this approach to characterize among other things, the effects of active ingredients on biological samples such as skin, to link and highlight biological and mechanical responses after application. Coupled to fluorescence approaches, AFM is a powerful tool to characterize products in cosmetic and pharmacological field.



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Silvia Letasiova is the managing director and senior scientist at MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia. She has background in biochemistry and microbiology and holds a doctoral degree in biochemistry. She joined MatTek in 2008 as a project manager and scientist and since then her main field of interest is the development and production of highly reproducible and predictive in vitro 3D reconstructed tissue models for in vitro topical toxicity testing. She is actively involved in the development and validation of assays aiming in reduction, corrosion, phototoxicity and sensitization. Silvia is a member of ESTIV, SETOX, EUSAAT and a full member of US SOT.

In vitro skin irritation tests using reconctructed epidermis tissue model, EpiDerm, for evalution of safety and efficacy testing of cosmetics.

The EpiDerm 3D human tissue model is used across a diverse range of applications including safety and risk assessment, and biological efficacy. Simple protocols and the evaluation of early cellular endpoints allow research to acquire data in few days. EpiDerm, a Reconstructed Human Epidermis, is a ready-to-use, highly differentiated 3D tissue model consisting of normal, human-derived epidermal keratinocytes cultured on specially prepared tissue culture inserts Cultured at the air-liquid interface, EpiDerm allows for the evaluation of topically applied compounds, chemicals, cosmetic/personal care product ingredients and final formulations. With multiple ECVAM validations and OECD accepted test guidelines, EpiDerm is a proven in vitro model system for chemical, pharmaceutical, cosmetics and skin care product testing. EpiDerm skin irritation test (SIT) is validated and accepted as OECD TG 439. EpiDerm Time-to-Toxicity assay [ET-50 assay] is used for screening, ranking and benchmarking of ingredients, for skin tolerance testing of final products (mildness testing) and for evaluation of minor changes in the formulations. This model can be used for different testing purposes, such as EpiDerm skin corrosion test (SCT), EpiDerm phototoxicity test, etc.

The presentation will provide an overview of the in vitro skin irritation tests using reconstructed epidermis tissue model, EpiDerm, for evaluation of safety and efficacy of cosmetics.



Prof. Dominique Lunter

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Dominique Lunter was appointed full professor of pharmaceutical technology and biopharmacy at the University of Tuebingen (Germany) in 2020. She held a guest professorship at the Paracelsus private medical University of Salzburg (Austria) in 2019. In same year she received the Venia Legendi from the University of Tuebingen, where she did her PhD in pharmaceutical technology in 2012. Her research interests are: sustained release dermal preparations, confocal Raman microspectroscopic investigation of the skin and skin penetration as well as 3D printing.

Studying the impact of emulsifiers on SC lipids by confocal Raman microspectroscopy

Emulsifiers are widely used in face washes, shower gels, body lotions and many more cosmetic and pharmaceutic products. Some of them are suspected to show irritating effects and to harm the skin barrier function. It is debated that this might be due to either extraction of lipids from the stratum corneum and/or by disturbance of their highly ordered structure. PEG-ethers are a group of emulsifiers which are widely used in pharmaceutics and cosmetics. We thus chose to investigate this group of emulsifiers regarding their impact on SC lipids. We hypothesized that a skin irritating effect may be related to the HLB-value of the emulsifiers. We thus selected emulsifiers with different chain length of the acyl group and different numbers of PEG groups. The emulsifiers were dissolved or dispersed in water and applied to the skin ex vivo in Franz diffusion cells. Subsequently, the stratum corneum was isolated and measured by confocal Raman microspectroscopy (CRM). This method was selected as it enables simultaneous measurement of SC lipids content and organization. We found that the amount of lipids extracted from the SC correlates with the PEG-chain length of the emulsifier. A maximum lipid extracting capacity was found for PEG-40-cetyl ether. Emulsifiers with shorter as well as longer PEG-chains extracted less lipids. Further, we were able to show that lipids extraction from SC was paralleled by a disruption of the lipid conformation. CRM was found to be a powerful tool in this context as it provided answers to both research questions at the same time while being a lot faster and convenient compared to conventional analytical methods.



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In 2015, I joined the Lip[Sys]2 team as an associate professor. The objective of my work is to set up a biological research axis allowing to pass from the tissue level to the cellular level to study the cutaneous lipids. Thus, my scientific research activity is carried out on in vitro cellular models in 2D and 3D, *ex vivo* and *in vivo*. This activity aims to access all the biological information of the skin barrier at the cellular level in order to better understand the biosynthesis of epidermal lipids, the biology of the skin, and the close relationship between the lipid composition and the physiological state of the skin tissue. As such, I had to develop analytical methodologies adapted to the epidermal lipidome with all its structural complexity.

Multi-scale analytical characterization of the skin barrier from in vitro to in vivo

Co-authors : Joudi Bakar, Ali Assi, Aline Rigal, Arlette Baillet-Guffroy & Ali Tfayli

The keratinocyte's differentiation is accompanied by profound modifications of the lipid composition to form the intercorneocyte lipid cement responsible for the skin barrier function. The aim of our work is to develop analytical approaches to study the biosynthesis of these lipids during keratinocytes differentiation.

Two analytical techniques were used:

1) Normal Phase Liquid Chromatography coupled with High-Resolution Mass Spectrometry. to separate the lipid classes of different polarities in one single run and to characterize the fine structures of lipids

2) Raman microspectral imaging; to follow the lipid evolution during differentiation and to provide information on their lateral packing within the *Stratum Corneum*.

Three models were used:

1) 2D cell model: to monitor the differentiation from the early stages

2) Epidermis reconstructed human model: to monitor the differentiation from *Stratum Granulosum* (SG) up to the *Stratum Corneum* (SC)

3) Native human SC: to study ceramide profiles In vitro, Ex vivo, and In vivo.

Results are as follows:

1) development of relevant analytical tools in the elucidation of complex lipid mixtures composition and their evolution at the cellular or tissue level

2) innovative application of Raman Microspectroscopy: several molecular modifications within the cell were observed indicating the transition between SG and SC

3) identification of two new subclasses of ceramides in the human reconstructed epidermis and in the SC.



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Mélanie Pedrazzani is working as an application engineer at DAMAE Medical since 2016. She obtained a PhD in physics in 2015 from the University of Paris Saclay in the field of optical microscopy for live imaging. Her current activities mainly focus on the implementation of LC-OCT optical technology, in clinical practice for non-invasive skin cancer diagnosis and in the field of dermo-cosmetics.

Line-field optical coherence tomography (LC-OCT) assisted by artificial intelligence for three-dimensional microscopic quantification of in vivo healthy epidermis

Line-field confocal optical coherence tomography (LC-OCT) is an optical technique based on a combination of confocal microscopy and optical coherence tomography, allowing threedimensional (3D) imaging of the skin in vivo with an isotropic spatial resolution of 1.3 µm and up to 400 µm in depth. The 3D cell-resolved images obtained by LC-OCT provide a considerable amount of information for the description and quantification of the upper skin layers using morphological metrics. This study presents metrics for quantifying the epidermis and uses them to describe the variability of the healthy epidermis between different body sites. These metrics include stratum corneum (SC) and epidermis thicknesses, dermal-epidermal junction (DEJ) undulation, and quantification of the keratinocyte network. An artificial intelligence approach was applied to automate the calculation of the metrics. We were able to fully describe and quantify the epidermis on a panel of eight volunteers on seven body areas including the head, the upper limbs and the trunk. Variations in SC/epidermal thickness and DEJ undulation were observed between body sites: the cheek had the thinnest SC and the least undulated DEJ, while the back of the hand presented the thickest SC. For all sites, the process of keratinocyte maturation was evidenced in vivo. These in vivo 3D quantifications open the door in dermo-cosmetics to understanding skin ageing and monitoring the impact of treatments.

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Ana-Maria PENA obtained her master's degree in biomedical and medical physics in 2002 (Babeş-Bolyai University, Romania) and her PhD in biophysics in 2006 (Ecole Polytechnique, France). Following postdoctoral research (2007 - CEA, France), she joined L'Oréal in 2007 as a scientist. She is currently working in the fields of multiphoton and fluorescence lifetime imaging microscopies and their application to the study of in vivo human skin, in vitro reconstructed human skin and human hair. She aims at developing imaging-based methods that allow characterizing the 3D structure of skin and hair and follow their changes during and after application of cosmetic ingredients or physical stimuli. https://www.linkedin.com/in/ana-maria-pena-60720754; https://orcid.org/0000-0001-9943-2513;

In vivo melanin 3D quantification and z-epidermal distribution by multiphoton FLIM, phasor and Pseudo-FLIM analyses

Characterizing melanins in situ and determining their 3D z-epidermal distribution is paramount for understanding physiological /pathological processes of melanin neosynthesis, transfer, degradation or modulation with external UV exposure or cosmetic/pharmaceutical products. Multiphoton fluorescence intensity- and lifetime-based approaches have been shown to afford melanin detection, but how can one quantify melanin in vivo in 3D from multiphoton fluorescence lifetime (FLIM) data, especially since FLIM imaging requires long image acquisition times not compatible with 3D imaging in a clinical setup? We propose an approach combining i) multiphoton FLIM, ii) fast image acquisition times, and iii) a melanin detection method called Pseudo-FLIM, based on slope analysis of autofluorescence intensity decays from temporally binned data. We compare Pseudo-FLIM to FLIM bi-exponential and phasor analyses of synthetic melanin, melanocytes / keratinocytes coculture and in vivo human skin. Using parameters of global 3D epidermal melanin density and z-epidermal distribution profile, we provide first insights into the in vivo knowledge of 3D melanin modulations with constitutive pigmentation versus ethnicity, with seasonality over 1 year and with topical application of retinoic acid or retinol on human skin. Applications of Pseudo-FLIM based melanin detection encompass physiological, pathological, or environmental factors-induced pigmentation modulations up to whitening, anti-photoaging, or photoprotection products evaluation.



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Education:

Ph.D in Applied Physics from the University of Twente, Enschede, the Netherlands Current position:

Managing Director of RiverD International B.V. (www.riverd.com), which develops Raman technology for tissue analysis and diagnostic applications and brings these to market; RiverD-products include the gen2-SCA family of in vivo skin analysis instruments.

Associate Professor at Erasmus-university Medical Center, head of the Raman spectroscopy laboratory, which focuses on cell and tissue characterization by Raman spectroscopy, with the aim to develop diagnostic applications.

Current research is focused on applications in dermatology (Raman-guided Mohs' surgery, in vivo detection of filaggrin mutations, melanoma diagnosis) and in oncological surgery (Raman guided surgery).

Comparing cultured skin, ex vivo skin, and *in vivo* skin by near-infrared confocal Raman spectroscopy

Skin cultures and *ex vivo* skin samples are increasingly used in tests of cosmetics and topical pharmaceutical products.

Near-infrared Confocal Raman Spectroscopy is a non-destructive technique for analysis of the molecular composition of the skin and analysis of skin penetration at high spatial resolution, which has achieved broad adoption.

Over the past 20 years, we have developed an easy-to-use and reliable instrument and dedicated methodology and data analysis software for *in vivo* skin analysis, which can also be applied to *ex vivo* skin samples and cultured skin.

This enables a critical comparison of the molecular anatomy of *in vivo* (human) skin, *ex vivo* skin samples and cultured skin, as well as their product penetration properties. The presentation will highlight differences between the molecular composition of *in vivo* skin measurements, cultured skin samples and *ex vivo* porcine skin samples, and will discuss how this information can support the further improvement and quality control of skin models.



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I graduated at Comenius University in Bratislava, Slovakia with Master's degree in genetics. Currently I'm working at MatTek In Vitro Life Science Laboratories, where I hold the position of Junior Scientist and my main focus is EpiDermTM and EpiDermFTTM 3D model tissues.

Evaluation of serum growth factors in wound healing using a full-thickness in vitro human skin model

Co-authors : Puskar M.¹, Armento A.², Stolper G.², Klausner M.², Letasiova S.¹, Hayden P.J.²

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Following skin injury, damaged tissue undergoes highly coordinated biological events to restore barrier function involving cross-talk between dermal fibroblasts and epidermal keratinocytes as well as their interaction with the extracellular matrix. A full-thickness in vitro human skin model (EpiDerm-FTTM) was used to evaluate the role of serum growth factors in cutaneous wound healing. This model is constructed from primary keratinocytes and fibroblasts and contains a functional barrier and fully developed basement membrane. Small epidermal-only wounds (3mm biopsy punch) or full-thickness wounds (cauterizer burns) were induced in the tissue model and monitored histologically from day 0 to day 6. Addition of 2% human serum demonstrated an increased rate of epithelial healing and fibroblast accumulation which could be abrogated in the presence of an EGFR tyrosine kinase inhibitor or a TGF-alpha neutralizing antibody. Gene expression analysis of the wounded area showed temporally regulated changes in mRNA expression of basement membrane components, collagens and genes involved in extracellular matrix remodeling on days 2, 4 and 6 post wounding. These results demonstrate that EpiDerm-FTTM is a useful in vitro skin model for investigating dermal-epidermal interactions during wound healing as well as for the evaluation of new therapeutics in the dermal wound healing process.



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Biography Dr. Mais Saleh is an Assistant Professor in Pharmaceutical science/Cosmetics science at the Department of Pharmaceutics and Pharmaceutical Technology, University of Jordan. She received her Ph.D. in Pharmaceutical Science in 2019 from King's College London. Her Ph.D. thesis focused on the Delivery of tocopherol phosphate nanomaterials into the skin to protect against ultraviolet radiation. During her Ph.D. project, she worked with Professor Stuart Jones's group in 2014 at the Institute of Pharmaceutical Science, Waterloo campus, and Professor Antony Young's group at St John Institute of Dermatology, Guy's Hospital. Recently, her research interests focus on the delivery of cosmetic actives into viable skin layers, and the applications of nanotechnology such as solid lipid nanoparticles and Iposomes in cosmetic formulations. Dr. Mais Saleh is an author of several peer-reviewed papers and a member of the Society of cosmetic science (SCS).

Nanostructured Vitamin E Phosphate: Characterizing its percutaneous Penetration into Excised Human Skin and release from cosmetic formulations

Co-authors : M.M. Saleh¹, R. Abu Hamdan¹, W. Alshaer², M. Amarin³

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Introduction: Modern topical sunscreens combine topical antioxidants with physical UV filters to achieve optimal skin protection¹⁻³. a-Tocopherol phosphate (a-TP), a new pro-vitamin E antioxidant, prevents UVA1 induced cell death and scavenges the reactive oxygen species that are produced in skin cells⁴. The aim of this study is to investigate if a-TP in candidate vehicles can penetrate excised human skin. This study also aims to prepare solid lipid nanoparticles [SLNs] that can act as a physical sunscreen, containing a-TP and evaluate for encapsulation efficiency (EE) and skin deposition.

Method: a-TP was prepared in different candidate formulations; 2% a-TP solution in a 20:20:60% (v/v/v) propylene glycol: ethanol: Tris buffer (0.1 M) vehicle at pH 7.4 (F1), 2% a-TP lotion at pH 6.8 (F2), and 2% a-TP carbopol gel at pH 6.4 (F3). A topical dose of 9 mg/cm2 from the three formulations was applied for 3 h to test the a-TP skin deposition in the stratum corneum (SC) and remained skin (RS) using the stripping method. Furthermore, skin deposition of a-TP from F1 was observed after 3, 6, and 24 hours. For sunscreen formulation, the SLNs are prepared by precipitation method⁵. The SLNs are evaluated for a-TP encapsulation, particle size, polydispersity index (PDI), zeta potential, and a-TP skin deposition.

Results: The skin deposition studies demonstrated that when a-TP administered in a pH 7.4 vehicle (F1, HD size 10 nm) showed a higher deposition in the SC at 3 hours although it was not statistically significant (31.58 \pm 13.19 µg/cm² vs. 25.36 \pm 3.93 µg/cm² vs. 24.13 \pm 10.50 µg/cm², p > 0.05) and a similar deposition in the RS (4.76 \pm 2.45 µg/cm² vs. 4.49 \pm 2.32 µg/cm² vs. 3.70 \pm 3.40 µg/cm², p > 0.05) compared to the lotion (F2) and gel (F3) formulations, respectively. The skin deposition of a-TP from F1 in SC increase when increasing the application time from 3 h to 24 h (31.58 \pm 13.19 µg/cm² vs. 63.65 \pm 13.32 µg/cm², p = 0.046, one-way ANOVA with Dunnett's multiple comparison tests]. This effect was not as pronounced with regards to penetration into RS (4.76 \pm 2.45 µg/cm² vs. 11.83 \pm 9.21 µg/cm² p > 0.05). Finally, a-TP loaded SLNs exhibited a size in the nanoparticulate range [41.81 \pm 0.74 nm), PDI of 0.20 \pm 0.01, zeta potential of -2.89 \pm 1.00 and demonstrated an interesting EE (87.50 \pm 17.50%).

Conclusion: The enhanced a-TP penetration into the SC from the vehicle at pH 7.4 when applied for 24 h was due to the formation of nanoaggregates which readily deposited into the SC. The a-TP loaded SLNs were characterized by valid size and



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Michal Szczepanczyk is an industrial Ph.D. student at Malmö University. His doctoral project is related to the investigation of the potential beneficial effects of hydrophilic extracts obtained from defatted algae biomass (DAB) on skin. Rich in polysaccharides, proteins, and vitamins DAB is a yet unexplored by-product of an omega-3 fatty acids extraction from fresh brown microalgae *Phaeodactylum tricornutum* grown by Simris Alg, a biotech company from south Sweden, where Szczepanczyk is also working since 2019 as a part of the R&D team. He holds M.Sc. in Biotechnology from Warsaw University of Technology.

Catalase activity in keratinocytes, stratum corneum, and defatted algae biomass as a potential skin care ingredient

Catalase is one of the most important antioxidative enzymes. Its main function is the decomposition of hydrogen peroxide belonging to the group of reactive oxygen species. Disbalance in hydrogen peroxide metabolism in the skin associated with reduced catalase expression can result in the development of skin diseases, such as vitiligo, polymorphic light eruption, and xeroderma pigmentosum. For some of these conditions, the topical application of exogenous catalases was suggested to support the natural antioxidative system. The purpose of our work was to develop an in vitro method allowing for the investigation of H_2O_2 decomposition at controlled conditions where the antioxidative enzyme is residing in a realistic biological matrix and to assess the catalase activity and the apparent kinetics of catalase in keratinocytes and stratum corneum (SC) samples and to make a comparison with the corresponding catalase function in defatted algae biomass (DAB), which may serve as a potential source of catalase and other antioxidative enzymes or metabolites to be used in skin care applications.

Catalases are the only group of enzymes able to generate gaseous oxygen therefore measurements of enzyme activity were based on the Clark oxygen electrode, which was employed to determine the concentration of oxygen produced by the catalase in various biological samples related to the skin organ or potential green sources of catalase to be used as a skin care ingredient.

In conclusion, the results of our work illustrate the advantages of the simple and highly available oxygen electrode method, such as its versatility and ability to provide fast and accurate measurements with small sample amounts of various biological samples of varying complexity, such as keratinocytes, excised SC, and DAB. By using this method, we demonstrated that DAB, a byproduct from food supplement manufacturing, retains quantifiable and hopefully valuable catalase activity after oil extraction and prolonged storage time.



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Lena Waszczuk is a PhD candidate in the start-up company DAMAE Medical, in collaboration with Laboratoire Charles Fabry (France). She holds an engineering degree from Institut d'Optique Graduate School and Ecole Supérieure de Physique et de Chimie (France). Her current research focuses on multimodal characterization of the skin using line-field confocal optical coherence tomography (LC-OCT) and confocal Raman microspectroscopy (CRM).

Morpho-molecular characterization of tattooed skin biopsies with adverse reactions using co-localized line-field confocal optical coherence tomography (LC-OCT) and confocal Raman microspectroscopy (CRM).

Line-field confocal optical coherence tomography (LC-OCT) is a non-invasive optical technique for imaging the skin at high resolution (~ 1 µm), based on a combination of OCT and reflectance confocal microscopy. LC-OCT generates three-dimensional (3D) images of skin tissues with a penetration depth down to 500 µm, providing a new tool for 3D morphological characterization of the skin. Confocal Raman microspectroscopy (CRM) is an optical modality that allows for point measurement of the molecular content of a sample by probing its molecular vibrations. We have developed a method to co-localize data acquired by separate LC-OCT and CRM systems, LC-OCT allows for recording 3D morphological overview images in which points of interest (POIs) can be localized for molecular analysis using CRM. Biopsies of tattooed skin with adverse reactions were analyzed using co-localized LC-OCT and CRM. The combination of morphological and molecular information revealed the presence of tattoo ink in specific areas of the dermis. The LC-OCT-CRM co-localization provided new information on the type of ink pigment used and its abundance in different regions of the dermis. Correlation of LC-OCT and CRM information also pointed out markers of inflammation with the presence of inflammatory cells. The morpho-molecular characterization provided by the combination of LC-OCT and CRM could allow better management of tattoo reactions, and contribute to warn against the use of certain pigments in tattoos.

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