19TH ANNUAL MEETING OF

SKIN FORUM

skinforum

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Tuesday June 25th

Wednesday June 26th

2024

UCL SCHOOL OF PHARMACY

19th Annual Meeting of Skin Forum

Date:	Tuesday June 25th and Wednesday June 26th 2024
Venue:	UCL School of Pharmacy

TUESDAY JUNE 25th

8.00 – 8.45am	**REGISTRATION**
9.00 am	**Opening remarks. Dr. Majella Lane UCL School of Pharmacy**
SESSION 1:	***THE SKIN LIPID BARRIER : METHODS AND MODELS***
	PLENARY LECTURE
9.15 – 9.55 am	**Professor Kateřina Vávrová, Charles University, Czech Republic**
	Formation of the skin lipid barrier
9.55 – 10.35 am	**Dr. Paulo Andrino, T&R Biofab, South Korea**
	The use of the 3D bioprinting technology in the development of specialized *in vitro* models
10.35 – 11.00 am	**BREAK AND POSTER VIEWING**
SESSION 2:	***INNOVATION IN TOPICAL FORMULATION: INDUSTRIAL PERSPECTIVE***
11.00 – 11.40 am	**Dr Leandro Santos, Incyte, USA**
	Points to consider during lead generation, optimization, and selection in topical dermatology
11.40 – 12.20 pm	**Dr Michael Herbig, RaDes GmbH, Germany**
	Design of Dermal Formulations: a Matter of Art – or Artificial Intelligence?
12.20 – 1.50 pm	**LUNCH AND POSTERS,**
SESSION 3:	***SHORT TALKS SELECTED FROM ABSTRACTS***
1.50 – 2.30 pm	**STUDENT AND ECR PRESENTATIONS**
SESSION 4:	***INNOVATION IN TOPICAL FORMULATION: ACADEMIC PERSPECTIVE***
2.30 – 3.10 pm	**Professor Ivana Pantelić, University of Belgrade, Serbia**
	Topical film-forming systems: Is the enhanced therapeutic effect worth the two-stage characterization hurdle?
SESSION 5:	***TRANSDERMAL DRUG DELIVERY***
3.10 - 3.50 pm	**Dr. Julia Lodder-Gadaczek, LTS Lohmann Therapie-Systeme AG, Germany**
	In vitro Permeation Design for Topical patches
SESSION 6:	***MOLECULAR SKIN CARE***
3.50 – 4.30 pm	**Professor Joachim Fluhr, Charité - Universitätsmedizin Berlin, Germany**
	Microbiome and the Barrier: Myth versus Reality
4.30 – 6.00 pm	**BREAK AND ATTENDED POSTER SESSION WITH WINE**
7.00 pm	**CONFERENCE DINNER**

WEDNESDAY JUNE 26th

SESSION 7	***BIOPHYSICAL METHODS – PROBING SKIN AT THE MOLECULAR LEVEL***
	OPENING LECTURE
9.00 – 9.40 am	**Professor Dominique Lunter, University of** Tübingen, Germany Bioequivalence of topical products - a Raman spectroscopic study
9.40 – 10.20 am	**Professor Christian Janfelt, University of Copenhagen, Denmark** Mass spectrometry imaging in studies of drug delivery and cancer in skin
10:20 – 10.40 am	**COFFEE**
10.40 – 11.00 am	**Dr. Peter Caspers, RiverD International B.V., The Netherlands** Quantitative assessment of topical formulations with in vivo Raman spectroscopy
SESSION 8	***SHORT TALKS SELECTED FROM ABSTRACTS***
11.00 – 12.20 pm	**STUDENT AND ECR PRESENTATIONS**
12.20 – 1.30 pm	**LUNCH**
SESSION 9	***NANOTECHNOLOGY AND FORMULATION***
1.30 – 2.10 pm	**Professor Silvia Tampucci, Università di Pisa, Italy** Curcumin-loaded nanomicellar formulations for skin cancer treatment
SESSION 10	***SHORT TALKS SELECTED FROM ABSTRACTS***
2.10 - 2.40 pm	**STUDENT AND ECR PRESENTATIONS**
SESSION 11	***REGULATORY UPDATE***
2.40 – 3.20 pm	**Dr Valentine Ibekwe, MHRA, UK** Quality aspects for topical formulation applications
3.20 pm – 4.00 pm	**Professor Guoping Lian, University of Surrey, Unilever, UK** Bioequivalence of topical formulations
4.00 pm -4.30 pm	**AWARD OF STUDENT PRIZES AND CLOSE OF MEETING**

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SPEAKER ABSTRACTS

Formation of the skin lipid barrier

Kateřina Vávrová

Skin Barrier Research Group, Charles University, Faculty of Pharmacy in Hradec Králové, Czech Republic

The uppermost layer of the skin, the stratum corneum, is our body's first line of defence against the external environment. The intercellular spaces are sealed by a complex lipid matrix that prevents excessive water loss and the penetration of potentially harmful substances or microorganisms. This lipid matrix is very different from conventional lipid bilayers: there are no phospholipids, the dominant lipids are a very heterogeneous group of ceramides, complemented by free fatty acids, cholesterol and minor lipids. Unlike cell membranes, the lipids of the stratum corneum do not form canonical bilayers surrounded by water, but minimally hydrated multilayered formations reinforced by ceramides in an extended conformation. In addition, the first lamella of this structure is covalently anchored to the surface of the corneocyte, forming the so-called corneocyte lipid envelope. In this talk I will present the fundamental elements of the lipid skin barrier, their likely arrangement and the mechanisms of formation of this unique barrier, including gaps in our current understanding of these processes as well as possible hypotheses.

I thank the project New Technologies for Translational Research in Pharmaceutical Sciences /NETPHARM, project ID CZ.02.01.01/00/22_008/0004607, co-funded by the European Union.

Dr. Paulo Andrino, T&R Biofab, South Korea

The use of the 3D bioprinting technology in the development of specialized in vitro models

Bioprinting holds immense promise in revolutionizing tissue engineering by offering unprecedented precision and customization in the fabrication of complex biological structures. Unlike traditional tissue engineering methods, bioprinting enables the deposition of cells, biomaterials, and bioactive factors layer by layer, mimicking the intricate architecture of native tissues. This technology allows for the creation of tissues with tailored properties, including mechanical strength, porosity, and vascularization, essential for promoting cell viability and functionality. Moreover, bioprinting facilitates the incorporation of multiple cell types and growth factors within constructs, enabling the development of heterogeneous tissues and organoids with enhanced biological functionality. By harnessing advances in material science, cell biology, and imaging technologies, bioprinting holds the potential to address critical challenges in regenerative medicine, such as organ transplantation shortages, personalized medicine, and disease modeling.

The lecture focuses on advancing skin bioprinting techniques to develop more realistic and functional skin models for tissue engineering applications. Extracellular matrix (ECM) derived from porcine skin (SdECM) is utilized as a scaffold, demonstrating its effectiveness in comparison to traditional materials like collagen. The developed skin models exhibit enhanced protein secretion, highlighting the importance of cell-ECM interaction. Moreover, the study explores the elasticity of the skin models, revealing higher elasticity in cultured samples due to the presence of elastin and collagen genes within SdECM. By employing a novel bioprinting approach, the study fabricates full-thickness skin equivalents (FTSEs) with a rete ridge structure, mimicking the native skin tissue morphology. Computational fluid dynamics and preset extrusion bioprinting techniques are utilized to achieve precise construction of the dermal layer with rete ridge architecture. These rete ridge FTSEs demonstrate preserved protein expression under UV irradiation, resembling human ex-vivo skin models. Additionally, vascularization and other annexes can be incorporated into the in-vitro skin models to make more predictable and complex structures.

This innovative methodology shows promise in replacing animal experiments and advancing in-vitro skin models for various applications in drug evaluation and safety assessments.

Points to consider during lead generation, optimization, and selection in topical dermatology

Leandro L. Santos, MSc, PharmD

Senior Director, Clinical Research, Incyte Corporation, USA

The development of molecules for topical dermatology has primarily relied on drug repurposing or on combination therapies, leading to an average of only one New Chemical Entity (NCE) approved per year by the FDA. Topical products offer benefits to patients by enabling localized treatment, while minimizing systemic exposure and the likelihood of adverse events. New therapies are further justified by the burden skin diseases cause on patients' quality of life. Notwithstanding the opportunities, the selection of a topical NCE presents challenges, primarily derived from a target product profile uncommon to oral drugs.

An alternative to increase the rate of discovery, and consequently, approvals of NCEs in dermatology, is the use of strategies tailored for the selection of drug candidates more likely to result in a successful topical product. As a result, the developability profile of a topical dermatological drug should account for pharmacological potency and ability to elicit a therapeutic response; acceptable solubility in solvents most commonly used in semi-solid and liquid-based formulations; good chemical stability in the solvents of choice; physicochemical properties that are conducive to skin permeation; and low likelihood of causing local skin irritation. Other aspects should also be considered and are a result of the interactions of the NCE and the topical formulation (vehicle) of choice: for example, physical stability of the formulation; dermal bioavailability; dermal (and sometimes) systemic metabolism; systemic exposure; and toxicological profile.

The purpose of this lecture is to present a novel framework intended to de-risk NCE selection in topical dermatology, based on four calculated physicochemical properties: molecular weight, clogP, topological polar surface area (TPSA), and aromatic ring count. Additionally, the use of topical-relevant solvents to assess the molecule's solubility profile, and a 2-day accelerated chemical stability methodology, are also considered as critical steps in early dermal development.

Design of Dermal Formulations: a Matter of Art – or Artificial Intelligence?

Dr Michael Herbig, RaDes GmbH, Germany

The development of topical dermatological formulations is complex and characterized by several specific features such as the nature of the barrier to be overcome, aspects of susceptibility to physical and chemical instability, the frequent use of complex excipients and a greater influence of sensory perception. At the same time, the number of industrial development projects for topical semi-solids is much lower than for peroral solids or injectable liquid formulations. As a result, benchmarking of excellence in formulation work, learning circles and the establishment of best practices are more difficult. For this reason, topical formulation development is sometimes still considered an "art" and lower development standards may be accepted.

In contrast, recent advances in artificial intelligence (AI) suggest that at least parts of formulation development may be supported by AI in the future which generally is enabled by a mechanistic understanding of formulations and their interactions with the skin barrier.

With or without AI, the basis for efficient topical formulation design is a systematic understanding of underlying physicochemical properties and thermodynamic driving forces. At RaDes, we call this a "rational design" approach that we've successfully applied to many development projects. Key elements are an API-centric formulation approach with a thorough understanding and characterization of a formulation as a drug delivery system. As such, it is primarily characterized by an understanding of i) the solubility of the active and functional ingredients, ii) the saturation and iii) the distribution and iv) the transformation upon application to the skin. For semi-solid formulations, understanding them as a physical body is also essential. As structured liquids, their solid-like properties dominate at rest, while their liquid-like properties dominate under shear, e.g. when processed, dispensed or spread on the skin. Rheological properties can affect stability, bioavailability, device compatibility, patient-conveniences and sensory properties as well as processability. Finally, like all pharmaceutical formulations, topicals are quality products which must meet comprehensive quality and regulatory standards in order to be marketed. Therefore, a "quality feasibility assessment" should be part of early formulation design. As complex excipients are often used in topical formulations, an adequate understanding of their composition and variability is important. Case studies of rational formulation design approaches will be presented.

The presentation will conclude with reflections on the overall opportunities and limitations of AI-assisted topical formulation development.

Topical film-forming systems: Is the enhanced therapeutic effect worth the two-stage characterization hurdle?

Ivana Pantelić
University of Belgrade-Faculty of Pharmacy, Department of Pharmaceutical Technology and Cosmetology, Vojvode Stepe 450, 11221 Belgrade, Serbia

Film-forming systems are drug delivery systems with enhanced skin substantivity, presumably offering improved sensory properties and therapeutic efficacy, jointly leading to less frequent dosing and overall favorable patient adherence.

Although a number of research groups are developing (trans)dermal film-forming formulations in the form of solutions, emulsions, sprays or gels, they still seem far from being listed as one of the EDQM's standard terms. The reason may lie in a rather complex set of characterization techniques necessary for full assessment of the target product profile.

Firstly, selection of both the film-forming polymer(s) and the volatile solvent(s) needs to be elaborated from the aspect of functionality, biocompatibility and eco-friendliness (e.g., innovative medicinal products cannot rely on microplastics-releasing excipients). Considering the presence of various functional groups within the core formulation components (namely, active pharmaceutical ingredients (APIs), film-forming polymers, solvents and plasticizers), a set of characterization methods is performed to elucidate the nature of the interactions. By coupling DSC, XRD, FT-IR, AFM and/or Raman spectroscopy wanted interactions may successfully be differentiated from unwanted ones. Subsequent evaluation commonly depends on the consistency of the primary formulation and may include critical rheological properties, pH, conductivity, to name a few.

Upon skin application, a dynamic evaporation process occurs, leaving a uniform film. Drying time, thickness and other mechanical, textural and sensory properties of the formed film need to be carefully studied. During this process, the incorporated API must not precipitate, but stay in a state of enhanced thermodynamic activity enabled by the presence of non-soluble excipients. It should be noted that the importance of studying such transformations was acknowledged within the EMA's Draft Guideline from 2018.

Practically, the initial dosage form (either a liquid or a semi-solid one) entails a set of characterization methods sometimes quite different from the set relevant for the final one, i.e., the film. The situation may be further complicated by introduction of micro- or nano-structured systems (e.g., microemulsions or self-microemulsifying drug delivery systems) or use of specially designed containers.

Although the enhanced efficacy of the film-forming formulations is usually demonstrated on an in vitro/ex vivo level, while offering comparison to marketed drugs, some in vivo data is available as well. Depending on the nature of the film-forming polymer used, or combination thereof, a variety of release profiles may be tailored, ranging from burst initial release to more sustained one. Nevertheless, due to the fast transformation of the formulation from a liquid/semi-solid state to the film, many of the aforementioned testing protocols require certain optimization.

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In vitro Permeation Designs for Topical patches

Dr. Julia Lodder-Gadaczek

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For the early development of a topical patch system the performance of in vitro permeation studies are indispensable. These studies give the possibility to differentiate between formulations, evaluate the best adhesive and, if necessary, give information about the best enhancer drug combination.

Normally human skin is used as standard model for these experiments. In most cases, this is the best model with a good *in vitro in vivo* correlation. But not only the choice of the barrier has an influence on the performance. For topical patches the design of the IVP setup must be chosen individually, caused by the potential of the API to penetrate, and expected concentration. Also, volatility of the API, solubility and diffusion in the skin caused by lipophilic properties must be considered and influences the design of the IVP setup. In this presentation different design options will be shown regarding the character of the API and formulation.

Microbiome and the Barrier: Myths vs. Reality

Prof. Dr. med. Joachim W. Fluhr [1, 2]

[1] Charité Universitätsmedizin Berlin, Institute of Allergology, Berlin, Germany

[2] Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Allergology and Immunology, Berlin, Germany

Introduction: The skin microbiome and epidermal barrier play critical roles in dermatology and cosmetic science. Their complex interplay is often misunderstood. The presentation aims to demystify prevalent uncertainties and showcase the beneficial interactions observed in studies of microbiome and epidermal function. The discussion extends to how the skin microbiome responds to exogenous stressors. It considers the effects of hygiene practices such as the use skin care and cleansing agents. The influence of life style patterns on the microbiome is also explored. Additionally, the microbiome's involvement in allergic conditions, particularly atopic dermatitis, is examined.

Content: The presentation explores the ambiguous effects of microbial populations on skin health and the effectiveness of skincare products. It emphasizes how microbial diversity contributes to enhancing skin resilience. The role of skincare products in improving skin hydration, protection, and overall health is discussed. These products' interactions with the microbiome are considered in detail, highlighting how they can support or hinder skin barrier function. The analysis includes a discussion on the emerging research linking microbiome diversity to reduced inflammation and better immune responses in the skin.

Conclusion: By addressing these uncertainties and focusing on positive research findings, the overview enhances understanding among dermatologists, skincare professionals, and the public. It advocates for evidence-based skincare practices and directs future research initiatives.

Good emulsifiers? Bad emulsifiers? Or the impact of emulsifiers on stratum corneum lipids.

Moritz Reuter[1], Hans Schönfelder[1],Yali Liu[1], Ziwei Zhang[1], Sebastian Volc[2], Dominique Lunter[1]
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[2] Department of Dermatology, University of Tuebingen, Germany

Purpose
Topical formulations like emulsions and creams necessarily comprise emulsifiers are frequently to stabilize the dispersed phase against coalescence during storage. Upon application to the skin, emulsifiers come in contact with the stratum corneum (SC) and may interact with it. The SC consists of keratinocytes embedded in highly ordered lipids which are a pivotal part of the skin barrier function. Emulsifiers applied to the skin may impar this barrier, especially when formulations are applied to diseased skin. To enable efficient therapy, such impairment must be avoided. It is thus of pivotal importance to characterize emulsifiers with regard to their effect on SC lipids and skin barrier function.
Methods
To this end, we analysed the impact of selected pharmaceutical emulsifiers ex vivo and in vivo with respect to their impact on SC lipids content and conformation by confocal Raman microspectrosopy (CRM) and liquid chromatography-mass spectrometry (LC-MS). CRM was used to investigate total lipid content and lipid conformation ex vivo while LC-MS was deployed to analyse the ceramides content of samples from the in vivo study in detail. Ex vivo experiments were performed in porcine skin as a surrogate for human skin while in vivo experiments were carried out in human volunteers. The study was performed according to the declaration of Helsinki, approved by the ethics committee of the University clinics of Tuebingen (221/2022BO2), and informed written consent was obtained from the volunteers. In vivo, the following physiological parameters were also measured to give an impression of the skin barrier function: transepidermal water loss (TEWL), skin hydration, erythema index and skin pH. Then, theSC was harvested by tape stripping, lipids were extracted and subsequently quantified.
Results
The ex vivo experiments showed that mostly, but not exclusively, hydrophilic emulsifiers exhibit a tendency to extract lipids from the SC which is paralleled by a shift in lipids conformation [1, 2, 3, 4, 5, 6]. In vivo experiments showed that lipid content could be correlated to physiological skin parameters.
Conclusions
Our results may serve as a basis to select appropriate emulsifiers for development of improved formulations for therapy of chronic skin diseases.

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Mass spectrometry imaging in studies of drug delivery in skin and in future treatment of skin cancer

Professor Christian Janfelt, University of Copenhagen, Denmark

Mass spectrometry imaging (MSI) provides compound-specific images of hundreds of compounds in one single experiment without the use of labeling by fluorescence or radioactivity. Drugs and metabolites are easily distinguished and can be imaged relative to endogenous compounds, such as lipids, which may serve as biomarkers of different tissue types for histological classification.

The presentation will provide an introduction to MSI and showcase examples of its application in imaging drugs in skin for drug delivery studies, including a study of treatment of skin cancer through topical delivery of anti-cancer drugs. In addition to this, imaging of the cancer itself will be presented, demonstrating how MSI through imaging of tumor biomarkers can be applied for accurate delineation of skin cancer (examples from excised human tissues and mouse models of keratinocyte carcinoma) and how these principles can be extended to the surgical room, offering mass spectrometry guided laser surgery of skin cancer.

Curcumin-loaded nanomicellar formulations for skin cancer treatment

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Purpose: The present study aimed at developing curcumin (CUR) loaded nanomicelles for the topical application in the treatment of melanoma. CUR is well known for its anti-inflammatory, antimicrobial, anticancer and antioxidant properties, but autoxidation and photodegradation reactions and the poor solubility in aqueous medium makes difficult to formulate this active in a patient-compliant vehicle. Recently, nanostructured drug delivery systems have been proposed in the treatment of dermatological diseases for their ability to increase the solubility, stability and bioavailability of the therapeutic agent, associated with targeted administration with consequent reduction in the dose and frequency of administration and improvement in the safety profile.

Methods: Different surfactants were investigated based on their ability to form stable nanomicelles, finally selecting Vitamin E-TPGS (TPGS) and Kolliphor ELP (ELP). A pre-formulative study and a design of experiment (DOE) were settled to evaluate the effect of the ratios of the two surfactants on both drug solubilisation and A375 melanoma cells viability. The quantitative determination of solubilized CUR in nanomicelles was carried out by HPLC analysis and the average hydrodynamic diameter (Dh) and polydispersity index (PDI) of the formulations were determined by Dynamic Light Scattering (DLS) technique. The DOE-selected nanomicellar formulation (TPGS60ELP30) was characterized by both ATR-FTIR and DSC. Furthermore, its stability was monitored over time for a period of three months in the dark at 4°C and at room temperature. Moreover, the cytotoxicity of curcumin-containing nanomicelles was evaluated on melanoma cell lines overtime and acquiring images with Operetta CLS high-content imaging device. Finally, a penetration study of CUR through porcine ear skin as a model of human skin after cutaneous application of TPGS60ELP30 formulated in a thermosensitive gel was performed. Confocal microscopy was applied to investigate CUR penetration profile.

Results: The pre-formulation and DoE studies allowed the evaluation of the influence of the two surfactants used on two dependent variables and the identification of optimal conditions for the desired response. The results highlighted the crucial role of TPGS compared to ELP in inducing cell death in melanoma cells. The solubilisation of CUR appeared to depend on both the concentrations of TPGS and ELP and to be a function of the total amount of surfactants in the system: the higher the concentration of surfactants, the greater the solubilisation of CUR. Based on DoE results, a nanomicellar formulation was then designed containing the appropriate amount of surfactants and an optimal TPGS:ELP molar ratio. The ATR-FTIR analysis showed that the 1510-1630 cm^{-1} bands due to C=O and C=C stretching of CUR are still present in the spectrum of the optimized TPGS60ELP30, while they are not observable in empty nanomicelles, confirming the encapsulation of CUR inside the lipophilic core of nanomicelles. The results of cytotoxicity studies carried out over time showed a remarkable time-dependent activity of the formulation in inducing cell death, with a value of cell viability at 7 and 14 hours of 50.56 ± 1.73 and 36.05 ± 2.28 % respectively, both statistically different from CUR 5µM alone at the same times (t test, $p<0.001$). Then, the good stability of TPGS60ELP30 was demonstrated both in terms of size and solubilized CUR at both room temperature and 4ºC. Finally, confocal microscopy showed that the thermosensitive gel containing TPGS60ELP30 produced effective CUR concentrations at the target site in the skin following topical application, thus representing a potential drug delivery system for skin cancer treatment.

Bioequivalence of topical formulations

Lian G[1,2]

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[2] Unilever R&D Colworth, Sharnbrook, Bedford, UK

Regulatory approval of generic dermatological drugs requires (a) in vivo tests in human to show that the rate and extend of active ingredients available at the site of action, or (b) well- controlled clinical trials that establish the safety and efficacy. In vitro testing is accepted only if has been correlated in vivo bioavailability data. In silico modelling is being reviewed with guidelines documents produced, yet it acceptance is still to be to be established[1, 2]. Discussed in this talk is how physiologically-based pharmacokinetics (PBPK) modeling can be developed for risk-informed mechanistic evaluation of the rate and extend of dermal absorption and bioavailability. Of particular interest is the recent progress in developing multiscale PBPK dermal absorption model where molecular dynamics, chemical informatics and quantum chemistry modelling are applied for ab initio simulation of how active ingredients partition in heterogenous skin tissues as well as complex formulations at microscopic level[3, 4]. Integration of the molecular level and microscopic level modelling with PBPK modelling provided not only detailed prediction of the rate and extend of active absorption and bioavailability at the site of action, but also mechanistic understanding of how active ingredients interact with critical attributes of complex formulation to affect the disposition of active ingredients in heterogeneous skin tissue microstructures [5, 6]. Verification and validation of PBPK modelling has been also discussed. The aim is to highlight the challenges and opportunities for adopting in silico modelling of bioequivalence of dermatological products as an accepted approach.

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POSTER ABSTRACTS

1.Understanding the Impact of Evaporation on Dermal Absorption through In-Silico Modelling

Benjamin N. Deacon[1], Samadhi Silva[1], Guoping Lian[2], Tao Chen[1]

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Understanding skin permeation is crucial for developing effective skincare and pharmaceutical products. In-vitro permeation tests have been widely used to understand safety and efficacy of active ingredients and formulations, recently there has been a shift towards performing finite dose, unoccluded experiments to account for more accurate in-use conditions. However, these are expensive and time consuming to perform, in-silico models have been suggested as a cost-effective alternative.

We use an in-silico evaporation model integrated with a physiologically based pharmacokinetics (PBPK) model of skin permeation to better understand the impact of evaporation on dermal delivery, permeability and pharmacokinetics. Allowing for the pharmacokinetics and permeability of volatile chemicals to be investigated under in-use conditions. We have validated the proposed model with published penetration and pharmacokinetics data from the Cosmetics Europe ADME Task Force. The evaporation model is in strong agreement with published experimental data, where the importance of cutaneous distribution and receptor fluid kinetics has been considered. Furthermore, a quantitative structure-property relationship is employed to benchmark the in-silico permeability results. Where the simulation results indicate the inclusion of evaporation reduces the amount of chemical delivered into the receptor fluid.

Investigating the complexities surrounding permeability with considerations for evaporation highlights the challenges faced to obtain accurate results for chemicals in in-use scenarios. This work has highlighted the significant impact evaporation has on the pharmacokinetics and permeability of volatile ingredients with the intrinsic volatility measured by vapour pressure providing a strong indicator of the extent the chemical will evaporate. We further discuss the model's effectiveness in assessing skin evaporation and dermal delivery, along with the potential abilities of the model to contribute towards risk assessment. Where we have highlighted the potential to aid in the reduction of IVPT experiments required for assessing the safety and effectiveness of formulations in industry.

2.Comparative Analysis of Pig Ear Skin and Human Skin Permeation: A Simulation Study Using PBPK Models

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In 2023, the FDA announced that animal testing will no longer be required before human trials for new medicines. As the safety and efficacy of the drug still need to be proven during pre-clinical studies, non-animal methods (NAMs) of drug evaluation are gaining importance. An example approach widely implemented to support informed decisions in drug discovery and development process is physiologically based pharmacokinetic (PBPK) modelling, which integrates drug characteristic, anatomical and physiological description of the system, and trial design, to simulate quantitative changes of drug amount in time in specific compartments.

Patel et al. (2022) published introduced the mechanistic multi-phase multi-layer model of dermal absorption (MPML MechDermA), which is a PBPK model that enables simulation of the pharmacokinetic behaviour of a topically applied drugs. The model is designed to account for body site- and sex- population variability of human skin. It is parametrised for nine locations: face (cheek), forehead, volar forearm, dorsal forearm, upper arm, abdomen, back (torso), upper leg (thigh), and lower leg. When combined with a full-body PBPK model, this model enables predictions of local and systemic bioavailability of dermal drugs. Yet, when employed independently, it allows to simulate experiments under ex vivo conditions, such as 'in vitro permeation tests' (IVPT). To extend the model utilisation, we parametrised it to describe anatomical and physiological properties of pig ear skin.

Although human skin is a gold standard in IVPT studies, pig skin is commonly used as a surrogate. It is generally accepted that these membranes can be treated as equivalents. However, some experimental data show significant differences in the permeability through humans and pig. In this project, we decided to study the differences in permeability of two substances: caffeine (logP = -0.07) and piroxicam (logP = 3.06) through pig skin and two most common body locations of human skin samples (back and abdomen).

All experiments were performed in silico, using PBPK models implemented in Simcyp® platform V22 (Certara Inc., Sheffield, UK). Caffeine and piroxicam are substances, which were presented in the manuscript of Patel et al. to show the performance of the MPML MechDermA model. The caffeine data were based on the data implemented in the Simcyp® compound library, and the piroxicam data was based on the mentioned manuscript. All experiments were simulated under unified conditions (same dose, duration of experiments, formulation), and each simulation consisted of ten trials with ten subjects each (in total 100 subjects). Six parameters were compared: cumulative amount in the receptor solution, flux, amount at the end of experiment in stratum corneum, viable epidermis, dermis, and sebum. The statistical analysis of the result was performed using R-studio. For normally distributed data, the student t-test was implemented, otherwise mann' whitney u-test was used. The statistically significant differences ($p<0.05$) between pig ear skin and human back and abdomen skin were observed for all parameters, except for flux and sebum amount in human back skin for piroxicam, and human back and abdomen skin for dermis. Further studies are needed to better understand the importance of different membrane types impact on the interpretation of IVPT results.

3.Thickening system for foaming antiseptic drugs

B. GAVINET, B. SOUYRI, A. ROSO

Easy application of a topical drug is key to an optimal patient compliance and treatment efficacy. In the case of foaming antiseptic drugs, most are formulated as solutions because of the presence of cationic APIs and high concentration of surfactants. This lack of viscosity is an obstacle to the pick-up of the drug and the treatment of a large body surface, as well as a cause of product loss and environmental exposure.

Povidone iodine 4% solution is commonly used as an antiseptic wash before surgery[1], with very low viscosity (less than 50 mPa.s, Brookfield LV S2S6). This work aim is to obtain a foaming Povidone iodine formulation with higher viscosity (more than 20,000 mPa.s, Brookfield LV S4S6), without compromising the bactericidal activity of this active.

Two stable solutions of 4% Povidone iodine were developed with different foaming systems: an association of an amphoteric and a non-ionic surfactant (ANI) and another one with addition of an anionic surfactant (ANIA). Thickening trials involved sodium chloride addition, a non-ionic polymer (Hydroxypropyl methylcellulose) and an associative anionic polymer (Polyacrylate crosspolymer-6), with physico-chemical follow-up and rheological characterisation. Selected formulas were tested according to standard NF EN 1040, a quantitative test evaluating basic bactericidal activity.[2]

Only Polyacrylate crosspolymer-6 alone or in combination with Hydroxypropyl methylcellulose has shown satisfying stability and viscosity results with both ANI and ANIA systems. Polymer addition and change in the surfactant system did not impact the efficacy of Povidone iodine, both tested products exhibiting the same efficacy as the market reference. This study provides opportunities for easier use by the patient.

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4.Application of physiologically based pharmacokinetic modelling to predict the in vivo and in vitro dermal absorption of caffeine.

Predicting Dermal Absorption of Caffeine: Applying the dermal PBPK model.
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Purpose: Dermal physiologically based pharmacokinetic (PBPK) modelling is increasingly recognised in pharmaceutical and personal care industries for its holistic approach. By incorporating human skin physiology, formulation dynamics, and trial design, it accurately predicts skin absorption. Caffeine, widely employed in pharmaceuticals and personal care products, serves as a notable case study, and this study showcases the effectiveness of a dermal PBPK model in accurately predicting its skin absorption, both in vivo and in vitro.

Methods: Simcyp's Multi-Phase Multi-Layer (MPML) MechDermA model V22 is used to develop the dermal PBPK model for caffeine. Skin partition and diffusion coefficients were determined using experimental measurements or QSAR predictors. The receptor solution: membrane partition coefficient (Kprec:mem) as a parameter of the IVPT module was derived from in vitro permeation data from water. The IVPT module was validated by simulating independent studies of caffeine permeation and skin retention, ranging from simple solutions to emulsions. This was followed by extrapolation to the in vivo situation by simulating stratum corneum (SC) profiles, measured using Confocal Raman Spectroscopy (CRS) and tape stripping, after the application of formulations. Predicted-over-observed (P/O) ratios are computed to assess the model's performance, with a ratio within twofold ($0.5 \leq P/O \leq 2$) considered satisfactory.

Results: The model's performance in predicting local skin concentration and permeation from aqueous solution was validated. By parameterising the emulsion model with reported formulation characteristics (Q2), pH, viscosity and globule size of dispersed phase, it accurately captured caffeine permeation for both reference and test creams (P/O = 1.43 and 1.45). Extrapolation to an in vivo situation allowed to effectively capture the SC concentration during uptake studies for both solutions with and without PG, as determined by in vivo CRS at different time points, as well as flux ($0.55 \leq P/O \leq 1.44$). However, the model underpredicted the flux values during depletion. The model accurately captured SC concentration determined in another study that used tape stripping at various time points ($0.91 \leq P/O \leq 1.45$), along with the penetration enhancement ratios due to the presence of PG in the gel formulations ($0.70 \leq P/O \leq 1.89$). In the same paper, the CRS investigation reported significantly higher permeation from the same formulation. The authors attributed this to insufficient sample size and high inter-subject variability. However, without further details on this variability, the model cannot accurately address the observed results discrepancies.

Conclusions: This case study illustrates the development of a dermal PBPK model of caffeine using a bottom-up approach. Initially the model was validated using in vitro permeation data from solutions. Subsequently, it was extended to emulsion formulations and further validated through in vivo permeation assessment using CRS and tape stripping. The successful application of the dermal PBPK model in predicting skin absorption of caffeine highlights a replicable workflow for investigating skin absorption of other chemicals of interest using the PBPK modelling approach.

5.ALTERED STRATUM CORNEUM BARRIER CHARACTERISTICS IN ATHLETES COMPARED TO A NON-ATHLETIC POPULATION

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Introduction: Competitive athletes are exposed to various stresses that may challenge skin barrier health. Excessive skin dryness is an issue athletes face and is widely reported in aquatic athletes (1). Natural moisturising factor (NMF) components play a key role in maintaining stratum corneum (SC) hydration and barrier function (2,3) and are removed from the SC following water exposure (4). Although the importance of NMF in skin barrier function and SC hydration is known, further elucidation of the mechanisms underpinning skin barrier challenges of athletes is required. Therefore, this study, assessed the differences in skin barrier health components between non-athletes and athletes.

Method: 30 non-athletes and 26 competitive athlete volunteers were recruited (female = 26, male = 30, mean age 38 ± 13). Athletes were characterised into aquatic athletes (swimmers and triathletes) and other athletes (runners and cyclists). Transepidermal water loss (TEWL), skin permittivity and skin pH were measured on the mid-volar forearm. Overall skin dryness (OSD) was visually graded from images captured with the C-cube device. Ten repeated tape strip samples were taken and a depth profile of the NMF components (histidine (His), pyrrolidone-5-carboxylic acid (PCA), trans-urocanic acid (trans-UCA) and cis-urocanic acid (cis-UCA) were quantified by HPLC (3). The amount of protein removed on each tape strip was quantified to normalise NMF levels (mmol NMF/g protein) and to assess SC cohesion. TEWL measurements were repeated after tape stripping to assess SC integrity. The skin microbiome was quantified from inner elbow swab samples and lifestyle data gathered through questionnaires.

Results: Skin barrier integrity was impaired in athletes compared to non-athletes (change in TEWL 45.5% vs 29.35%). Athletes had higher SC cohesion, mostly accounted for by aquatic athletes who had lower protein removal across sequential tapes. Aquatic athletes showed a greater tendency towards visual skin dryness with 67% presenting with OSD of ≥ 1, compared to 33% and 42% in other athletes and non-athletes, respectively. Athletes had higher total NMF levels, again mostly accounted for by aquatic athletes compared to non-athletes. Aquatic athletes had significantly higher His and PCA and proportionately higher cis-UCA compared to non-athletes, while both athlete groups had significantly lower trans-UCA levels. A general trend showed an association between increased His and PCA levels with increased mean SC permittivity values. Baseline TEWL, skin pH and shower frequency were comparable between all groups, while moisturiser use was highest in aquatic athletes.

Conclusion: Athletes, especially aquatic athletes have altered SC characteristics. Impaired SC integrity and increased skin dryness in aquatic athletes likely reflect repeated disruption to their skin from sporting-associated activities. Increased NMF and skin permittivity suggest a SC compensatory response to environmental stress and may partly explain why aquatic athletes had increased SC cohesion despite impaired integrity.

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6.DEVELOPMENT OF AN *IN-VITRO* DERMAL TRANSFER MODEL TO ELUCIDATE PROTOCOLS OF SAFE CONTACT FOR TOPICAL MEDICAMENTS.

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Purpose: Testosterone gels, predominantly used by men on low hair areas such as the abdomen or shoulders, are volatile in nature and progress through dynamic formulation changes to leave a dried film at the skin application site (Kamal, 2020). There are potential safety concerns, raised by the FDA and UKMHRA, over inadvertent secondary transfer of testosterone gel to other individuals. To date, clinical studies have been conducted to investigate the degree of transfer from skin to skin contact (Stahlman, 2012). However, no *in-vitro* model exists to evaluate such secondary transfer (De Ronde, 2009). This study will seek to develop an *in-vitro* model to characterise the impact of application protocols on the secondary transfer of Testogel (a testosterone gel product), with a view to developing a body of evidence to characterise and understand secondary dermal transfer.

Methods: Secondary transfer was modelled with primary skin (modelling patient skin, n=6) or secondary skin (contact skin, n=6) using 500 μm dermatomed porcine skin in a Franz diffusion cell. The primary skin sections were dosed with a finite amount of the formulation and standardised contact between the two skin samples was initiated at 4 different time points including a non-contact primary condition representing a therapeutic dose. Sweat conditions included dosing the primary skin with artificial sweat before or after dose. Testosterone permeation and distribution (unabsorbed, stratum corneum, epidermis, dermis and receptor fluid) after 24 hours was determined by reverse-phase HPLC-UV. Raman microscopy and light microscopy were used to image the disruption to the primary skin film from contact.

Results: Greater amounts of testosterone were transferred from primary skin to secondary skin when contact happened at earlier time points. However, despite lower levels of transference, drug delivery to primary skin was lower at these later contact times, with a greater proportion of testosterone being unabsorbed. For example, with contact occurring 10 minutes after dosing, 22 $\mu g/cm^2$ of testosterone was delivered to primary skin compared to 45 $\mu g/cm^2$ when contact was made immediately after dosing ($p<0.05$). Raman microscopy showed evidence of the dried primary skin testogel film being moved and disrupted because of contact occurring at these later time points. Testosterone delivery through secondary skin was 1.5% of the therapeutic dose when contact was made 10 minutes after dosing compared to 35.2% following contact immediately after dosing ($p<0.05$). Presence of sweat on primary skin, followed by contact, resulted in greater amounts of testosterone being transferred to secondary skin and less unabsorbed testosterone on primary skin compared to the absence of sweat, resulting in an increase in delivery of testosterone to primary skin. For example, dosed primary skin with sweat had 43 $\mu g/cm^2$ of testosterone delivered compared to 22 $\mu g/cm^2$ without sweat, when contact was made 10 minutes after dosing ($p<0.05$). Testosterone delivery was greater in secondary skin exposed to sweat-administered primary skin than that without sweat, with 29.1% and 16.3% of the therapeutic dose delivered in the 10- and 30-minute sweat contact conditions, respectively.

Conclusions: Decreased testosterone delivery to primary skin following contact at later time points was attributed to disruption of the dried drug film hindering delivery of testosterone. Reduced testosterone transfer to secondary skin occurred at later contact times due evaporation of alcoholic vehicle in the dried film on primary skin. The presence of sweat increased the amount of testosterone transferred and delivered to secondary skin, suggesting evidence for exercise-induced sweat increasing the transfer of testosterone from dried testosterone gel films.

7.Percutaneous absorption of Fentanyl *in vitro*

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Fentanyl (CAS 437-38-7) is a synthetic opioid used in clinical practice as an analgesic and anaesthetic. Public health concerns have been raised as to the potential of fentanyl and it's analogues to exhibit toxicity after dermal absorption following unintentional contamination. The current study measured the percutaneous absorption of free base fentanyl through human and rat skin using Franz type *in vitro* static diffusion cells. Franz type diffusion cells are an established methodology routinely used to determine the penetration of substances through the skin. The approach provides information on chemical hazard and risk assessment without reliance on animal toxicity testing.

The work used Phoenix® diffusion cells with automated receptor fluid sample collection over a 24-hour study duration. Topical dosing was performed by the direct application of fentanyl free base (diluted in isopropyl alcohol (IPA)) across the entirety of the skin surface within the donor chamber to give a range of contamination densities from 20 to 100 $\mu g.cm^{-2}$. The cells used had a 1 cm^2 diffusional surface area and a receptor fluid volume of 10 ml. The receptor fluid used was 50% aqueous ethanol which was stirred at 400 rpm using a magnetic stirrer. Skin surface temperatures were maintained at 32±1°C for the study duration. Samples (250 µl) were taken at hourly intervals up to nine hours and then three hourly to 24 hours. Sample analysis was by LC-MS. Maximum penetration rates (J_{max} $ng.cm^{-2}.h^{-1}$, average ± SEM of n=12 replicates) for each contamination density for human skin were: 78 ± 25 (20 $\mu g.cm^{-2}$), 119 ± 350 (30 $\mu g.cm^{-2}$), 199 ± 44 (50 $\mu g.cm^{-2}$), 359 ± 121 (100 $\mu g.cm^{-2}$). For rat skin J_{max}'s were: 107 ± 14 (20 $\mu g.cm^{-2}$), 173 ± 11 (30 $\mu g.cm^{-2}$), 286 ± 28 (50 $\mu g.cm^{-2}$), 795 ± 96 (100 $\mu g.cm^{-2}$).

Comparison of penetration rates with literature toxicology values indicate that dependant on the contamination density and residence time, skin decontamination and medical countermeasures may be required to ameliorate toxicity from the percutaneous absorption of free base fentanyl. This work is in agreement with, and, builds on previous work with free base carfentanil (Dalton et al, 2021).

Reference

Dalton, C.H., Watkins, R., Pritchard, S. and Graham, S. "Percutaneous absorption of Carfentanil in vitro" Toxicology *In Vitro* 72 (2021) 105100 DOI: 10.1016/j.tiv.2021.105100

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8.The effect of novel *Acmella oleracea* extract-containing, alkyl polyglucoside-based anti-wrinkle cream on the facial skin microtopography

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Purpose: Keeping pace with growing consumer demands for healthy aging, personal safety, efficacy, and innovation, coupled with sustainability and environmental considerations, the current study has addressed the development and evaluation of novel, non-invasive, topical, anti-aging product, based on naturally grown, ethically sourced, sustainably produced ingredients. The particular objective was to assess the influence and *in vivo* effectiveness of formulated oil-in-water cream, stabilized by mixed alkyl polyglucoside (APG) emulsifier (arachidyl alcohol/behenyl alcohol/arachidyl glucoside), and containing *Acmella oleracea* (L.) plant extract (*Asteraceae*), as a model anti-aging active, on skin surface topography using objective, non-invasive methods. **Methods:** The randomized, open-label, split-face trial was carried out on the group of 13 healthy, female volunteers (aged 42–56 years), who applied the investigated cream to the area around the eyes (crow's feet) and mouth (bitterness folds), twice a day, during two weeks. Visioscan® VC 20plus apparatus (Courage+Khazaka, Germany) was employed to determine the changes in skin topography/microrelief on the assigned periorbital and perioral areas after 14 days of continuous treatment. The parameters measured were: SELS parameters (Surface Evaluation of the Living Skin – skin roughness-SEr, scaliness-SEsc, smoothness-SEsm, and wrinkles-SEw); roughness parameters (R1, R2, R3, R4, R5); texture parameters (energy, variance, contrast, entropy, and homogeneity); surface and volume parameters. **Results:** During the study period, significant ($p < 0.05$), beneficial changes in the skin topography parameters were noticed after only two weeks of the tested cream application: decrease in the values of SEsc (about –40%), SEsm (–10% to –15%), SEw (about –10%), R1–R4 (–4% to –8%), contrast (–7% to –14%), variance (about –10%), volume (–6% to –9%) and surface (–4% to –9%), and increase in SEr (20–40%), energy (20–30%), entropy (about 2%) and homogeneity (about 3%), suggesting the smoother and more homogeneous, visibly milder skin relief in both, periorbital and perioral areas. **Conclusions:** The present findings confirmed the positive effect of the developed *A. oleracea* extract-containing, alkyl polyglucoside-based cream on the overall condition and appearance of facial skin, further anticipating its promising anti-wrinkle activity.

9.Comparing Nanocarriers for Topical Delivery: Cutaneous Bioavailability of Bakuchiol from (Micro/Nano)emulsion and Polymeric Micelle Formulations

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Bakuchiol (BAK) is a meroterpene phenol derived from *Psoralea corylifolia* with antioxidant, anti-inflammatory, antibacterial, and anti-acne activities that is reported to mimic the effects of retinoids in the skin by promoting extracellular matrix and collagen synthesis. BAK is an oil at room temperature, and its topical delivery is problematic due to its oily form, high lipophilicity, and poor aqueous solubility (Log P = 6.1; aqueous solubility = 0.132 ± 0.041 mg/mL), resulting in low cutaneous bioavailability. Despite their ability to solubilize BAK, lipophilic formulations (e.g., ointments and creams) prove suboptimal – their high affinity for BAK impairs its partitioning into the stratum corneum. The aims of this project were to create innovative, aqueous formulations using polymeric micelles and micro- and nanoemulsions to improve BAK cutaneous delivery and to compare their efficacy by quantifying BAK skin deposition and determining its cutaneous biodistribution.

Initial studies involved the development and characterization of the nanocarrier formulations. Two polymers were used to prepare micelles: TPGS and Poloxamer 407 (0.5%; 200 mg of BAK per g of polymer). Micro- and nanoemulsions were developed using the same pseudo-ternary phase diagram. Transcutol® and Labrasol® were used as a surfactant mix (S_{mix}), and oleic acid (OA) was used as the oil/penetration enhancer. Three emulsion-based formulations were selected: microemulsion (ME: 0.5% BAK, 7.5% OA, 42% S_{mix}, 50% water), nanoemulsions with and without OA (NE1: 0.5% BAK, 1% OA, 8.5% S_{mix}, 90% water; and NE2: 0.5% BAK, 9.5% S_{mix}, 90% water). In NE2, BAK acted as the oil phase in the system. All nanocarriers were characterized with respect to size, morphology, BAK content, and stability. Cutaneous delivery of BAK from the nanocarrier formulations was evaluated using porcine skin mounted in vertical Franz diffusion cells under infinite (1.25 mg/cm² of BAK) and finite dose conditions (50 µg/cm² of BAK). Formulations were applied to the skin for 8 h to quantify BAK cutaneous deposition and to determine the BAK cutaneous biodistribution. Quantification was carried out using validated UHPLC-UV and UHPLC-MS/MS methods.

Optimized TPGS and Poloxamer 407 micelles showed encapsulation efficiencies of 96.6±3.2% and 101.5±0.3%, respectively, with sizes of 27.98 nm (PDI 0.21) and 26.74 nm (PDI 0.092). NE2 showed a higher EE (106.8 ± 5.7%) than ME and NE1 (86.3 ± 4.6% and 90.2 ± 2.4%, respectively). Micro-and nanoemulsion nanocarriers had larger diameters than micelle formulations (ME:108 nm, PDI 0.071; NE1: 128.8 nm, PDI 0.299; NE2: 68.3 nm, PDI 0.243). Despite the large size and PDI of NE1 and NE2, both nanoemulsions resulted in higher BAK deposition than the other nanocarriers, whether applied at infinite or finite doses. BAK deposition after applying NE1 and NE2 was 3-4 fold higher than ME and micelle-based formulations. Moreover, BAK deposition and cutaneous biodistribution from NE1 and NE2 were similar, indicating no significant impact of OA on BAK delivery – clearly an advantage. Nanoemulsions are systems with high BAK thermodynamic activity, facilitating BAK partitioning and enabling the highest BAK cutaneous delivery. The biodistribution profile revealed that the nanoemulsions delivered more BAK into the viable epidermis and dermis, which are the target sites for BAK activity.

In conclusion, the nanoemulsions were superior to the microemulsion and micelle-based formulations. As evidenced by NE2, OA was not necessary to improve BAK delivery. The results confirmed the importance of thermodynamic activity in skin delivery and showed that use of a "smaller" nanocarrier does not necessarily mean better delivery.

10.Subcutaneous versus intradermal microneedle delivery of biopharmaceuticals: A literature review on differences in pharmacokinetic parameters

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Biopharmaceuticals are essential in the treatment of various diseases but most of them require parenteral administration. As patients associate intravenous and subcutaneous (*s.c.*) injections with discomfort and pain, other routes have been investigated including intradermal (*i.d.*) microneedle delivery. Since microneedles are shorter than hypodermic needles, they minimize contact with nerves in deeper skin layers and thereby reduce discomfort during administration.

To document the current knowledge of pharmacokinetic differences between *s.c.* injections and the *i.d.* delivery by hollow microneedles (HMN), dissolvable microneedles (DMN), and coated microneedles (CMN) of biopharmaceuticals, studies that compare both routes were collected.

There is a vague trend towards a quicker and higher plasma peak with similar bioavailability following the delivery of biopharmaceuticals by HMN compared to *s.c.* injections. The fast onset may be due to a rapid drug uptake by the extensive network of lymphatic and blood vessels in the dermis. In case of DMN and CMN, trends are less explicit. Their pharmacokinetics may be altered by the swelling and dissolution behaviour of base and coating materials. Hence, about half of the DMN studies reported a delayed absorption compared to *s.c.* injections. However, excipient effects might be less relevant in CMN due to a rapid dissolution of the thin films. Therefore, the plasma peak in CMN studies was reached earlier or at the same time compared to *s.c.* injections.

Overall, the mechanistic understanding of absorption, permeation and metabolic processes in the skin that lead to differences in pharmacokinetic profiles following *i.d.* and *s.c.* administration is limited and requires further investigation.

11.Enhancing Photodynamic Therapy Effects: a Novel *In Vitro* Skin Tissue Phantom Approach

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The overall purpose of this project is to explore the possibility of increasing the efficacy and reduce the recurrence rate of photodynamic therapy (PDT). PDT employs a combination of light energy and photosensitizing agents to treat certain cancer types and other medical conditions like psoriasis, acne, and bacterial infections. In the case of skin cancer treatment, PDT mostly relies on the generation of singlet oxygen and downstream reactive oxygen species (ROS), via excitation of the well-known photosensitizer protoporphyrin IX (PpIX). To achieve successful clinical PDT protocols, the development of reliable and realistic *in vitro* methods that allow for easy and flexible screening and optimization is crucial. The *in vitro* methodology employed here is a new skin tissue phantom based on the simple two-component system of water and monoolein. In the aqueous dermal environment, this lyotropic system forms a stable inverted liquid crystalline cubic phase that can be utilized to incorporate PpIX and thereby mimic PDT conditions. The tissue phantoms colloidal properties are evaluated by SWAXS. With this novel set-up, we have investigated how parameters, such as concentration of the photosensitizing agent PpIX and degree of tissue oxygenation, influence the kinetics of ROS generation. Considering the highly reactive nature and transient existence of ROS, we utilize the Skin Covered Oxygen Electrode (SCOE) [1] to probe the consumption of molecular oxygen, which is the main source of ROS and thus indirectly reflects the ROS generation. In addition, we explore the possibility of using a combined approach of adding hydrogen peroxide to increase skin tissue oxygenation via native epidermal catalase, which converts hydrogen peroxide into oxygen [2]. Building on the outcome from initial studies employing tissue phantoms, tissue oxygenation of skin membranes will be further explored in vitro with the aim to optimize ROS generation for skin cancer treatment.

The main conclusions from our preliminary results are:

- PpIX can successfully be incorporated in liquid crystalline cubic phases of water and monoolein, enabling a realistic skin tissue phantom for PDT studies *in vitro*.
- The developed *in vitro* setup can probe the oxygen depletion caused by the presence of PpIX in the tissue phantom under photo illumination.
- Addition of H_2O_2 increases oxygen levels due to native epidermal catalase, which potentially can be used to enhance ROS generation and improve PDT efficacy.

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12.Innovative Transdermal Delivery System for Enhanced Doxorubicin Efficacy in Breast Cancer Treatment

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Purpose: A novel approach was devised to address the challenges in delivering doxorubicin (DOX) for breast cancer treatment. This involved the development of a non-invasive transdermal delivery system, aiming to minimize side effects associated with its administration.
Methods: Transdermal patches were formulated by incorporating carbopol 971 (CP) and polyvinyl alcohol (PVA), which were loaded with starch-coated iron oxide nanoparticles coupled with DOX (DOX@St-IONPs). The study assessed the patches' physicochemical properties, *in-vitro* drug permeation, as well as the intrinsic characteristics of DOX@St-IONPs and their effects on the viability of triple negative breast cancer cell lines (TNBC-MDA-mb 231).
Results: The M2 patch, formulated with a 1:1 ratio of CP to PVA loaded with greenly synthesized DOX@St-IONPs (particle size: 38.1 nm, PDI: 0.5, zeta potential: -12.3 mV) exhibited promising in vitro anticancer activity. They displayed significant cytotoxicity (95%, IC50: 0.26 µg/mL) against TNBC-MDA-mb 231, surpassing free DOX cytotoxicity (48%, IC_{50} of 4.58 µg/mL). The M2 patch retained uniform thickness (0.38 mm), weight (0.26 mg), and achieved a remarkable 97% DOX loading and 58.3% of swelling after 90 min. Additionally, it demonstrated flexibility and maintained an acceptable surface pH (7.4). Notably, the M2 patch showcased a fivefold enhancement in drug penetration compared to free DOX patches (M3), with a flux of 14.85 µg/cm^2/h and permeability of 0.95 cm/h versus 2.95 µg/cm^2/h and 0.19 cm/h, respectively.
Conclusion: This approach proposed promising non-invasive DOX delivery through the developed transdermal patches.
Key words : Iron oxide nanoparticles; Doxorubicin; Transdermal patches; Breast cancer.
Short title : Transdermal Patches for Enhanced Doxorubicin Efficacy

13.Transdermal supplementation of a novel vitamin D analogue mimics sun exposure with enhanced vitamin D binding protein production

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Background: Vitamin D (VD) deficiency has been associated with various health disorders, but most clinical trials have failed to demonstrate the benefits of oral VD supplementation due to variable inter-subject bioavailability (1). Administering VD via the skin may overcome this issue as the vitamin D binding protein (VDBP) helps to absorb and distribute VD from the cutaneous tissue (2), mimicking the transport mechanism of VD generated from sun exposure. A new phosphate analogue of VD (VDP) has demonstrated adequate skin permeation for effective VD supplementation (3), and this study aimed to better understand transdermal VDP metabolism and in vivo absorption through comparison to oral VD supplementation and UV exposure.
Methods: In vitro metabolism of VDP in the skin and intestinal epithelium were studied using rat epidermal keratinocytes (REK) and human epithelial Caco-2 cells respectively. Cells were treated with 10 μM VDP over 24 h. In vivo absorption of VDP was studied in adult male rats treated with a VDP patch compared to VD capsules, and in adult female mice treated with a VDP patch compared to a single UVB dose of 1.9 J/cm^2 (280 – 320 nm). VD and metabolites levels were analysed using GC-MS with a limit of detection of ca. 10 ng/ml. Skin and blood serum VDBP levels were quantified by a sandwich enzyme-linked immunosorbent assay.
Results: VDP was converted to VD rapidly in both REK and Caco-2 cells, with detectable levels at 1 h, and > 50% dose conversion at 24 h. VDP dosing increased VDBP levels in REK cells, but not in the Caco-2 cells. VDP patch and oral VD administration in rats showed a similar 25(OH)D_3 response at 72 h after dosing, but only the skin induced a significantly higher local (420.4 ± 48.4 μg/g tissue) ($p < 0.01$) and circulating (480.4 ± 64.3 μg/ml) ($p < 0.001$) levels of VDBP compared to untreated animals (110.0 ± 14.9 μg/g tissue and 281.6 ± 29.0 μg/ml). VDP patch administration was superior to UV exposure in raising 25(OH)D_3 response in mice, with a 15-fold increase in 25(OH)D_3 level (304.7 ± 74.3 ng/ml) compared to untreated animal (19.2 ± 9.4 ng/ml) ($p < 0.001$) at 24 h. However, both treatment groups produced a similar increase in local (patch 385.9 ± 39.8; UV 316.1 ± 42.3 μg/g tissue) and systemic (patch 520.2 ± 49.7, UV 412.9 ± 53.7 μg/ml) VDBP at 24 h post-dosing compared to control (169.8 ± 38.3 μg/g tissue and 277.9 ± 41.3 μg/ml) ($p < 0.01$).
Conclusion: This work suggested that transdermal vitamin D supplementation using VDP was effective in improving VD status. This supplementation by the skin increased VDBP levels akin to UV exposure. Therefore, it is proposed that transdermal vitamin D supplementation could represent an innovative approach to address vitamin D deficiency.

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14.Lidocaine transport into the dermis, adipose and muscle tissue after multiple application of a lidocaine-containing topical delivery system (SP-103) in in-vivo pigs

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Purpose

For the treatment of local musculoskeletal pain, clinicians frequently prescribe lidocaine-containing topical delivery systems because these systems ensure site-specific drug delivery, pain relief at low daily doses, and avoidance of first-pass effect. Because data on the transport behavior of lidocaine into muscle tissue after application of topical delivery systems (TDS; aka "patches") is lacking, we monitored lidocaine concentrations in dermis, subcutaneous tissue, muscle tissue, and serum to assess the extent of lidocaine transport into muscle tissue via redistribution or direct diffusion after application of a lidocaine-containing TDS (SP-103).

Methods

Lidocaine concentrations in dermis, adipose tissue, muscle tissue, and serum after multiple applications of systems-103 were assessed in vivo for 72 hours in eight pigs. In four of these pigs the lidocaine concentrations were monitored directly after the first SP-103 application (at t = 0), in two pigs after the second application (at t = 24 h), and in the other two pigs after the third application (at t = 48 h). SP-103 were applied for 12 hours each. Lidocaine bioavailability was monitored in treated dermis, adipose tissue, and muscle tissue, and in untreated muscle tissue by sampling diluted interstitial fluid every four hours from the respective tissues using open-flow microperfusion (OFM). Concurrently, serum samples were collected every 4 hours from all pigs for 72 hours. Lidocaine concentrations were analyzed from the collected samples with HPLC MS/MS.

Results

Four hours after the first SP-103 application, the lidocaine concentration in serum was 34.1 ± 22.7 nM, and lidocaine concentrations in the treated sites decreased from dermis (1,535.5 ± 2,032.3 nM), adipose tissue (26.5 ± 25.8 nM) to muscle tissue (23.7 ± 14.4 nM). The lidocaine concentration in untreated muscle tissue was comparable to that of treated muscle tissue four hours after the first SP-103 application and its concentration-time profile followed the pattern of the concentration-time profile measured in serum over the entire study duration.

However, after the second SP-103 application lidocaine concentrations in the treated muscle tissue started to exceed those measured in untreated muscle tissue, and at the end of the study (at t = 72 hours) the lidocaine concentration in treated muscle was higher (16.6 ± 14.3 nM) than that in serum (3.6 ± 1.9 nM) and in untreated muscle (3.0 ± 1.0 nM).

Conclusions

Lidocaine was transported via the blood circulation into the muscle tissue, as was supported by the concentration-time profile recorded in untreated muscle. In addition to circulatory distribution, after multiple SP-103 applications lidocaine transport into targeted muscle tissue was also accomplished via direct diffusion. Thus, utilizing an additional pharmacokinetic path for more effective drug delivery to the underlying tissue. TDS with higher lidocaine content and deeper tissue penetration may provide desired analgesic effect when applied over a site of musculoskeletal pain.

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15.Needle-free Interstitial Fluid Sampling Investigation of Skin Inflammaging

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Purpose

Inflammaging, the phenomenon of the low-grade chronic inflammation, which occurs as a consequence of aging, is considered a highly significant risk factor for chronic morbidity, frailty and premature death (Franceschi and Fabbri 2018). It contributes to the pathogenesis of numerous age-related non-communicable diseases including cardiovascular diseases, chronic kidney disease, neurodegenerative diseases, diabetes mellitus and cancer (Franceschi et al., 2018; Ferrucci et al., 2018). However, it is difficult to detect the low-level cytokines released from the skin and thus detect, monitor, and understand this process during aging. The aim of this study was to develop a non-invasive approach to sample skin interstitial fluid (ISF) using controlled tissue stretching. It was anticipated that the direct sampling of the skin ISF could provide an innovative means to monitor the changes in inflammatory markers during aging to better understand inflammaging.

Method

Skin interstitial fluid was extracted from the forearms of healthy participants draw from three different age groups (20-40, n=13, 40-60, n=13, 60-80, n=14). The ISF extraction was achieved by controlled skin stretching through the application of hypobaric pressure (- 4.5 psi) for 10 min. The extracted ISF was analysed for 10 pro-inflammatory cytokines using MSD multiplex assay. The skin barrier and elastic properties were also measured using transepidermal (TEWL) meter (Biox Ltd) and Cutometer® (EnviroDerm Ltd), respectively.

Results

Controlled skin stretching facilitated the extraction of 0.7 ± 0.2 ul of ISF (n= 33) across the skin of the healthy volunteers in all three age groups with no statistical difference in amounts extracted per group. The skin barrier function, indicated by the TEWL, was statistically similar ($p > 0.05$) across the three age groups (19.3 ± 5.7 g/m2/h (20-40 y/o) vs 23.5 ± 6.5 g/m2/h (40-60 y/o) vs 21.6 ± 6.2 g/m2/h (60-80 y/o) and the tissue elasticity was also similar ($p > 0.05$), Uf at 0.9 ± 0.1 mm (20-40 y/o), 0.8 ± 0.2 mm (40-60 y/o) and 0.9 ± 0.2 mm (60-80 y/o). Despite a lack of change in barrier function the level of pro-inflammatory cytokines IL-1b, IL-17, TNF-a, IL-2 and IL-12p70 in the extracted ISF were significantly higher ($p < 0.05$) in the 60-80 y/o age group compared to the younger age group (20-40 y/o).

Conclusion

The direct non-invasive extraction of ISF across the skin successfully detected low-grade cytokine release, which is typically associated with inflammaging. The absence of notable differences in skin barrier function suggested that barrier function deterioration did not cause the cytokine release. The types of cytokines released matched the cell senescence-associated secretory phenotype (SASP) and this suggested that cell senescence, not barrier disfunction, was the primary driver of inflammaging in the skin.

16.Feasibility of confocal Raman spectroscopy (CRS) for penetration and bioequivalence studies of topical products

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Purpose
When it comes to approval of topical generic drug products, to date, in vivo studies of efficacy remain the only option. High costs and ethical burdens make them unappealing [1]. Lately, the authorities have been considering a limited number of suitable methods for establishing equivalence. According to the "EMA draft-guideline on quality and equivalence of topical products", the stratum corneum (SC) sampling technique (Tape Stripping, TS) can be used in lieu of a clinical endpoint study [2]. Unfortunately, TS lacks spatial resolution, is time-consuming [1] and difficult to standardise [3]. Contrary to TS, CRS enables to investigate the chemical composition of a SC sample at microscale resolution, and the distribution of the substances contained therein can be directly analysed [4]. In combination with its simple data acquisition and easy sample preparation, CRS is currently enjoying a significant increase in interest [5]. Hence our work focuses on providing more pivotal data and showing the suitability of non-invasive CRS as a promising alternative to TS with respect to bioequivalence studies.
Methods
Comparative studies between CRS and TS using a marketed salicylic acid (SA) containing formulation (=comparator formulation) were conducted on porcine postauricular skin of the same subject. A reverse engineered - allegedly bioeqvuivalent - SA formulation served as test formulation. Different application amounts (low, medium, high) and incubation times (for invasion and depletion phase) in Franz diffusion cells were analysed for both methods in parallel.
Results
Comparison of the results obtained by TS and CRS shows a clear similarity in terms of the penetrated SA amounts into the SC. CRS is not only able to generate similar data to TS for different application amounts on the same skin samples, but also for different incubation times in invasion and depletion phase. The results for the reverse engineered - allegedly bioeqvuivalent - SA formulation met the requirements of the EMA as the 90 % confidence interval fell within 80-125 % of the comparator formulation [2].
Conclusions
In view of the fact that similar quantitative penetration profiles can be obtained as with TS, this is a further step towards establishing CRS as a method to obtain pivotal bioequivalence data.
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17.Initial refinement of dermal absorption risk assessments: Quantifying epidermal turnover and protein binding

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Epidermal turnover and desquamation are typically not observed in studies with excised skin since it is no longer metabolically active. These processes are known to reduce the bioavailability of dermally absorbed compounds since some material may be lost with the desquamated cells. By accounting for the impact of epidermal turnover with computational modelling, the aim of this work is to obtain more realistic dermal absorption values for risk assessments.

An *in silico* model is being developed based on the equations by Reddy *et al.,*[1] who added a convective term to Fick's second law to account for epidermal turnover. The model will be evaluated using published literature data and the collection of new *ex vivo* permeation data, which will support modelling other dermal absorption processes even if it does not fully capture epidermal turnover. The fraction of a compound that is bound to skin proteins is a critical part of modelling epidermal turnover. In addition to slowing diffusion, binding to skin proteins can reduce the bioavailability of a compound, as desquamation removes these skin proteins and any compounds bound to them. Current proposed equations do not reliably predict the fraction unbound in the epidermis as they are based on plasma protein binding data,[2,3] which is not equivalent to the protein composition and binding in the upper layers of skin. Planned experiments to obtain new protein binding data more relevant to the cutaneous composition will be used to inform the fraction unbound in the upper layers of skin for a range of compounds.

The proposed work should give insight into the effect of epidermal turnover relative to diffusion and binding processes, which has not been fully addressed in currently published models.

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18.A novel in vitro method for evaluating formulations interactions with the cutaneous hydrolipidic film

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Purpose Human skin is composed of two layers: the epidermal outer layer, highly cellular, providing the barrier function and the inner dermal layer, ensuring strength and elasticity and nutritional support to the epidermis. Nevertheless, hydrolipidic film is considered as a sort of ''fourth skin layer''. It is composed by Skin Surface Lipids (SSL) and sweat that cover the stratum corneum and it can represent the ultimate skin barrier towards the environment and the first substrate for cutaneous application drug. The purpose of this study was to introduce a novel method to assess formulation affinity toward the skin, enabling prediction of formulation behaviour following skin application, by measuring contact angle formed between a drop of formulation and an artificially prepared hydrolipidic film under conditions similar to the physiological ones of the skin surface. This approach seeks to optimize formulation design for enhanced efficacy and patient outcomes in skin therapy.
Methods A synthetic hydrolipidic film (S-HF) has been prepared according to the literature by mixing artificial sweat and sebum in a ratio 1:1. Artificial sweat composition was obtained as reported by Villegas et al. (2019) according to standard NIHS 96-10, adding 0.1% glycine to mimic the natural composition of sweat, that includes also amino acids. After complete solubilisation of the components (sodium chloride, ammonium chloride, lactic acid, urea, acetic acid, and glycine) in deionized water, the pH has been adjusted to 4.7 by addition of sodium hydroxide 1M. Artificial sebum was prepared following an extensive literature review to closely mimic the human counterpart in terms of constituent categories and concentration ranges (Wertz, 2009). The components (triglycerides, fatty acids, wax esters, and squalene) were melted in a hot bath at 60°C. Measurements of the static contact angle were carried out by an optical contact angle instrument using the sessile drop method. To ensure reproducibility of the measurements and avoid water evaporation from the drop of formulation, we designed a closed system consisting of a glass box filled for a half of silica beads immersed in deionized water and closed with a perforated plexiglass foil to allow the entry of the syringe used by the instrument. For this study, different products were selected based on their differences in terms of chemical structure, HLB (Hydrophilic-Lipophilic Balance), and CMC (Critical Micellar Concentration) or in excipient function. To investigate the differences in affinity between formulations and S-HF over time following application to the skin surface, the decrease in contact angle was measured immediately after application and at predetermined time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 minutes).
Results Our findings revealed distinct behaviours among the different products, particularly in terms of wettability kinetics, which were influenced by HLB and CMC. Vitamin E-TPGS demonstrated superior efficiency in wetting the hydrolipidic films compared to other products, regardless of the concentration tested. This was evidenced by consistently lower values of contact angle over time and can bring out new insights in the use of TPGS in drug delivery systems.
Conclusions This system can help in the screening of new formulations intended for cutaneous use to select the one that has a better interaction with the skin in terms of affinity for the tissue and which can be subjected to skin penetration and permeation studies.

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19.Skin retention of luteolin from binary and ternary solvent systems

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Background Luteolin is widely reported for its anti-inflammatory properties and its capacity to alleviate arthritis-induced pain. It is commonly included in various oral supplements available on the market at doses ranging from 50 to 100 mg per day. Targeting localized inflammation, topical application has many advantages compared with oral administration. Dermal drug delivery offers a non-invasive route for administering drugs. It avoids the challenges encountered in other delivery methods, such as the need for swallowing in oral administration, and enables targeted drug delivery to specific sites. To evaluate the suitability of luteolin for dermal delivery, the physicochemical properties of this permeant need to be understood since limited scientific literature is currently available. Although *in vivo* dermal absorption studies in human subjects are regarded as the gold standard experimental model for assessing drug delivery systems, the use of human tissue requires ethical approval and patient consent. Porcine ear skin is reported to be a suitable surrogate for human skin and can be used to evaluate the *in vitro* performance of simple luteolin topical formulations by Franz diffusion cell studies and mass balance studies.
Methods The Shake flask method was used for Log P and Log D determination, according to OECD guidelines. The Log P was determined using water and 1-octanol; similarly Log D was evaluated using PBS and 1-octanol. For Franz diffusion cell studies and mass balance studies, the solvents investigated included Transcutol® (TC), propylene glycol (PG) and isopropyl mysritate (IPM). 1% (w/v) of luteolin formulations were prepared in various solvent systems, including TC/PG 25/75, TC/PG 50/50, TC/PG 75/25, TC/IPM 35/65, TC/IPM 50/50, TC/IPM 75/25, TC/PG/IPM 60/10/30, TC/PG/IPM 70/10/20 and TC/PG/IPM 80/10/10. 10 µl of each formulation was applied to an approximate 1 cm^2 application area on the surface of porcine ear skin. 24 h Franz diffusion cell and mass balance studies were conducted at 32 ± 1 °C. Thermodynamic activity, solubility parameter and TC/PG/IPM miscibility were also investigated.
Results The Log $P_{o/w}$ value of luteolin was determined as 3.73 ± 0.05 (n=3, pH of water = 6.40 ± 0.02). Log D value of luteolin was determined as 3.11 ± 0.04 (n=3, pH of PBS = 7.33 ± 0.02). Although no permeation of luteolin was observed in our initial work, different amounts of the permeant were retained in the skin for various solvent systems after 24h. No correlation between solubility parameter and skin retention was evident. The highest percentage of drug was retained in the skin for the TC/PG 25/75 (7.01%), TC/IPM 35/65 (6.09%) and TC/PG/IPM 60/10/30 (6.45%) solvent systems.
Conclusions The Log P and Log D value confirm the high lipophilicity of luteolin. This correlates well with the results from Franz diffusion cell and mass balance studies, in which only skin retention could be observed. The highest value of skin retention was 7.01% after 24h Franz diffusion cell studies. This indicates that luteolin may be a potential candidate for dermal delivery targeting with optimised skin formulations. Future work will expand the range of candidate formulations investigated and confocal Raman studies will also be explored to understand how specific formulations influence luteolin skin delivery.

20.Investigation of piroctone olamine delivery to the skin from single, binary and ternary solvent systems

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Objectives

The aim of the present work was to investigate several solvent systems for their ability to promote piroctone olamine (PO) delivery to the skin. The formulations examined comprised of 1% (w/v) PO in six single solvent systems, one binary solvent system and three ternary solvent systems were evaluated.

Methods

The solvents used in this study were propylene glycol (PG), diethylene glycol monoethyl ether or Transcutol® (TC), propylene glycol monolaurate (PGML), isopropyl myristate (IPM) and caprylic/capric triglyceride or Labrafac™ Lipophile WL 1349 (LAB). PG and TC were chosen based on previous solubility and stability studies. In addition, PGML, IPM and LAB are used in existing shampoo formulations. The concentration of PO used for *in vitro* permeation studies was 1% (w/v). Heat separated human epidermis was used for *in vitro* permeation experiments performed as finite dose studies. The receptor fluid used in the Franz cells was PBS with the addition of Brij® O20. The duration of the experiments was 24 h, which was then followed by a full mass balance evaluation. PO deposited on the skin and in the skin was determined by swabbing and extraction. All permeation and mass balance samples were analysed by HPLC.

Results

The permeation of PO was investigated for single, binary and ternary solvent systems. For the single solvent systems, PO was not observed to permeate. The majority of the PO recovered was from the skin surface. Skin permeation of OPX was observed for binary and ternary solvent systems. The highest permeation for all PG:TC binary solvent system ratios tested was from the PG:TC (75:25) system. The amount of PO permeated from this system was10.50 ± 1.49 $\mu g/cm^2$. However, the highest PO extraction from inside the skin was observed for PG:TC (25:75) with a value of $22.52\% \pm 3.44\%$.

Conclusion

To our knowledge, this is the first study to examine the permeation behaviour of PO for a range of single, binary, and ternary solvent in human skin. The combination of PG and TC clearly promoted skin permeation of the active. This likely reflects synergistic effects of these solvents on the skin.

21.Low-energy nanoemulsions with ibuprofen – interfacial interplay of oils, surfactants and cosolvents

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Purpose: The aim of this work was to elucidate how oils and stabilizers of different properties affect the phase behavior of low-energy nanoemulsions while serving as carriers for active pharmaceutical ingredients (API) with both lipophilic and hydrophilic characteristics (ibuprofen as the model drug).
Methods: A solubility study was conducted via the shake flask method in order to select oils, surfactants and cosolvents with the highest ibuprofen saturation solubility. Influence of natural and synthetic oils varying in composition and polarities, low molecular surfactants (polysorbate 80 and sorbitan oleate) in different ratios, and cosolvents with different geometry (propylene glycol, glycerol, and polyethylene glycol 400) was taken into account. Nanoemulsions were prepared by emulsion phase inversion (EPI) methods using a vortex mixer/magnetic stirrer with medium stirring speeds. Ibuprofen was incorporated at an early stage of the (pre)formulation study in order to determine whether it remains in the proximity of the stabilizer layer, affecting the nanoemulsions' formation. The microstructural and stability aspects of the obtained nanoemulsions with/without ibuprofen were simultaneously analyzed by a number of techniques. Any sign of phase separation or a hint of emulsion ring formation were among the early sample exclusion criteria.
Results: Among the tested natural oils, eucalyptus oil (475 mg/ml) and olive oil (31 mg/ml) showed the highest saturation solubility of ibuprofen, while isopropyl myristate (IPM) (67 mg/ml) was singled out from the assessed synthetic oils. Long-chain fatty acids (linoleic and linolenic acid) from olive oil could not be incorporated into small droplets without the use of high energy, which resulted in nanoemulsions with droplet sizes above 200 nm. Despite maintaining its small amounts in the formulation (up to 0.1%), eucalyptus oil destabilized nanoemulsions due to higher polarity of volatile terpenes, resulting in phase separation 24 hours after preparation. The optimal properties in terms of droplet size ($d \sim 110$ nm), polydispersity index ($PDI < 0.25$) and preliminary stability were shown for nanoemulsions with C17 oil - IPM, which was used for further tests. By varying the selected stabilizers, it was determined that the HLB value has a great influence on the effective coating of oil droplets and that for IPM, the optimal HLB value of the surfactant mixture was 12. The addition of the cosolvents has a marked effect on the formation of the interface layer because it positions itself near the stabilizer layer, changing its properties and mobility of its constituents, improving the targeted nanoemulsion's attributes (significantly lower droplet size and PDI value). PEG 400 complemented the selected oil/surfactant mixture, providing the samples with the most satisfactory stability. Ibuprofen was successfully incorporated into the carriers at a maximal concentration of 2.5%, rendering these nanoemulsions with a significantly smaller droplet size and much higher homogeneity in comparison with placebo formulations, indicating the amphiphilic API's influence on the nanoemulsion's stabilization. Conductivity measurements confirmed oil in water (O/W) type microorganization, while the pH values obtained in the range of 4.5–6.0 implied preliminary suitability of these formulations for cutaneous application.
Conclusions: The IPM/polysorbate 80/sorbitan oleate/PEG 400 mixture provided an ibuprofen-loaded nanoemulsion with the most desirable characteristics. Also, this research has shown that it is necessary to consider the influence of all potential components that have a tendency to position themselves near the stabilizer layer, even if they do not stabilize nanoemulsions directly.

22. Characterisation of nifedipine and development of candidate formulations for novel topical preparations

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Purpose

Nifedipine, a calcium antagonist, was investigated because of its reported efficacy in treating cutaneous lesions caused by peripheral vascular diseases, diabetes, and hypertrophic scars. The specific aims of the research were to characterize the physicochemical properties of nifedipine and to conduct the necessary pre-formulation experiments for the development of an optimized topical nifedipine formulation.

Methodology

An HPLC method was developed and validated to analyze nifedipine. The melting point of nifedipine was determined by Differential Scanning Calorimetry (DSC). The physical form of the nifedipine sample was analyzed by X-ray diffraction (XRD). The solvents investigated in the solubility study of nifedipine were dimethyl sulfoxide (DMSO); Transcutol®; dimethyl isosorbide (DMI); polyethylene glycol (PEG) 400; PEG 200; tripropylene glycol (TriPG); methanol (MeOH); 1,2-butanediol; water; isopropyl alcohol (IPA); propylene glycol (PG); 1,5-pentanediol; 1,3-butanediol; isopropyl myristate (IPM) and oleic acid. Nifedipine solubility was determined by adding excessive amounts of nifedipine to the various solvents selected for study as reported previously.

Results

The HPLC method for nifedipine was validated according to ICH guidelines. The melting point of nifedipine was determined to be 172.23 °C. XRD analysis confirmed that the nifedipine sample was stable form I (the commercially used form). Nifedipine showed the highest solubility in DMSO (286.35 mg/ml) with comparatively high solubilities also in Transcutol®, DMI, PEG 200, PEG 400 (123.76, 122.62, 98.69 and 69.53 mg/ml respectively). In contrast, nifedipine was extremely insoluble in water (0.014 mg/ml).

Conclusion

The physicochemical properties of nifedipine were characterized and an analytical HPLC method was successfully validated. The next steps will involve permeation studies in porcine and human skin to identify the best solvents to take forward for development of novel topical formulations of this compound.

23.Using non-invasive skin stretching to monitor longitudinal changes to the tumour microenvironment

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Background: Malignant melanoma is a prevelant, deadly skin cancer (1). Current diagnostic methods rely on invasive biopsies, limiting their utility for longitudinal monitoring. Alternatives such as blood liquid biospies are hindered by poor sensitivity as a result of the dilution of chemical signals leaking from the tumour tissue into the systemic circulation. Interstitial fluid (ISF), the non-clotting liquid that carries chemical signals from the cells into the lymph and blood, presents a promising alternative biofluid for melanoma biomarkers, as it can be sampled un-diluted, but non-invasive sampling of ISF remains challenging. The aim of this work was to assess the capability of skin stretching to repetitively, non-invasivly extact ISF from melanoma tissue to generate longitudinal data to help understand disease progression.
Methods: C57BL female mice, 6-8 weeks of age, were purchased from Charles River, Kent, UK. To initiate the melanoma, 1×10^6 B16F-10 cells in 100 μL sterile PBS (pH 7.4, Thermofisher, Loughborough, UK) were subcutaneously injected into dorsal skin of the mice. Skin was stretched at -4.5 psi for 20 min by the application of a novel 3D printed stretching device (Formlabs, Massachusetts, USA). Liquid biopsy ISF samples were collected pre and post tumour inoculation. Serum and tumor lysate samples were collected towards the study endpoint. The collected samples underwent analysis for the presence of multiple cytokines using the MSD multiplex analyser (Mesoscale Diagnostics™, Maryland, USA) and for proteins using LCMS (Thermofisher Scientific, UK).
Results: The stretching device was successfully directly applied to the melanoma tissue of mice, and ISF was sampled longitudinally without visible skin damage. Sample analysis showed a total of 30 proteins were shared between ISF and the tumour lysate, but only 2 proteins were shared between the serum and the tumour lysate. Among the 30 proteins found in the ISF, Lactate dehydrogenase (LDH), an approved serum melanoma biomarker, released due to the hypoxic tumour microenvironment (2) spiked on day 7 post-tumour implantation, but it was absent in the serum of mice. Multiplex cytokine analysis revealed that ISF shared 9 cytokines (IL-6, IL-33, IL-10, MCP-1, IL-9, MIP-1, IL-15, IP-10, and IL-12) with the tumour lysate, whereas serum exhibited only 4 cytokines (IL-6, MCP-1, KC-Gro, and MIP-2) shared with the tumour lysate. The findings revealed a sequential release of cytokines, including spikes in cytokine levels such as IL-33 on day 2 , IL-6 on day 4, and IL-10 on day 7.
Conclusion: Skin stretching was a useful non-invasive method for the longitudinal extraction of ISF from melanoma-bearing mice. The analysis of extracted ISF showed that during the dynamic release of putitative chemical biomarkers over time transitions of the tumour from early to late stage melanoma was evident. This suggested that ISF biomarkers could be useful in tumour staging and thus could offer both diagnostic and prognostic potential.

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24.Development of microneedle patches for the treatment of psoriasis

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Purpose

Psoriasis is a chronic inflammatory skin disease that effects about 2% of the world's population.[1] A topical treatment is necessary in all disease stages but often inadequate due to poor substantivity of the formulation and insufficient penetration of the APIs. We investigated how the use of microneedle patches can improve the skin penetration of the API betamethasone dipropionate (BDP) out of a topical formulation. Using continuous liquid interface production (CLIP) 3D printing we manufactured round microneedle array patches (MAPs) with varying geometries. The aim was to elucidate the MAPs ability to improve delivery of BDP into ex-vivo pig skin from a novel Oleogel-based formulation.

Methods

All MAPs with a diameter of 14 mm were designed using Onshape. MAPs were manufactured on a prototype S1 CLIP printer from Carbon using a PEG based raisin. Needle geometries were varied from square pyramidal, conical, to obelisk, with varied needle lengths of 400µm, 600µm, 800µm, or 1000µm. The printed MAPs were rinsed with isopropanol, air dried, and post cured using ultraviolet light.[2]

The MAPs were characterized regarding needle strength and robustness while pressing them into the skin with a texture analyzer. Furthermore, differences in skin damage produced by the different shapes and lengths of the needles were investigated using tissue marking dye. Franz diffusion cells were used for the ex-vivo penetration testing into pig skin, and the skin was divided into the different layers (stratum corneum, viable epidermis, dermis) using a cryotome. The skin layers were extracted and the amount of BDP was detected via LC-MS.

Results

All MAP designs could be successfully produced using CLIP printing. The damage on the skin surface caused by the microneedles increases with a thicker base with all designs. The size of the holes created by the application decreased with decreasing length and diameter of the needle designs. Only the obelisk shaped microneedle could resist the penetration into the skin without breaking or bending in all four needle lengths and was therefore chosen for penetration testing. The amount of BDP that penetrates the skin was significantly improved using the 600 µm, 800 µm and 1000 µm MAPs compared to the control without the use of microneedles.

Conclusion

With the obelisk shaped microneedle patches, MAPs in lengths of 400 µm, 600 µm, 800 µm and 1000 µm have been successfully developed, which have both, a thin flexible patch for easy handling and robust microneedles themselves that are suitable for use in the skin. BDP delivery was significantly enhanced which may improve treatment of patients.

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25.Long-acting nano hydrogels for wound healing and anti-aging purposes

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Purpose: Wounds are defined as damage to the physical and physiological integrity of the epidermal or dermal layer, which can lead to a variety of other pathologic problems if not properly treated. Wound healing is a complicated physiological process that includes inflammatory, proliferative, and remodeling stages, as well as diverse processes such cellular migration, cytokine, growth factor, and collagen formation. Wound healing is governed by a controlled system including transforming growth factor-beta (TGFB1), vascular endothelial growth factor (VEGF), and others. As a result, ginseng-loaded nano hydrogel (NHG) was produced, which enhanced ginseng's wound healing capacity. Methods: In order to create NHG, particle size, PDI, zeta potential, and encapsulation efficiency were first determined. Furthermore, FT-IR, PXRD, SEM, TEM, rheology, swelling, and diffusibility tests were carried out. Furthermore, a diffusion study was conducted. Then, to establish the therapeutic effectiveness of NHG, an in vivo efficacy study was conducted. Results: The optimal nanoformulation had particle sizes of 420.11 ± 5.21 nm, PDI of 0.424 ± 0.013, zeta potential of 0.006 ± 0.002 mV, and encapsulation efficiency of 89.051 ± 0.022%. The FT-IR and PXRD analyses revealed the nanostructure of the improved nanoformulation. SEM and TEM investigation revealed the nanoformulation's ultra-morphology, which consisted of spherical nanoparticles containing encapsulated ginseng extract. The discovered NHG exhibited thixotropic behavior, allowing for facile cutaneous administration. Conclusions: The diffusion research results suggested that ginseng diffusion from NHG lasted more than 20 hours. The in vivo wound closure research demonstrated good outcomes for NHG. Furthermore, the antioxidant investigation yielded outstanding outcomes for NHG. Other wound healing indicators, including EGF, VEGF, hydroxyproline levels, and genetic expression of TGFB1, Col4A1, and Col1A1, showed considerably higher outcomes ($p < 0.05$) for NHG. Thus, the multifactorial pharmacological mechanism of optimized new NHG has effective wound healing capability.

Short title: Wound healing nanohydrogel-

Keywords: Ginseng, nanohydrogel, genetic expression, VEGF, hydroxyproline

26.An ex-vivo study of the human nail barrier: Insights to aid the development of novel topical antifungal preparations

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The human nail is composed of hard and soft keratin (1). These keratins come together to form the functional region of the nail, known as the cortex (1). The cortex consists of two elements, the inner element known as the intermediate filaments (IFs), and the outer, protective element known as the intermediate filaments associated proteins (IFAPs) bound together by disulphide bonds (2,3). Due to the lack of understanding of the relationship between the structure of the nail and its barrier properties, the development of novel topical antifungals for the treatment of nail disease is problematic.

Keratin of human nails, rhino horns and porcine trotters were studied by measuring the disulphide bond reduction, protein amide I structure and water uptake (% swelling) upon exposure to chemicals that modify the human nail structure including thioglycolic acid (TA) and tris(2-carboxyethyl) phosphine (TCEP). The barrier properties of the rhino horn was assed by measuring the permeation enhancing effect of thioglycolic acid on penetration of nitric oxide and rhodamine B as model compounds in rhino horn was tested with Franz diffusional cells.

Human nails, rhino horn and porcine trotters all showed the breakage of the keratin disulphide bond (S-S) through appearance of a S-H peak in the traces using TA and TCEP. However, ATR-FTIR showed little change in the amide I structure in the three tissues, with the exception of denaturation in the turn proteins in the human nail and the rhino horn after the application of TCEP. The human nail and the rhino horn showed minimal water uptake when treated with TCEP, whereas in the TA solution, they both swelled. The porcine trotter did not show any significant change ($p \leq 0.05$) in the swelling upon chemical treatment. Nitric oxide permeation in TA solution (5%w/w) showed a flux ($p \leq 0.05$) across rhino horn (3.02 ± 0.058 mM/cm2/hr) in comparison to nitric oxide alone (0.894 ± 0.12 mM/cm2/hr). Rhodamine B in TA solution showed a superior flux ($p \leq 0.05$) across rhino horn compared to rhodamine B alone (0.208 ± 0.43 mM/cm2/hr and 0.11 ± 0.017 mM/cm2/hr respectively).

The study findings suggested that the disulphide bonds in keratin tissues, regardless of their origin, could be broken using TA and TCEP. As the secondary structure of the protein was largely unchanged by TA, but it increased the tissue swelling, this data suggested that it modified the tissue through the IFAPS. In contrast, TCEP modified the secondary protein structure and had less effect on tissue swelling suggesting a larger impact compared to TA on the IFs. Opening the keratin tissues disulphide bonds decreased its barrier properties and this effect was larger with a small molecule such as NO.

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27.Knowledge, Attitude and risk perception in Oral Isotretinoin use: A cross sectional study from Jordan

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Purpose: The most prevalent skin condition is acne vulgaris. Recent clinical practice guidelines recommend oral isotretinoin to treat moderate-to-severe acne. The aim of this study is to assess patient knowledge and use of oral isotretinoin for acne treatment. This is a cross-sectional descriptive study conducted in the country of Jordan.

Methods: The study sample includes people resident in Jordan aged ≥ 14 years who have been treated with oral isotretinoin for acne. The study involved 373 participants who previously used oral isotretinoin for skin disorders.

Results: Most were Jordanian (89.3%), aged 19-25 (37.3%), and from the central region (82.8%). Mostly, they used isotretinoin for severe or mild acne (25.2% and 24.1%, respectively), Rosacea (4.1%), or to alleviate acne scars. Surprisingly, 58.1% did not consult their specialist for side effects, and 20% shared their treatment. The average proper use score was 9.98 out of 16. A link was found between higher risk knowledge scores and proper use scores. Side effects like nausea, irregular heartbeat, and pancreatitis affected some users (11.5%, 10.5%, 7.0%, and 3.2% respectively). Knowledge about isotretinoin's risks varied, with percentages recognizing teratogenicity (57.7%), liver damage (52.6%), lipid profile effects (37.2%), while 25% believed it had no side effects.

Conclusion: The study revealed partial adherence to oral isotretinoin guidelines, with gaps in monitoring and consultation. A positive correlation emerged between risk knowledge and proper usage, emphasizing the need for comprehensive education and monitoring strategies in isotretinoin therapy for skin disorders.

28.Optimization of the TR146 cell model for buccal permeation

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Purpose

The study of buccal permeation is challenging due to limited availability of human buccal tissues and difficulties in obtaining fresh and intact porcine buccal tissue. The focus of this work is to develop and optimise a TR146 cell model for buccal permeation. The performance and integrity of the model is generally assessed by measuring the barrier integrity via transepithelial electrical resistance (TEER).

Methods

TR146 cells were cultivated under six different conditions. The cultivation of TR146 in Ham F12 media and DMEM media was investigated. Differentiation of the TR146 cells was triggered by exposing the top layer of cells to air – termed "airlift". The effect of differentiation markers such as Human Keratinocyte Growth Factors (HKGS) was also studied with regards to improving the permeation barrier. TR146 cells were seeded onto Transwell® plates at a density of 1×10^5 cells/mL. 0.2 ml of the cells were suspended in the media and transferred into the apical compartment or inserts of the Transwell® plates. 1 ml of complete media was then added to the basal compartment of the Transwell® plate. The plates were then placed in an incubator at 37°C with the media changed for basal and apical compartments every 2-3 days. After 5 days, some cells had the media removed from the apical compartment to trigger differentiation. The barrier integrity of the cells in these conditions was analysed over a period of 1 month by measuring TEER values.

Results

The use of DMEM media resulted in consistently higher TEER values than observed for cells that were cultivated in Ham F12. The difference in TEER values of cells that were subject to airlift and submerged was statistically insignificant ($P > 0.05$) when the Ham F12 and DMEM media was used. The supplementation of 1% HKGS to complete DMEM media in which the cells were airlifted after day 5 resulted in the highest observed TEER at 243 $\Omega*cm^2$. This TEER was significantly higher ($P < 0.05$) and was obtained on day 27 after which the TEER values plateaued.

Conclusions

Optimising the TR146 cell model is important for studying buccal permeability and drug delivery. TR146 cells have historically proven to be leakier than porcine buccal tissue therefore developing a model with optimal barrier integrity is critical before new *in vitro* models may be developed. This work has concluded that the addition of HKGS and airlifting the cells results in a significantly improved barrier integrity that should mimic better the *in vivo* buccal environment.

29.Evaluation of Algae Extract-Based Nanoemulsions for Photoprotection against UVB Radiation: An Electrical Impedance Spectroscopy Study

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Skin cancer is one of the most common types of cancer globally and UVB radiation is a significant risk factor for its development[1]. To prevent skin cancer, there is a continuous research for finding suitable photoprotective ingredients from natural sources within formulations that are also environmentally friendly. Additionally, there is a growing interest in nanoemulsions due to the versatility of incorporating different lipophilic substances in cosmetic formulations. In this work oil-in-water nanoemulsions containing potentially photoprotective marine macroalgae extract from the Colombian Caribbean were developed and characterized. For this, a Box-Behnken experimental design was used for establishing the optimal formulation composition and the physical properties such as droplet size, polydispersity index (PDI) and zeta potential were evaluated by dynamic light scattering (DLS). Nanoemulsions showed an average droplet size of 128.5 ± 8.6 nm, a PDI of 0.25 ± 0.06 and zeta potential of 45.14 ± 0.02 mV. To evaluate the photoprotection capacity of the formulations, we utilized electrical impedance spectroscopy (EIS) to assess alterations in the electrical characteristics of excised pig skin membranes placed in Franz cells equipped with a 4-electrode set-up. The in vitro methodology developed in our previous study[2] was used, involving both UVB irradiation (180 J/cm2) and oxidative stress conditions. Oxidative stress was achieved by adding hydrogen peroxide (1 mM H2O2) as a source of reactive oxygen species, while sodium azide (10 mM NaN3) was used as an inhibitor of the antioxidative enzymes such as catalase, which are naturally present throughout the epidermis. The initial skin membrane integrity was examined prior to exposure to UVB and oxidative stress conditions with the purpose of establishing reference values. Next, a standard dose of formulation (2 mg/cm2) was topically applied. Three different controls were included: 1) no product on top (nothing applied), 2) a nanoemulsion without algae extract (only vehicle), and 3) a commercial sunscreen with SPF 100. The photoprotective capacity of the oil-in-water photoprotective nanoemulsions was statistically significant compared with the control groups. In general, 15% of change in the resistance of the membrane was observed when using the formulation containing algae extract, while a 50% change was obtained for the vehicle. Therefore, a suitable nanoformulation based on natural ingredients was developed and its promising photoprotective capacity was demonstrated.

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30.Characterization of metronidazole for topical delivery to the skin

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Purpose

Rosacea is a chronic condition that includes redness of the face accompanied by pimples and pustules. Metronidazole is a nitroimidazole derivative commonly used to treat rosacea. The development of targeted and effective metronidazole skin preparations is important because long-term oral administration of this drug will cause tolerance and obvious side effects. The aim of this work are to (i) characterize the physicochemical properties of metronidazole and (ii) use a rational formulation design approach for the development and testing of novel metronidazole topical medicines.

Methods

An HPLC method suitable for metronidazole analysis was developed and validated. Thermal analysis was performed using the DSC Q2000 system (TA Instruments, USA) and TGA (TA Instruments, USA). The structure of metronidazole was determined by infrared analysis using ATR-FTIR (Perkin-Elmer, Massachusetts, USA). The distribution coefficient (Log $P_{o/w}$) of metronidazole in deionized water was determined by the Shake Flask method. Polyethylene glycol - 200 (PG 200), 1,3-Butanediol (BG), 1,2-Propanediol (PG), Hexylene glycol (HG), Benzyl alcohol (BA), Transcutol® P (TP), Isopropyl myristate (IPM), PBS, PBS (with 5% Brij O20) were selected as solvents for solubility experiments. The drug solubility was determined by adding excess metronidazole to different solvents and stirring for 48 h. In addition, the stability of the compound in selected solvents was determined by preparing a solution of 10 mg/ml in the relevant solvent. Samples were taken at 0, 24, 48, 72 and 96 h. For all solubility and stability experiments samples were placed in amber tubes and stored in a water bath at 32 ± 1 ℃.

Results

The HPLC method was successfully validated according to ICH guidelines with excellent linearity, precision and accuracy. Thermal analysis indicated that the melting point of metronidazole was 160.4 ℃ with weight loss commencing at 213.54 ℃. The ATR-FTIR results confirmed that the sample of metronidazole was consistent with the known chemical structure. The Log $P_{(o/w)}$ was determined to be -0.072. Metronidazole had the highest solubility in BA (87.86 ± 0.43 mg/ml) and TP (44.67 ± 0.31 mg/ml). The lowest solubility of metronidazole was in IPM, with a value of 1.2 ± 0.32 mg/ml. Over 96 h, metronidazole showed stability in methanol:water (40:60), TP, BA, and PBS.

Conclusion

The physicochemical properties of metronidazole were characterized and a suitable HPLC method was developed and validated. Candidate solvents were also screened in terms of solubility and stability of the drug. Building on these findings future work will focus on developing novel topical preparations of metronidazole for targeted delivery of the active to the skin.

31.Permeation of 2-phenoxyethanol in human skin – *in vivo* studies

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Purpose: A number of baby wipe formulations contain 2-phenoxyethanol (PE) as a preservative and cetylpyridinium chloride (CPC) as a surfactant with antimicrobial activity. However, no studies have reported the cutaneous absorption of PE and CPC from topical formulations in the scientific literature.

Methods: Previously, we reported the skin absorption of PE in porcine skin and human skin *in vitro.* In the present work, the permeation of PE from preparations with CPC and without CPC was investigated in human skin *in vivo*. The studies were conducted using Confocal Raman Spectroscopy (CRS) and tape stripping (TS).

Results: For the CRS studies, a higher area under the curve (AUC) of PE for the formulation containing CPC was observed in comparison with the formulation without CPC ($p>0.05$). The TS data indicated that the amount of PE recovered from tapes 1-6 for the preparation with CPC was higher compared with the formulation without CPC ($p>0.05$). These results align with the corresponding *in vitro* permeation data, where the PE solution with CPC showed greater permeation of PE compared with the formulation without CPC. Both the CRS and TS techniques have limitations for assessment of distribution of PE and CPC in the skin *in vivo*, primarily attributed to the Raman signal intensities of compounds under investigation and the variability in the amount of SC collected by TS.

Conclusions: While acknowledging the methodological constraints of CRS and TS, the results from the present study are consistent with *in vitro* permeation data, where the presence of CPC resulted in higher PE permeation. Additionally, the findings of the present study provide further support for the use of CRS for non-invasive evaluation of PE-containing products *in vivo*.

32.Introducing confocal Raman spectroscopy as a suitable alternative to tape stripping in skin penetration studies investigating bioequivalence

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Purpose: Since its publication in 2018, the EMA draft guideline on quality and equivalence of topical products has provided a precise guide for carrying out skin penetration tests. The method currently used for penetration studies is tape stripping as the gold standard, with the suggestion that at least two measurements should be taken [1-4]. Those measurements should include uptake measurements taken ideally in the steady state as well as clearance measurements. Furthermore, the draft guideline describes criteria by which two formulations can be assessed as bioequivalent, such as the 90 % confidence interval being within a range of 80 – 125 % of the comparator formulation [5, 6]. Yet tape stripping is an elaborate destructive method that lacks spatial resolution and also generates a high degree of variability due to the low precision of the results. Therefore, the aim of this study was to investigate the suitability of the non-destructive confocal Raman spectroscopy (CRS) as a more efficient substitute for tape stripping in skin penetration studies, especially concerning bioequivalence testing of topical products.
Methods: Effekton as an on-the-market gel containing ketoprofen was used as the reference formulation as well as a bioequivalent test formulation. By diluting Effekton 1:2 with carbomer gel, a non-bioequivalent test formulation was produced and tested for validation purposes as required by the EMA. The steady state was determined by measuring three points in time with the premise that the resulting increase in the total penetrated API amount over time is equal to 0 in the steady state. Those measurements also represent the uptake phase, while two further measurements were carried out for clearance, after the product was removed from the skin. For all points in time, all three formulations were measured to show the influence of reaching the steady state on bioequivalence determination. All formulations were applied to the skin and incubated for the predetermined time period. A 785 nm-wavelength NIR laser was used to perform CRS measurements. Ketoprofen concentration in the skin was investigated to a depth of 6,4 µm into the skin using 3 sec accumulation time and a spectrum acquired every µm. Afterwards, tape stripping was performed and the tapes sonicated in methanol to extract ketoprofen [7, 8]. The ketoprofen content of the resulting solutions was then quantified by HPLC [9]. Both methods were conducted on the same skin samples in order to demonstrate a direct correlation between tape stripping and CRS.
Results: This study shows a clear correlation of the total penetrated amounts measured by either tape stripping or CRS Similarly, bioequivalence was demonstrated with both methods. Furthermore, it was possible to show an influence of the selected incubation time with regard to reaching the steady state on the outcome of the bioequivalence test.
*Conclusions:*Overall, we were able to demonstrate that CRS is a suitable method for the investigation of skin penetration and is particularly suitable for bioequivalence studies.

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33.Green emollient/emulsifier based-emulsion with bioactive milk peptides: exploring the preliminary *in vivo* effects on skin properties

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Purpose: The burgeoning concerns among today's consumers regarding sustainability and personal and environmental safety have led to a continuous quest for new, improved, cleaner, safe, and efficacious skincare products derived from natural, environmentally friendly ingredients. In line with this, the present study aimed to formulate and evaluate the *in vivo* efficacy of innovative oil-in-water cosmetic emulsion comprising "green" emollient and emulsifier components, alongside milk protein hydrolysate as a model active. **Methods:** The selected model emulsion characterized by suitable rheology, stability and texture profiles, and with proved *in vitro* activity and safety, containing 30% of emollient (C15-19 alkane), 2% of mixed emulsifier (lauryl glucoside/myristyl glucoside/polyglyceryl-6 laurate) and 5% of bioactive milk peptides, was prepared by cold process and preliminary evaluated *in vivo*, in healthy human volunteers (2 males, 10 females, 45 ± 7 years). Using non-invasive bioengineering and visualization techniques, several skin biophysical and topography properties were assessed on the volar forearm, before (baseline) and 2 hours after active and placebo emulsions' application on assigned skin areas. The parameters measured were: stratum corneum hydration (SCH), transepidermal water loss (TEWL), erythema index (EI), melanin index (MI), skin pH value, roughness (SEr), smoothness (SEsm), and wrinkles (SEw). **Results:** After the conducted short-term study, both investigated emulsions, with and without milk protein hydrolysate, demonstrated improvement in skin hydration and appearance. While no significant modifications in TEWL, EI, MI, and pH values were seen, a significant ($p < 0.05$) increase in SCH (20%–30%) and SEr (about 10%) values was demonstrated after emulsions' application, when compared to parameters' baselines and non-treated control, proposing preserved skin barrier function, good skin tolerability, and enhanced skin hydration and smoothness. In addition, there were no significant differences detected in the evaluated skin parameters' changes between active and placebo emulsions, probably due to the short evaluation time. **Conclusions:** The present preliminary findings indicated a promising potential for the developed "green" emulsion containing bioactive milk peptides as a skincare product. However, the ongoing long-term efficacy study will help to clarify the benefits of this formulation, contributing to our understanding of its suitability as an effective skincare option.

34.Fluid-dependent release and permeation kinetics of timolol from intelligent hydrogel patches for wound healing

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Purpose

Hydrogels are popular for wound management and mostly used in a hydrated state, in which they exhibit high flexibility [1]. In contrast, dry hydrogels have higher absorption capacities for exudates but typically behave very brittle, making them inapplicable. To address these challenges, the novel, intelligent hydrogel patches with tunable stiffness and water uptake can be used in dry and hydrated states [2]. The vehicle is combined with the β-adrenergic receptor antagonist timolol to obtain a controlled drug delivery system that promotes re-epithelialization and wound healing [3].

Methods

The hydrogels were developed by copolymerization and crosslinking of N-isopropylacrylamide and oligo(ethyleneglycol) comonomers [2]. The drug loading of the material was performed by swelling in an aqueous solution of timolol maleate. The hydrogel was used in the hydrated or conditioned state after additional equilibration at a defined humidity of 25 % r.h.. In vitro drug release and permeation studies were performed over 48 hours using Franz diffusion cells equipped with synthetic membranes or porcine skin, respectively. To further investigate the behavior of the patches on wounds, the skin was injured by removal of the outermost skin layers (0.2 mm) using a dermatom. The absorption and release of fluid was analysed on the basis of the calculated degree of swelling Q.

Results

Drug release from the hydrogel in the hydrated state followed Higuchi kinetics [4], whereas the conditioned patch showed a biphasic, swelling-dependent release mechanism. Drug permeation over the non-wounded skin occurred only from the hydrated hydrogel during the period under consideration, which reached a steady state after 24 hours. Due to the lack of barrier function in the wounded skin, faster drug permeation was observed. The absolute amount of permeated drug over the wounded skin resulted in a similar order of magnitude for the hydrated and conditioned hydrogels. The present moisture from the skin was absorbed, which accelerated the swelling, release and permeation rates.

Conclusions

The possibility of using hydrogels in a hydrated and conditioned state was shown to be feasible in individual wound management. Depending on the amount of fluid, swelling and deswelling of the hydrogel and thus controlled drug release is possible, providing different pharmacokinetic patterns. This enables individualised drug release according to the needs of the wound and its moisture status. Moreover, moisture management provides a microenvironment and favorable conditions for tissue regeneration.

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35.Enhancing Local Effectiveness of Baricitinib (BNB) in Topical Diseases Treatment: A Permeation Study

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Baricitinib (BNB) is an orally administered immunosuppressant that selectively targets specific molecules, such as Janus kinase 1 and 2, resulting in reduced inflammation, cell activation, and immune cell proliferation. BNB is currently utilized in the treatment of various conditions, including moderate to severe atopic dermatitis (AD), rheumatoid arthritis, and COVID-19, with recommended oral doses ranging from 2 to 4 mg per day.

In the context of AD treatment, BNB has shown effectiveness in improving skin symptoms, often noticeable from the first day of treatment. With the purpose to avoid systemic effects from the immunosuppressant BNB and to enhance its local effect, five different chemical enhancers have been explored to facilitate skin penetration and retention: Nonane, Lauryl acrylate, Squalene, Sebacic acid and menthol. A novel approach involves direct application to the skin.

For our study, permeation and diffusion of different solutions containing BNB at a concentration of 2 mg/mL, with the addition of 5% of the permeation chemical enhancers and the through human skin were assessed using Franz diffusion cells obtained from Crown Glass Company, Inc. (NJ, USA). Following completion of the permeation study, tissue samples were meticulously cleansed with distilled water to eliminate any residual BNB solution on the tissue surface. BNB retained in the tissues was extracted by excising the permeation area, which was then immersed in 1 mL of Transcutol® P and subjected to sonication for 10 minutes using an ultrasonic water bath. The resulting supernatant was subsequently filtered and quantified via HPLC with a UV/Vis detector.

Results showed that among the enhancers tested, lauryl acrylate demonstrated the highest retention of BNB in the skin, multiplying the retention of BNB on the skin up to 3 times from a BNB solution without enhancers, followed by sebacic acid and menthol.

This finding holds promise for improved treatment outcomes by limiting the amount of permeated drug while effectively retaining it on the skin surface, thereby minimizing systemic effects.

36.Skin permeability of niacinamide and its effects on stratum corneum hydration and molecular structure.

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Purpose

Niacinamide (NAM), or nicotinamide, is a common cosmeceutical used in various skincare products. Clinical studies utilizing skincare formulations containing NAM have indicated that this compound demonstrates antioxidant properties, enhances skin hydration, and promotes improvements in skin appearance. However, the precise mechanisms underlying these beneficial effects are not yet fully understood. In this study, we investigate the influence of pH on the permeation and skin distribution of NAM in human skin and 3D skin models. In addition, the effects of NAM on stratum corneum (SC) hydration and molecular structure are investigated.

Methods

MatTek EpiDerm models and full-thickness human skin were utilized to investigate the permeation of NAM (5% concentration, infinite dose, Franz cell set-up, 24h). The effect of pH was studied by employing either citrate buffer saline (CBS, pH 5.0) or phosphate buffer saline (PBS, pH 7.4) as donor solutions. The water uptake of SC samples was examined with dynamic vapor sorption (DVS). The molecular structure of the SC lipid matrix and keratin filaments was analyzed through small- and wide-angle X-ray scattering (SWAXS). In these measurements, the SC samples were pretreated for 24h by incubation in the donor solutions, with and without NAM.

Results

The findings from the permeation study demonstrate that pH significantly impacts the permeability of NAM across both human skin and 3D skin models. At pH 5.0 (CBS), a significantly lower NAM permeation is observed compared to pH 7.4 (PBS). The DVS data (n=5) show that pretreatment with NAM solutions enhances the SC water uptake at 95 % relative humidity (RH) and this effect is consistent irrespective of pH and buffer salt content. The SWAXS data (n=2) show that pretreatment with NAM has little effect on the SC lipid matrix, while a clear effect is observed on the soft keratin structure. In brief, NAM results in increased swelling of the spacing between keratin filaments when the water content of the SC is low (60% RH). However, when the SC hydration is high (95 % RH), this effect is obscured by swelling due to water uptake.

Conclusion

Our results show that skin permeation of NAM is significantly affected by pH, underscoring the relevance of adjusting pH in skincare products for optimal NAM delivery. The DVS data show that pretreatment with NAM formulations increases skin hydration levels. Moreover, the SWAXS results offer novel insights into the impact of NAM on the SC molecular structure, showing that NAM selectively interacts with keratin filaments by inducing a state akin to hydration without direct water involvement.

37.Assessing Dose Influence on the Rheological Properties of Hidroxytirosol Ointments

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Purpose:
The present work reports the effect of Hydroxytyrosol (at different doses) on the rheological properties (viscosity and viscoelasticity) of a model hydrophilic (polyethylene glycol) waterless ointment [1].
Methods:
The model ointment contains polyethylene glycol (PEG)-1500 (15%), PEG-400 (75%), and virgin olive oil (10%). The doses used were low (1 %), medium (2.5 %), and high (5 %). Four ointments (placebo PP, PH1, PH2.5, and PH5) were prepared under the same conditions at 60 ºC.
The viscosity and oscillatory experiments were performed at 25°C in a controlled stress Kinexus Lab+ Rheometer (Malvern) using a cone-plate geometry (CP-40). In the viscosity method, 10 samples were taken per decade while the shear rate ranged from 0.1 to 100 s^{-1}. The frequency sweep method was conducted between 0.1 Hz and 100 Hz, with a shear strain of 0.2%.
Viscosity, storage *modulus* (G´), and loss *modulus* (G´´) values were statistically evaluated through ANOVA coupled with a Tukey-Kramer analysis ($\alpha = 0.05$).
Results:
Table 1 summarizes the results. All the Tukey p-values for viscosity were minor to 0.05,
Table 1. Viscosity, viscoelasticity parameters and ANOVA results.

	PP (mean ± SD)	PH1 (mean ± SD)	PH2.5 (mean ± SD)	P5 (mean ± SD)	ANOVA p-value
Viscosity (at 1 s^{-1})	39.23 ± 2.26 (n = 4)	53.11 ± 2.34 (n = 3)	30.98 ± 2.99 (n = 3)	21.08 ± 2.54 (n = 3)	5.66 E-07
Storage *modulus* (G´) (at 1 Hz)	386.7 ± 21.4 (n = 3)	143.3 ± 18.5 (n = 2)	131.8 ± 24.8 (n = 2)	136.4 ± 25.7 (n = 2)	1.14 E-04
Loss *modulus* (G´´) (at 1 Hz)	826.2 ± 51.8 (n = 3)	212.2 ± 27.1 (n = 2)	242.7 ± 28.9 (n = 2)	192.4 ± 19.7 (n = 2)	1.88 E-05

especially between PH1 and PH5. The introduction of Hydroxytyrosol in the model formulation decreased viscosity at low and medium doses but increased it at high dose. Viscosity within the active group increased with the dose. For all ointments, G´´ values were higher than corresponding G´ values, which indicates a dominant liquid-like behaviour. The Tukey p-values for viscoelastic parameters were less than 0.05 between the placebo and the three active ointments.
Conclusions:
Hydroxytyrosol affected the viscosity and viscoelasticity of the model placebo ointment. The higher the dose, the higher the viscosity. Hydroxytyrosol significantly decreased the two viscoelasticity parameters when compared to the placebo.

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38.Bicontinuous cubic mesophases for epicutaneous patch testing of contact allergy

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Patch testing for allergies is a common diagnostic method used to identify sensitivities to various substances. However, traditional carriers like petrolatum, commonly employed in patch testing, encounter challenges in dissolving hydrophilic substances such as salts[1]. This limitation hampers the effectiveness of patch testing procedures. Lyotropic liquid crystalline mesophases offer a promising alternative due to their ability to accommodate both hydrophobic and hydrophilic substances[2]. In this study, we investigate inverse cubic mesophases formed by glycerol monooleate (GMO), diglycerol monooleate (DGMO), and water as potential vehicles for nickel patch testing.

In vitro release experiments were performed to determine the release profile of the salt from the formulation. In a clinical setting, one of the challenges encountered is the necessity for the formulation to possess suitable viscosity to ensure simple syringe application onto the patient's skin. Therefore, we have benchmarked our formulations versus petrolatum by determining the force required to handle each vehicle using a texture analyzer. Structure-rheology relationships and effects from adding DGMO were further evaluated using SWAXD and a Bohlin Rheometer.

Release experiments reveal a higher nickel release from lipid-based carriers compared to petrolatum indicating the potential to lower the dose and shorten the application time of the patch on the patient from today's 48h to less than a day. Texture analysis showed that the addition of DGMO leads to lower forced required for handling the formulation.

We hypothesize that bicontinuous cubic mesophases can serve as a universal vehicle for patch testing of a wide range of allergens with varying physicochemical properties. Next, we will initiate a clinical trial on patients with potential nickel allergy.

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39.Anti-inflammatory efficacy of flurbiprofen nanosuspensions

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Purpose: The objective of this study is to assess the anti-inflammatory efficacy of flurbiprofen nanosuspensions for topical application. Flurbiprofen was encapsulated within poly-ε-caprolactone (PεCL), further stabilized using poloxamer 188. Then, the formulations were either freeze-dried (FD) or lyophilized and sterilized (IR), using two different cryoprotectants trehalose (TRE) or PEG 3550 (PEG), resulting in a total of four formulations: (1) freeze-dried nanoparticles with trehalose (FD-NSTRE), (2) lyophilized and sterilized with TRE (IR-NSTRE), (3) freeze-dried nanoparticles with PEG (FD-NSPEG) and (4) lyophilized and sterilized with PEG(IR-NSPEG).

Methods: The anti-inflammatory effects of each NS were evaluated by topically applying 12-O-tetradecanoylphorbol-13-acetate (TPA) on mouse ears to induce oedema formation. The experiment involved groups of six adult male Wistar CD-1 mice weighing between 20 and 25 g. A volume of 50 μL of the NSs, along with the standard drug indomethacin as a reference, was applied simultaneously to both sides of the right ear along with TPA. All experiments were conducted in compliance with NOM-062-ZOO-1999 and approved by the Academic Committee of Ethics of the Vivarium of the Autonomous University of the Morelos State of Mexico (Approval No. 0122013).

Results: FD-NSPEG and FD-NSTRE notably decreased inflammation triggered by TPA, exhibiting approximately twice the inhibition compared to both the reference drug indomethacin and sterilized formulations.

Conclusions: It can be concluded that the anti-inflammatory capacity of flurbiprofen stands out in effectiveness in the lyophilized formulations: FD-NSTRE and FD-NSPEG.

40.A New topical formulation of baricitinib for the treatment of psoriasis.
***In vivo* studies in mouse models**

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Purpose:

Baricitinib (BCT) has been widely used in dermatology as a new molecular-targeted therapy. Increasing evidence suggests that baricitinib is effective against atopic dermatitis, alopecia areata, psoriasis, and vitiligo[1]. Psoriasis is an autoimmune, recurrent inflammatory skin disease with an estimated incidence of 2 % of the world's population[2]. Many inflammatory dermatoses are driven by inflammatory mediators that rely on JAK's (Janus Kinases)/STAT's (Signal Transducers and Activators of Transcription Signals), and the use of JAK inhibitors has become a new strategy for the treatment of diseases for which conventional drugs have not been effective[1]. The JAKs transduce cytokine signalling through the JAK-STAT pathway, which regulates the transcription of several genes involved in inflammatory, immune, and cancer conditions. Targeting the JAK family kinases with small-molecule inhibitors has proved to be effective in the treatment of different types of diseases. The purpose of this work is to test a new topical formulation of BCT for the treatment of psoriasis in an *in vivo* mouse model by evaluating the biomechanical properties of the formulation developed.

Methods:

T4 formulation was selected by factorial design and has the following composition: Transcutol®, Labrafac-lipophile® 1349, Lauroglycol® 90 and Surfadone® LP100. This work was conducted to investigate the effectiveness of T4 for the treatment of psoriasis on BALB/c mice. The experimental group was made up of 3 groups (5 mice for each group): negative control group mice were untreated healthy animals (no formulation or imiquimod) and positive group mice were induced psoriasis with 5 mg/ml imiquimod (IMQ) topically applied once daily for 6 consecutive days. The third group of mice were induced psoriasis with topical 5 mg/ml IMQ for 6 days and then treated with T4 formulation (5 mg/ml) once daily for another 6 days. On day 13 of this study, we measured the biomechanical properties on the dorsal skin of mice using DermaLab (Cortex Technology, Aalbord, Denmark) to evaluate the Trans Epidermal Water Loss (TEWL) and a Corneometer CM-825 (Courage & Khazaka Electronics GmbH, Germany) to assess the Stratum Corneum Hydration (SCH).

Results:

The results showed a significant statistical difference ($P < 0.05$) in TEWL versus positive control and negative control. Also, there was a significant difference between the positive control and T4. However, no significant difference was observed between the negative control and T4 ($P > 0.05$). The hydration results (SCH) showed a significant difference between positive control and negative control and a significant difference was observed between the negative control and T4, but no significant difference was observed between the positive control and T4.

Conclusions:

According to the obtained results, it was shown that T4 recovers the properties of the stratum corneum and improves skin hydration. However, further investigation should be conducted on this model.

41.Can assessment of key critical quality attributes predict human sensorial perceptions?

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In topical drug product development, a positive sensorial experience, when applying the product on the skin, can play a vital role in patient perception and acceptance. Human sensorial panel tests can be pricey, and have challenges associated with training human subjects, and the outcomes can be subjective in nature. In this work, we aimed to study the possibility of predicting sensory attributes of topical semi-solid gels using in vitro instrumental tests characterizing physical critical quality attributes (CQAs), which may relate to the sensorial behaviour of products during use.

Eight gels manufactured with hydroxyethyl cellulose (HEC) or carbomer homopolymer (Carbopol® 980P (CBP)) were selected from 26 gel formulations to conduct sensory panel test. Specifically, for in vitro assessments, rheological tests were performed by an AR-G2 rheometer. Texture and frictional properties of the gels were examined by a TA.XTplus texture analyzer and a HR1 Discovery Tribometer, respectively. An infrared thermal imaging (IRT)-based technique was used to assess in vitro cooling potential of the gels. For in vivo sensory panel test, skin biophysical properties of 46 subjects (n=46, ethics ID: 2020/HE001995) were firstly examined using a non-invasive Courage + Khazaka (C+K) instrument. Then, subjects were trained on the concepts and assessment criteria of the 6 different sensory attributes classified as during and after-feel sensations. To start the panel test, at time 0, 25 µL of each gel sample was placed onto a marked forearm area (19.6 cm^2) of each subject, and spread by subject's forefinger at rotational speed of 1 circle/s for 15 s. After 15 s, subjects stopped spreading, and assessed cooling sensation, shine and slipperiness. The after-feel attributes of stickiness and smoothness were evaluated after waiting for 1 and 2 min, respectively. Gel sensorial attributes were evaluated by a continuous 1-9 scale, from very low (1) to very high (9) intensity. The results from the in vivo sensory panel tests were compared with the in vitro characterization data and formulation composition of the gels to understand the correlation (if any) between formulation, in vitro characterization data and sensory observations.

Skin biophysical parameters (by the C+K) exhibited high interindividual variability, representing a true sample from the general population. The two sets of control samples (HEC-08 and CBP-02, blinded replicates) were perceived consistently, by the subjects despite huge differences in skin biophysical properties among 46 subjects. Cooling sensation, shine and smoothness sensory attributes of gel formulations were perceived with only slight differences across the 8 gels. However, dissimilarities in spreadability, slipperiness and stickiness perceptions between the gels were sensed well by the subjects, which meaningfully correlate with the formulation composition (e.g. polymer and ethanol content) and in vitro characterization data such as coefficient of friction (tribology), rate of evaporation (cooling in vitro) and stringiness (texture profile).

The findings show that the CQAs of topical gels assessed instrumently in vitro may be valuable for understanding and predicting some of the sensorial characteristics of these formulations following application. Significant differences in instrumental attributes, such as rheological, tribological behaviour and texture properties are likely to be perceptible to human subjects.

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42.Effects of selected ionic liquids on the permeability of human skin

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Transdermal drug delivery provides advantages such as bypassing drug metabolism and improved patient compliance. However, this delivery method is limited by its ability to penetrate the skin barrier [1,2]. Ionic liquids (IL) have great potential to facilitate transdermal and topical drug delivery [3]. The aim of the study was to investigate the effect of two imidazolium based IL: 1-methyl-3-octylimidazolium bromide (C8MIM) and 3-dodecyl-1-methylimidazolium bromide (C12MIM) to the permeability and retention of Theophylline (TH) and Diclofenac Sodium (DIC) through human skin. Two different approaches were used; initially, IL were co-applied with the model permeants and in a second experiment human skin was pretreated with IL before the application of the permeants. Co-application of IL with TH indicated that both IL significantly enhanced the permeability of the permeant in comparison with the control (no IL) while C12MIM was a significantly more potent permeation enhancer than C8MIM. No significant differences were observed for the skin retention of TH between the two IL and the control samples. On the other hand, co-application of IL with DIC indicated that the two IL are not favoring the permeation of the permeant against the control while C8MIM resulted significantly higher skin retention of DIC in the skin tissue in comparison with C12MIM. Both IL deposited significantly higher amounts of DIC in the skin over the control. When human skin was pretreated with IL before the application of the permeants, the permeation of both model compounds was significantly higher for the samples pretreated with C12MIM over those pretreated with C8MIM and the controls. In addition, TH amount in the skin was significantly higher when the tissue was pretreated with C8MIM but not when it was pretreated with C12MIM. No significant differences were found between the DIC amount in the skin tissue regardless the pretreatment with IL or not. Thus, the effects induced by IL showed drug as well as application specificity (co-application or pre-treatment). Further studies are required to explore the interactions of IL with skin lipids and to understand the full potential of IL in the field of transdermal and topical drug delivery.

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43.Quantitative assessment of topical formulations with in vivo Raman spectroscopy

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The in vitro conditions and the physiological aspects in skin permeation testing are generally very different from in vivo conditions. Therefore, the need exists for methods to assess the permeation of topical formulations in the skin in vivo. Confocal Raman spectroscopy is a well-established technology, which enables in vivo measurements of concentrations of skin constituents and topical formulations with a high spatial resolution. The gen2-SCA (RiverD International, Rotterdam, The Netherlands) is a dedicated instrument that employs confocal Raman spectroscopy for quantitative measurement of the permeation of topical formulations through the stratum corneum in vivo.
Intrinsic skin constituents and ingredients of topical formulations have unique Raman signals. For two materials the ratio of the signal intensities is proportional to their mass ratio. For skin permeation measurements we have chosen protein as one of the materials, which makes the signal ratio material/protein of a Raman spectrum proportional to the mass ratio, g material per g protein. Based on this principle we have established a collection of reference spectra of various ingredients of topical formulations. In addition, we determined the proportionality factors for conversion of Raman signal ratio to material/protein mass ratio. Multiplication by the protein concentration as a function of depth in the stratum corneum resulted in quantitative concentration in g/cm3. We used the gen2-SCA to rapidly measure in vivo Raman spectra across the stratum corneum before and after topical application of various formulations. Quantitative concentration profiles as a function of depth in the stratum corneum were created with the analysis suite SkinTools (RiverD). The quantification method is applicable to materials that are soluble in water, ethanol, or other solvents. The methods to quantify materials in the skin and to determine the limit of detection, as implemented in SkinTools, have been published1,2. Integration of concentration profiles over the thickness of the stratum corneum yielded the total amount of material in the stratum corneum. Monitoring of the total amount as a function of time resulted in an in vivo flux through the stratum corneum.
We will discuss the method of quantification of topical materials and the methods to determine in vivo flux and limit of detection. We present examples of in vivo permeation results using confocal Raman spectroscopy of caffeine and other topical ingredients. The measurements are performed in vivo and directly on the human skin. Moreover, skin permeation, bioavailability and flux can be measured at the anatomical location where a product will be applied. Product penetration and flux vary widely between different anatomical locations.
In vivo confocal Raman spectroscopy enables quantitative assessment of skin permeation of topical formulations. The method has great potential to assess in vivo dermal bioavailability and bioequivalence.

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44.Exploring Baricitinib Efficacy in Alopecia Management: A Focus on Targeted Immunotherapy of Baricitinib in an Olive oil-based Formulation Rich in Hydroxytyrosol

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- **Purpose**: Conventional systemic treatments for alopecia typically include glucocorticoids or immunosuppressants. Baricitinib (BCT), a potent Janus kinase (JAK) 1 and 2 selective inhibitor, has recently been approved for alopecia areata management. However, the treatment has some side effects that could be avoided by a topical application. For this, the purpose of this study was to test the efficacy of a topical Baricitinib formulation based on extra virgin olive oil rich in hydroxytyrosol and its potential in treating alopecia. Extra virgin olive oil (EVOO) is known for its antioxidant and anti-inflammatory properties and its capacity to moisturise the skin promoting natural skin's barrier.

- **Materials and Methods**: BCT was dissolved in Transcutol® P and mixed with EVOO rich in hydroxytyrosol. Permeation tests were conducted on Franz cells with human skin, followed by sample collection within 24 hours and analysis via HPLC. Rheological measurements, including viscosity and flow behaviour, were determined using a Haake Rheostress 1® rheometer through rotational tests. Finally, the formulation was tested on HLA-DQ8-Dd-villin-IL-15tg mice. Two drops (50 µL) of the EVOO-formulation were applied daily on the mice's back and head using a Pasteur pipette for three weeks.

- **Results**: In this study, the viscosity of the formulation was found to be consistent, exhibiting Newtonian behaviour. Investigations into the penetration of BCT through human skin revealed a gradual absorption rate with preserved skin integrity. Notably, the EVOO-based formulation displayed excellent capacity in retaining the drug in the skin (Q_{ret} = 468.75 ± 54.38 ng/cm^2). The results of in vivo experiments revealed remarkable tolerance and substantial improvement in hair growth in mice following the application of the formula. Furthermore, the formulation based on Extra Virgin Olive Oil (EVOO) showed no alteration in the skin's biophysical properties, thereby confirming its suitability as a safe topical agent.

- **Conclusion**: The study highlights the potential of a formulation composed of EVOO and BCT to promote hair growth for alopecia treatment offering a safe and effective topical.

45.Assessment of the Biomechanical Properties and Cytotoxicity of an Anti-inflammatory Gel

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Purpose: The main objective of this study was to assess the biomechanical characteristics on the flexor side of the left forearm following the application of a gel formulation with an anti-inflammatory active. The gel, based on Lutrol, was prepared and loaded with pranoprofen (Pra-Gel), a potent NSAID used to alleviate inflammation, fever, and pain. Additionally, this study aimed to investigate the cytotoxicity of the gel.

Methods: Transdermal water loss (TEWL) and stratum corneum hydration (SCH) have evolved as biomechanical metrics. The transepidermal water loss (TEWL) was measured using a DermaLab® module. The measurements of stratum corneum hydration (SCH) were carried out on ten volunteers by a Corneometer® 825. MTT cytotoxicity assay was carried out employing human keratinocytes cell line, HaCaT.

Results: After applying Pra-Gel to the skin, a statistically significant reduction in Transepidermal Water Loss (TEWL) values was observed, indicating an occlusive effect without compromising skin integrity. However, Stratum Corneum Hydration (SCH) experienced a slight increase. Furthermore, there were no visible signs of skin irritation when Pra-Gel was applied to the volunteers' skin, suggesting good tolerance of the hydrogel. Additionally, the impact of various concentrations of Pra-Gel on human keratinocytes was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium cytotoxicity assay. After 24 hours of incubation, it was noted that the tested dilution of the formulation (1/400) did not affect cell viability, which remained close to 95%.

Conclusions: The *in vitro* cytotoxicity findings suggest that Pra-Gel does not induce significant toxic effects on cells, indicating high biocompatibility with the human keratinocyte cell line (HaCaT). These results were corroborated by evaluating the biomechanical properties of the skin, including Transepidermal Water Loss (TEWL) and Stratum Corneum Hydration (SCH) following the application of Pra-Gel.

46.Harnessing the Power of Platelets: Platelet-Based Gels for Enhanced Wound Healing in Diabetic Foot Ulcers

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Purpose Chronic wounds, such as diabetic foot ulcers (DFUs), pose significant challenges in for healthcare systems worldwide due to their complex aetiology and limited treatment options. With a growing emphasis on innovative therapies the research community is turning to novel treatment options from advanced dressings to cell-based therapies. Platelets and their derivatives have been explored for their healing properties. It is particularly the growth factors released from activated platelets (α-granules), that have been shown to play a role in cell proliferation, angiogenesis, and differentiation, thus tissue healing, remodelling and regeneration. The aim of this work was to investigate the potential of leukocyte and platelet-rich plasma gels as innovative therapies for promoting wound healing in DFUs.

Methods Two platelet-based gels were investigated – a standard platelet-rich plasma (PRP) gel prepared using a double spin centrifugation process, and a commercial leukocyte and platelet-rich plasma (L-PRP) gel (RAPID^{TM}). The standard PRP gel was prepared using a double spin centrifugation procedure. Whole blood was spun at 220 g for 10 min upon which the PRP layer was gently removed, the remaining two layers were spun again in a fast spin (2000 g for 10 min) which resulted in a platelet-poor plasma layer. To generate the RAPID L-PRP gel a pre-programmed Angel centrifuge was used to separate the blood into its respective layers. To generate the PRP or L-PRP gels, the respective PRPs were combined with autologous or recombinant human thrombin and vitamin C. Calcium chloride was used in some experiments as an adjuvant platelet activator alongside thrombin. Rheological analysis and thromboelastography were used to characterize the gels, while *in vitro* cell proliferation assays on human immortalized keratinocytes (HaCat cells) and growth factor release studies were conducted as indicators of therapeutic efficacy.

Results The PRP and L-PRP showed supraoptimal platelet concentrations ($7.23 \pm 3.27 \times 10^8$ and $8.60 \pm 4.85 \times 10^8$ cells/mL, respectively). RAPID L-PRP gels demonstrated faster gelation compared to the standard formulation (33 ± 10 vs. $158 \pm 10\ s$). Both formulations showed substantial quantities of PDGF-bb and VEGF, with the standard gels containing 229 ± 56 mg/mL and 534 ± 172 pg/mL of PDGF and VEGF, respectively, and RAPID releasing 322 ± 154 mg/mL and 446 ± 119 pg/mL of these growth factors. Thromboelastography revealed that the combination of autologous thrombin and calcium chloride resulted in the strongest and fastest-forming PRP gel. All tested dilutions of the fluid released by PRP stimulated HaCat cell proliferation, with 5-15% releasate inducing a 2.7-8.9-fold increase in cell growth over 48 h.

Conclusions The findings of this study highlight the potential of platelet-based gels, as promising therapies for promoting wound healing in DFUs. These gels show substantial release of key growth factors that are instrumental in tissue regeneration. Further studies are needed to assess their efficacy and optimize their application in clinical settings, but findings to date offer new hope for patients suffering from chronic wounds, including DFUs.

47.EFFECT OF ULTRASOUND ON SKIN PERMEATION OF CLINDAMYCIN HYDROCHLORIDE

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The aim of this study was to apply the sonophoresis technique to the hydrophilic molecule clindamycin hydrochloride to increase its penetration through human skin. According to the physicochemical properties of this active, it is difficult to penetrate the skin, and low frequency ultrasound (US) is an effective physical mechanism for skin permeation enhancement (Mitragotri *et al.* 1995a).

METHODS

Ex vivo percutaneous studies were carried out using vertical diffusion cells with automatic sampling (Microette R, Hanson Research). Two sets of experiments (n=4) were carried out: without and with ultrasound waves. The experimental conditions were the followings: donor compartment, clindamycin hydrochloride solution (1% P/V); receptor solution, PBS pH 7.4; temperature, 32°C ± 1°C; agitation, 450 rpm; sample times 0-2-4-6-8-12-16-20-22-24 h; membrane, human skin (0.4mm thickness from Biopredic Int.).

In the experiment with ultrasonic waves (Q500 sonicator, probe diameter 12.7mm, 3.98 cm^2, QSONICA), the temperature of the donor compartment was recorded continuously during sonication to avoid temperatures > 38ºC. The ultrasound conditions were the followings:

-frequency: 20kHz; intensity: 3 W/cm^2; amplitude: 20% ; pulse mode: （3s on/3s off)x 3; cycles: 14 (total time ultrasound: 4.2 min; total time：25 min).

The concentration of the drug in the samples was determined by HPLC (UV 195 nm).

RESULTS

Without ultrasounds, clindamycin was not detected in the receptor solution, except at 24h. However, when US were applied, clindamycin was quantified from 16h post-dosing. Considering the amount of drug retained in the skin, the US had an impact on the retention decreasing ($p<0.05$) the amount retained (median 242.82 µg/g (150.0-272.84) *vs*. median 123.54 µg/g (102.92-141.41) without and with US, respectively).

CONCLUSIONS

The application of ultrasound had an effect of increasing the transdermal permeability of clindamycin and decreasing the amount retained in the skin.

Reference: Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. Sciences 269, 850-853, 1995.

48.TRANSDERMAL PERMEATION OF KETOPROFEN FROM HEXOSOMES AND REVERSE HEXAGONAL LIQUID CRYSTAL FORMULATIONS

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Hexagonal lyotropic liquid crystals consist of nano-scaled hydrophilic and hydrophobic domains, which are separated by surfactant self-assembled layers. Hexosomes are aqueous dispersions of reverse hexagonal liquid crystals and have been proposed for pharmaceutic and cosmetic applications as they can protect the encapsulated molecules against physical, chemical, and enzymatic degradation enhancing molecular absorption through the skin [1]. In the present research, transdermal permeation of a lipophilic model drug, ketoprofen (KP), solubilized in diglycerol monoisostearate hexosomes was evaluated. For comparative purposes, a reverse hexagonal liquid crystal formulation containing KP was also developed and evaluated.

Methods. Hexosomes were prepared using similar approach as in [2]. Liquid crystalline structures characterization has been performed by Small-angle X-ray scattering (SAXS) and cryo-TEM.

Both formulations (0.5% wt. KP) were tested through two types of membranes (n=3 of each): human skin (0.4 mm, Biopredic Int.) and Strat-M^R (Millipore). Franz-type vertical diffusion cells with automatic sampling (Microette, Hanson Research), and same conditions (32.5 ±1°C, 450 rpm, receptor PBS pH 7.4, applied dose ~870 mcg KP) were used in this study. The duration of the assays was 24h for skin permeation, and 48h for Strat-M^R. At the end of the assays, the rest of the formulation remaining on the membrane was removed, the membrane was properly washed and the KP retained on the membranes was extracted and quantified. KP in the samples was evaluated by HPLC UV detection.

Results. The permeated percentages (24h) are always higher from hexosomes, although the behavior is different depending on the membrane. Permeation through Strat-M^R is maximum at 20h from hexosomes, while it continues to increase from the liquid crystal formulation. In the case of skin, a higher steady-state flux is obtained for hexosomes (median (range) 4.26 $\mu g \cdot cm^{-2} \cdot h^{-1}$ (3.55-5.65) *vs.* 0.77 $\mu g \cdot cm^{-2} \cdot h^{-1}$ (0.49-1.08), respectively), as well as the amount retained in the skin (median (range) 75.28 µg/g (69.02-95.88) *vs.* 58.62 µg/g (43.61-61.63), respectively). The results confirm that hexosomes are a novel and promising formulation for topical application.

References

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49.Waste material from sunflower, wheat and corn in skin toners – quality and efficacy contribution

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In order to meet the demand for sustainable cosmetics use of waste material is being explored. Our aim was to develop skin toners with ethanol extracts obtained from waste materials of sunflowers, wheat and corn that are left behind after harvest and then to investigate the potential of these ingredients to contribute to quality and efficacy of cosmetics.

With this aim 36 water based toners was prepared. All extracts (of wheat, corn and sunflower) were mixed in equally amounts in each toner in overall 0.1% or 1.0% concentrations, with different solubilizers. Samples 1 to 26 were prepared to determine the individual influence of substances on the physicochemical and organoleptic properties of water-based formulations with extracts. While, samples with ordinal numbers from 27 to 36 were considered as final formulations of skin tonics. Characterize and investigation of transparency and stability of samples was performed by organoleptic characterization, pH, transmittance and conductivity measurements, initially and after 1, 3 and 6 months. The effect of final formulations on the cell survival of normal human keratinocytes (HaCaT) was determined using the MTT assay.

In the case of preliminary formulations, samples with higher extracts concentration were opalescent, and with lower more transparent. All samples had a pH range of 4–6, and for the final formulations only slight differences in pH values were observed. The most stable transmittance values were observed with the final formulations and after six months, conductivity values were not altered for the final formulations. Physicochemical and microbial quality was obtained in water-based toners without preservatives with extract mixture, together with adequate sensory characteristics, pleasant smell and colour.

The tested samples showed no cytotoxic effects on the HaCaT cell line in all applied concentrations. The effect of the samples on the cell survival of the HaCaT cell line was dose dependent. The proliferation of HaCaT cells ranged from 94.4- 145.3 %. Samples with higher extract concentrations were the most effective.

Developed final samples remain transparent and showed good stability. All tested samples showed a positive effect on the cell survival of normal human keratinocytes in vitro. Samples with higher extract concentration showed the best activity and stimulated the proliferation of HaCaT cells at all tested concentrations indicating the skin rejuvenating effect of developed toners.

50.Enhancing Skin Permeation and Retention of Plant Extract through Nanoparticle Encapsulation: A Comparative Study on Healthy and Damaged Skin

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The use of plant extracts in skincare formulations is well-established due to their diverse bioactive compounds with potential therapeutic benefits for skin health. However, challenges such as limited stability, poor skin penetration, and controlled release have prompted the exploration of innovative delivery systems. Nanoparticles have emerged as effective carriers for enhancing the delivery of plant extracts to the skin, offering improved bioavailability and targeted delivery of plant extracts to the skin. In the context of dermocosmetic applications, the use of nanoparticle-based delivery systems holds great potential for addressing various skin conditions. This study focuses on investigating the delivery and retention of a plant extract in human skin using nanoparticle encapsulation.

For this reason, we prepared PLGA nanoparticles loaded with *Phlomis crinita* plant extract and evaluated their size. Using Franz diffusion cells, we conducted a permeation study to assess parameters such as flux and permeation constant (*Kp*) of the extract through the skin. After 27 hours, the amount of extract permeated was quantified via HPLC-DAD. Additionally, at the end of the permeation study, we investigated the amount of extract retained in the skin. The study was conducted on both healthy and damaged skin to assess the impact of skin condition on the delivery and retention of the plant extract.

In our study, we successfully developed PLGA nanoparticles with an average particle size of 59.5 ± 2.876 nm. These nanoparticles exhibited notable permeation capabilities through both healthy and damaged skin, with a higher flux observed in the damaged skin, indicating increased permeability. The calculated permeation constant (*Kp*) values further confirmed improved skin penetration facilitated by the PLGA nanoparticles. Additionally, the permeation study demonstrated successful delivery of the encapsulated plant extract, as evidenced by the quantity of extract permeated after 27 hours (27,80 ± 2,67 and 38,49 ± 0,02 for healthy and damaged skin, respectively). These findings provide strong evidence supporting the efficacy of the PLGA nanoparticles in sustaining the release of the encapsulated extract. Moreover, the nanoparticles exhibited greater retention of the extract in healthy skin compared to damaged skin, highlighting their potential for sustained therapeutic effects.

These findings highlight the promising potential of nanoparticle encapsulation, with their optimized size, in enhancing the delivery and efficacy of plant extracts for skincare applications. Furthermore, our results establish a strong foundation for future *in vivo* studies to assess the efficacy and potential therapeutic applications of the developed PLGA nanoparticle formulation.

51.Isolates of *Pinus sp.* in liposomes: influence of the carrier on antimicrobial activity against *Cutibacterium acnes*

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In an attempt to develop a product based on natural ingredients for potential local treatment of acne-prone skin, the purpose of this study was to examine antimicrobial activity against *Cutibacterium acnes* of the *Pinus sp*. green cones' isolates (essential oil, EO and the extract, E) incorporated into liposomal dispersions.

EO was isolated from the crushed plant material by steam distillation using a Clevenger apparatus, while E was obtained *via* Soxhlet apparatus using 70% (V/V) ethanol. Four liposomal dispersions were prepared using purified water and Phosal 40 IP (Lipoid, Germany): blank dispersion (sample L), dispersion with encapsulated EO (sample L-EO), blank dispersion with the addition of E in the outer phase (sample L-E) and with encapsulated EO (sample LEO-E). Zetasizer Lab Blue Label was used for liposome size and zeta potential evaluation, while measurements of pH and electrical conductivity were performed using pH-meter HI 9321 and conductometer CDM 230, respectively. In order to check the preliminary physico-chemical stability of the prepared samples, measurements of Ph, electrical conductivity, liposome size, and zeta potential were re-evaluated after 30 and 90 days of room temperature storage. The antibacterial effect of the examined EO, E, and liposomal dispersions was tested by agar well diffusion assay on the standard strain *C. acnes* ATCC 6919, while minocycline was used as a positive control. Thereby, EO and E were tested in the same concentrations used in the liposomal dispersions.

The droplet size of the prepared liposomes was in the range of 197.4 to 250 nm. The average droplet size in the sample L was 217.0. The addition of EO led to a decrease in droplet size, while the addition of E had an inverse effect. In all samples, the polydispersity index, as a measure of droplet size distribution uniformity was lower than 0.2 indicating that the liposomes were relatively monodisperse. The zeta potential of tested liposomes ranged from – 36.63 to -41.16 mV, suggesting good kinetic stability. pH from 4.0 to 5.01 in all tested samples indicated their applicability in skin products. The addition of EO led to the decrease of conductivity compared to the sample L, while the addition of E led to its increase. Repeated measurements of tested physicochemical characteristics of the investigated liposomes did not change applicability, suggesting satisfactory preliminary stability. Neither of the tested *Pinus sp*. isolates nor liposomal samples L, L-EO, and L-E exhibited antimicrobial activity against *C. acnes*. However, encapsulation of EO into liposomes along with the addition of E in the outer phase of the dispersion resulted in inhibition zones measuring 11.47±0.51 and 12.26±0.98 mm (for undiluted and liposomes diluted with brain heart infusion (BHI) in a ratio of 1:2, respectively) suggesting that both encapsulation of EO into liposomes and synergism between tested isolates (EO and E) enhanced biological activity related to the potential application of the prepared sample on the skin. It should be emphasized that minocycline yielded inhibition zones of 20.10±4.24 and 18.28±3.60 mm used in the concentration of 8 and 4 μg/m, respectively.

In conclusion, in the present study, liposomes with EO and E isolated from green cones of *Pinus sp*. revealed satisfactory physico-chemical characteristics/stability, suggesting their prospective usage in the preparation of the products based on natural ingredients for potential local treatment of acne-prone skin.

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52.Bolalipids and Skin

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Purpose:

The impact of two synthetic single-chain bolalipids on skin barrier function was investigated. Bolalipids are a new class of surfactants with potential applications in pharmaceutical formulations. They consist of two phosphatidylcholine head groups separated by either a 24-carbon atom (PC-C24-PC) or a 32-carbon atom (PC-C32-PC) alkyl chain. Bolalipid formulations at various concentrations were prepared and tested ex vivo using porcine ear skin. Data were compared with reference surfactants including a phosphatidylcholine phospholipid mixture (lipoid S-75), sodium dodecyl sulfate (SDS), polyethylene glycol 12 hydroxystearate (PEG-HS), and water as a negative control.

Method:

Preparation and characterization involved surfactants formulations loaded with sodium fluorescein (SF) as a model drug. Parameters tested including micellar size, PDI, and pH. Tape stripping was used to assess protein content through NIR-densitometry after applying the formulation on the porcine ear skin. Drug quantification via fluorescence spectroscopy was conducted after extraction from tapes using ultrasonication and centrifugation. Release studies were performed using Franz-type diffusion cells to explore surfactants influence on fluorescein sodium permeation.

Results:

To facilitate comparisons, the stratum corneum (SC) was divided into three zones: outer, middle and inner depth (0-20%, 20-40%, 40-60% of total SC depth respectively). Both bolalipids inhibited the penetration of the hydrophilic drug SF into the SC during tape stripping experiments. The gel-forming PC-C32-PC formulation increased SF penetration in the outermost SC layer compared to micellar PC-C24-PC, but both formulations resulted in lower SF penetration into the middle region compared to the aqueous control. Both bolalipids exhibited a keratolytic effect by removing higher protein amounts from the skin surface compared to water, suggesting a potential keratolytic effect of bolalipds, as confirmed by their comparison with salicylic acid, a known keratolytic agent. Despite having much lower molar concentrations, the bolalipids formulations showed similar protein removal profiles. Reducing bolalipid concentration slightly increased SF penetration into the inner SC, but it remained lower than the reference surfactants. Release studies showed that bolalipids significantly inhibited SF release compared to other surfactants. These results are in line with the tape stripping data, confirming that bolalipids appear to hinder SF release from the aqueous surfactant systems.

Conclusion:

Bolalipids do not promote skin penetration/permeation of a hydrophilic permeant using tape stripping and franz diffusion cell experiments, but cause stronger protein removal from the skin surface. Bolalipids penetration is mainly confined to the outermost skin layer (20% of SC). Trends towards enhanced protein removal resemble the effect caused by salicylic acid as keratolytic drug.

53.Chitosan based Mucoadhesive bilayer patches for Oral lichen planus

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Objective: Oral lichen planus (OLP) is a chronic inflammatory condition often characterized by erosive and/or painful oral lesions that have a considerable impact on quality of life. Current treatment necessitates the use of steroids and immunosuppressants in the form of mouthwashes, creams, ointments or gel, but these are very less effective due to inadequate drug contact time with the lesions. Recurrence of disease and progression to malignancy is another challenge. To overcome the limitations of current therapies, we developed and evaluated mucoadhesive bilayer patches containing synthetic drugs and phytochemicals for targeted drug delivery against OLP.

Methodology: Formulations of mucoadhesive bilayer patches containing Clobetasol propionate, Tacrolimus, Curcumin and AKBA (3-acetyl-11-keto-beta boswellic acid) were prepared using chitosan and polyvinyl alcohol (PVA). The effect of cross-linking on drug release profile, mucoadhesive characteristics of films, % swelling, flexibility, and in vitro residence time (h) was investigated. An *in vitro* cell line study was also conducted to find out suitable drug and phytochemical combinations in downregulating the STAT3 (Signal transducer and activator of transcription 3) in LPS-induced primary gingival keratinocyte cell lines.

Results: The developed buccal films were uniform in terms of drug content and thickness. The formulations demonstrated desired release profile of up to 8 hours. *Ex vivo* mucoadhesion strength was in the range of 0.33 ± 0.035 to 0.35 ± 0.014 N and all the formulations were flexible with controlled swelling of 25 to 30%. Results of cell line studies suggested that combination is better than individual drugs in downregulating the STAT3 than the individual drugs in LPS-induced primary gingival keratinocyte cell lines.

Conclusion: The developed bilayer mucoadhesive patches are better than the current line of treatments for the management of OLP. There are tremendous opportunities for more detailed studies, scale-up and commercialization of the formulations.

54.Design of liposomal Pro-Vitamin E phosphate for dermal application

Mais M Saleh, Nowar Sarayra, Walhan Alshaer, Nour Aladaileh, Marzouq Amarin

Long-exposure to UV radiation causes photoaging which can be reduced by topical applied antioxidants. The most commonly used antioxidant in sunscreens is vitamin E (VE) and its derivatives. The major challenge is that significant amount need to reach the viable skin layers for effective photoprotection. Vitamin E phosphate (VEP) was encapsulated in liposome to enhance stability and enhance its topical delivery. The VEP-loaded Liposome were prepared from VEP, hydrogenated soybean lecithin, and cholesterol by thin film hydration method. These liposomes were characterized for size, polydispersity index, and encapsulation efficiency (EE), and surface charge. *In-vitro* release testing (IVRT) and *ex-vivo* skin penetration studies was performed in Franz diffusion cells. The anti-aging effect of optimized liposome formulation was performed using *in-vitro* scratch assay on human dermal fibroblast (HDF). The optimized liposome formulations were homogeneous dispersions with a diameter of 100-124 nm (Fig. 1) and EE of 30-45.3%. The zeta potential of liposome was reduced after the addition of VEP. The optimized liposome formulations (pH 8, pH 6.7) demonstrated 1.2 and 12-fold, respectively higher cumulative release across synthetic membrane compared to the control. The flux of VEP through human skin membrane was comparable to the control at pH 8 (151.84 ± 38.72 vs. 166.19 ± 52.07 µg/cm^2/h)., whereas 7-fold higher at pH 6.7 compared to the control (87.06 ± 93.62 vs. 12.49 ± 16.73 µg/cm^2/h). The optimized liposome at pH 8 delivered higher VEP in *stratum corneum* at 24 h compared to the control (90.61 vs. 59.3 µg/cm^2). The wound closure was higher for VEP-loaded liposome compared to the negative control (Fig.2).

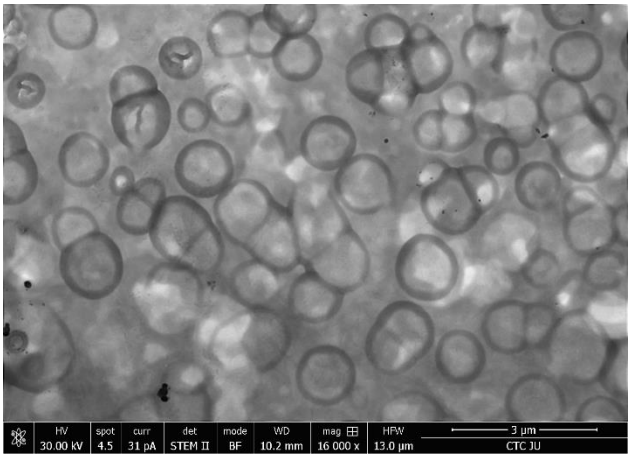

Fig.1: Bright field transmission electron microscopy (TEM) images of optimized liposome formulation.

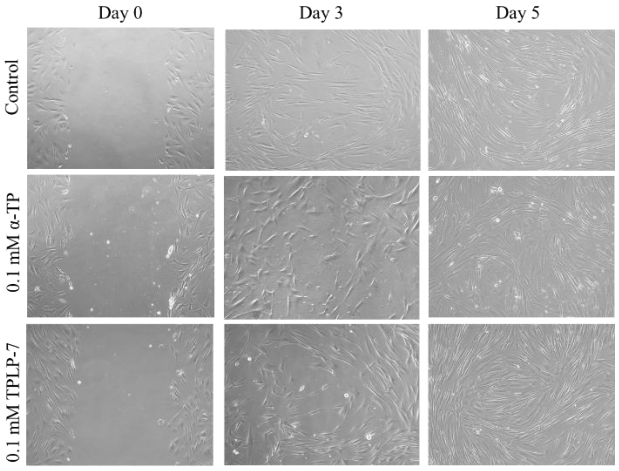

Fig.2: Effect of optimized VEP-loaded liposome formulation (TPLP-7), free VEP (α-TP), and control on the wound healing of human dermal fibroblasts (HDF).

55.Understanding chemical extraction across the skin using skin stretching

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Background: The skin is a major physical, immunological, and sensory barrier between the bodies internal and external environment. Evidence to suggest that the hair follicles are an important pathway for percutaneous penetration are increasing and methods such as follicle occlusion have been used to show the importance of this penetration pathway into the skin (Pereira et al 2023; Teichmann et al 2006).

The stretching of the skin that occurs during massage has been shown to open the skin appendages and enhance the delivery of agents into the skin after topical application (Benaouda *et al.*, 2022). Skin stretching has the advantage over micropore formation, when attempting to administer therapeutic agents into the skin, in that it is non-invasive but also rapidly reversed by the energy stored in the skin collagen, which relaxes the tissue after the application of tissue strain. However, unlike delivery into the skin, the effect of the appendages on extraction of chemicals from the skin has been less well studied. The aim of this work was to understand if hair follicle opening using skin stretching can facilitate chemical extraction from the skin tissue.

Methods: Skin appendage occlusion using aluminium chloride was used to evaluate the impact of the appendageal pathway on the extraction of chemicals across the skin ex-vivo. Fresh full-thickness porcine ear skin and rat skin were used in Franz diffusion cells. FITC-dextran of 10 and 150 kDa was used as a receiver solution. For follicle occlusion 20% aluminium chloride was applied on top of the skin for 15 min. The receptor phase was placed underneath the skin. The hypobaric chamber was attached to the apical surface of the skin mounted cells in a pre-heated water bath (Grant Instruments, Cambridge, UK) set at 37˚C to obtain a temperature of 32˚C at the skin surface. The duration of the experiment was 1h under -4.5 psi hypobaric pressure. The extracted amount was analysed on a Spark plate reader (Tecan Ltd).

Results: Extraction efficiency across the porcine skin without alumunium chloride application for 10 kDa was 9.6% (SD +/-6.8), and for 150 kDa 5.4% (SD+/-3.2) respectively. For the rat skin the 10 kDa extraction efficiency was 13.5% (SD+/- 2.2), and for 150 kDa it was 20.5% (SD +/- 10.4) respectively. The application of 20% aluminium chloride demonstrated effective skin appendage blockage with the extraction efficiency of 0% for both 10 kDa and 150 kDA for both the rat skin and porcine skin.

Conclusion: Skin appendage occlusion demonstrated that the skin appendageal route was instrumental in chemical extraction using skin stretching. Skin appendage blockage with 20 % aluminium chloride resulted in a significant reduction in the extraction of FITC dextran evidencing that sweat ducts and hair follicles are important in the percutaneous chemical extraction pathway.

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56.Effects of selected ionic liquids on skin lipid model membranes

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Lipid matrix of Stratum Corneum represents the major barrier for topical and transdermal drug delivery [1]. To overcome this barrier, permeation enhancers are commonly used to enhance drug permeation [2]. Ionic liquids (IL) represent a promising group of permeation enhancers [3]. The aim of this study was to investigate the interaction of two imidazolium based IL: 1-methyl-3-octylimidazolium bromide (C_8MIM) and 3-dodecyl-1-methylimidazolium bromide (C_{12}MIM) using simplistic skin lipid models. Initially, the IL were incorporated in the lipid models and their microstructure was studied using X-ray diffraction (XRD). In addition, the alterations to the barrier properties of the model membranes containing the IL were compared to control membranes (non-containing IL) for three different permeants: H_2O, Theophylline (TH) and Diclofenac sodium (DIC). XRD data indicated that both IL mostly affected the reflections of cholesterol (reduced intensity). Concerning permeability, both C_8MIM and C_{12}MIM significantly reduced the membranes' resistance to water loss while C_8MIM showed significantly higher water loss values than C_{12}MIM. The presence of C_8MIM – but not C_{12}MIM - in the lipid membranes was proved to significantly increase the permeability of TH. On the other hand, the presence of C_8MIM or C_{12}MIM significantly increased the permeation of DIC in comparison with the control membranes but they didn't show significant differences between them. In an additional study, we evaluated the permeability of two permeants (TH and DIC) when they were co-applied with the IL on control skin lipid membranes. The co-application of IL with TH and DIC gave roughly comparable effects for the two permeants. In contrast with the case of incorporation of IL to the lipid membranes, the C_{12}MIM proved to be a better enhancer in comparison with C_8MIM for both permeants. Further studies are needed to explore the full potential of ILs as novel permeation enhancers for topical and transdermal drug delivery.

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57.Ultrasound-Compatible Franz Diffusion Cell for Ultrasound-Mediated Topical Drug Delivery Systems

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- Purpose:

Sonophoresis is a topical drug delivery system that uses ultrasound (US) as the physical enhancer. Franz-type diffusion cells have been developed to measure the conventional *in vitro* skin permeation of drugs, resulting in a lack of low US-reflection conditions and difficulty in achieving desired acoustic parameters. The aim of the present work was to develop and manufacture transparent Franz-type diffusion cells that are compatible with the ultrasound and US-mediated drug delivery systems.

- Methods:

The US-compatible Franz cell donors were prepared using an online 3D model design program. 3D-printing was used for fabricating the donors with two types of transparent resins (Formlabs® Clear or Accura® ClearVue). Comparative studies between glass and 3D-printed Franz cell donors were conducted including sealing performance and imiquimod absorption. Ultrasound at an excitation frequency of 1.1MHz was employed to measure the acoustic characteristics of 3D-printed donors. The preparation and characterization of imiquimod-loaded microbubbles were conducted to establish a US-mediated drug delivery system. Consequently, *in vitro* permeation studies were carried out with Strat-M membrane to assess the differences between resin and glass donors, as well as to investigate the enhancement of drug permeation through US-mediated microbubbles.

- Results:

No significant leakage and imiquimod absorption were observed in two transparent resins. The moisture evaporation was increased in Formlabs® Clear which was suggested by a greater porosity of the material. The spatial characteristics of the sound field in the 3D-printed donor was virtually undistorted, indicating the low US-reflection condition in the donor. However, standing waves were observed to be generated by the reflection of the glass receptor bottom, resulting in an average peak-to-peak acoustic pressure offset of 1.7%. An ultrasonic transducer with a frequency of 1.1 MHz was employed to activate the imiquimod-loaded microbubbles, and the oscillation and collapse of microbubbles were stimulated by a high intensity acoustic field characterized by an acoustic pressure of 1 MPa. The ultrasound exposure resulted in a 98.87% of microbubble destruction and a tolerable temperature increase of 3.26°C on Strat-M membrane after 60-second ultrasound treatment. Permeation studies with Strat-M membrane also showed the same performance between 3D-printed and glass donors as indicated by the nearly overlapping curves in the permeation amount. The imiquimod-loaded microbubbles resulted in 1.74 ± 0.29 μg imiquimod permeation and 2.29 ± 0.32 μg imiquimod partition, and the perturbation of the skin model induced by US-mediated cavitation was demonstrated by the observed increase in drug permeability, with 2.96 ± 0.25 μg of drug permeation and 3.84 ± 0.39 μg of drug partition in 24 hours.

- Conclusion:

Conventional glass Franz-type diffusion cells suffer from a lack of low US-reflection conditions and difficulty in achieving desired acoustic parameters. To address these issues, we developed a US-compatible Franz cell donor with the same performance as the conventional glass donor for evaluation of US-mediated imiquimod delivery systems.

58.An *ex vivo* human skin explant model for preclinical evaluation of subcutaneously administered biomacromolecules: A case study using hyaluronic acid-based soft tissue fillers

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Subcutaneous injection is the principal method of administration for both locally- and systemically-acting biomacromolecules. However, few preclinical models are available to evaluate post-injection behaviour and to facilitate formulation development. Recently, we described the development of an *ex vivo* "full thickness" porcine skin model containing hypodermis with an extended 5-day culture period, which was used to investigate the localization and interaction of subcutaneously injected HA-based soft tissue fillers [1]. Porcine skin is a well-recognised surrogate, but it still has limitations for the evaluation of biological responses in human skin. We present (i) the development of an *ex vivo* human skin explant model with extended viability, and (ii) its application in the preclinical evaluation of the bio-stimulating effect of Teosyal RHA®1 after subcutaneous administration.

Human abdominal skin harvested immediately after surgery was cut into ~18 mm diameter explants with the hypodermis conserved (thickness of ~10 mm). Explants incubated with optimized medium and PBS (negative control) were collected at Day 0, 3, 5, 7, 9 and subjected to H&E staining for histological evaluation of epidermal structural integrity over a 9-day culture period. Immunofluorescent staining of Ki67 and Claudin-1 assessed epidermal cell proliferation and barrier function, respectively. Explants received subcutaneous injection of 50 µL Teosyal RHA®1, NaCl (0.9%, injection control), and TGF-β3 (40 ng/mL, positive control) and were harvested after incubation for 5 days. The expression of type I/type III procollagen and elastin was assessed by immunofluorescent staining.

H&E-stained images showed that spongiosis (primary endpoint) and epidermal detachment (secondary endpoint) were significantly delayed in the optimized medium group compared to PBS, with <6.8% of positive spongiosis area and no detachment of the basal layer from the underlying dermis detected after 7 days. Metabolic activity of the explants was confirmed by the expression of Claudin-1 and Ki67 up to Day 5, which was therefore selected as the endpoint for the biological evaluation. Immunofluorescent stained images showed no significant differences between the “no-treatment” and injection control groups in terms of the expression of type I/type III procollagen and elastin, indicating that mechanical stress from the injection had no major impact on ECM production. TGF-β3 injection triggered the production of type I procollagen which, given a previous report that the TGF-β family regulates fibroblast function, supports the hypothesis that it stimulates the synthesis of ECM proteins. Teosyal RHA®1 exhibited a superior effect on the production of type III procollagen as compared to TGF-β3. Thicker elastin fibres with an increased density were observed in both positive control group and Teosyal RHA®1 treated skin explants.

In conclusion, we successfully established an *ex vivo* "full thickness" human skin explant model that retained viability during an extended culture period and enabled the visualization of ECM protein production triggered by Teosyal RHA®1 within the timeframe of the experiment, pointing to a possible bio-stimulating effect in the clinic. This preclinical platform will now be employed in combination with quantitative bioanalysis methods, e.g. ELISA or qPCR, to evaluate diffusion of subcutaneously administered biomacromolecules away from the injection site or local reactions in the tissue microenvironment.

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59.Glucocorticoid Delivery in Wound Care: A Study on Bacterial Cellulose and Microemulsion Technologies

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Addressing the complexities of treating inflammatory wounds, such as pyoderma gangrenosum, requires innovative therapeutic strategies to overcome the adverse effects of systemic anti-inflammatory drugs, the limitations of conventional semi-solid formulations and the inherent difficulties of wounds as drug targets [1–3].

In this study, we introduce a novel approach that combines the unique properties of bacterial cellulose wound dressings as a carrier and a microemulsion formulation technique, for the topical delivery of anti-inflammatory glucocorticoids, thereby creating active wound dressings. Five microemulsions were formulated, loaded with hydrocortisone (HC) or dexamethasone (DEX), and extensively characterized for microstructure, biocompatibility, sterilisation, and shelf-life stability. The drug-loaded formulations were incorporated into bacterial cellulose using an absorption loading technique, as confirmed by freeze-fracture transmission electron microscopy. Finally, permeation through Strat-M® membranes and the anti-inflammatory activity of permeated glucocorticoids were evaluated *in vitro* to assess key aspects of active wound dressings.

Upon evaluation, the microemulsions exhibited regular oil-in-water (o/w), water-in-oil (w/o), or bicontinuous microstructures and proved to be highly effective in incorporating therapeutic concentrations of poorly water-soluble glucocorticoids HC or DEX, in addition to their drug stability and biocompatibility. The successful integration of these formulations into bacterial cellulose was confirmed by freeze-fracture transmission electron microscopy, which revealed a homogeneous distribution of the microemulsions within the hydrophilic three-dimensional cellulose network, surprisingly even for w/o microemulsions. Furthermore, Strat-M® permeation testing indicated controllable and significant drug permeation, with the glucocorticoids maintaining their anti-inflammatory potency *in vitro.*

These findings suggest that the combination of bacterial cellulose with microemulsion technology holds great promise for the development of active, anti-inflammatory wound dressings that can contribute significantly to the effective management of inflammatory skin conditions such as pyoderma gangrenosum.

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60.Influence of Ceramide Class on Water Permeability of Skin Lipid Membranes

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Purpose: Molecular Dynamics (MD) simulations of the stratum corneum (SC) have emerged as a powerful tool for the modelling of skin barrier properties, facilitating the prediction of the interactions between the skin barrier function and a wide range of compounds [1]. Integral to the skin barrier function is the brick-and-mortar structure of the SC, with the keratin-rich corneocytes (bricks) being embedded in a matrix (mortar) comprised of cholesterol, ceramides, and fatty acids in equimolar parts [2]. This lipid matrix forms a continuous lamellar phase throughout the entire SC, providing a diffusion barrier against ingress of xenobiotics as well as water loss from within [3]. Necessarily, MD simulations of this complex system need to simplify and generalize certain aspects to build applicable models, such as reducing the system size to one lipid bilayer and substituting a range of similar lipid species with one representative species [4]. This substitution of a variety of lipid species with a singular species is especially common with respect to ceramides, which exist as over 16 different classes in the human skin [5]. In simulations, they are mostly represented by a single class, with this overwhelmingly being the ceramide classes based on non-hydroxylated fatty acids linked to either sphingosine of phytosphingosine, or NS and NP according to ceramide nomenclature [6]. Reported in this study is the progress in applying MD simulations to investigate the effects of the ceramide classes as well as the parameters of the ceramide species on the barrier properties of the SC lipid membrane, measured by the water permeability of the bilayer. *Methods:* Four fully hydrated lipid bilayers were built, comprising cholesterol, lignoceric acid and either ceramide NS24 or NP24 in equimolar amounts. Two different forcefield parameter sets of the forcefield CHARMM36, the native set for sphingolipids as well as a modified version for better representation of ceramide NP, were used for the simulations of each ceramide class. All simulations were run in the MD simulation software GROMACS. The water permeation coefficients were calculated by the diffusion coefficients and the mean force acting on inserted water molecules with fixed z-position across the whole bilayer. *Results:* Comparison of the results show a strong dependence of the water permeabilities of the bilayer on the forcefield parameters of the ceramides, with a significant divide in the values obtained from the simulations, as the force field using the native parameters predicted significantly higher water permeabilities for both systems than the revised field parameters. Furthermore, the comparison of the choice of ceramide class to incorporate into the lipid bilayer also yielded notable differences in the resulting water permeability of the membranes. *Conclusions:* The findings of this study highlight the importance of two aspects in simulating skin lipid membranes: Firstly, the choice of force field parameters has significant impact on simulation studies, returning significantly different values of water permeability. Additionally, as only the parameters of the ceramides were altered, these changes also underline the importance of the ceramides for the barrier function. To build on this finding, the choice of ceramide class to use in the simulations also affects the water permeability of the bilayer, implying a profound influence of the chosen ceramide species on the bilayer properties. This mirrors observations from experimental studies regarding the impact of changes of the ceramide composition in the SC [7] and warrant further investigation into the mechanistic influence of ceramides on the SC lipid barrier.

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61.Non-invasive topical sampling of tryptophan, kynurenine, phenylalanine and tyrosine from melanoma and benign skin: a pilot study

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Purpose. To escape immunoediting, a high number of cancer phenotypes convert tryptophan (Trp) to immunotolerance inducing kynurenine (Kyn). Kyn upregulation has been found in blood of patients with skin melanoma. The main goal of this study was to investigate if higher abundance of Kyn can be detected on the surface of melanoma lesions compared with adjacent benign skin.
Methods. Sixteen patients with suspected melanomas were enrolled in this study at Skåne University Hospital, Sweden. Seven suspected skin lesions were diagnosed as malignant melanomas (MM), 6 – melanomas in situ (MIS), and 3 – non-melanomas (NM). Sampling from the patients was performed by tape stripping (3 consecutive tape strips) suspected skin lesion and adjacent healthy non-lesional (NL) skin. The collected samples were analysed by HPLC-MS/MS for amounts of Trp and Kyn as well as tyrosine (Tyr), and phenylalanine (Phe) for comparison. The latter two amino acids are found in amounts similar to Trp on healthy skin. Non-invasive sampling was supplemented by skin imaging with dermatoscope and electrical impedance spectroscopy (EIS) measurements.
Results. Comparison between melanoma and adjacent healthy NL skin showed higher abundance of all four analytes collected from melanoma lesions. Significantly higher amounts of Tyr, Phe and Trp were collected from MM lesions compared to NL skin ($p < 0.01$). Levels of Kyn in samples collected from MM lesions were ~2 fold higher, but not statistically significant. The increase of Trp in melanoma skin was lower compared to Tyr and Phe. Subsequently, Trp/Phe ratio in melanoma lesions, MM and MIS ($p < 0.01$ and $p < 0.05$), was lower compared to NL healthy skin. Evaluation of skin barrier by EIS showed that skin resistance of MM lesions was significantly lower than NL skin, 35.1±16.3 kOhm and 80.3±30.4 kOhm (mean±SD, n=7, $p < 0.01$), respectively. The results of skin resistance suggest that MM skin is damaged and may be 'leaky' for metabolites. Skin resistance of MIS lesions was lower than adjacent NL skin, but the difference was not significant. Spearman's correlation analysis for NL skin adjacent to MIS demonstrated strong negative correlation between analytes collected and skin resistance ($r = -0.9$) and revealed no correlation for NL skin adjacent to MM lesions. Interestingly, skin resistance of NL skin adjacent to MIS and MM was not significantly different, whereas abundance of analytes on NL skin adjacent to MM was ~2 fold lower compared to NL skin adjacent to MIS. Principal component analysis of compiled analytes and EIS measurements showed clustering of healthy skin (NL and NM) and melanoma lesions (MIS, MM).
Conclusions. The abundance of Kyn in melanoma lesions was not significantly higher compared to adjacent healthy skin. However, abundance of Tyr, Phe and Trp was ~5-6 folds higher in melanoma lesions compared to adjacent NL skin. Measurements of skin resistance and abundance of analytes from NL healthy skin adjacent to MIS or MM skin, revealed that NL skin appears to be different depending on the melanoma lesion it is adjacent to, and sampling proximity. Discrimination between NL and cancerous skin based on non-invasive tape sampling of Tyr, Phe, Trp and Kyn is feasible. Differentiation between melanoma subtypes requires further studies with optimized sampling on larger study cohorts.

62.Enhancing Transdermal Delivery of Highly Lipophilic Drugs: Insights from Cannabinoid-Based Cream Formulations

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Purpose: Among painkillers, morphine compound in hydrogels is widely researched as a topical analgesic for wound-related pain management. With concerns over opioid side effects, wound healing inhibition, and accidental overdoses, the U.S. FDA has encouraged non-addictive alternatives for acute pain management. The study focuses on exploring transdermal cream formulations containing cannabinoids, specifically Delta-9-tetrahydrocannabinol (D9-THC) and Cannabidiol (CBD), as potential alternatives to reduce opioid reliance in pain and wound management. With a particular emphasis on the early-stage clinical development of Pyoderma Gangrenosum, a rare inflammatory neutrophilic skin disease, this research offers hope for patients worldwide.

Methods: Skin absorption kinetics were evaluated through in vitro finite dose models on ex vivo human torso skin using the Phoenix RDS Automated Diffusion system from Teledyne Hanson. Each formulation was tested on skin samples from six donors, with samples collected over 48 hours. Receptor solution samples were taken at various time points for permeation kinetics analysis. Tape stripping was employed to assess analyte distribution across skin layers. Cannabinoid quantification was conducted using the *Waters Xevo TQ-S Acquity UPLC-MS/MS* system.

Results: Our results underscore the challenges in the transdermal delivery of highly lipophilic cannabinoids, necessitating the development of specialized carriers to enhance permeation and stability. Various carriers including nanocomposite polymeric multi-layered lipid vesicles and liposomes were tested to improve drug bioavailability. Experimental evaluation of four cream formulations, despite employing distinct technological bases, displayed remarkably consistent performance. The most effective carriers facilitated consistent and controlled transdermal cannabinoid delivery over extended periods. The product performance and the rate and extent cannabinoids permeate through the skin are presented in the accompanying figures and table.

Conclusion: In vitro skin permeation studies are valuable for formulation development, aiding in selecting optimal candidates and assessing product quality. Results from these studies align with in vivo findings, proving useful in preclinical development and facilitating regulatory approval of topical products. IVPT serves as a surrogate method to screen formulations for generic dermal/transdermal products and assess bioequivalence. The study successfully achieved targeted therapeutic delivery of highly lipophilic cannabinoids, encouraging further exploration into novel topical drug delivery systems for molecules with high lipophilicity. Research now extends to the transdermal administration of vitamin D3 and its derivatives.

63.Towards elucidating the molecular mechanisms of the skin penetration enhancer propylene glycol

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Transdermal drug delivery (TDD) is a non-invasive method for the controlled delivery of drugs through skin directly into the blood stream. However, a major limitation of TDD is that very few drugs have the correct physicochemical properties to cross the skin's main permeability barrier, the stratum corneum (SC). The SC is the outermost layer of the skin, and contains layers of corneocytes embedded in a lipid matrix composed of ceramides, fatty acids, and cholesterol. One of the most common strategies employed in TDD to temporarily overcome the barrier properties of the skin is the use of chemical penetration enhancers (PEs). PEs may facilitate the transport of drugs across the skin by altering the permeability of the SC.

Propylene glycol (PG) is a widely used skin PE that is known to diffuse into the SC[1] and interact with the SC lipids to increase the permeation of drugs.[2] Experiments have demonstrated that PG increases the mobility and disorder of SC lipids, and may extract cholesterol from the SC. However, little is known about the molecular mechanisms of drug permeation enhancement by PG.

In this work, we have performed molecular dynamics (MD) simulations to investigate the molecular-level effects of PG on the structure and properties of model SC lipid bilayers.[3] The model bilayers were simulated in the presence of PG concentrations over the range of 0−100% w/w PG, using both an all-atom and a united atom force field. PG was found to localize in the hydrophilic headgroup regions at the bilayer interface, to occupy the lipid-water hydrogen-bonding sites, and to slightly increase lipid tail disorder in a concentration-dependent manner. We showed with MD simulation that PG enhances the permeation of small molecules such as water by interacting with the bilayer interface; the results of our study may be used to guide the design of formulations for transdermal drug delivery with enhanced skin permeation, as well as topical formulations and cosmetic products.

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64.Analysis of Topical Formulations: Investigating Inactive Ingredients and Their Volatility

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Purpose:

Understanding the composition and properties of inactive ingredients present in topical formulations is crucial for ensuring the safety and efficacy of these products. Additionally, examining the impact of inactive ingredient volatility on formulation stability is essential for optimizing drug delivery to and through the skin. Currently, the multiphase, multilayer mechanistic dermal absorption (MPML MechDermA) model within the Simcyp Simulator is capable of predicting the local and systemic absorption of drugs applied to the skin accounting for dynamics such as formulation evaporation and precipitation. However, recognizing the significance of individual component evaporation, this research aims to investigate the inactive ingredients commonly used in topical formulations, emphasizing the importance of tracking the evaporation of individual components. This research aims to investigate the inactive ingredients commonly used in topical formulations and assess their volatility.

Methods:

Data was extracted from the FDA Orange Book, focusing on Reference Listed Drugs (RLD) applied topically. The list was refined based on dosage forms, and research was conducted on the inactive ingredients present in these products. For creams, lotions, and gels, the 20 most commonly used inactive ingredients were selected from a pool of 852. Their volatility was estimated using the evaporation QSAR in the Simcyp Simulator (US EPA, 1987).

Results:

The majority of products analysed were classified as creams (31.6%), followed by gels (17%), lotions (7.2%), ointments (10.6%), and solutions (9.8%). Water and propylene glycol emerged as the two most commonly used inactive ingredients across creams, lotions, and gels, with the highest volatility. Additionally, benzyl alcohol, another commonly used ingredient, exhibited relatively higher volatility compared to other inactive ingredients.

Conclusions:

This study provides insights into the composition and volatility of inactive ingredients in topical formulations. Considering the importance of inactive ingredients in formulation metamorphosis, their volatility plays a critical role in determining drug delivery to and through the skin. It is noteworthy that the Simcyp MechDermA model already has the ability to predict changes in the total formulation volume. This research represents an effort to identify important ingredients to target in model development and verification when accounting for evaporation of individual components in simulations, enhancing its utility in formulation optimization and drug delivery prediction.

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Corporate Profile (As of September 2023)

Company name	Maruho Co., Ltd.
Founded	July 1915
Incorporated	October 1949
Capital	382.53 million yen (unlisted)
Fiscal year	Ends September
Sales	85.72 billion yen (2022/10-2023/9)
Number of employees	1,566

Our Engagement Tools

Information on Maruho's business activities and various initiatives is provided for our wide range of stakeholders.

For people who want to know more about Maruho

Corporate website
https://www.maruho.co.jp/english/

For people who want to work at Maruho

Recruiting (in Japanese)
https://www.maruho.co.jp/recruit/

For medical professionals

Website for medical professionals (in Japanese)
https://www.maruho.co.jp/medical/check.html

For people who want to know about Maruho's future and strategies

Maruho Report
https://www.maruho.co.jp/english/about/corporate/report/

For more information about our drugs

Website for patients and general customers (in Japanese)
https://www.maruho.co.jp/kanja/

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maruho

Corporate Profile

Achieving further growth and contribution to society as a pharmaceutical company specializing in dermatology

Atsushi Sugita
Representative Director,
President & CEO

To achieve well-being, we will continue to appreciate every moment of life

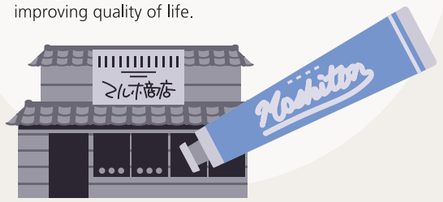
As a pharmaceutical company specializing in dermatology, the Maruho Group aims to create a society where everyone can live with a smile. By actively collaborating and cooperating with the Group companies in Japan and overseas, we are evolving into a group of companies with a diverse business portfolio.

In October 2022, we renewed our corporation philosophy in order to declare our commitment to make even more "high-quality contributions." Our goal is to increase the "smiles" of patients who are filled with joy at being able to live their normal lives after their symptoms have improved. Imagining such smiling faces, we will strive to create technical and medical innovations and build a corporate group capable of responding to society's expectations.

Maruho by the Numbers

More than
100 years in business

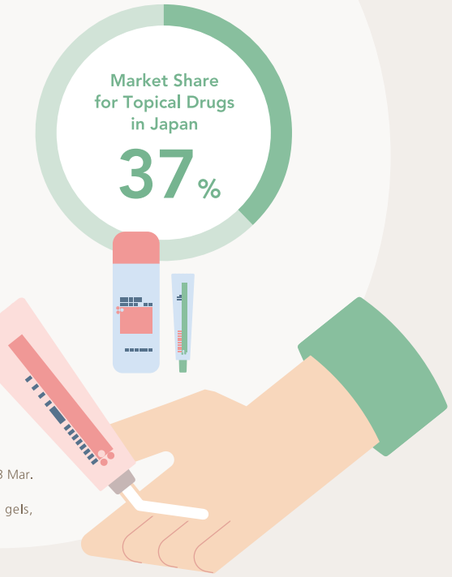
Maruho's history began with a commitment to "contribute to society through medicine" by our founder Eikuma Koba. For more than a century, we have continued to support people's daily lives through pharmaceuticals and contributed to improving quality of life.



More than
60 facilities in Japan

We have a well-developed corporate structure with a network of sites nationwide. In addition to disseminating appropriate medical information to medical professionals across Japan, we are able to meet the needs of many patients.

Sales of Prescription Topical Drugs in Japan
No.1



We possess unique know-how in topical drugs and contribute to patients with a wide range of skin diseases such as atopic dermatitis, acne (acne vulgaris), psoriasis, hyperhidrosis, and infectious diseases.

Copyright © 2024 IQVIA.
In-house analysis based on IQVIA JPM 2022 Apr.–2023 Mar. (drug price base) / Reprinted with permission
Topical drugs: ointments, liquids, creams, lotions, gels, sprays, and topical foam agents

Business partnerships with more than
20 companies

We are promoting collaboration with overseas pharmaceutical companies and licensing activities for research institutes and others. These partnerships empower us to expand new possibilities.

Equity-to-asset ratio
85.8%

R&I rating
A-

We are building a robust management foundation with the aim of being a company that engages in sound management while growing and developing sustainably.

Maruho medical representatives ranked among dermatologists in Japan
No.1*

In addition to supplying high-quality products, Maruho continues to contribute to medical care through its Medical Representatives (MRs), having established a compelling reputation and trust among dermatologists in Japan.

*Percentage of dermatologists who answered "No.1 / High" in survey
Rep-i web survey for dermatologists in Japan (Aug. 2023) by INTAGE Healthcare Inc.
Aug. 2023 survey: 498 respondents

Mission

More smiles, brighter life for you.

Maruho is always searching for what brings your personal happiness. By listening to you and appreciating every moment of life, we will seek the truth that makes you smile.

Values

- Be genuine
- Notice the signs
- Grow your curiosity and change the world
- Exceed your limits
- Inspire each other

These five Values are the standards of behavior that each one of us should uphold in order to realize our Mission.

Maruho's Businesses

In order to respond to the various needs of patients, Maruho expands its business portfolio beyond prescription drugs to diagnostic drugs, medical devices, and skin care cosmetics. We are expanding the areas where we contribute to medicine close to people's skin concerns, from prevention to diagnosis, treatment, and aftercare.

Prescription Drug Business

We develop a variety of products for a wide range of skin diseases. We are also expanding treatment options for patients by adding efficacy and dosage forms to existing products.

Diagnostic Drug Business

In addition to contributing to the early detection of symptoms, we promote a care cycle that provides comprehensive information, including diagnosis, which is indispensable for treatment.

Medical Devices Business

In recent years, we develop new products and projects by combining Maruho's expertise in prescription drugs with Maruho Hatsujyo Kogyo's precision metal processing technology.

Self-Care Business

With the desire to contribute to the field of skin care involving many people every day, we have developed our own brand of products to help even more people living with skin problems.