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SKIN @ BATH NETWORK
S@BNS
SYMPOSIUM

The 2nd
Skin@Bath Network Symposium

12th–13th December 2019

In the **Old Theatre Royal in Bath, UK**
(http://oldtheatreroyal.com/venue/).

Abstract Book

Skin@Bath Network Symposium 2019

Welcome Message

Dear Friends,

On behalf of the members of the Organising Committees we would like to welcome you to the "Second Skin@Bath Network Symposiun".

We have been overwhelmed by the number of delegates, both early-career and more established clinical and non-clinical scientists and physicians, who have registered for this meeting and have expressed a willingness to give oral or poster presentations. We also appreciate the encouragement you have given us to organise what we hope is meeting that brings together clinical and non-clinical scientists from many different disciplines and research areas, all of whom have interests in the skin.

Many of you have travelled long distances to attend this meeting in the historic city of Bath. Some of you are visiting Bath for the first time whereas others have been fortunate to visit the city before. Although we have a very intensive scientific programme over the next two days, we have also been able to organise a reception dinner on the 12th which we trust will provide a relaxing networking opportunity at what is a particularly special time of the year in the run-up to the Festive period.

We trust that during the meeting you will have plenty of opportunities not only to renew old friendships with long-term collaborators but also to establish new collaborations. We also hope that in the future we will be able to look back on this meeting in Bath as the second one of a regular series of Symposiun devoted to skin.

We wish you a fruitful and pleasant stay in Bath.

Best wishes,

Charareh Pourzand and Caoimhe Fahy

SKIN@BATH 2019 Acknowlegments

The Skin@Bath organising committee gratefully acknowledges the valuable support from:

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Main Program

Thursday 12th December 2019

8:25-8:30 – Welcome message (**Dr Caoimhe Fahy** and **Dr Charareh Pourzand**, co-Chairs of the Symposium).

8:30-8:40 – Inaugural Speech (**Prof Bernie Morley**, Deputy Vice-Chancellor and Provost, University of Bath)

Plenary Lecture (8:40-9:05)

Chair: Steve Ward, PhD, FBPhS (Professor of Leukocyte Biology, Department of Pharmacy and Pharmacology & Vice-Chair for Centre for Therapeutic Innovation, University of Bath, UK)

8:40-9:05 - Banafshe Larijani, PhD (25 min) (Professor of Cell Biophysics, Department of Pharmacy and Pharmacology and Department of Physics, University of Bath, Co-founder, Joint Managing Director and CSO of Fastbase solutions, Spain & Director of Centre for Therapeutic Innovation, University of Bath, UK)
'The signalling pathways and the clinical translational work'

Inflammatory Skin Disorders I (9:05-10:10)

Chair: Steve Ward, PhD, FBPhS

9:05 - 9:30 Kieron Leslie, FRCP (25 min) (Prof of Dermatology, University of California San Francisco, USA)
'Autoinflammatory skin diseases'

9:30 - 9:50 - Neil McHugh, MBChB, MD, FRCP, FRCPath (20 min) [Prof of Pharmacoepidemiology, Honorary Rheumatologist in Royal National Hospital for Rheumatic Diseases (RNHRD), Head of Department of Pharmacy and Pharmacology, University of Bath, UK]
'Epidemiology of early psoriatic arthritis (PsA)'

9:50 - 10:10 - Andrew Franklin, BSc (Hons), PhD (Med Sci) (**20 min**) [Medical Scientific Liaison (Immunology, Hepatology & Dermatology), Novartis Pharmaceuticals UK Limited]

'Immunopathology of psoriatic disease'

*******10:10 - 10:40 Coffee/Tea Break******

Approaches for Skin Therapy I (10:40 -12:00)

Chairs: Ian M. Eggleston, PhD (Reader in Medicinal Chemistry, Department of Pharmacy and Pharmacology & Centre for Therapeutic Innovation, University of Bath, UK)
& Nikoletta Fotaki, PhD (Reader in Pharmaceutics, Department of Pharmacy & Pharmacology, University of Bath)

10:40 - 11:00 - Paul Winyard, PhD (20 min) (Professor of Experimental Medicine, University of Exeter Medical School, Exeter, UK)

'Using redox-active compounds to enhance photodynamic killing of cultured human squamous carcinoma cells'

11:00 – 11:20 - Alison Curnow, MD, PhD (20 min) [Professor, Director of Medicine (Academic), University of Exeter Medical School, Exeter & Royal Cornwall Hospital, Truro, UK]

'Insights gained from regression analysis of PpIX fluorescence imaging undertaken during routine dermatological PDT'?

11:20 - 11:40 - Ewan Eadie, PhD (20 min) (Honorary Lecturer & Head of Scientific Services for Photobiology and Optical Radiation at NHS Tayside, The Scottish Photodynamic Therapy Centre; The University of Dundee, Dundee, United Kingdom)

'Daylight Photodynamic Therapy in the UK – it works… honest '

11:40 - 12:00 - Ben Novak, PhD (20 min) (Head of Exploratory Development, Drug Development Department, Biofrontera Bioscience GmbH, Leverkusen, Germany)

'Optimisation strategies for ALA-PDT of non-melanoma skin cancer: results of a translational research approach'

*******12:00 – 13:20 Lunch Break & Poster Presentation ******

Developments in the Extraction of Biomarkers from the Skin (13:20-14:35)

Chair: Stuart Jones, PhD (Senior Lecturer in Pharmaceutics, Department of Pharmacy, Faculty of Life Sciences & Medicine, King's College London, UK)

13:20 – 13:40 - Robert Chilcott, PhD, CBiol, FSB, FRSC, ERT (20 min) (Head of Toxicology. University of Hertfordshire, Hatfield, Hertfordshire, UK)
'Application of sebomics for the analysis of residual skin surface components to detect potential biomarkers of type-1 diabetes mellitus'

13:40 – 14:00 - Ryan Donnelly, PhD (20 min) (Professor of Pharmaceutical Sciences, Queen's University Belfast , School of Pharmacy, Medical Biology Centre, Belfast, Northern Ireland)
'Microneedles as a means of cutaneous drug monitoring'

14:00 – 14:20 - Faiza Benaouda, PhD (20 min) (Research Fellow, Institute of Pharmaceutical Science, King's College London)
'Hypobaric extraction of molecules out of the skin'

14:20 – 14:35 - Luca Lipani (15 min) *(Department of Pharmacy and Pharmacology, University of Bath)*
'Non-invasive, transdermal, path-selective and specific glucose monitoring via a graphene-based platform'

******5 min break ********

Inflammatory Skin Disorders II (14:40-15:40)

Chair: Prof John Westwick, PhD, DSc, Hon BPharmS (Imperial College, London)

14:40 - 15:00 - William Tillet, MD, PhD (20 min) (Consultant Rheumatologist at the Royal National Hospital for Rheumatic Diseases & Senior Lecturer in the Department of Pharmacy & Pharmacology, University of Bath, UK)
'The assessment of Psoriatic Arthritis (PsA) – past, present and future'

15:00 – 15:20 - Oliver Fitzgerald, MB BCh BAO, MRCPI, MRCP, FRCPI, MD, FRCP (20 min) (Consultant rheumatologist and Newman Clinical Research Professor at St Vincent's University Hospital and the Conway Institute, University College Dublin (UCD), Ireland.
'Identifying Psoriatic Arthritis (PsA) in patients with Psoriasis; might prevention of PsA be far away?'

15:20 – 15:40 - John Pauling, MD, PhD (20 min) (Consultant Rheumatologist at the Royal National Hospital for Rheumatic Diseases & Senior Lecturer in the Department of Pharmacy & Pharmacology, University of Bath, UK)
'The measurement of sclerosing skin disease'

*******15:40 – 16:10 Coffee/Tea Break ******

Approaches for Skin Therapy II (16:10 -17:55)

Chairs: Albert Bolhuis, PhD (Senior Lecturer in Microbiology, Department of Pharmacy & Pharmacology, University of Bath, UK); ***Ian S Blagbrough, PhD*** (Senior Lecturer in Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, UK)

16:10-16:35 - *Keynote Lecture:* Marvin Edeas, MD, PhD (25 min) (Visiting Professor, Cochin Institute-INSERM, University Paris Descartes , Department of Cancer, Development and Reproduction, Founder and Executive Chairman of the International Society of Microbiota, Chairman of the scientific committees of Targeting Mitochondria and Microbiota World Societies)
'An introduction to Microbiota and skin Microbiota: Recent Advances and Perspectives'

16:35 – 16:45 - Ka Ho Ho (10 min) (PhD student, Department of Pharmacy & Pharmacology, University of Bath, UK)
'Film-Forming Agents as a Protective Barrier to Fungal Skin Infection'

16:45 – 17:00 - Robert Kelsh, PhD (15 min) (Professor of Developmental Biology, Department of Biology and Biochemistry, University of Bath, UK)
'Modelling human diseases notably melanoma with Zebrafish and medaka'

17:00 - 17:20 - Martin Malmsten, PhD, Cchem FRSC (20 min) (Professor in Biopharmaceuticals, Biophysics and Drug Delivery, University of Copenhagen, Director, LEO Foundation Centre for Cutaneous Drug Delivery, Guest Professor in Pharmaceutical Physical Chemistry in University of Lund, Norway)
'Host defence peptides for combatting infection - From mode of action to drug delivery'

17:20 – 17:35 - Gernot Walko, PhD (15 min) (Lecturer in Cell and Developmental Biology, Department of Biology and Biochemistry, University of Bath, UK)
'Targeting YAP/TAZ as a novel therapeutic strategy in cutaneous squamous cell carcinoma.'

17:35 – 17:55 - Jun Lu, PhD (20 min) (Professor of Pharmaceutical Sciences, Key laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing, China)
'Topical therapeutic efficacy of ebselen against multidrug-resistant Staphylococcus aureus targeting thioredoxin reductase'

******5 min break ********

18:00-18:15 – Welcome Speech (**Prof Ian H. White,** Vice-Chancellor and President, University of Bath)

*******18:15 - 20:00 Drink, Music & Dance Show ******

*******20:00- 22:00 – Dinner Buffet Reception ******

Friday 13th December 2019

Inflammatory Skin Disorders III (8:30-9:50)

Chair: Prof John Westwick

8:30-8:55 - Alan Irvine, MD, DSc, FRCPL, FRCPI, FAAD (25 min) (Prof of Dermatology, St James Hospital, Trinity College Dublin)
'Emerging treatments in Atopic Dermatitis'

08:55-9:10 – Li Jian (15 min) MD, PhD *(The First Affiliated Hospital of the Army Medical University, Chongqing, China)*
'Introducing a new concept for the treatment of Rosacea'

9:10-9:35 - Padraic Fallon, MRIA, FTCD (25 min) (Professor of Translational Immunology, Clinical Medicine, Interim IMM Director, Molecular Medicine Ireland, School of Medicine, Trinity College Dublin)
'Atopic dermatitis and filaggrin: of mice and men'

9:35 - 9:50 - Expert Patient talk- Huo Yan MD (15 min) (Dermatologist, BiShan People's Hospital, Chongqing , China)
'Psoriasis in China'

*******5 min break*******

Iron, Mitochondria, Ferroptosis & Skin I (9:55-10:40)

Chairs: Prof Robert C. Hider & Prof Fudi Wang (MD, PhD, Qiushi Chair Professor, Director of Institute of Nutrition and Food Safety, Director of Nutrition Discovery Innovation Centre, School of Public Health, Zhejiang University School of Medicine, Hangzhou, China)

9:55-10:20 - *Keynote Lecture:* Ioav Cabantchik, PhD (25 min) (Professor Emeritus of Biochemistry and Biophysics, President, IBIS: International Bioiron Society (2017-19), International Society for the Study of Iron in Biology and Medicine, Institute of Life Sciences, Hebrew University of Jerusalem, Israel)
'The labile side of iron and of iron sulphur clusters in health, evolution and disease'

10:20–10:40 - Xi Huang, PhD (20 min) (Professor of Environmental Medicine, New York University & CEO of Fe I Beauty Tech Inc, Montvale, NJ, USA)
'Removing iron for skin protection'

*******10:40 - 11:10 Coffee/Tea break ******

Iron, Mitochondria, Ferroptosis & Skin II (11:10-12.40)

Chairs: Prof Ioav Cabantchik & Prof Robert Evans (Brunel University, School of Engineering and Design, London, UK)

11:10-11:35 - *Keynote Lecture:* Carsten Culmsee, PhD (25 min) (Professor of Clinical Pharmacy, Vice Dean of the Department of Pharmacy, Center for Mind, Brain and Behavior – CMBB; Institute of Pharmacology and Clinical Pharmacy, Department of Pharmacy, Philipps University Marburg, Germany)
'Mitochondrial regulation of ferroptosis in health and Disease'

11:35-11:55 - Rachel Nechushtai, PhD (20 min) (Professor and co-director of The Alexander Silberman Institute of Life Sciences, Faculty of Science, The Hebrew University of Jerusalem, Israel)
'Wolfram Syndrome, mitochondrial iron overload & skin'

11:55-12:05 – Claudio Raimondi, PhD (10 min) (Lecturer, Queen Mary University of London, United Kingdom)
'The role of Neuropilin-1 in adult vasculature homeostasis and regulation of mitochondrial function'

12:05 – 12:30 - *Keynote Lecture:* Robert C. Hider, PhD, FRSC (25 min) (Emeritus Professor in Medicinal Chemistry, King's College London & Honorary Professor, Department of Pharmacy and Pharmacology, University of Bath, UK)
'Therapeutic iron chelation'

12:30-12:40 - Agostino Cilibrizzi, PhD (10 min) (Lecturer, Institute of Pharmaceutical Sciences, King's College London, UK)

'Mitochondrial iron overload and skin photosensitivity'

*******12:40 – 13:50 Lunch Break & Poster Presentation ******

Skin Photoaging, Photodamage & Photoprotection I (13:50-14:50)

Chair: Prof Rex Tyrrell *(Honorary Professor, Department of Pharmacy and Pharmacology, University of Bath, Bath UK; Formerly President of the European Society of Photobiology)*

13:50 - 14:10 Hugo Corstjens, PhD (20 min) (R&D and Innovation independent consultant, founder of Novigo +, Formerly executive manager of basic science research and advanced technologies in Estee Lauder Laboratories, Belgium)
'Circadian rhythm in the skin in relation to skin ageing and disorders'

14:10-14:25 - Bethany Ferris, MD, PhD (15 min) (The University of Edinburgh)
'Ultraviolet radiation lowers blood pressure in a large haemodialysis cohort'

14:25-14:40 - Karl Lawrence, PhD (15 min) (St John's Institute of Dermatology, King's College London, UK)
'Wavelengths longer than 380nm cause photodamage to skin cells that is not adequately protected by application of conventional sunscreens'.

14:40-14:50 – Shida Chen (10 min) (*Department of Bioengineering Department, Chongqing University, Chongqing, China*)
'Ultraviolet A-induced endoplasmic reticulum stress mediated heme oxygenase 1 expression in skin epidermal cells'

******* 5 min break *******

Topical Drug Delivery (14:55-16:00)

Chairs: Prof Richard Guy (Professor of Pharmaceutical Sciences, Department of Pharmacy and Pharmacology, University of Bath, UK) ***& Dr Begona Delgado-Charro*** (Reader in Pharmaceutics, Department of Pharmacy & Pharmacology, University of Bath)

14:55-15:10 - Andrea Pensado, PhD (15 min) (Department of Pharmacy and Pharmacology, University of Bath, UK)
'The skin pharmacokinetics of topical drug delivery'

15:10-15:30 - James Clarke, PhD (20 min) (Certara UK Limited, Simcyp Division, Sheffield, UK)
'Predicting dermal absorption in diseased or damaged skin using PBPK modelling'

15:30-15:45 - **Pauline Vitry, PhD (15 min) (**Department of Pharmacy & Pharmacology, University of Bath, Bath, UK)
'Evaluating topical drug bioavailability in the skin using Raman spectroscopy '

15:45 – 16:00 - Alice Maciel Tabosa, PhD (15 min) (Department of Pharmacy & Pharmacology, University of Bath, UK)
'Tracking excipients from a topical drug product through the stratum corneum in vivo'

******* 16:00 – 16:25 Coffee/Tea Break ******

Ethnopharmacology & other Skin-related approaches (16:25-17:30)

Chairs: Dr Matthew Lloyd (Senior Lecturer in Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, UK) *&* ***Prof Julia Li Zhong*** *(Professor of Skin Photobiology and Dermatology, Department of Bioengineering Department, Chongqing University, Chongqing, China).*

16:25-16:40 Lori Bystrom, PhD (**15 min**) (Lecturer in Food Entreprise, Bath Spa University, UK)
'Ethnopharmacology and skincare'

16:40-17:00 Mojgan Moddaresi, PharmS, PhD, FRSB, CBIOL (20 min)
[Director of Personal Care Regulatory (Dublin, Cambridge)]
'EU and UK Cosmetic regulations for synthetic and Natural-based products '

17:00-17:10 Mustafa Varcin, PhD MPharm ARPharmS (10 min) (Honorary treasurer, Society of Cosmetics Scientists, UK)
'Society of Cosmetic Scientists '

17:10 – 17:20 - Chunli Wang, PhD (10 min) (Bioengineering College, University of Chongqing, Chongqing, China)
'Single-Cell Map of Patellar Tendon Reveal Diverse Participator Involving in the Injured Microenvironment'

17:20 – 17:30 – Davide Califano (10 min) *(Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK; Centre for Sustainable Chemical Technologies, University of Bath, Claverton Down, Bath, BA2 7AY, UK)*
'Continuous production of hydrogen peroxide by a self-activated multienzyme cellulose composite: a promising material for wound dressing'

POSTER Sessions: Thursday 12th and Friday 13th at lunch break:

P1 **MOLECULAR STRUCTURE OF PHARMACEUTICAL AND COSMETIC CREAMS**
Delaram Ahmadi, Dave Barlow[1], Jayne Lawrence

P2 **EVALUATING RAPID FORMULATION CHANGES *IN SITU* WHEN ELOCON CREAM AND EMOLLIENTS ARE APPLIED TO THE SKIN AT SIMILAR TIMES**
M. T. Beebeejaun, M. B. Brown, V. Hutter, L. Kravitz, W. J. McAuley

P3 **IMPROVING THE EFFECTIVENESS OF AMINOLEVULINATE-BASED PHOTODYNAMIC THERAPY (ALA-PDT) OF SKIN CELLS WITH ULTRAVIOLET A-INDUCED LABILE IRON RELEASE**
Dana Beiki, Tina Radka, Olivier Reelfs, Ian M Eggleston, Charareh Pourzand

P4 **'HAPPY BOTTOMS'–BEST NAPPY CARE IN PAEDIATRIC ONCOLOGY**
Sophie Constantinou, Madeleine Adams

P5 **SYNTHESIS OF HIGHLY MODULAR MULTIMODAL IMAGING PROBES FOR RECOGNITION OF CANCER BIOMARKERS**
Ruediger M. Exner, Fernando Cortezon-Tamarit, Haobo Ge, Stephen Paisey, Charareh Pourzand and Sofia I. Pascu

P6 **PREPARATION OF POLYSACCHARIDE-BASED NANO-EMULSION AS A TARGETED DELIVERY SYSTEM FOR MALIGNANT MELANOMA CELLS**
G. Hatami Fard, T. Keshavarz, S. Getting, M. Dwek, H.M.N Igbal

P7 **NON-INVASIVE EXTRACTION OF MACROMOLECULES ACROSS THE SKIN USING HYPOBARIC PRESSURE**
Heeaun Park, Stuart A. Jones, Faiza Benaouda

P8 **CONCERNS WITH VITAMIN D SUPPLEMENT QUALITY AND THE POTENTIAL FOR TRANSDERMAL DELIVERY**
Anish Patel, Makiko Kawashita, Mandy Wang, Mandy Wan, Jignesh P Patel, Greta Rait, Stuart A. Jones

P9 **OPTIMIZATION OF A NANOFORMULATION FOR SKIN DELIVERY: THE INFLUENCE OF STRUCTURE AND COMPOSITION**
Simone Stefani, Ana Isabel Barbosa, Tânia Moniz, Sofia A. C. Lima and Salette Reis

P10 **EFFECTS OF LOX INHIBITION ON HEMIN-INDUCED CELL DEATH**
Melanie Merkel, Ina Eisenbach, Carsten Culmsee

P11 **CHEMICAL CHARACTERIZATION OF THE $PVPA_{SC}$ MODEL: THE EFFECT OF SURFACTANTS AND DIFFERENT PH ON BARRIER PERMEABILITY**
Tânia Moniz, Sedef Kaplan, Sofia A. Costa Lima and Salette Reis

P12 **UNDERSTANDING THE DISTRUBUTION OF NITROUS OXIDE IN HUMAN AND PORCINE SKIN UPON THE TOPICAL APPLICATION OF S-NITROSOGLUTATHIONE**
Qalander Khan, Yi Jiazhuo, Faiza Benaouda, Sara Nasereddin and Stuart A. Jones

P13 **DESIGN OF Hydroxypyridinones and tetrapyrrole macrocycles TO Life Sciences**
Maria Rangel

P14 **A NANOTECHNOLOGICAL APPROACH FOR THE TOPICAL DELIVERY OF CYCLOSPORINE A**
Maria Inês Silva, Ana Isabel Barbosa, Sofia A. Costa Lima, Salette Reis

P15 **DERMATOLOGICAL PRODUCT METAMORPHOSIS FOLLOWING APPLICATION OF BETAMETHASONE SPRAY FORMULATIONS.**
P. Zarmpi, A. Pensado-Lopez, S. Gordeev, J. White, A. Bunge, R. Guy, B. Delgado-Charro

Abstracts

Invited Lectures /Oral Presentations

Plenary Lecture

IMAGING ONCOPROTEIN ACTIVATION AND DYNAMICS IN CANCER

Banafshé Larijani[1,2,3] James Miles[2,3], Lissete Sanchez-Magraner[3], Christopher Applebee[1,3] , Pierre Leboucher[3], Somaia Elsheikh[4] , Selvaraju Veeriah[5], Stephen G Ward [6], Peter J Parker [7];

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3-FASTBASE SOLUTIONS, Astondo bidea, Kabi 612 Scientific and Technology Park of Bizkaia 48160 Derio, Spain

4-Department of Cellular Pathology, Queens Medical Centre, Nottingham, NG7 2UH, United Kingdom

5- Cancer Research UK Lung Cancer Centre of Excellence, University College London Cancer Institute, University College London, London, UK

6- Leukocyte Biology Laboratory, Centre for Therapeutic Innovation & Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY UK.

7- Protein Phosphorylation Laboratory, The Francis Crick Institute, London, UK

Currently there is a transition point in cancer research, towards a need for a more profound understanding of molecular heterogeneity in various types of tumours as well as a sensitive and specific quantitative methodology for analysis of the activation status of biomarkers.

Our team has developed an innovative, photophysics imaging approach to assess proteomic heterogeneity as well as activation status of biomarkers. Two-site amplified time resolved FRET (A-FRET) allows *in-situ* analysis of biomarkers and successfully correlated protein kinase B (PKB) activation state with a worsened patient outcome in breast, head and neck cancers, clear cell renal cell carcinoma and melanoma. A-FRET is able to accurately determine the interaction of immune biomarkers such as that of PD-1 and PD-L1, dimerisation of receptors, HER2/HER3, with this dimerisation being correlated to a worsened clinical outcome in a subset of breast cancer patients.

The outcome of analysing biomarker functionality is a stratification of patients which is unobtainable when using classical clinical analysis; this will ultimately lead to more personalised medicines and therapies being available to patients.

Invited Lecture

AUTOINFLAMMATION AND THE SKIN.

Kieron Leslie

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Autoinflammation is characterized by aberrant regulation of the innate immune system and often manifests as periodic fevers and systemic inflammation involving multiple organs, including the skin. Mutations leading to abnormal behavior or activity of the interleukin 1 beta (IL-1ß)-processing inflammasome complex have been found in several rare autoinflammatory syndromes, for which anticytokine therapy such as IL-1 or tumor necrosis factor-alfa inhibition may be effective. It is becoming clear that features of autoinflammation also affect common dermatoses, some of which were previously thought to be solely autoimmune in origin (eg, vitiligo, systemic lupus erythematosus). Recognizing the pathogenetic role of autoinflammation can open up new avenues for the targeted treatment of complex, inflammatory dermatoses.

Invited Lecture

CAN THE DEVELOPMENT OF PSORIATIC ARTHRITIS BE PREVENTED?

Neil McHugh

Department of Pharmacy and Pharmacology, University of Bath, UK; prsnjm@bath.ac.uk

The presence of skin psoriasis is one of the highest risk factors known for the development of chronic inflammatory arthritis. Once established psoriatic arthritis carries a high burden of disease and considerable comorbidity. As skin psoriasis precedes psoriatic arthritis in the majority of cases with a median interval of 8 years, there is a major window of opportunity for both early screening and potentially prevention. Certain risk factors are not modifiable such as known genetic risk factors, gender and family history. However lifestyle factors such as smoking, alcohol intake and obesity are worthy of consideration. Our own work has shown that obesity is a major risk factor, and furthermore weight reduction may mitigate that risk. Even should psoriatic arthritis not be able to be prevented our ongoing work including a randomised clinical trial as part of a NIHR programme grant is addressing whether early detection and intervention is an effective mechanism for improving long-term outcome.

Invited Lecture

IMMUNOPATHOLOGY OF EARLY PSORIATIC ARTHRITIS

Andrew Franklin

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IMMUNOPATHOLOGY OF PSORIATIC DISEASE

Andrew Franklin, BSc (Hons) PhD

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Inflammation has physiological purposes, but it can also have pathological consequences.[1] Psoriasis is a chronic, systemic inflammatory condition that is commonly associated with significant comorbidity that confers a disease burden which is more than 'skin deep'.[2] The most common comorbidity of psoriasis is psoriatic arthritis, a chronic inflammatory spondyloarthropathy affecting axial and peripheral skeleton.[3] Today, the IL-23–Th17–IL-17 axis is the focus of considerable research with respect to the development of molecules that have therapeutic effect in plaque psoriasis and psoriatic arthritis.[4] The term psoriatic disease is increasingly being used to encompass the manifestations of tissue and organ involvement observed in many psoriasis patients,[5] extending the concept of a disease confined to the skin and joints.[6] The pathogenesis of comorbid conditions associated with psoriasis and psoriatic arthritis is largely affected by inflammation as well.[7] Whether targeted systemic therapies for skin and joint disease are able to reduce inflammation in associated comorbid conditions remains to be established.[7]

References

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Invited Lecture

USING REDOX-ACTIVE COMPOUNDS TO ENHANCE PHOTODYNAMIC KILLING OF CULTURED HUMAN SQUAMOUS CARCINOMA CELLS

Winyard PG, Ferguson DCJ, Smerdon GR*, Curnow A

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Protoporphyrin IX (PpIX)-based photodynamic therapy is a common clinical treatment for non-melanoma skin cancers and precancers, which uses the photosensitive properties of PpIX to induce oxidative stress in targeted skin cells when activated by irradiation with red light (λ_{max} 635 nm).

We have performed *in vitro* testing of a range of redox-active compounds, for their potential adjunctive capacity as enhancers of PpIX-based photodynamic killing of the epidermoid carcinoma cell line, A431. As part of this screen, we tested the adjunctive effects of compounds in cultured cells which had already been either: (a) adapted, long-term (48 hours), to hyperoxia (18.6% O_2; i.e. "standard" laboratory cell culture conditions), or (b) adapted, long-term, to physioxia (2% O_2; i.e. cells cultured in a gas-tight cabinet containing a regulated concentration of O_2).

Upon photodynamic irradiation, cells which had undergone long-term adaptation to hyperoxia generated higher concentrations of mitochondrial reactive oxygen species, compared with cells which had been in a physioxic (2% O_2) environment. However, after photodynamic irradiation, there was no significant difference in viability between hyperoxic and physioxic cells. On the other hand, the expression of several genes encoding the activity of key antioxidant enzymes was higher after the long-term culture of hyperoxic cells, compared with physioxic cells. Importantly, when putative adjunctive compounds were tested in cells which had undergone long-term adaptation to physioxia, several thioredoxin reductase inhibitors (e.g. auranofin) were observed to possess adjunctive activity, by increasing the efficacy of photodynamic treatment in the cells which had been adapted to physioxia. In contrast, cells which had undergone a long-term adaptation to hyperoxia did not exhibit an adjunctive effect of thioredoxin reductase inhibitors, on photodynamic treatment.

We conclude that, when testing the cell killing effects of redox-active compounds, used in conjunction with photodynamic irradiation, a key factor is the O_2 concentration-dependent "pre-adaptation" of cells, which is associated with an induction of antioxidant genes and increased antioxidant enzyme activity. This cellular adaptation to standard cell culture O_2 conditions appears to contribute to the development of a phenotype that is resistant to oxidative stress-induced cellular damage and death. Therefore, long-term cell culture under physioxia is required, prior to exposure to putative adjunctive compounds and photodynamic irradiation, in order to reveal the adjunctive potential of certain redox-regulating compounds.

Invited Lecture

INSIGHTS GAINED FROM REGRESSION ANALYSIS OF PPIX FLUORESCENCE IMAGING UNDERTAKEN DURING ROUTINE DERMATOLOGICAL PDT

J. Tyrrell, C. Paterson, A. Curnow

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Good clinical outcomes and excellent cosmesis can be achieved with dermatological photodynamic therapy (PDT) when treating superficial Basal Cell Carcinoma, (sBCC), Bowen's Disease (BD) and Actinic Keratosis (AK) with protoporphyrin IX (PpIX) precursors. A licensed topical dermatological PDT protocol has been standardised for these applications to good effect. The detailed mechanism of action underlying this treatment process is complex but through further understanding, it may be possible to further improve outcomes and widen application.

Clinical PpIX fluorescence imaging was conducted using a pre-validated, non-invasive imaging system (Dyaderm, Biocam, Germany) during routine methyl aminolevulinate (MAL)-PDT treatment of 172 patients with licensed dermatological indications (37.2% AK, 27.3% sBCC and 35.5% BD). Linear and logistic regressions were employed to model any relationships between variables that may have affected PpIX accumulation and/or PpIX photobleaching during irradiation and thus clinical outcome at three months.

Patient age was found to be associated with lower PpIX accumulation and photobleaching, however only a reduction in PpIX photobleaching appeared to consistently adversely affect treatment efficacy. Clinical clearance was reduced in lesions located on the limbs, hands and feet with lower PpIX accumulation and subsequent photobleaching adversely affecting the outcome achieved (OR: 0.5 (0.2, 0.9; $p<0.05$). If air cooling pain relief was employed during light irradiation, PpIX photobleaching was significantly reduced ($p < 0.05$) and this resulted in an approximate three-fold reduction in the likelihood of achieving clinical clearance (OR: 0.4 (0.2, 0.7; $p<0.01$). Clinical outcome was collectively observed to be 75.6%.

PpIX accumulation and photobleaching are therefore concluded to be important indicators of dermatological MAL-PDT treatment success and anything that adversely effects them has the potential to reduce treatment efficacy. PpIX photobleaching during the first treatment was found to be an excellent predictor of clinical outcome across all lesion types. Non-invasive imaging of PpIX fluorescence during MAL-PDT therefore provides insights, which can inform future treatment protocols to potentially improve outcomes where these are currently limited (e.g. within acrally located lesions) or alternatively, to extend this treatment modality's indications.

Invited Lecture

DETERMINATION OF LIGHT DOSE IN DAYLIGHT PHOTODYNAMIC THERAPY

Ewan Eadie[1], Paul O'Mahoney[2,3], Luke McLellan[3], Marco Morelli[4], Emilio Simeone[4], Michael Higlett[5], Marina Khazova[5], Sally Ibbotson[1,2,3]; ewan.eadie@nhs.net

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Daylight Photodynamic Therapy (dPDT) is an effective, well tolerated and patient-preferred treatment for field change pre-malignant photodamage. Despite an extensive evidence base, uptake of this therapy in the UK has lagged behind the rest of Europe. Weather concerns are likely to contribute to this apparent inertia, with the perception that sufficient light exposure could not be achieved for effective treatment. The Photobiology Unit at Ninewells Hospital in Dundee, Scotland, has been investigating light delivery for dPDT in the UK and has worked towards introducing tools to improve the convenience of and confidence in this important therapy.

Over 500,000 measurement data from multiple locations across the UK were analysed, over a three year period, reporting average Protoporphyrin-IX (PpIX) weighted radiant exposure ("dose") for two-hour periods throughout the day and year. Temperature, sunscreen and ultraviolet radiation (UVR) exposure were also taken into consideration. Results were compared to minimum threshold criteria indicating typical times-of-day and months-of-year when dPDT could be performed in the UK. On average, the minimum threshold criteria for light delivery in dPDT can be achieved January to November in the UK, although temperature limits treatment April to October. UVR exposure during treatment can be minimised by either treating in Spring or Autumn or later in the day during Summer. Sunscreen is an important component of treatment but must be carefully chosen as it could impact on effective light dose.

The historical data, whilst indicative of appropriate treatment periods, does not provide prospective accurate determination of dose. Computational modelling from weather forecast data and Earth Observation Satellite (EOS) imaging were compared to ground-based measurements to determine if light dose could be accurately predicted and subsequently reported in real-time. On average, deviation of EOS modelling from ground-based measurements was 0.2 ± 4.9% , whilst forecasting was 20 ± 16% different from measured data.

Our early results suggest that, whilst dPDT can be successfully performed in the UK without light measurements, computational modelling could increase confidence in effective delivery of this important therapy with predictive treatment planning and convenient, accurate guidance to both clinicians and patients.

Invited lecture

OPTIMISATION STRATEGIES FOR ALA-PDT OF NON-MELANOMA SKIN CANCER: RESULTS OF A TRANSLATIONAL RESEARCH APPROACH

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Background: 5-aminolevulinic acid based photodynamic therapy (ALA-PDT) has long been proven clinically useful for a variety of skin diseases, most notably epidermal neoplasia. We developed a preclinical research programme to better understand critical success factors such as prodrug stability, skin penetration, treatment emerged pain, the influence of illumination parameters, and treatment resistance. This talk describes how a translational research programme informs clinical development and how early discoveries can be mapped to therapeutic reality.

Methods: We characterised how a nanoemulsion-based drug delivery system (BF-200) improves ALA stability and penetration, as investigated in cell cultures, nude mice and minipig *in vivo*, and porcine and human skin *in vitro*. Primary sensory neuron cells were treated with ALA *in vitro* to understand pain mechanisms. Squamous cell carcinoma (SCC) cell lines were analysed for ALA uptake / PpIX formation, resistance mechanisms, and the influence of different illumination parameters on photodynamic efficacy. Further investigations covered the interaction of ALA-PDT with putative SCC cancer stem cell subpopulations

Results: Combining ALA with BF-200 greatly increased stability and penetration into cell cultures, porcine and human skin and was superior to other formulations. We could identify both direct and indirect mechanisms of cutaneous sensory neuron activation by PDT, identifying potential analgesic targets. We discovered differential gene expression of ALA uptake transporters and heme synthesis enzymes in two SCC lines that show different PpIX formation kinetics and phototoxic response, which uncovered potential resistance mechanisms. In the same cell lines, we found that total light dose is the key factor for efficacy as opposed to fluence rate. A stemness associated gene expression panel was implemented to investigate the susceptibility of cancer stem cells to PDT *in vitro*. ALA stability and penetration were significantly enhanced by the nanoemulsion BF-200. The results from the different *in vitro* approaches can be translated into future development programmes to further improve efficacy and tolerability of ALA-PDT.

Conflict of interest: HL, BN, BS, MG & MF are employees of the Biofrontera group which developed an ALA containing drug and a PDT light source. LS, AKH, LH, NS & TD declare no conflict of interest.

Invited Lecture

MICRONEEDLES AS A MEANS OF CUTANEOUS DRUG MONITORING

Ryan F. Donnelly

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This talk describes the use of hydrogel-forming microneedle (MN) arrays for minimally-invasive extraction and quantification of drug substances and glucose from skin *in vitro* and *in vivo*. MN prepared from aqueous blends of hydrolysed poly(methyl-vinylether-co-maleic anhydride) (11.1% w/w) and poly(ethyleneglycol) 10,000 daltons (5.6% w/w) and crosslinked by esterification swelled upon skin insertion by uptake of fluid. Post-removal, theophylline and caffeine were extracted from MN and determined using HPLC, with glucose quantified using a proprietary kit. *In vitro* studies using excised neonatal porcine skin bathed on the underside by physiologically-relevant analyte concentrations showed rapid (5 min) analyte uptake. For example, mean concentrations of 0.16 µg/mL and 0.85 µg/mL, respectively, were detected for the lowest (5 µg/mL) and highest (35 µg/mL) Franz cell concentrations of theophylline after 5 min insertion. A mean concentration of 0.10 µg/mL was obtained by extraction of MN inserted for 5 min into skin bathed with 5 µg/mL caffeine, while the mean concentration obtained by extraction of MN inserted into skin bathed with 15 µg/mL caffeine was 0.33 µg/mL. The mean detected glucose concentration after 5 min insertion into skin bathed with 4 mmol/L was 19.46 nmol/L. The highest theophylline concentration detected following extraction from a hydrogel-forming MN inserted for 1 h into the skin of a rat dosed orally with 10 mg/kg was of 0.363 µg/mL, whilst a maximum concentration of 0.063 µg/mL was detected following extraction from a MN inserted for 1 h into the skin of a rat dosed with 5 mg/kg theophylline. In human volunteers, the highest mean concentration of caffeine detected using MN was 91.31 µg/mL over the period from 1 to 2 h post-consumption of 100 mg Proplus® tablets. The highest mean blood glucose level was 7.89 nmol/L detected 1 h following ingestion of 75 g of glucose, while the highest mean glucose concentration extracted from MN was 4.29 nmol/L, detected after 3 hours skin insertion in human volunteers. Whilst not directly correlated, concentrations extracted from MN were clearly indicative of trends in blood in both rats and human volunteers. This work strongly illustrates the potential of hydrogel-forming MN in minimally-invasive patient monitoring and diagnosis. Further studies are now ongoing to reduce clinical insertion times and develop mathematical algorithms enabling determination of blood levels directly from MN measurements.

Invited Lecture

BIOMARKER EXTRACTION ACROSS THE SKIN USING HYPOBARIC PRESSURE

Faiza Benaouda

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Background: Biomarkers can be valuable prognostic and diagnostic tools. They can also improve clinical trial success rates [1]. However, owing to difficulties associated with biomarker discovery, extraction and quantification their use is often associated with a significant financial cost. One means to reduce the costs associated with the use of biomarkers is to develop rapid non-invasive techniques for their extraction. Sampling the interstitial fluid via the skin is one promising approach that can achieve non-invasively biomarker sampling, but the current techniques need refinement to allow rapid, low cost procedures to be performed.

Hypobaric pressure: Researchers at King's College London have developed a novel hypobaric pressure device to extract macromolecules and nanomaterials from the skin. The device applies a small chamber to the surface of the skin from which it removes air to generate hypobaric pressure conditions. This change in hypobaric pressure stretches and thins the skin, it opens up skins pores and it increases blood supply to the local area without pain. The hypobaric device can be loaded with an extraction buffer to facilitate biomarker extraction. The hypobaric device is a simple system that could be mass-produced with very little cost and it could be used by researchers and healthcare staff. In addition, the temporal changes induced to the skin by the application of hypobaric pressure are reversed almost immediately upon removal of the device and it is therefore considered a non-invasive biomarker extraction method.

Macromolecule extraction: The presentation will discuss data from both in vitro and in vivo experiments that assess the capability of hypobaric pressure to extract macromolecule across the skin. A series of experiments will be presented that utilize both large molecular weight model agents and cytokine biomarkers in order to understand how skin stretching facilitates interstitial fluid extraction from the tissue. Measurements of skin stretching *in-situ* using confocal microscopy will be used to support the theory that the stretching of the skin makes changes deep within the skin tissue such that even the basement membrane permeability is modified by hypobaric pressure to facilitate biomarker extraction.

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Oral Presentation

TRANSDERMAL, PATH SELECTIVE AND CONTINUOUS GLUCOSE MONITORING DEVICE BASED ON A GRAPHENE MULTIPLEXED PLATFORM.

Luca Lipani

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Currently, there is no available needle-free approach for diabetics to monitor glucose levels in the interstitial fluid. Here, we report a path-selective, non-invasive, transdermal glucose monitoring system based on a miniaturized pixel array platform (realized either by graphene-based thin-film technology, or screen-printing). The system samples glucose from the interstitial fluid via electroosmotic extraction through individual, privileged, follicular pathways in the skin, accessible via the pixels of the array. A proof of principle using mammalian skin ex vivo is demonstrated for specific and 'quantized' glucose extraction/ detection via follicular pathways, and across the hypo- to hyper-glycaemic range in humans. Furthermore, the quantification of follicular and non-follicular glucose extraction fluxes is clearly shown. In vivo continuous monitoring of interstitial fluid-borne glucose with the pixel array was able to track blood sugar in healthy human subjects. This approach paves the way to clinically relevant glucose detection in diabetics without the need for invasive, finger-stick blood sampling.

Invited Lecture

THE ASSESSMENT OF PSORIATIC ARTHRITIS, PAST, PRESENT AND FUTURE

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Psoriatic arthritis is a heterogeneous disease that can manifest in several ways including arthritis, spondylitis, enthesitis, dactylitis, iritis as well as skin and nail disease. Historically the primary outcome measure in Psoriatic arthritis trials have been measures that focus solely on the articular manifestations of disease such as the Disease Activity Score 28 (DAS 28) or American College of Rheumatology 20% improvement criteria (ACR20). It is now recognised that this approach is insufficient to truly capture the totality of disease burden. In response there has been an international effort to devise a composite measure that captures all domains of disease. Several candidate measures have now been developed including the Composite Psoriatic Arthritis Disease Activity Index (CPDAI), GRACE measure (initially named the Arithmetic Mean of Desirability Function- AMDF the re-named the GRACE after the original development study) and Psoriatic Arthritis Disease Activity Score (PASDAS), Disease Activity in Psoriatic Arthritis (DAPSA) and RAPID3. In this session we will review the evolving field and novel tools for the assessment of Psoriatic Arthritis.

Invited Lecture

IDENTIFYING PSORIATIC ARTHRITIS IN PATIENTS WITH PSORIASIS; MIGHT PREVENTION OF PSA BE FAR AWAY?

Oliver FitzGerald

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Psoriatic Arthritis (PsA) is a complex, polygenic autoimmune disease with diverse clinical features. PsA is a clinical diagnosis, there are no diagnostic criteria or laboratory tests. The CASPAR criteria are used for classification not diagnosis. The diagnosis of PsA can be difficult and often depends of the expertise of the treating physician. As a result, the diagnosis of PsA is commonly missed with one study showing that 29% of Psoriasis patients attending a dermatology clinic having undiagnosed PsA. This leads to a diagnostic delay which if more than 6 months, in turn contributes to poor radiographic and functional outcome.

As PsA frequently develops in the setting of longstanding psoriasis, patients with psoriasis represent a high-risk group for the development of PsA. In this presentation, the known clinical, genetic, imaging and biochemical risk factors or biomarkers associated with the development of PsA in a population of patients with skin psoriasis are reviewed. Novel approaches required to address this issue are proposed with the hypothesis that it will be a combination of biomarkers rather than a single marker that will ultimately prove sufficiently sensitive and specific, opening up the possibility of considering disease prevention clinical trials.

Invited Lecture

John Pauling, MD, PhD (20 min) (Consultant Rheumatologist at the Royal National Hospital for Rheumatic Diseases & Senior Lecturer in the Department of Pharmacy & Pharmacology, University of Bath, UK)

THE MEASUREMENT OF SCLEROSING SKIN DISEASE

John Pauling

Royal National Hospital for Rheumatic Diseases & Department of Pharmacy & Pharmacology, University of Bath, UK

Clinical thickening of the skin (scleroderma) occurs in a number of sclerosing skin disorders. An important sclerosing skin disease is systemic sclerosis; a potentially life-threatening multisystem disorder characterised by autoimmunity, vasculopathy and aberrant tissue remodelling. The skin is the most commonly affected organ in systemic sclerosis manifesting as cutaneous fibrosis. Whilst not life-threatening, the cutaneous manifestations of systemic sclerosis are a major cause of disease-related morbidity. Assessing the different cutaneous manifestations of systemic sclerosis is challenging and existing clinical endpoints have not performed well in clinical trials of systemic sclerosis resulting in few treatments achieving marketing authorisation. This presentation shall review the clinical features and impact of skin thickening in systemic sclerosis and existing methods for assessing such features. A review of novel approaches to assessing sclerosing skin disease shall be presented including recent work to validate high-frequency ultrasound as an objective non-invasive method of assessing dermal fibrosis.

Oral Presentation

FILM-FORMING AGENTS AS A PROTECTIVE BARRIER TO FUNGAL SKIN INFECTION

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Superficial fungal infections are one of the most common causes of human diseases caused by dermatophytes. Although the infections are mild and rarely life-threatening, they are frequently recurring, and the incidence has increased continuously, especially in the urban region. Simultaneously, there is a growing resistance to antifungal drugs, exposing, in particular, the immunosuppressed patient to higher levels of risk. The project aims to develop a physical barrier that can prevent the early stages of infection to the skin, to avoid the development of antifungal resistance and cross-contamination.

To identify the effectiveness of film-forming agents to prevent and treat fungal skin infections. An *ex vivo* porcine skin infection model was developed to study the potential of film-forming agents in preventing and treating fungal infections caused by dermatophytes (the moulds *Trichophyton rubrum* and *T. interdigitale*, and the yeast *Candida albicans*). Cell viability assays and microscopy were used to study the effects of film-forming agents on dermatophytes. Through studying the interaction with different compounds and chemical elements, the mechanism of action of the film-forming agents was determined by chemical analytical methods, e.g. QTOF-LCMS, NMR and ICP-OES.

Two cationic polymers used in pharmaceutical and cosmetic products inhibited the growth of *T. rubrum*, *T. interdigitale* and *C. albicans* on porcine skin. Viability assays and microscopy imaging indicated that the polymers have fungicidal activity against the *Trichophyton* species, while they act as fungistatic against *C. albicans*. These cationic polymers appeared to coat the cells and inhibit fungal growth by removing the carbohydrate content and essential metal ions (*via* chelation) from the surrounding.

Invited Lecture

ZEBRAFISH AS A MODEL SYSTEM FOR HUMAN DISEASES OF THE SKIN AND PIGMENT CELLS

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From their first exploration as a model organism in the 1980s, zebrafish have now become a mainstream model for biomedical research. Their rapid generation time, large clutch size and ease of maintenance, and with external fertilisation allowing full access to all stages make them very convenient, and their lower sentience than mammalian models makes them desirable from a 3Rs perspective. Crucially, their optical transparency and ease of genetic manipulation makes them outstanding for *in vivo* examination of cell biological and genetic mechanisms. The system benefits from an excellent genome sequence, well-organized online resources, and an open, collaborative research community. Research on fish pigment cells, especially the neural crest-derived cells in the skin, has long received attention. Mutants modelling a wide-range of human pigmentation diseases, including albinism, Hermansky-Pudlak Syndrome, Waardenburg Syndrome have been well-documented. Mutations affecting any candidate human disease-causing gene can be readily generated using CRISPR-Cas9 approaches, and targeted induction of changes directly equivalent to those in human disease alleles are now the focus on these efforts. Embryological observations in these models have illuminated the endogenous function of these genes and helped understand the pathologies of these conditions. The genetics of skin has only recently begun to be examined in the fish, but these same approaches can be readily applied. A major area for biomedical modelling in the zebrafish is in the field of melanoma biology, where transgenic expression of human oncogenes has been used to efficiently generate melanoma models, for example allowing investigation of the genetic basis for their invasiveness.

Invited Lecture

HOST DEFENSE PEPTIDES TO COMBAT INFECTIONS – FROM MODE-OF-ACTION TO DRUG DELIVERY

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Membrane interactions play an important role for host defense peptides, including their antimicrobial, anti-inflammaory, and anticancer effects. The present overview exemplifies some of our recent work on how biophysical investigations with model lipid bilayers and bacterial lipopolysaccharides can be combined with biological experiments to clarify modes-of-action, and allow peptide optimization towards therapeutic development. In addition, since efficient delivery of such peptides is challenging due to their size, cationicity, and amphiphilicity, delivery systems are important for their development into therapeutics, e.g., for controlling peptide release, for reducing infection-related peptide degradation, for suppression of toxicity, or for increasing bioavailability. In addition, numerous nanomaterials display potent and triggerable antimicrobial effects on their own. When combined with host defense peptides, combinatorial and synergistic effects have been observed. The mechanistic origin of these effects are poorly understood that present, however, precluding rational design of mixed nanoparticle/peptide antimicrobials and nanoparticulate delivery systems for host defense peptides. Reporting on some of our recent work in this area, membrane interactions and antimicrobial effects of selected nanomaterials as peptide delivery systems are outlined, ranging from nanoparticles as passive peptide containers to systems providing additional functions for combatting infection.

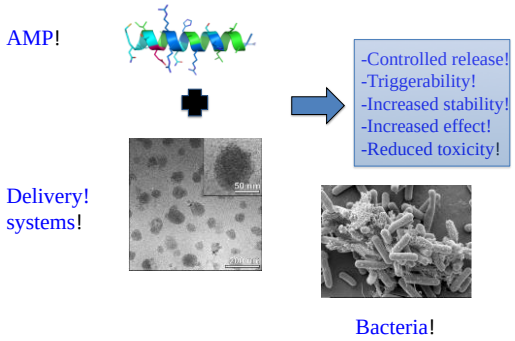

Figure 1. Nanoparticulate drug delivery systems provide various advantages for antimicrobial peptides.

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Invited Lecture

FIGHT IR-INDUCED AGING : NOVEL CONCEPT TO SKIN PHOTOPROTECTION

Gernot Walko

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Skin epidermis is essential for our survival as terrestrial beings. It provides protection from harmful microbes and other assaults from the external environment and retains body fluids. To withstand normal wear and tear, the epidermis constantly self-renews. This ability resides in tissue-resident stem cells, which self-renew, preserve, and repair their tissue during homeostasis and following injury. Epidermal cancers have become by far the most frequently diagnosed cancers in the UK and world-wide. There is ample evidence that in epidermal cancers, neoplastic cells hijack the homeostatic controls that operate in normal stem cells, eliminating those that inhibit differentiation and upregulating those that exert a positive effect on proliferation. By performing genome-wide functional screens I have previously obtained an extensive catalogue of genes involved in the control of normal and neoplastic epidermal cell proliferation. In my seminar, I will focus on two essential epidermal stemness-regulating proteins identified in my screens: the closely related transcriptional co-activators YAP and TAZ. I will summarize our knowledge about how YAP/TAZ drive self-renewal of stem cells to repair epidermal tissue defects, and how epidermal carcinoma cells exploit YAP/TAZ's functions to sustain their uncontrolled proliferation and to maintain an undifferentiated stem-like cell state. I will end my seminar by giving an overview about my lab's current and future research directions.

Invited Lecture

TOPICAL THERAPEUTIC EFFICACY OF EBSELEN AGAINST MULTIDRUG-RESISTANT STAPHYLOCOCCUS AUREUS TARGETING THIOREDOXIN REDUCTASE

Jun Lu

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Multidrug resistant (MDR) bacterial infection is a major public health threat worldwide. Thus there is an urgent need to find new antimicrobial agents and new antibacterial targets to overcome this threat. Thioredoxin (Trx) and glutathione systems are two major thiol-dependent antioxidant systems maintaining bacterial cellular redox balance. The Trx system is widespread in eukaryotes and prokaryotes, but the GSH system is deficient in many bacteria, such as Staphylococcus aureus, Helicobacter pylori, Bacillus subtilis, etc. Furthermore, there is great difference in structure and reaction mechanism between bacterial TrxR and host mammalian TrxR. These properties make bacterial TrxR act as a possible antibacterial drug target. Recently, we have found that selenium-containing organic compound ebselen has a bactericidal effect against GSH-negative bacteria by attacking the active site of bacterial TrxR, leading to intracellular redox imbalance and bacterial cell death. A clinically isolated MDR S. aureus exhibited to be sensitive to ebselen. Moreover, ebselen displayed therapeutic efficacy in the treatment of rats with the MDR S. aureus skin infection. The bactericidal effect of ebselen was correlated with the inhibition of bacterial TrxR activity. Taken together, these results suggest that ebselen is a potent antibacterial agent and TrxR may emerge as a potential drug target for topical treatment of skin staphylococcal infections.

Invited Lecture

EMERGING TREATMENTS IN ATOPIC DERMATITIS

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Atopic Dermatitis is the most common inflammatory skin disease and, when severe, has a significant impact on the quality of life of patients and their families. The treatment landscape for AD patients is changing rapidly now with the development of pathogenesis targeted small molecule and biologic therapies that offer hope for better disease control for patients with fewer off target adverse effects. This talk will review recent developments in AD pathophysiology and the progress of targeted therapies to the clinic

Oral Presentation

INTRODUCING A NEW CONCEPT FOR THE TREATMENT OF ROSACEA

Jian Li

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Treatment of erythematotelangiectatic rosacea (ETR) is extremely challenging, because of the severe facial flushing and anxiety, which form a vicious cycle of reciprocal causation in the pathogenesis. There are no known specific treatments available, some cases have reported that beta-blockers play a role in the treatment of rosacea, but there are not yet any randomized controlled prospective, with larger series, clinical studies to evaluate the effects of systemic beta-blocker therapy in treating ETR. Tetracycline such as minocycline is the standard systemic therapy of rosacea. However to date no study has assessed its effect on patients with only ETR or persistent centrofacial erythema with transient flushing.

Objective: the aim of this study was to compare the efficacy and safety between the monotherapies of carvedilol and minocycline and identify which treatments have the largest or fastest impact on persistent centrofacial erythema with transient flushing and anxiety.

Materials and Methods: This is a prospective, single-centre, single-blind, randomized, controlled clinical study. Patients received carvedilol 5mg bid or minocycline 100mg qd for 3 months. The rosacea-specific Qol instrument(RosaQoL),Patient self assessment(PSA), Clinicians erythema assessment(CEA), Generalized anxiety disorder(GAD-7) and Patient health questionnaire (PHQ-9) were performed with questionnaires and taking pictures of face every 2 weeks.

Conclusion: Our study found that compared to minocycline, carvedilol could significantly reduce flushing and erythema. In addition carvedilol had a maintenance effect after drug withdrawal, as well as significantly reducing the skin temperature. The use of this drug could be a novel effective systematic treatment for ETR.

Invited Lecture

ATOPIC DERMATITIS AND FILAGGRIN: OF MICE AND MEN.

Padraic Fallon.

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One of the predisposing genetic factors for the development of atopic dermatitis (AD) are gene mutations that affect the integrity and functions of the skin barrier. Loss-of-function mutations in the filaggrin gene, leading to a defective skin barrier, predispose to the development of AD in patients as well as progression to AD-associated asthma. A mouse strain with a natural mutation that developed flaky skin was shown to have frameshift mutation in the *Flg* gene, analogous to mutations in *FLG*, resulting in filaggrin-deficiency and a disrupted skin barrier. *Flg* mutant mice have neonatal skin inflammation, become atopic and develop spontaneous AD-like skin inflammation that progresses with age to secondary compromised lung function. In this presentation the use of filaggrin mutant mice to gain insights on the underlying mechanism that contribute to the development of allergic skin and lung inflammation will be explored.

Invited Lecture

PSORIASIS IN CHINA-EXPERT PATIENT TALK - YAN HUO, BISHAN PEOPLE'S

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Psoriasis is a life-long term skin disease which impacts on the overall quality of life of patients. Patients not only suffer from physical discomfort but also social exclusion and low self-esteem. As for myself, I am an expert patient who suffered from psoriasis for over 20 years and also a dermatologist. In this article I will discuss my experience of psoriasis and the difference in therapies between the U.K. and China.

Keynote Lecture

LABILE IRON AS THERAPEUTIC TARGET IN IRON DYSHOMEOSTASIS
from concept to clinical application

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The concept of labile cell iron or labile iron pools (LIP) was introduced in order to describe transitory forms of cell iron that are of both physiological, pathological and pharmacological importance.

Physiologically, LIP is at the crossroads of cell iron metabolism, serving as metabolic source of metal but also as indicator of iron levels that cells sense and regulate by balancing uptake with utilization or storage. LIP is per se a generic term used to describe the labile iron in the cell, mostly in the cytosol, where it is neither chemically homogeneous nor constant over time. ***Pathologically***, an excessive and persistent rise in LI can compromise cell integrity, since excessively accumulated labile iron can also engage in the catalysis of ROS formation when acting on reactive-O- intermediates to generate noxious reactive O species (ROS). Thus as LIP attains "excessive" levels and ROS formation surpasses cellular anti-oxidant capacities, particularly when GSH resources are depleted, serious chemical damage ensues leading cells to necrotic or ferroptotic death.

The above noxious reactions are often initiated in mitochondria, which not only tend to accumulate excessive LI in various pathologies but also suffer the consequences of ROS formation and ensuing damage. The mitochondrial iron accumulation is mostly associated either with major infiltration of labile iron originating from iron overloaded plasma (LPI) or with an intracellular **misdistribution** of the metal caused by an inherited inability of mitochondria to use incoming iron for heme or for iron sulfur cluster production (as in Friedreich ataxia- FRDA or Wolfram syndrome 2-WS2). Iron misdistribution disorders are characterized by accumulation of iron in given cells or cell compartments with concomitant depletion elsewhere in the system. In inherited disorders like Friedreich ataxia and Wofram syndrome 2-WS2, faulty ISC-biogenesis causes iron to accumulate in mitochondria with concomitant cystolic iron depletion and ensuing GSH depletion, leading to ferroptotic death. Similar features were observed in PD-neurons.

Pharmacologically. To the extent that mitochondrial siderosis and GSH depletion are primary or secondary contributors to FRDA, WF2 or PD pathologies, one can consider mitochondrial LIP as bona fide target of chelation for iron detoxification, provided the treatment is properly controlled so as not to attain a stage of cell or systemic metal deficiency and supplementation with precursors of GSH synthesis. Thus, chelation was used conservatively by aiming to scavenge the labile metal from sites of excessive accumulation and deliver the chelated metal sites of deprivation via biological chaperons (e.g. from cells to plasma transferrin or from organelles to cytosol components). GSH production was stimulated by supplementation with N-acetyl-cysteine-NAC.

We have taken conservative iron chelation and stimulated GSH production as concepts tested at the laboratory bench (cell and animal models of disease) to clinical bedside in 3 different siderotic disorders: FRDA, WS2 and PD.

We will provide a progress report on ongoing trials and our longstanding and more recent (realistic) expectations.

The above is the result of fruitful collaborations with Arnold Munnich (Paris) in FRDA, David Devos (Lille) in PD and Rachel Nechushtai (Jerusalem) in WS2.

Invited Lecture

PREVENTION OF SKIN AGING BY REDUCING IRON FROM THE SKIN

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Reactive oxygen species (ROS) produced by endogenous and exogeneous sources are considered the major contributor of skin aging, which is characterized by dryness, wrinkles, and atypical pigmentation. UV enhances ROS production in cells and, thus, causes premature skin aging, which is termed as "photo-aging". The use of antioxidants is a current therapy to repair symptoms and damages related to ROS-induced aging of the skin. Because ROS is so reactive that oxidizes often very fast with the biomolecules nearby, this renders antioxidants almost ineffective. A better understanding of the root cause of ROS formation and a more effective approach are needed in order to prevent ROS-mediated skin aging and photo-aging.

Iron is the most abundant transition metal in the human body for many essential physiological functions. There are multiple pathways of iron absorption from the diets into the body. Once iron is inside the body, menstruation and epidermal desquamation of the skin are important routes to get rid of it. However, when menstruation is no longer available, iron accumulates more in the skin of older women. Iron catalyzes ROS formation through Fenton/Haber-Weiss reactions. Without iron, this reaction goes so slow and ROS production becomes unnoticeable. Therefore, removing iron from the skin is more effective and one step earlier than antioxidant therapy. It changes the mode of action on skin aging from repair by antioxidants to prevention by iron removal.

In this talk, molecular mechanisms of iron excretion through skin will be discussed. Transferrin and transferrin receptor axis are important to transport iron from the body to the skin. Accumulation of iron in the skin increases with age and causes ROS formation, leading to the skin aging, such as wrinkles and hyper-pigmentation, and even more skin thinning in the presence of UVA. By lowering iron in skin, dark circle under the eye can be significantly reduced. Elasticity and water retention in the skin are significantly increased. Taken together, our results indicate that high level of iron accumulations in the epidermis is one of the most critical endogenous etiological factors in skin aging and photo-aging, particularly in older women. By identifying iron as the cause with further investigation, we may provide the appropriate prevention and treatments solutions for skin aging according to the cause.

Keynote Lecture

MITOCHONDRIAL REGULATION OF FERROPTOSIS

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Mitochondria are key regulators of energy metabolism, redox balance, calcium homeostasis, and programmed cell death. In the past, we characterized a pivotal role for mitochondria in caspase-dependent and in caspase-independent death signalling in neurons. For example, we identified mitochondrial damage in caspase-independent neuronal death involving the release of AIF from mitochondria to the nucleus in oxidative cell death, i.e. ferroptosis, in vitro and these findings are highly relevant for neuronal cell death in vivo, in paradigms of ferroptosis, cerebral haemorrhage, and after cerebral ischemia. Using pharmacological compounds and genetic approaches, e.g. targeting regulators of mitochondrial fission such as Drp1 or pro-apoptotic members of the bcl-2 family such as BID we confirmed the conclusion that mitochondria represent the “point of no return” in paradigms ferroptosis.
More recently, we found that protective interference with mitochondrial pathways of programmed cell death was frequently attributed to metabolic switches, i.e. reduced mitochondrial respiration and increased glycolytic activity in the protected cells. Thus, according metabolic switches may serve as a general strategy for mitochondrial protection and, thereby, neuroprotection, but may also affect mechanisms of neuroinflammation involving activation of microglia. The deeper understanding of the underlying mechanism of such metabolic protection may reveal novel therapeutic targets in neural diseases featuring oxidative dysregulation, mitochondrial impairments, neuronal death and also neuroinflammation.

Invited Lecture

WOLFRAM SYNDROME, MITOCHONDRIAL IRON OVERLOAD & SKIN

Ola Karmi[1], Henri-Baptist Marjault[1], Yang-Sung Sohn[1], Ioav Cabantchik[1], U. Najwa Abdulhaq[2], Gil Leibowitz[3], David H. Zangen[2] and **Rachel Nechushtai**[1]
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NAF-1 belongs to the NEET proteins which are [2Fe-2S] proteins. localized inside the mitochondria (MiNT, CISD3) and at the outer mitochondrial membrane (OMM - mNT, CISD1) and the Endoplasmic Reticulum (ER) and the ER-mitochondrial associated membranes (MAM – NAF-1-CISD2)[1]. A homozygous mutation in the NAF-1 encoding gene, *cisd2*, that is located on chromosome 4q22-24, causes an autosomal recessive disorder named **Wolfram Syndrome Type-2** (**WFS-T2**)[2]. The single missense mutation at nucleotide 109 where G substituted with C (G109C), leads to exon skipping, frame shift and premature stop codon that practically lead to the absence of the NAF-1 protein. This mutation appears to be abundant (1:40) in our regional Palestinian populations. WFS-T2 patients suffer, among other pathologies, from optical nerve atrophy, sensorineural hearing loss, psychiatric episodes and β-cell dysfunction[2]. The latter results from cellular stress and apoptosis leading to sever insulin deficiency.
NAF-1 was shown to be involved in Fe/Fe-S/ROS/Ca^{+2}/redox homeostasis. Suppressing of NAF-1 expression (via shRNA), results in misdistribution of cellular iron, where iron accumulation in the mitochondria occurs and the later leads to ROS induction, oxidative damage with ensuing the key cellular processes of autophagy, mitophagy, ferroptosis and apoptosis[4]. To avoid these pathophysiological situations, we tested treatments of an iron chelator with/without antioxidant agent on **skin fibroblasts** obtained from four patients confirmed with the absence of NAF-1Like in the fibroblasts of WFS-T2 patients, mitochondrial labile Iron and ROS accumulation occurred in INS-1E – NAF-1(-) cells. Combining optimized levels of chelation/anti-oxidant treatments, induced a near-complete correction of the mitochondrial membrane potential, mitochondrial labile iron and ROS levels in the WFS-T2 patient skin fibroblasts cells or the β-pancreatic cellular model.
A mice model of *cisd2-/-* was generated[3]; In the latter similar symptoms to WFS-T2 patients e.g. eye and muscle atrophy, shorter life span and a major decrease in mitochondrial integrity were observed. In addition, a decreased density of hair follicles containing hair in the *cisd2-/-* mice was detected compared with wild-type **skin**. Histological analyses of the skin from 12-mo-old wild-type and *cisd2*-/- mice, indicated that the ***cisd2*-/- skin exhibits a phenotype involving a hyperplastic epidermis, hair follicle atrophy, a decrease in subcutaneous fat and muscle, and an increased thickness of the dermis layer.**

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Oral Presentation

NEUROPILIN-1 CONTROLS ENDOTHELIAL HOMEOSTASIS BY REGULATING MITOCHONDRIAL FUNCTION AND IRON-DEPENDENT OXIDATIVE STRESS VIA ABCB8

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The transmembrane protein Neuropilin-1 (NRP1) promotes vascular endothelial growth factor (VEGF) and extracellular matrix signalling in endothelial cells (ECs). Although it is established that NRP1 is essential for angiogenesis, little is known about its role in EC homeostasis. Here, we report that NRP1 promotes mitochondrial function in ECs by preventing iron accumulation and iron-induced oxidative stress through a VEGF-independent mechanism in non-angiogenic ECs. Furthermore, NRP1-deficient ECs have reduced growth and show the hallmarks of cellular senescence. We show that a subcellular pool of NRP1 localises in mitochondria and interacts with the mitochondrial transporter ATP-binding-cassette-B8 (ABCB8). NRP1 loss reduces ABCB8 levels, resulting in iron accumulation, iron-induced mitochondrial superoxide production and iron-dependent EC senescence. Treatment of NRP1-deficient ECs with the mitochondria-targeted antioxidant compound mitoTEMPO or with the iron chelator deferoxamine restores mitochondrial activity, inhibits superoxide production and protects from cellular senescence. This finding identifies an unexpected role of NRP1 in EC homeostasis.

Keynote Lecture

THERAPEUTIC IRON CHELATION

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There are three iron chelators in current therapeutic use, desfen•ioxamine, deferiprone and desferasirox. All three are associated with difficulties; desferrioxamine is not orally active, deferiprone is associated with reversible agranulocytosis in 1-2% of patients and desferasirox is associated with skin and renal side effects. So there is a continuing effort to identify efficient chelators which lack side effects. Two such compounds will discussed namely Deferitazole, a desferrithiocin analogue and CN 128, a deferiprone analogue.

In addition to their role in the treatment of systemic iron overload, resulting from regular blood transfusion, iron chelators have potential for treating neurodegeneration and skin disease. The presentation will cover current interests in the treatment of Parkinson's disease, photosensitivity and psoriasis.

Oral Presentation

MITOCHONDRIA-TARGETED IRON CHELATORS

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Mitochondria are important producers of reactive oxygen species (ROS) which are generated as a result of leakage of electrons from the electron transport chain. The coexistence of both ROS and iron in mitochondria renders them particularly prone to oxidative damage. For instance, mitochondrial labile iron (LI) is a major contributor to the susceptibility of skin fibroblasts to ultraviolet A (UVA)-induced oxidative damage, leading to necrotic cell death via ATP depletion. We have designed fluorescent mitochondria-targeted peptide-based iron chelators functioning as biosensors of the mitochondrial LI pool. These dual probes demonstrated selective accumulation in the organelle and LI-sensitive fluorescence emission. Mitochondria iron overload is a key feature of the neurodegenerative disease Friedreich's ataxia (FRDA). We have shown that cultured primary skin fibroblasts isolated from Friedreich's ataxia (FRDA) patients are 4 to 10-fold more sensitive to UVA-induced death than their healthy counterparts. Furthermore, using one of our fluorescent probes (BP19), we have demonstrated that FRDA cells display higher levels of mitochondrial LI (up to 6-fold on average compared to healthy counterparts) and show a larger increase of mitochondrial generation of ROS after UVA irradiation, consistent with their differential sensitivity to UVA. In addition, pre-treatment of FRDA cells with a hexadentate mitochondrial iron chelator (BP29) fully abrogates the UVA-mediated cell death and reduces UVA-induced damage to mitochondrial membranes. These results reveal a link between FRDA as a disease of mitochondrial iron overload and sensitivity to UVA of skin fibroblasts. Our findings suggest that the high levels of mitochondrial LI in FRDA cells, which contribute to high levels of mitochondrial ROS production after UVA irradiation, are likely to play an important role in the marked sensitivity of these cells to UVA-induced oxidative damage. Our results pave the way to the development of topical mitochondria-targeted iron chelators as skin photoprotective agents.

Invited Lecture

CIRCADIAN RHYTHM IN THE SKIN IN RELATION TO SKIN AGEING AND DISORDERS

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Almost all plants and animals exhibit circadian rhythms. These intrinsic time keeping mechanisms align biological functions with regular and predictable environmental patterns to optimise function and health. In this presentation we will introduce the concepts of circadian timing in mammals with an emphasis on the peripheral clock network in different organs including skin. Disruption of these rhythms can be caused by many factors such as shift work, LAN, long distance travel, etc. We will discuss the association between disrupted circadian clocks and the increased risk for diseases such as cancer. In depth knowledge of the interplay between chronodisruption and the onset and progression of cancer offers new perspectives for personalised treatments.

Invited Lecture

EFFECTS OF ULTRAVIOLET RADIATION ON BLOOD PRESSURE IN A LARGE CHRONIC HAEMODIALYSIS PATIENT COHORT

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Hypertension remains a leading global cause for premature death and disease. Population blood pressure correlates with latitude and is lower in summer than winter. Seasonal variations in sunlight exposure account for these differences, with temperature believed to be the key contributor. Recent research has indicated that ultraviolet (UV) light enhances nitric oxide availability by mobilising storage forms in the skin, suggesting that incident solar UV radiation may lower blood pressure. We tested this hypothesis by exploring the association between environmental UV exposure and systolic blood pressure (SBP) in a large cohort of chronic haemodialysis patients in whom SBP was determined regularly. We studied 342,457 patients (36% Black, 64% White) at 2,178 U.S. dialysis centers over 3 years. Incident UV radiation/temperature data for each clinic location were retrieved from public databases. Pre-dialysis SBP varied by season and was ~4 mmHg higher in Black patients. Temperature, UVA and UVB were all linearly and inversely associated with SBP. Intriguingly, this relationship remained statistically significant after correcting for temperature. In hemodialysis patients, incident solar UV radiation is associated with lower SBP, independent of temperature. This raises the possibility that lack/avoidance of sunlight is a new risk factor for hypertension, perhaps even in the general population.

Invited Lecture

WAVELENGTHS LONGER THAN 380NM CAUSE PHOTODAMAGE TO SKIN CELLS THAT IS NOT ADEQUATELY PROTECTED BY APPLICATION OF CONVENTIONAL SUNSCREENS.

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The adverse effects of solar UVR (~295 – 400nm) on the skin are well documented, especially in the UVB region (~295-315/320nm), and sunscreens have been demonstrated to be beneficial in inhibiting a wide range of photodamage. The effects of long-wave UVA1 (>380nm) and visible radiation on the skin are much less well known. Most sunscreen formulations provide very little protection in the long wave UVA region (380-400nm) and almost none from shortwave visible wavelengths (400-420nm). We demonstrate photodamage in *vitro* and *in vivo* in this region with high irradiance, narrowband LED arrays at 385nm and 405nm, using environmentally relevant doses. This is also relevant for UVA1 phototherapy. The endpoints include cell viability, DNA damage (cyclobutane pyrimidine dimers – CPD), differential gene expression and oxidative stress *in vitro* in HaCat keratinocytes, and pigmentation, erythema, DNA damage and gene expression changes *in vivo* in human volunteers. For most endpoints we found a clear dose-response relationship for both sources. There was a highly significant reduction in cell viability, increase in reactive oxygen species, and a number of genes associated with adverse effects were significantly upregulated including genes for inflammation (IL-1a, IL-6, IL-8, IL-10, IL-20, PTGS2), photoageing (MMP-1, MMP-3, MMP-9, MMP-10, MMP-12) and oxidative stress (PON-2, HMOX-1) *in vitro* and *in vivo*. At high doses of 385nm radiation there was an increase in CPD production *in vitro*, but no such effect at 405nm. We also demonstrate the production of delayed or 'dark' CPD *in vivo* in healthy volunteers. In addition we demonstrate that these sources induce skin-type dependent changes in erythema, immediate pigment darkening (IPD) and persistent pigment darkening (PPD) *in vivo* measured immediately, 6 and 24 hours post exposure.These endpoints were subsequently used to demonstrate that there is inadequate protection provided by a conventional sunscreen (SPF=15) labelled as UVA protective in the USA and Europe. The addition of a new filter, C1332 to the formulation, which absorbs in this region (380-420nm), to a formulation of similar SPF (SPF=15.8) in most cases provided significantly more protection when compared to the conventional formulation, returning damage to levels comparable with the unirradiated control. This work provides new insight into photodamage and may lead to new strategies to provide improved photoprotection in the currently poorly protected UV/visible radiation boundary region. This work was funded by BASF GmbH. BASF who produces the raw ingredients used for the sunscreens used in this study.

Oral Presentation

THE ROLE OF HEME OXYGENASES-1: A MULTIFUNCTIONAL PROTEIN THAT PARTICIPATED IN THE ENDOPLASMIC RETICULUM STRESS AND CUTANEOUS THERMAL INJURY HEALING

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Ultraviolet irradiation is known as a crucial environmental factor that can cause skin-related diseases. Recent studies have shown that Ultraviolet A (UVA) exposure induces numerous antioxidant genes, including heme oxygenase-1 (HO-1). In the present study, we found that the reduction of HO-1 and HO-2 expression in HaCaT cell line could lead to cell shrinkage, enhanced LDH leakage and increased cellular reactive oxygen species (ROS) level following UVA irradiation. We have previously demonstrated that Acetyl-11-keto-β-boswellic acid (AKBA) is an antioxidant agent that protects the skin cells against UVA radiation by modulating the inflammatory response and ROS production. In the present study, we further demonstrate that AKBA could increase both HO-1 and HO-2 expression, suggesting that AKBA's protection against oxidative stress may be through modulation of HO system. The vital cyto-protective properties of HO-1 and HO-2 against UVA radiation warrant further investigation.

UVA also causes endoplasmic reticulum stress by causing the phosphorylation of a subunit of eIF2. Meanwhile, UVA is also known inducers of nuclear factor erythroid-derived 2-related factor 2 (Nrf2) and HO-1, both of which belong to a vital protective signaling family against oxidative stress. We have recently found that UVA irradiation activates the phosphorylation of eIF2a and Nrf2-HO-1 pathway in a dose-dependent manner. Modulation of eIF2a phosphorylation status with a selective inhibitor of eIF2a de-phosphorylation (Salubrinal) could alter expression pattern of Nrf2-HO-1 signaling and affect the cell cycle in mouse Keratinocyte cell line, JB6. As a main sensor in the ER membrane, PERK could phosphorylate eIF2a resulting in ATF4 induction and ATF4 could also regulate HO-1. Meanwhile, PERK could also phosphorylate and activate Nrf2, which also provides us a new insight to explore the HO-1 transcriptional regulation under the crosstalk of ER stress and Nrf2 pathway. Besides, this study offered us the possibility to investigate the cascade of HO-1 regulation in response to different stimuli conditions.

In the latest research, we also observed that in mouse cutaneous thermal injury, HO-1 could be rapidly induced around the wounded tissues and it could accelerate cutaneous wound healing, which revealed that HO-1 may not only protect against inflammatory, but also mediate proliferation that is helpful to accelerate wound healing.

Invited Lecture

ASSESSMENT OF THE DERMAL BIOAVAILABILTY OF TOPICAL GLUCOCORTICOIDS: STRATUM CORNEUM SAMPLING VERSUS THE VASOCONSTRICTION ASSAY

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Background: The clinical efficacy of topical glucocorticoids (immunosuppression, anti-proliferation and anti-inflammation) is crucially dependent upon the rate and extent at which these compounds reach their pharmacological targets in the viable skin. To-date, steroid-induced vasoconstriction has been used as a surrogate marker of potency (and as a metric for bioequivalence) even though the blanching response is not linked to clinical effect. To determine whether an effective level of steroid is achieved locally following topical application, knowledge of the drug's input rate to the basal epidermis and its clearance therefrom is required. The goal of this work, therefore, was to examine whether stratum corneum (SC) sampling could provide quantification of steroid delivery and to compare the results with pharmacodynamic measurements of vasoconstriction. **Methods**: Betnovate® 0.1% cream (betamethasone valerate -BMV-) was applied at doses of 2, 5 and 10 mg/cm^2 to the ventral forearms of 12 healthy volunteers (ethical approval EP 17/18 154). At separate skin sites, the mass of drug in the SC was measured by the adhesive tape-stripping technique (a) after a 4-hour 'uptake' period, and (b) after removal, subsequent to a further 6-hours of 'clearance'. The BMV-induced vasoconstriction was assessed by a chromameter over 22-hours post-application of the cream for 4-hours.
Results: SC uptake of BMV was significantly higher ($p < 0.05$) when 5 mg/cm^2 of the cream was applied compared to the 2 and 10 mg/cm^2 doses. In all cases, ~30% of the BMV in the SC at the end of the 4-hour uptake period was cleared in the subsequent 6-hours. The BMV flux into the viable epidermis was ~4 ng/cm^2/hr, corresponding to a first-order elimination rate constant from the SC of ~0.07 hr^{-1}. The vasoconstriction results were highly variable and insensitive to the amount of BMV cream applied, regardless of whether non-responders were excluded from the data analysis or not.

Conclusions: Evaluation of BMV dermato-pharmacokinetics from SC sampling enabled quantitative assessment of drug delivery into the viable skin. In contrast, the pharmacodynamic data based on the vasoconstriction assay were dose-insensitive and very poorly reproducible.

Invited Lecture

PREDICTING DERMAL ABSORPTION IN DISEASED OR DAMAGED SKIN USING PBPK MODELLING

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Physiologically Based Pharmacokinetic (PBPK) modelling is a mechanistic modelling framework that uses a bottom-up approach to describe the relevant physiology of the system and physicochemical properties of the drug, separately. One of the major strengths of this approach is the ability to extrapolate between populations, this extrapolation can be between ethnic groups, to a paediatric or diseased population.

The aim of the present research was to collect data describing changes in skin physiology for various skin diseases. A summary of the collected data for Plaque Psoriasis, such as changes in corneocyte dimensions and hydration, number of layers in the stratum corneum, epidermal thickness, and blood flow are presented.

Following incorporation of these parameters in to the PBPK model framework, one can predict how dermal absorption and pharmacokinetics of various drugs and formulations may be affected by this altered physiology. Example simulations with Methoxsalen and Caffeine are presented to illustrate how the physiological changes can affect drug disposition, and how this is dependent on the physicochemical properties of the drug.

Invited Lecture

RAMAN SPECTROSCOPIC ASSESSMENT OF TOPICAL DRUG BIOAVAILABILITY IN THE SKIN

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Quantitative evaluation of the bioavailability (BA) of a topically applied drug at or near its site of action in the skin represents an unmet scientific challenge. Most dermatological drug targets are located in the epidermis/upper dermis, below the stratum corneum (SC, which is the principal barrier to drug absorption). While a few alternatives to clinical assessment exist, identification and validation of surrogate approaches for evaluation of local BA represents a work-in-progress. The central hypothesis of this research is that spectroscopic imaging (specifically, Raman) may offer a non-invasive, accurate, sensitive and reproducible method for determination of the rate and extent to which a topically administered drug becomes available at or near its site of action below the SC.
Using a Renishaw inVia microscope we investigated two molecules of interest: crisaborole, a recently approved drug for the treatment of eczema and a model compound, 4-cyanophenol (CP); both have strong Raman signals (-C≡N vibration) in a frequency range where skin is spectroscopically 'transparent'. It was possible to monitor the clearance of the molecules up to a depth of ~70 □m into the skin. The results from the uptake experiments showed strong correlations between the Raman signal intensity and the amount of drug extracted from the SC tape-strips that were analysed by High Performance Liquid Chromatography (HPLC). The effect of the attenuation of the Laser as a function of depth into the skin was also investigated and a correction proposed using the Amide I signal (1650 cm^{-1}) originating (primarily) from keratin.
Raman spectroscopy possess a great potential to track drug penetration as a function of depth into the skin (and beyond the SC), and can be used to track drug clearance from the SC. Furthermore, we were able to compare drug delivery into the skin from different formulations, and to follow the penetration of certain formulation excipients. Artifacts due to signal attenuation by absorption/scattering of radiation can be mitigated by normalization, and correlation with complementary techniques (e.g., SC sampling) may offer the opportunity for at least a semi-quantitative assessment of drug BA using the Raman approach.
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Invited Lecture

TRACKING EXCIPIENTS FROM A TOPICAL DRUG PRODUCT HROUGH THE STRATUM CORNEUM *IN VIVO*

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For topical formulations, drug disposition can be influenced by the time-dependent skin concentrations of certain excipients, which may modify drug solubility in the formulation, or in the stratum corneum (SC), or enhance drug diffusivity across the barrier. However, skin uptake and penetration of excipients are infrequently monitored and their local pharmacokinetics are rarely quantified. Among the most commonly used excipients in topical products are propylene glycol (PG) and 1,3-butylene glycol (BG), the uptake of which into human skin *in vivo* has been measured in this research using the minimally invasive approach of SC sampling to better understand their role in drug delivery from an approved drug product. Sections of the plaster (cut to 5 cm^2) were applied to the forearms of healthy volunteers (n = 4) and PG and BG uptake into the SC was measured after 0.5, 1, 2 and 6 h of wear. Quantitation of the glycols extracted from the acquired series of tape-strips permitted SC-concentration versus depth profiles to be deduced. The glycols were assayed by HPLC-UV after derivatisation using p-toluenesulfonyl isocyanate.

The results obtained from the uptake of PG and BG suggests that these volatile solvents were rapidly taken into the SC and it is likely that they would transiently change the structure of the SC and assist in transferring the drug quickly from the formulation into the barrier. In parallel, evaporation of the solvents was identified. From a thermodynamic point of view, the loss (by evaporation and SC uptake) of PG and BG will eventually reach the point at which the amount of drug remaining at the skin surface can no longer be fully solubilised and drug transfer into the SC will slow down. In broad terms, the results obtained suggest that tracking key excipients – not only the drug – would be helpful in determining drug bioavailability from a topical formulation.

Invited Lecture

ETHNOPHARMACOLOGY AND SKINCARE

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Ethnopharmacology is an area of research that focuses on the traditional uses of natural products (e.g, plants, minerals, insects etc.) and their medicinal properties. This discipline builds on the understanding that some traditional uses of natural products are probably inspired by non-human animal behaviour. Several studies have indicated that traditional plant preparations have exhibited pharmacological effects consistent with their topical uses by people and/or animals (e.g, birds, orangutans etc.) in their natural environments. Such evidence indicates that there is much to learn about skincare from our ancient ancestors, folklore and animals in the wild. Some of the natural products highlighted in these types of studies also have the potential to revolutionise treatment strategies for different skin conditions.

Invited Lecture

A BRIEF REVIEW ON COSMETIC REGULATION IN THE EU/US AND REQUIRED COMPLIANCE TESTS

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The EU regulation 1223,2009 came to place to ensure harmonisation of cosmetic products placed on the market in all the EU states. In this presentation, we briefly looked into the different between pharmaceutical and cosmetic definition and EU regulatory system for cosmetic products. Then the required test before they are placed on the market will be discussed.

Oral Presentation

Single-Cell Map of Patellar Tendon Reveal Diverse Participator Involving in the Injured Microenvironment

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Tendon is the connective tissue that connect muscles to bones and it drives bone movement by pulling the contraction of the muscle. Deficient understanding of the constituents of tendon resident cells and how these cells participate in the damage repair directly compromise the clinical treatments for the healing of injured tendon. For identifying the tendon derived cells, previous investigations with genetic tracing and flow cytometry were limited in the ability to assess stromal cell heterogeneity and address differentiation trajectories, since these approaches require the selection of limited gene products to tag and trace cells. While such methods can definitively identify the cell types that can contribute to tissue plasticity, the analysis is limited to markers discovery, and is thus biased and not comprehensive. Here we report on single-cell RNA sequencing (scRNA-seq) to high-throughput investigate the stromal cell heterogeneity in patellar tendon. Combining tissue based RNA-seq, immunohistological analyses with single-cell RNA-seq profiling reveals evidences for 15 clusters of resident cells in health tendon tissue, whereas 11 clusters in injured tendon. These complicated heterogeneity of resident cells rendered various microenvironments for tendon regeneration. Therefore, these results demonstrate the availability of scRNA-seq to deconstruct tenogenic niches and suggest novel functional interactions among resident stromal cell subpopulations.

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Oral Presentation

CONTINUOUS PRODUCTION OF HYDROGEN PEROXIDE BY A SELF-ACTIVATED MULTIENZYME CELLULOSE COMPOSITE: A PROMISING MATERIAL FOR WOUND DRESSING

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Antibiotic resistance is considered a threat for human health worldwide.[1] In particular, the appearance of multi-drug resistant microorganisms makes chronic wound management very challenging for healthcare systems.[2] For this reason, the use of antimicrobial enzymes as an alternative to antibiotics is broadly studied. Here, we describe the development of self-activated biodegradable films based on the coupled enzymatic reaction of cellobiohydrolase from *Trichoderma reesei* (*Tr*CBHI) and cellobiose dehydrogenase from *Myceliophthora thermophila* (*Mt*CDHA) immobilised in cellulose films regenerated from ionic liquid solution (RCFs). Herein we provide crucial information for designing enzyme-based antimicrobial cellulose films such as material toughness, surface porosity, adsorption kinetics, maximum loading and binding isotherm constants for different proteins. Moreover, enzyme coupled reactions between *Tr*CBHI and *Mt*CDHA for H_2O_2 production in RCFs was characterised. Resistance to compression and porosity at a μm scale were found to be tunable by changing cellulose concentration before film regeneration. Both kinetic and binding isotherm experiments showed high affinity of the cellulose binding module (CBM) possessing enzymes for RCFs, suggesting that specific interactions occurred. A sequential reaction was triggered by stacking *Tr*CBHI and *Mt*CDHA, previously immobilised onto separate RCFs, which produced 0.16 nmol/min·cm^2 of H_2O_2. In this system cellulose served both as support for enzyme immobilisation and substrate for the sequential reaction leading to the production of H_2O_2 at a μM level revealing efficacy of functionalised RCFs as antimicrobial agents. The material herein designed exhibits important traits such as biocompatibility, good mechanical stability, adjustability and simplicity in manufacturing which highlight its potential use in wound dressing applications.

Abstracts

Poster Presentations

P1

MOLECULAR STRUCTURE OF PHARMACEUTICAL AND COSMETIC CREAMS

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Skin cream formulations are widely used in pharmaceutical, cosmetic, and personal care products, and yet their internal architectures are poorly characterized at the molecular level. Moreover, while their long-term stability and functional utility are critically dependent upon their molecular structure, there is no real understanding yet of the structural mechanisms underlying the destabilizing effects of additives like drugs, anti-oxidants or preservatives, and no structure-based rationale to guide product formulation.

Here we report studies wherein we sought to secure a detailed understanding of the molecular structure of simple creams based on Aqueous Cream BP, and how this is influenced by addition of an antimicrobial preservative.

Creams containing cetyl and stearyl alcohol (co-surfactants), sodium dodecyl sulfate (surfactant), water and liquid paraffin were prepared as specified in the British Pharmacopoeia, with and without the preservative, 1,5-pentanediol. The macroscopic properties of the creams were studied using rheology and microscopy and their molecular architecture determined via small-angle neutron scattering (SANS) and small and wide-angle X-ray scattering (SAXS/WAXS) experiments.

Aqueous creams with excess surfactant and co-surfactants showed clear evidence of a lamellar structure – both directly as Bragg-peaks in SANS and SAXS profiles and indirectly as Maltese crosses in polarised-light micrographs. Rheological measurements showed that the creams exhibit shear-thinning behaviour and that the lamellar structure enhances consistency. The lamellar *d*-spacings of the creams with and without preservative varied as ~240 vs ~270 Å, each with oil-containing bilayers ~48 Å thick. SANS studies using individually perdeuterated cream components showed that the co-surfactants and the preservative co-locate in the lamellar bilayers. The surfactant, however, arranges (primarily) as a monolayer surrounding the oil droplets, and also combines with the co-surfactant(s) to form ellipsoidal bicelles. WAXS measurements indicated hexagonal close-packing of the co/surfactant alkyl chains, with the paraffin forming a liquid-crystalline phase.

The convolved findings from these studies have thus furnished an unprecedented level of detail on the molecular structure of a simple aqueous cream and reveal that the textbook view of cream structure is in some respects naïve and in other respects wholly incorrect, and this could have implications for the rational design of future skin creams.

P2

EVALUATING RAPID FORMULATION CHANGES *IN SITU* WHEN ELOCON CREAM AND EMOLLIENTS ARE APPLIED TO THE SKIN AT SIMILAR TIMES

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Topical corticosteroids (TCS) and emollients are the first-line treatments for atopic eczema. Despite their common usage, there is a lack of evidence supporting the optimal application protocol for both products, resulting in considerable variation in clinical opinion on how to apply the products together. The Eczema Priority Setting Partnership (2013) identified "what are the best and safest ways to use TCS?" to be one of the top ranked unanswered questions by both patients and HCPs. This question remains unanswered. Thus, the aim of this work was to evaluate the emollient effects on the percutaneous absorption and skin retention of a commonly used, potent TCS (Elocon cream, 0.1 % w/w mometasone furoate). Excised human skin samples mounted in Franz cells were dosed with Elocon cream alone and Elocon cream before or after an emollient (with a five or thirty-minute interval between applications). The emollients selected were Diprobase cream, Diprobase ointment and Hydromol Intensive cream. TCS quantification was performed using HPLC. Raman microscopy was employed to analyse the mixed TCS and emollient formulations. Total drug absorption (sum of percutaneous absorption and skin retention) was significantly altered to varying extents depending on the emollient investigated and the application protocol employed, ranging from a 2 fold decrease when Elocon cream was applied after Diprobase cream or Diprobase ointment, to a 2.4 fold increase when Elocon cream was applied with Hydromol Intensive cream irrespective of the order of application. Decreased TCS absorption was attributed to a multitude of complex formulation changes, including mixing of the TCS *in situ* (on the skin surface) with an emollient dissimilar to the TCS base, changes in drug thermodynamic activity and rapid drug crystallisation when the TCS was mixed with Diprobase cream. Inclusion of emollient excipients with penetration enhancing capabilities, such as urea and IPM in Hydromol Intensive cream was found to contribute to increased TCS absorption. These findings contradict clinical recommendations that the order of product application is unimportant or that leaving thirty minutes between product applications is sufficient to mitigate emollient effects on drug absorption. Instead, patients may need to leave longer time intervals between product applications and the practicalities of this warrant exploration.

P3

IMPROVING THE EFFECTIVENESS OF AMINOLEVULINATE-BASED PHOTODYNAMIC THERAPY (ALA-PDT) OF SKIN CELLS WITH ULTRAVIOLET A-INDUCED LABILE IRON RELEASE

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The effectiveness of topical ALA-PDT has been established in the therapy of superficial skin cancer lesions, notably actinic keratoses. The application of ALA causes the accumulation of photosensitising concentrations of protoporphyrin IX (PpIX) which following irradiation with an external light source (usually blue or red light) catalyses the generation of reactive oxygen species, resulting in cell death. The major drawback side effect of topical ALA-PDT is the pain experienced by patients that may cause non-compliance to terminate the course of the treatment. To improve the efficiency of ALA-PDT of skin cells, (i) we changed the conventional light source to UVA (320-400nm) that is absorbed more efficiently by PpIX and is 40-fold more potent in killing skin cells than red light; (ii) we aimed to exploit the damaging effects of rapid release of labile iron by applying short pulses of low UVA doses instead of a continuous source of light following ALA treatment. This is because the labile iron released in ALA-treated cells following the first irradiation acts as a catalyst to exacerbate the oxidative damage upon subsequent exposures. The HaCaT keratinocytes were treated with two therapeutic doses of 0.5 and 1mM ALA for 2h and then irradiated with a range of UVA doses of 1-5 kJ/m^2 with 1 or two hours dark intervals. The UVA doses are equivalent to 0.5-1.5 min sunlight. In clinical settings these are short pulses of ca 5-25 seconds. Cell death was examined 24h after UVA by MTT, Annexin V-propidium iodide and colony forming assays. The results showed that both ALA concentrations significantly increased the level of PpIX and sensitized keratinocytes to very low non-cytotoxic UVA doses. Moreover applying short pulses of UVA to ALA-treated keratinocytes was a fast and effective way to promote cell death, therefore, improving the current modality for topical ALA-PDT, through a reduction of the irradiation time and the length of pain endured during the treatment.

P4

‘HAPPY BOTTOMS’–BEST NAPPY CARE IN PAEDIATRIC ONCOLOGY

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Napkin dermatitis (ND) can be particularly severe for paediatric oncology patients. Due to a lack of available research, the assessment and management of ND often differs between healthcare professionals. This project aimed to create a guideline for ND care, to enable staff to make consistent, accurate assessments of skin and to provide a consensus on the best management regime. It was also designed to improve documentation, so that outcomes can be compared when further changes to ND management are made in future.

Staff completed a survey of their current knowledge and practice treating ND. After consultation with other Children’s Cancer and Leukaemia Group centres, local Paediatric Oncology Pharmacists and adult TVNs, a 2-page guideline and documentation chart were produced. Education of staff was undertaken via ward-based sessions. Patient information leaflets and a poster board for families were created. An audit of the documentation chart was conducted for new admissions over a 2 month period. A second survey of staff and patients’ families was subsequently performed.

53% of patients (n=25/47) had a documentation chart used during their admission. Of those, data was completed on 73% of the days during admission (148/203). 59% of the time ND was ‘the same’ or ‘improving,’ and only ‘worsening’ 7% of the time. 92% of staff found the guideline useful and 83% stated it improved their confidence in recognising and managing ND. On 88% of occasions barrier cream was used correctly, whereas barrier film was only used correctly 26% of the time. All families who received the patient information leaflet stated it improved their ability and confidence in recognising nappy rash.

Although staff feel that their confidence in assessing and managing ND has been improved by having a guideline, their engagement with the documentation chart was poor with nearly half the patients not having any data recorded. Additionally, even when the chart was used management did not always follow the guidance. We intend to organise further teaching sessions for ward staff and are altering the documentation chart so that it will be included as part of the patient’s admission booklet.

P5

Synthesis of Highly Modular Multimodal Imaging Probes for Recognition of Cancer Biomarkers

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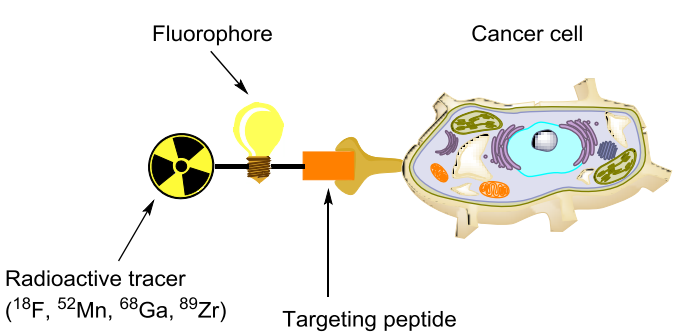
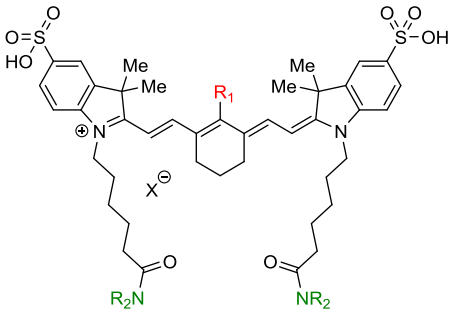
Multimodal imaging techniques (e.g. PET/CT or PET/MRI) have led to remarkable improvements in cancer diagnosis and treatment.[1] Nevertheless, certain approaches are, so far, severely understudied. One such approach is the combination of positron emission tomography and fluorescence imaging.[2] The latter has received a surge of attention over the last decade, after the first proof of principle studies in humans indicated the usefulness of fluorescence guided surgery.[3] The aim of this work is the synthesis of targeted, highly fluorescent near-infrared dyes amenable to straightforward radiolabelling, either through incorporation of prosthetic groups for labelling with fluorine-18 or ligands for labelling with various radioisotopes, and the investigation of the *in vitro* behaviour of the resulting probes in various cell types, including human skin fibroblasts such as FEK4. The fluorophore has intense absorption and emission in the NIR region of the electromagnetic spectrum, as well as multiple functional groups, which allow for the straightforward, orthogonal introduction of functional molecules. Two derivatives of the dye were radiolabelled successfully with zirconium-89. Further conjugation to LysUreaGlu, a small molecule targeting the prostate specific membrane antigen (PSMA), was successful. Other targets, which are currently under investigation are the vascular endothelial growth factor (VEGF) and the gastrin releasing peptide receptor (GRPR). All resulting probes are being screened in cancerous (e.g. PC-3,) as well as non-cancerous cell lines including FEK4.





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P6

Preparation of polysaccharide-based nano-emulsion as a targeted delivery system for malignant melanoma cells

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As an intrinsic multi-drug resistant cancer, malignant melanoma is one of the common skin cancer types. Melanoma is capable of initiating alternative pathways to avoid apoptosis and continue proliferation. Patients diagnosed with progressed stages of melanoma have a survival period of 7 months to 2 years depending on the stage of their condition. Hence the treatment process is challenging. Unfortunately, various chemotherapeutics have been barely successful so far in overcoming drug resistance and treating melanoma.

This research aims to address malignant melanoma drug resistance using nano-emulsion preparations. Chitosan, pullulan and alginate were chosen, based on previous studies, for the initial formulation and optimization step. The first objective was to construct a formulation with lowest initial burst and optimum steady release over a period of 21 days. Following the release test of single polymer blends, the three chosen polymers were blended with each other in specific ratios using a crosslinker for encapsulation of coumarin-6 and doxorubicin. Subsequently, in vitro release tests were conducted for drug loaded blended nano-emulsions. Toxicity test was also conducted for blank polymer blends and drug loaded nano-emulsions during the optimization process. Followed by toxicity test, cellular uptake, and apoptosis induction ability of the formulations were studied and optimized nano-emulsion in terms of release and optimal toxicity were chosen to continue.

The modification of the Nano-emulsion as a targeted delivery vehicle for malignant melanoma, was done afterwards. To reach this objective, the formulation was surface-modified to target folate receptors on malignant melanoma. Followed by preparation of targeted formulation (doxorubicin and decarbazine loaded), melanoma and keratinocytes were treated as mono and co-culture. The treatments were done to compare the effect of formulation on both cell lines. According to all observations and tests conducted, the formulation was found to be effectively reducing melanoma cells while leaving keratinocytes intact.

P7

Non-invasive extraction of macromolecules across the skin using hypobaric pressure

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Purpose: Biomarkers can be powerful tools in the process of medicines development as they can aid the understanding of diseases and they can indicate the success of medical interventions. However, when the biomarker requires chemical quantification, sampling the molecules can be challenging because they are often present in bodily fluids at very low concentrations. One new method of biomarker sampling is direct extraction through the skin using hypobaric pressure. This approach is a needle-free, non-invasive method of extracting biomarkers present in interstitial fluid (ISF). However, the means by which skin stretching facilitates this process is not clearly understood. The aim of this study was to use high molecular weight fluorescent probes as model biomarkers to examine the means by which local hypobaric pressure facilitates the extraction of molecules across the skin.

Methods: The biomarkers were modelled using 10,000 (FD-10S) and 150,000 (FD-150S) FITC- dextran in PBS solution at pH 7.4. The extraction efficiency (%) was determined by the concentration of either FD-10S or FD-150S extracted through porcine and rat skin mounted on Franz cell. The hypobaric pressures applied were 610, 510 and 310mbar. Live imaging of the hair follicle size upon the application of hypobaric pressure was performed using a Flex-Stager cell with a Nikon confocal microscope.

Results: The higher hypobaric pressures resulted in a higher extraction efficiency for both dextran markers, e.g., for FD-10S, the extraction efficiency, across porcine skin, at 310 mbar was 28.5 ± 5.41% (n =5), but was 2.54 ± 0.65 % (n =5) at 610 mbar. The extraction efficiency of both dextrans was significantly higher ($p < 0.05$) through rat skin (e.g. 10.97% ± 2.22% (FD-150S), n=5) compared to porcine skin (3.50 ± 1.22% (FD-150S), n=5). The confocal microscopy images showed that an increase in hypobaric pressure enlarged the hair follicle diameter (e.g. by ~44 μm at 610 mbar and by ~85 μm at 610, for porcine skin), but it was evident that the dextran markers passed through the skin both via the hair follicles and directly through the stratum corneum.

Conclusion: The application of hypobaric pressure to the skin extracted model biomarkers across the tissue both through the hair follicles and across the stratum corneum. As molecules with a molecular weight of up to 150,000 Da were drawn out of the skin, the application of hypobaric pressure to the tissue appears to be a promising method to non-invasively sample the cutaneous tissue.

P8

CONCERNS WITH VITAMIN D SUPPLEMENT QUALITY AND THE POTENTIAL FOR TRANSDERMAL DELIVERY

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Background: Vitamin D is fat-soluble seco-steroid that plays a multi-purpose role in skeletal health and calcium homeostasis in the human body. Deficiency in vitamin D is becoming more prevalent globally and consequentially supplement use is increasing. However, as vitamin D is not chemically stable and food manufacturers are not required to adhere to strict guidelines as to vitamin D concentrations in supplements there is concern that the currently available supplements are of variable quality. The aim of this study was to determine the vitamin D content of vitamin D_3 supplements on sale in the UK and consider the opportunities to reformulate these supplements as transdermal patches.

Methods: Vitamin D_3 supplements sourced as food and pharmacy grade products were assayed using both a reversed phase high performance liquid chromatography (RP-HPLC) and normal phase HPLC (NP-HPLC) owing to the difficulties in vitamin D extraction from the supplements. RP-HPLC was performed using a Phenomenex C18 Synergi Hydro-RP column (250 x 4.6 mm, 4µm particle size, 80 Å diameter) with an acetonitrile: methanol (55:45) mobile phase at a flow rate of 1.5 mL/min. Whilst NP-HPLC was performed using a Phenomenex-Luna Silica column (150 x 4.6 mm, 5 µm particle size, 100 Å diameter) with a hexane: pentan-1-ol (99.2:0.8) mobile phase at a flow rate of 1.0 mL/min.

Results: A total of 13 vitamin D_3 UK supplement products were analyzed, of which eight were liquid products, two were soft gelatin capsules and three were tablet formulations. Two of the 13 products were pharmacy grade products and the remainder were food grade supplements. Both the pharmacy products, Fultium-D3 drops and Vigantoletten tablets were found to contain the appropriate amount of vitamin D, but only 1 of the 11 food grade supplements, Natures Aid vitamin D_3 drops, contained the appropriate vitamin D content. The vitamin D_3 percentage of the label claim for all the products varied from 0.11 to 165.32% using the two different analytical methods.

Conclusions: The wide range of vitamin D products on sale and the variability in quality suggest that there is an opportunity for alternative delivery forms in the UK market. The transdermal delivery of vitamin D would be an attractive option if a chemically stable formulation that could deliver appropriate concentrations across the skin could be produced.

P9

OPTIMIZATION OF A NANOFORMULATION FOR SKIN DELIVERY: THE INFLUENCE OF STRUCTURE AND COMPOSITION

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The optimization of skin delivery strategies is necessary in research of topical and transdermal applications towards improvement of bioavailability and efficacy. In recent years lipid nanoparticles called Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) have been extensively investigated and applied as promising tools for skin delivery of bioactive compounds for pharmaceutical and cosmetic applications. In this context, the main goals of this study are to develop and characterize different formulations of lipid nanoparticles, SLNs and NLCs, produced with different combinations of two solid lipids (cetyl palmitate and witepsol E85) and two liquid lipids (oleic acid and miglyol 812). Through the Dynamic Light scattering (DLS) technique, nanoparticles have been characterized in terms of size, polydispersity index and zeta potential, while a morphological survey has been carried out using cryo-SEM. The formulations evaluated in terms of storage stability (at room temperature and 4°C), and of colloidal stability. The skin permeation profile of the developed lipid nanoparticles labelled with calcein was assessed using two *in vitro* models: pig ear skin using the Franz diffusion cell and Phospholipid Vesicle-Based Permeation assay (PVPA) simulating the human stratum corneum. All six nanoformulations led to good results and a clear increase in permeation compared to free calcein. In particular, the NLCs produced using as solid lipid cetyl palmitate and as liquid lipid oleic acid showed a marked increase of permeation and thus promising potential for transdermal application. Further biocompatibility assays were performed with fibroblasts and keratinocytes, and even though the highest tested concentrations containing with oleic acid were toxic towards both cell lines, it was possible to find safe working concentrations for transdermal delivery.

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P10

Effects of LOX inhibition on hemin-induced cell death

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Ferroptosis is a form of caspase-independent regulated cell death mediated by iron-dependent accumulation of lipid peroxidation products and reactive oxygen species (ROS) derived from iron metabolism. Ferroptosis can be induced by small molecules like erastin or glutamate through inhibition of the X_c^- system (cysteine/glutamate antiporter). Recently, hemin, the oxidized product of hemoglobin has been linked to ferroptotic cell death signaling, but so far, the underlying mechanism leading to oxidative cell death induced by hemin is not clarified. Former studies identified that the toxicity of hemin is caused by iron that is released when hemin is decomposed and it is hypothesized that hemin-induced cell death shares molecular and cellular mechanisms of ferroptosis.

The major aim of this study was to identify the molecular mechanisms of hemin-induced cytotoxicity in comparison to ferroptosis. Hemin toxicity in comparison to ferroptosis was investigated in neuronal HT-22 cells exposed to hemin (150 and 200 µM) and erastin (0.5 µM). Inhibitors of ferroptosis (ferrostatin-1) and necroptosis (necrostatin-1), 5-LOX inhibitors (e.g., zileuton, C06, and ST1853), the 12/15-LOX inhibitor PD146176, the Bid-inhibitor BI6c9 and Pifithrin-alpha (PFTα) were used to investigate protective effects in the model system of hemin-induced cell death.

We found that hemin decreased the metabolic activity of HT-22 cells in a concentration-dependent manner. Ferrostatin-1 and Necrostatin-1 rescued the metabolic activity, measured by the MTT assay, after hemin exposure to a similar extent as the 5-LOX inhibitors ST1853 and Zileuton. The 5-LOX inhibitor C06 was only protective at concentrations of 20 and 50 µM regarding the metabolic activity but was not preventing hemin-induced cell death as assessed by the AV/PI assay. The 5-LOX inhibitors were not able to prevent ATP depletion, loss of mitochondrial membrane potential nor the increase in lipid peroxidation caused by hemin, but they reduced the mitochondrial ROS formation. The BID inhibitor and PFTα were able to increase the metabolic activity, whereas the 12/15-LOX inhibitor PD146176 showed only moderate protection against hemin. In comparison, all applied inhibitors were protective against erastin-induced cell death.

Overall, our findings suggest that the hemin-induced cell death largely depends on 5-LOX activity rather than on 12/15-LOX activity. Similar to ferroptosis inducers such as erastin and RSL-3, hemin also increased mitochondrial ROS formation, decreased metabolic activity and mediated ATP depletion in HT-22 cells. In conclusion, this study identified a key role of 5-LOX in hemin-induced cell death which is in contrast to the predominant role of 12/15 LOX activity in paradigms of ferroptosis.

P11

CHEMICAL CHARACTERIZATION OF THE $PVPA_{SC}$ MODEL: THE EFFECT OF SURFACTANTS AND DIFFERENT PH ON BARRIER PERMEABILITY

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Skin is the biggest organ in human and other animal bodies and it represents an important drug target and place for the application of many drug treatments and cosmetics. Since every new pharmaceutical drugs and cosmetic agents must be tested in terms of their biological effect and toxicological profile in the skin, adequate testing systems should be defined and developed. Moreover, in the last decades, several ethical questions have been raised and the establishment of new and more strict rules regarding the prohibition of studies in animals have been implemented. Thus, the development of efficient skin mimetic models became crucial and stimulated the scientific research in this field of application. Many skin substituents have been developed and nowadays some of them are already available in the market, while the research continues for further improvements in the quality, complexity, stability, price and mimetic properties of the skin models.

In this context, our research group recently developed and characterized an alternative *stratum corneum (SC)* mimetic model $PVPA_{SC}$ [1] that simulates this human skin layer, inspired on a phospholipid vesicle-based permeation assay (PVPA).

In the present work, permeability experiments were performed using $PVPA_{SC}$ barriers and the hydrophilic model compound calcein to study: a) the effect of presence of different surfactants commonly used to enhance drug solubility on the permeability of the drug and b) the influence of pH on the permeability of calcein, by adjusting the pH to 5.0, 6.2 or 7.4. Moreover, the permeability of different nanoparticle formulation containing cyclosporine A as a drug of interest was investigated in the new skin mimetic model. As a final goal and to validate our new model, the same topics were also studied in a commonly used model of *SC* isolated from pig ear skin.

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P12

UNDERSTANDING THE DISTRUBUTION OF NITROUS OXIDE IN HUMAN AND PORCINE SKIN UPON THE TOPICAL APPLICATION OF S-NITROSOGLUTATHIONE

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Donors such as S-Nitrosoglutathione (GSNO), which chemically decompose to release nitrous oxide (NO), can be applied to the skin in order to administer NO as a therapeutic gas[1]. The successful delivery of NO into the skin has been shown to illicit an antinociceptive response, local vasodilation, antimicrobial effects and facilitate wound healing[2]. However, the manner in which NO distributes in the skin tissue to achieve its various biological effects is unknown and this renders optimal topical formulation design for particular therapeutic applications problematic [1]. The aim of this project was to understand how NO distributes into both porcine and human skin after release from a topically applied formulation. NO release from GSNO was measured using UV spectroscopy. GSNO was presented as an aqueous solution (PBS pH 7.) and a GSNO hydrogel patch (GSNO/HPMC/PEG/PG in 1:5:5:1 w/w ratio) to the surface of both porcine and human skin. Franz diffusion cells were used to determine both the transdermal NO transport and skin deposition. The Griess assay was used to determine the NO in the skin layers by quantifying the increase in nitrate in the Franz cell receiver fluid and each layer of the skin tissue. The GSNO hydrogel patch showed a significantly ($p < 0.05$) lower GSNO degradation rate than the solution formulations at the same concentration (0.84 ± 0.03 mM/h vs 1.24 ± 0.01 mM/h). The hydrogel patch exhibited the same transdermal nitrite flux as the solution across porcine (0.30 ± 0.06 ug/cm^2/min vs 0.28 ± 0.05 ug/cm^2/min), but a lower flux in comparison to the solution ((0.02 ± 0.02 ug/cm^2/min vs 0.16 ± 0.01 ug/cm^2/min) in human skin. Skin deposition studies showed that the nitrite localised in the viable skin layers for both the GSNO hydrogel patch and solution formulations (Table 1).

Table 1. The nitrate skin deposition of two different GSNO formulations in porcine and human skin. SC represents stratum corneum, EP represents epidermis and D the dermis.

Form	Human SC	Human EP	Porcine SC	Porcine EP	Porcine D
30mM sol.	0.583± 0.0577	12.6453 ±0.8459	0.317±0.094	3.401±0.764	4.739±0.553
30mM patch	0.4081±0.1800	9.4322±0.4124	0.125±0.050	3.779±0.244	4.232±0.323

Through the detection of nitrite, a surrogate marker of NO, data was gathered that suggested NO preferentially deposited in the viable skin layers after topical administration. This aligned well with the potent vasodilation effects observed after the topical administration of NO in other studies [2,3].

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P13

DESIGN OF HYDROXYPYRIDINONES AND TETRAPYRROLE MACROCYCLES TO LIFE SCIENCES

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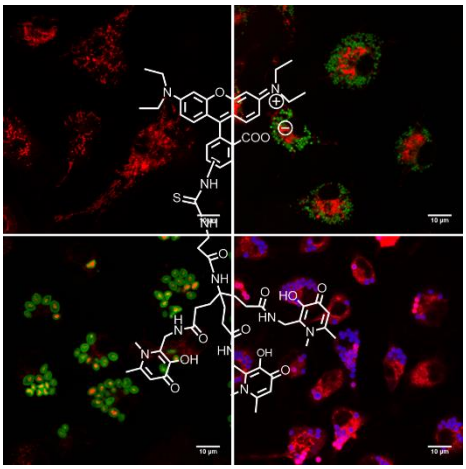
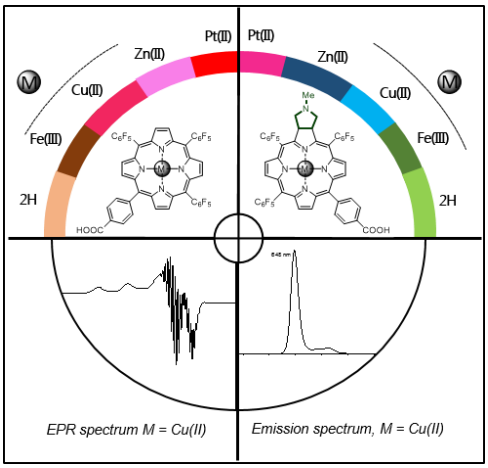
Research performed in our group is focused on the design of molecules for biomedical, agricultural and environmental applications. Several classes of molecular frameworks, namely hydroxypyridinones ligands and tetrapyrrole macrocycles, are functionalized with chemical groups to produce molecules whose physicochemical properties are fine-tuned according to the application in view. Both classes of ligands possess excellent chelating properties and the synthesis of a variety of metal ion complexes that also find application in the biomedical and agriculture fields is also performed. Regarding 3-hydroxy-4-pyridinones, particular attention is given to the design of molecules to be tested in (a) novel therapeutic strategies such as, Infection and Diabetes Mellitus (b) Metal Ion Sensing and (c) Plant Nutrition. Concerning tetrapyrrole macrocycles, research is focused on the synthesis and photo-physical properties of new functionalized porphyrin and chlorin macrocycles aiming the development of new photosensitizers for photodynamic therapeutic approaches to address Cancer and Infection. The characterization of the molecules produced is performed in the solid state and in solution, using several spectroscopic methods. Speciation studies in aqueous solution are particularly important for all the fields of application. The partition and permeation properties of the synthesized molecules in biological membranes are evaluated by spectroscopic methods.An overview of the work will be presented taking into special consideration the design and properties of molecules with potential therapeutic use in the treatment of skin diseases





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P14

A NANOTECHNOLOGICAL APPROACH FOR THE TOPICAL DELIVERY OF CYCLOSPORINE A

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According to the Journal of the European Academy of Dermatology and Venereology, skin diseases are ranked as the fourth most common cause of human illness, affecting both children and adults. The existing treatments often consist on immunosuppression therapy with oral corticosteroids or cyclosporine. However, the associated side effects due to prolonged systemic therapy compromises the effectiveness of the applied treatment. This adverse outcome justifies the need for reliable and efficient topical therapies, making the design of affordable treatments as a routine healthcare requirement.

Cyclosporine A (CsA) is an immunosuppressant drug effective in the treatment of many dermatological diseases. However, its has been used mainly in oral delivery due to its high molecular weight, hydrophobic nature and low permeability through skin barriers. In the current study, CsA was encapsulated in Solid Lipid Nanoparticles (SLNs) to achieve a system capable to perform a CsA topical application. SLNs were designed as drug vehicles, since they have been reported as biocompatible, easily produced and capable of transporting hydrophobic drugs to the main target.

SLNs were prepared by hot emulsification using Tween 80® and Softisan®, a non-animal derived semisolid lanolin substitute with good adhesion to the skin, widely used in the cosmetic industry. The designed nanoparticles presented a size of 200 nm, negative surface potential, high CsA entrapment efficiency (88%), spherical morphology, cellular biocompatibility in fibroblast and keratinocyte cell lines and confirmed storage stability for 8 weeks. CsA-loaded SLNs also permeated the pig ear skin at a higher extent than free CsA, possibly due to the characteristics of Softisan®. Our findings suggest that CsA-SLNs may present a safe key-treatment for skin-related disorders.

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P15

DERMATOLOGICAL PRODUCT METAMORPHOSIS FOLLOWING APPLICATION OF BETAMETHASONE SPRAY FORMULATIONS.

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Background

Topical drug products metamorphosis resulting from the evaporation and absorption of inactive ingredients may cause supersaturation and crystallization of the active and impact significantly on the drug release and bioavailability. This study aimed to characterize the changes in the physical form of betamethasone-17-valerate (BMV) due to solvent evaporation following application of a spray formulation.

Methods

Ethanol solutions with BMV (1%, 3%, 5% and 10% w/w) were prepared containing 5% w/w polymer (hydroxypropyl cellulose), 1% w/w plasticizer (triethyl citrate). Films were sprayed on glass substrates (2x2 cm^2) using aerosol technology (spray nozzle: 0.3 mm, distance from substrate: 10 cm) and equilibrated at 32˚C (24 hours) and 35% RH allowing solvent evaporation. Drug crystallization was first evaluated with polarized optical microscopy. The distribution of BMV in the films was tracked using Raman mapping (area: 80x70 μm^2, wavelength: 532 nm, 10% of maximum laser power, exposure time: 10s, magnification: x20). The physical state of BMV in the films was assessed using the characteristic Raman peaks of BMV (1666 cm^{-1} and 1659 cm^{-1} for the dissolved and crystalline drug, respectively).

Results

All films were transparent, homogeneous and dry after 24 hours. Optical images showed smooth films with some areas of greater roughness. BMV crystals were not observed in the 1% w/w films but became visible for higher drug loading suggesting BMV precipitation. Pyramidal crystals and crystal aggregates were more obvious as the drug load increased from 3 to 10%. Raman mapping suggested an even distribution for BMV and a complete dissolved status for the drug in the 1% films and confirmed the presence of BMV crystals in films with higher drug loadings. In addition, there was a shift in the position of the BMV peak from 1666.38 (± 0.29) cm^{-1} in areas without crystals to 1659.84 (± 0.59) in sites with drug crystals.

Conclusions

Evaporation of the volatile solvent induced crystallization of BMV in polymeric films that was dependent on the initial drug loading. Future experiments will characterize BMV release from these residual phases in order to investigate the link between the physical status of the drug and its release and subsequent absorption.

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