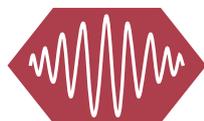


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# Nail Forum One Day Meeting

The School of Pharmacy, University of London, UK - 28 June 2011

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# AGENDA

- Date: Tuesday June 28th, 2011
- Venue: The School of Pharmacy (University of London)
- 9:30 - 10:00 **Registration**
- 10:00 - 10:10 Prof. Adrian F. Davis (Limeway Consulting)  
**Opening Remarks**
- Session 1: Nail Physiology and Function**
- 10:10 - 10:50 Dr. Richard Weller (University of Edinburgh)
- Session 2: Formulations to Target the Nail**
- Chair: Prof. B. Delgado-Charro
- 10:50 - 11:30 Dr. Kenneth A. Walters (KAW Consulting Ltd.)
- 11:30 - 12:10 Prof. Adrian F. Davis (Limeway Consulting)
- 12:10 - 14:00 **Lunch and Poster Viewing**
- Session 3: Overcoming the Nail Barrier**
- Chairs: Dr. Daniela Monti and Dr. Majella Lane
- 14:00 - 14:40 Dr. Begoña Delgado-Charro (University of Bath)
- 14:40 - 15:20 Dr. Clive Roper (Charles River)
- Session 4: Biophysical Characterisation of the Nail**
- 15:20 - 15:50 Dr. Perry Xiao (London South Bank University)
- 15:50 - 16:20 **Coffee**
- Session 5: Nail Forum**
- 16:20 - 17:30
- 17:30 **Close of Meeting**

# Speaker Abstracts

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## S1. Nail Physiology and Function

Dr. Richard Weller, Department of Dermatology, University of Edinburgh

Nails provide protection for the terminal phalanx and increase fine touch sensitivity and the handling of small objects. The nail plate is made of hard keratin which is formed in the nail matrix. The nail matrix lies in an invagination of the epidermis on the dorsal surface of the terminal phalanx and runs distally to the lunule. The nail fold is protected from the environment by the cuticle.

The nail is part of the integument, and as such frequently affected by skin disease, in particular psoriasis, lichen planus, alopecia areata, and eczema. Nail specific diseases such as paronychia, dermatophyte infections and local tumours produce distinctive signs reflecting disruption of the nails anatomy and physiology. Systemic disease frequently presents with signs in the nails such as clubbing, koilonychia, telangiectasia, Beau's lines and colour changes.

This presentation will review illustrative examples of the nail diseases which require clinical intervention.

## S2. Formulations to Target the Nail

Dr. Kenneth A. Walters, KAW Consulting Ltd., UK.

The human nail can be afflicted by several disease states including psoriasis and infections due to bacteria, viruses or fungi. Whilst rarely life threatening, these generate self-consciousness and psychological stress. Approximately 50% of all problems affecting the nail result from fungal infections, onychomycoses. For many years dermatologists believed that topical treatment for anything other than the most superficial fungal infections of the nail plate was futile. The nail plate was viewed as an impermeable barrier only to be reached *via* the blood supply to the germinative nail matrix and prolonged oral dosing with powerful antifungal agents was the order of the day. Investigation of the penetration and distribution of chemicals into and through the nail plate, however, demonstrated that it was possible to deliver drugs to the nail following topical application and this led to the development of slightly more effective topical products. Nail lacquers containing amorolfine and ciclopirox have shown limited clinical effectiveness in onychomycoses. Attempts have been made to treat psoriasis of the nail plate using a lacquer formulation containing clobetasol-17-propionate and a gel formulation containing tazarotene, again with limited but partially successful outcomes.

Attempts to increase the penetration of drugs into the nail plate have included physical techniques such as iontophoresis, sonophoresis and abrasion, all with some degree of experimental success but unlikely product potential. The concept of sandpaper abrasion followed by lacquer application is attractive but it would be more realistic and cost effective to include a chemical enhancer within the formulation. Although skin permeation enhancers that interact with intercellular lipids in the stratum corneum are unlikely to have a direct effect on the nail plate, they may possess properties that can influence the release of antifungal or anti-psoriatic agents from lacquer formulations. Perhaps of greater interest for nail drug delivery are those chemicals that can interact with keratin to enhance the efficacy of existing topical therapeutic modalities or perhaps form the basis of novel formulations.

## S3. Formulation of Topical Products for Onychomycosis: Lessons from Skin

Professor Adrian F. Davis, Limeway Consulting Ltd. UK

This talk gives a personal reflection on the major learnings from the last 60 years in the science of drug delivery to the skin, especially those with relevance to drug delivery to the nail. Study of the composition, structure and function of the skin, especially the stratum corneum barrier, has led to insights into structure-penetration relationships that inform new drug discovery. Although the nail has a very different composition and structure compared with the skin, similar insights underlaid the development of AN-2690, a small water-soluble antifungal boron compound for the treatment of onychomycosis. In contrast, drug clearance from the skin and the roles of blood flow and lymphatic drainage is relatively poorly understood.

In 1960 Higuchi published his physicochemical analysis of the percutaneous absorption process which laid the foundation for rational topical formulation. Processes such as dissolution and diffusion within the applied phase, which influence drug release, were distinguished from processes of partitioning into and diffusion through the stratum corneum which influence drug transport. These distinctions are important, and the nail literature contains examples in which lipids, acting as nail lacquer plasticisers to increase drug release, are wrongly described as nail transport enhancers. The physicochemistry of nail lacquers is similar to that of transdermal patches and USP Apparatus 5 (Paddle over disc) may be appropriate to measure *in vitro* release. From Higuchi's theoretical basis, Poulsen *et al.* from Syntex described simple aqueous glycol systems in which drug-vehicle interactions and the importance of degree of saturation of the drug in the vehicle and the potential of supersaturation were illustrated. Mertin and Lippold have shown similar effects on nail transport.

The vast challenge, for the majority of drugs, of transdermal delivery led to the development of passive co-enhancer systems containing functional excipients which act to affect both partition and diffusion coefficients and in a synergistic manner and which are being introduced into topical therapy. Similar commercial stimulus seems unlikely with passive drug delivery to the nail. Drug dosage in topical dermatologicals has been, and remains, driven by history and consumer habits. Comparisons of drug concentrations in receptor fluid and in target tissue from *in vitro* studies with  $IC_{50}$  free drug potency, especially in closed systems, are as clinically irrelevant in nail penetration as they are in skin penetration studies. The related science of bioequivalence is a bottleneck for product development, despite the great attention to methodological detail that has been focused on, for example, skin stripping protocols. The requirement for pharmaceutical equivalence, that is the same dose of drug, limits potential for innovation in dose rationalisation, although there are some indications that regulators are open to science-based arguments for dose reduction. Also, the assumption that bioequivalence limits applied to topical dermatologicals, broadly  $\pm 20\%$ , should be the same as for oral drugs seems to have little basis in science. As always, learnings are based on successes and failures; the hope is that these learnings from skin will help improve the success rate in nail drug delivery development.

## S4. Overcoming the Nail Barrier

M. Begoña Delgado-Charro, Department of Pharmacy and Pharmacology, University of Bath.

Transungual drug delivery is limited by the thickness and impermeability of the nail plate. Our recent work has looked into the mechanisms by which iontophoresis and chemical enhancers may enhance transport of drugs into and across the nail plate. Recent research on nail iontophoresis has addressed the following topics: (a) permselective properties of human nails, (b) transungual iontophoretic transport numbers, (c) feasibility of transungual iontophoresis. *In vivo* work on the effect of current application on ionic transport and on transonychia water loss (TOWL) will be presented. Briefly, the clear enhancement of ionic transport observed, the feedback from volunteers, the small effects in TOWL, and the magnitude of voltages indicate that nail DC current iontophoresis is feasible and probably a safe technique.

*In vitro* work has investigated the iontophoretic and passive transungual fluxes of sodium and lithium and the effect of pH and co-ion competition on the cations' transport numbers providing valuable information about nail permselectivity and transungual transport. Sodium and lithium iontophoretic fluxes show low inter-nail variability and are significantly greater than passive fluxes. Cationic transport numbers responses to modifications in the cations' molar fraction and pH in the donor solution were remarkably similar to those observed during transdermal iontophoresis. On the contrary, experiments with mannitol suggest a modest and highly variable contribution of electroosmosis to the iontophoretic transungual flux. Laser scanning confocal microscopy has been used to investigate the depth, uniformity and pathways of penetration of fluorescent markers into human nail during passive and iontophoretic experiments and suggest the contribution of both the transcellular and intercellular pathways.

Recent work has looked into the effects of current application, hydration and penetration enhancers on the nail plate *via* different techniques such as infra-red (FTIR) and impedance spectroscopy. The latter suggest that iontophoresis causes reversible changes to the nail and that treatment with N-acetyl-L-cysteine causes greater, irreversible, changes in the nail structure. Finally, mercury intrusion porosimetry and SEM image analysis has provided a new insight into the microstructure and porosity of human nail and bovine hoof and into the effects of hydration and of N-acetyl-L-cysteine treatment on the microstructure of both membranes. Tridimensional structures having percolation characteristics comparable to nail and hooves were generated with Pore-Cor™. Microstructural changes determined via mercury intrusion porosimetry and modeled by Pore-Cor™ were related to drug diffusion.

## S5. An *In Vitro* Model for Determination of Human Ungual and Transungual Drug Delivery

Clive S. Roper, Charles River, Edinburgh, UK.

Onychomycosis (fungal nail infection) affects about 6-8% of the adult population. Drugs used to treat may be taken orally or applied directly to the nail. Oral drugs can affect the body's natural microbiological balance and lead to unwanted side effects. Drugs applied topically to the nail are often not efficacious due to poor delivery into and/or through the nail and as such do not reach the nail bed. Testing novel formulations or new chemical entities in clinical trials, with little understanding of drug delivery prior to instigation of the testing, often leads to failure of the formulation or drug (expensive in materials and time). Charles River has developed an *in vitro* model designed to compare delivery of drugs in different formulations into and through the nail plate as a response to this need. The test is based upon standard skin penetration methodologies.

The curvature of the nail is removed by immersion in physiological saline until pliable. The nail is then placed into a modified stainless steel flow through diffusion cell (Scott/Dick, Newcastle University) with an exposure area of 0.64 cm<sup>2</sup>. A non physiological receptor fluid (methanol: water; 3:1, v/v) is pumped underneath the nail plate at a flow rate of 0.75 mL/h and collected daily. The test system is maintained at a temperature of 32°C. Radiolabelled test preparations (formulations) are applied according to planned clinical use procedures (e.g. daily, weekly etc). The nail is washed daily with dilute soap solution and dried with tissue swabs. The experiment is terminated up to 4 weeks after initial dosing by washing and harvesting the nail. The nail is stored at -80°C in a clamp designed to maintain it in a flat conformation. The nail is then blocked in CMC paste and sectioned (typically at 30 to 150 µm) using a cryomicrotome. Experimental samples (including nail sections, washings, tissue swabs, receptor fluid and representative formulation mock doses) will be analysed for total radioactivity by liquid scintillation counting. The analysis may be performed using bioanalytical techniques for non radiolabelled test items using suitably validated methods.

The presentation will provide details of the methods and some results obtained from typical studies both as comparison of formulations and investigating the delivery of new chemical entities. Due to client confidentiality, the test item names and formulation details will not be shared.

## S6. *In Vivo* Nail Measurements by Using Photo-thermal Radiometry and AquaFlux

Perry Xiao<sup>ab</sup>

<sup>a</sup>Faculty of ESBE, London South Bank University, UK, <sup>b</sup>Biox Systems Ltd, UK

Human nails are a skin appendage that is made of keratin. Water content and water diffusion coefficient of nails are two key parameters in drug permeation studies through nail tissue<sup>[1,2]</sup>. However, to measure water content and water diffusion coefficient of *in vivo* human nails is very difficult. In this paper, we present our latest study on *in vivo* human nail measurements by using opto-thermal transient emission radiometry (OTTER)<sup>[3-5]</sup> and a condenser-chamber TEWL (trans-dermal water loss) method - AquaFlux<sup>[6]</sup>. We will present the theoretical background, and experimental results on water concentration depth profile in nail, as well as topically applied solvent penetration through nail. Combining OTTER results with nail transonychia water loss (TOWL) results measured by using the condenser-chamber TEWL method, we can also get the water diffusion coefficient of nail. By measuring the water diffusion coefficient of nail at different water concentration level, we can also get information on how water diffusion coefficient depends on water concentration.

We have also developed a new method for studying *extra vivo* human nails water holding capabilities by using the condenser-chamber TEWL method. Healthy nails always contain certain amount of water, and they will lose this water to the environment naturally through evaporations. We monitored this desorption process by continuously measuring the water vapour flux density from nail samples using the condenser-chamber TEWL method. We have also developed mathematical models for modelling the nail desorption process. By fitting the normalized nail desorption data with the mathematical models, we can get the water diffusion coefficient information, which can then be related to the water holding capabilities of the nail samples.

### References

- <sup>[1]</sup> B Forslind, M Lindberg, *Skin, Hair, and Nails: Structure and Function*, Taylor & Francis; ISBN-10: 082474313X, (2007).
- <sup>[2]</sup> D. A. R. de Berker, J. André and R. Baran, *Nail biology and nail science*, *Int J Cosmet Sci*, Vol 29 Issue 4, 241 – 275, 2007.
- <sup>[3]</sup> R.E. Imhof, A.D. McKendrick and P. Xiao, "Thermal emission decay Fourier transform infrared spectroscopy", *Rev. Sci. Instrum.*, Vol. 66, No. 11, 5203-5213, (1995).
- <sup>[4]</sup> P Xiao JA Cowen and R E Imhof, "*In Vivo* Transdermal Drug Diffusion Depth Profiling - A New Approach to Opto-Thermal Signal Analysis", *Analytical Sciences*, Vol 17 Special Issue, pp s349-s352, 2001.
- <sup>[5]</sup> P Xiao, H Packham, X Zheng, H Singh, C Elliott, EP Berg and RE Imhof, "Opto-Thermal Radiometry and Condenser-Chamber Method for Stratum Corneum Water Concentration Measurements", *Appl. Phys. B*, 86, 715-719, (2007).
- <sup>[6]</sup> R E Imhof, M E P De Jesus, P Xiao, L I Ciortea and E P Berg, Closed-chamber TEWL measurement:- microclimate, calibration and performance, *Int J Cosmet Sci*, accepted, to be published.

## P1. Effect of Hydration and N-acetyl-cysteine on the Structure of Human Nail Plate and Bovine Hooves

L. Nogueiras Nieto<sup>1,2</sup>, J.L. Gomez Amoza<sup>1</sup>, M.B. Delgado Charro<sup>2</sup> and F.J. y Otero Espinar<sup>1</sup>

<sup>1</sup>Departamento de Farmacia y Tecnología Farmacéutica. University of Santiago de Compostela, Spain.

<sup>2</sup>Department of Pharmacy and Pharmacology, University of Bath, UK.

This work investigated the structural changes induced by N-acetyl-L-cysteine (AC) and hydration on human nail plate and bovine hooves via scanning electronic microscopy (SEM) and mercury intrusion porosimetry (MIP). Veal hooves (sourced from the local abattoir) and human nail clips (donated by healthy human volunteers after informed consent) were treated with either water or 10% AC aqueous solution. The samples were removed at predetermined times, rinsed with water to eliminate any residual AC residues, dried and frozen by immersion in liquid nitrogen to avoid structural modifications. Finally the samples were lyophilized at -30°C for 48 hour (Labconco Corp.,USA). First, the surface of untreated and treated samples were examined by SEM with a LEO-435VP microscope and the size distribution of superficial pores estimated via the module "Grain analysis" provided by SPIP v4.6.0 (Image Metrology). Secondly, the microporous structure of treated and untreated samples was investigated *via* MIP using an AutoPore IV 9500 V1.09 (Micromeritics) and a 0.004-172.4 MPa pressure range. The porous structure of the samples was modelled based on the MIP data and using the software Pore-Cor™ (University of Plymouth) and as described by Gomez-Carracedo *et al.* 2010. The water permeability through the structures was predicted assuming that liquid flow only takes place in the z-direction accordingly to Peat *et al.* 2000.

SEM images indicated that both hydration and 10% AC treatment altered significantly the surface of nails and hooves; the changes being more manifest for the chemical enhancer. The average superficial pore size as determined by image analysis of the SEM pictures was ~10 µm for both the untreated nails and hooves. This superficial pore size determined via image analysis of SEM microphotographs was higher than that obtained *via* MIP, and is expected to reflect fissures, micro-fractures and deformations at the surface of the nail. The results of the image analysis also suggested: (a) the number of superficial pores to be higher in hooves than in human nail clips, (b) the porosity of hooves and nail clips to increase upon treatment, specially for the samples treated with 10% AC and (c) the average size of the surface pores not to be significantly modified by 10%AC or hydration.

MIP results and the simulated microstructures suggested the nail clips and hooves to present a low porosity. The microporous structure was similar for both membranes: pores distributed along the surface of nail and hooves and a inner section which is not porous and would represent the major barrier to drug permeation. Upon hydration and AC treatment of the two membranes there was a significant increment in the number and size of pores as well as in their connectivity. Further, the dimensions of the non-porous central area decreased with time of treatment. Again, this effect was more evident for the samples treated with 10% AC. The permeability of water molecules across the samples was predicted using Core-Por™ and, in agreement with the other observations, it was predicted to increase for treated samples.

Overall, these results suggest that hydration and treatment with 10% AC provokes microstructural changes in nails and hooves which result in an increased porosity of the systems and an increased water permeability. It is expected that these changes will result in modified permeation of actives.

## P2. Investigation of Nail Water Content *In Vivo* Using Confocal Raman Spectroscopy

Diar Mohammed, Haydar Abdalghafor, Jonathan Hadgraft, Majella E. Lane.  
The School of Pharmacy, University of London, U.K.

Nail is a keratinised tissue in which water is the principle plasticiser. A number of researchers have reported the importance of altering nail plate barrier to increase its permeability by the means of chemical treatments (Kobayashi *et al.*, 1998) and use of penetration enhancer (Hui *et al.*, 2003), but to date the influence of water on nail barrier function has attracted the most attention (Wessel *et al.*, 1999; Barba *et al.*, 2010). Hydrating the nail makes this keratin rich tissue more elastic, swollen and more permeable to topically applied substances such as ketoconazole (Gunt, 2006). In this work we report for the first time an investigation of water content of human nails *in vivo* using Confocal Raman Spectroscopy (CRS).

6 healthy volunteers aged 24–30 years with no history of nail disease or application of nail polish were recruited (3 males, 3 females). The right and left medial figures were selected. Fingernails were soaked in water for 0, 2.5, 5, 10 and 15 min before the measurements were taken. Fingernails were also hydrated for 2hrs with water and measurements were collected at time 0, 10, 30 and 60 min. *In vivo* measurements were conducted using a CRS microscope equipped with two fibre-coupled diode pumped lasers operating at two wavelengths, 671 nm (high wave number region) and 785 nm (fingerprint region). Nail water content measurements were obtained from the 671 nm laser (a 1  $\mu\text{m}$  spot of  $\leq 20$  mW power).

The baseline water content at 0  $\mu\text{m}$  within the nail was  $\sim 8$  % w/w. This value increased with depth to  $\sim 21$  % w/w at a depth of 40  $\mu\text{m}$  in the nail, from which it showed a plateau phase. Hydrating the nail for 2.5 min elevated the water content within the nail to  $\sim 12$  mass % at 0  $\mu\text{m}$  and  $\sim 23$  mass % at 40  $\mu\text{m}$ . The greater the hydration time the higher the water content within the nail up to 15 min where there is no further increase even after 2 hours of hydration. The water content within the nail returns back to the baseline 30 min after 2 hrs hydration. The data are in line with the literature when hydration of the nail was measured using NIR-FT Raman spectroscopy (Wessel *et al.*, 1999) or portable NIR spectrometer (Egawa *et al.*, 2003). Human nail is stable against outer influences, but it becomes soft and flexible after soaking in water, which can be observed during washing and bathing. This may partially be due to water and protein changes within the nail that eventually affect its structure (Wessel *et al.*, 1999), hence it becomes more permeable to substances. However, recently some commonly used organic solvents have also been shown to reduce water up take into the nail (Xiao, 2011).

This study shows that water content within the nail can successfully be determined *in vivo* using confocal raman spectroscopy. The values obtained are in good agreement with nail water content determined using other techniques. Further work is underway to investigate enhancer penetration into the nail and related effects on nail structure.

## P3. Dynamic Vapour Sorption-Near Infrared (DVS-NIR) Spectroscopic Investigation of the Human Nail

Haydar Abdalghafor, Diar Mohammed, Jonathan Hadgraft and Majella E.Lane.  
The School of Pharmacy, University of London, U. K.

The water content of the nail is essential to maintain its structure and properties. Assessment of the nail water content may be used as a tool to understand the nail barrier function and how formulation excipients affect the nail. In this study, Dynamic Vapor Sorption (DVS) and Near Infra Red (NIR) spectroscopy were used to probe the nail water content and changes associated with it<sup>[1, 2]</sup>. This is the first report where both techniques are simultaneously used to monitor changes in the human nail both gravimetrically and at the molecular level.

6 nail clippings from 5 volunteers were tested using a DVS apparatus (DVS-1, SMS, UK). Study conditions involved subjecting each nail clipping to a fixed temperature of 32°C and a range of relative humidities (RH%) ranging from 40%-90%, with a 10% increment. During the DVS run, real time NIR spectra were acquired using a NIR fibre optic probe (Foss NIRSystems, U.K. The spectra were pre-treated by taking the 2nd derivative (D2) followed by correction using Standard Normal Variance (SNV).

The increase in water content of the nail in response to the increase in RH% was observed gravimetrically (increased sample weight) and spectroscopically using NIR. A good relationship was observed for the weight changes and NIR spectra at 1422 nm ( $r^2 = 0.95$ ) and at 1908 nm ( $r^2=0.97$ ) as RH% was increased. The recorded wavelengths were in line with the literature<sup>[1]</sup>, where 1422nm represents free water while 1908nm is related to the change of bound water.

This is the first report of the application of DVS-NIR to characterise human nail tissue. The data confirm the utility of this technique to monitor the water sorption/water content of the nail *in vitro*. Future work will investigate the effect of other permeation enhancers on nail water content. In the long term the effects of selected solvents on the nail structure and its hydration properties will be elucidated towards the rational design of nail formulations.

## P4. Application of Dynamic Vapour Sorption-Near InfraRed (DVS-NIR) Spectroscopy to Probe Solvent Modulation of Nail Hydration

Haydar Abdalghalfor, Diar Mohammed, Jonathan Hadgraft, Majella E. Lane.  
The School of Pharmacy, University of London, UK.

The nail covers the dorsal aspect of tip of the fingers and toes and it comprises mainly of hard keratin and lipids. Therefore it is important to monitor nail hydration levels to understand the nail properties. Previously we have monitored the nail hydration behaviour in response to changes in relative humidity (RH %) both gravimetrically and spectroscopically using DVS-NIR.

The aim of this investigation was to demonstrate the use of DVS combined with NIR spectroscopy to probe IPM and decanol effects on nail hydration *in vitro*.

Nail clippings from 3 human volunteers were examined using a DVS apparatus (DVS-1, SMS, UK). The samples were soaked for 24 hours in each of the tested solvents. Excess solvent was then blotted with a tissue. Nail clippings were then subjected to a fixed temperature of 32°C and a range of relative humidities (RH%) ranging from 40%-90%, with a 10% increment. During the DVS run, real time NIR spectra were also acquired using a NIR fibre optic probe (Foss NIRSystems, U.K.). The spectra were pre-treated by taking the 2nd derivative (D2) followed by correction using Standard Normal Variance (SNV).

Results showed that both solvents improved nail hydration. IPM treatment increased the overall nail mass with improved water uptake after 70% RH. Decanol treatment did not make result in any changes in the nail mass, however, the bulk water uptake (at 1422 nm) was significantly greater compared with IPM ( $p < 0.05$ ). The data suggest that these solvents act via different mechanisms. It is possible that decanol permeated into the nail inducing molecular changes in the nail structure which promoted the tendency to absorb water. This is in line with previous literature reports<sup>[1]</sup>. For IPM one possible explanation for its effects on nail properties are its partition properties. Once it is taken up by the nail it may disrupt nail cellular arrangement<sup>[2]</sup>. The influence of IPM effect nail hydration was only noticeable when RH% levels exceeded 70%. Neither of the solvents showed any effect on the bound water properties at 1908 nm.

In conclusion, the findings confirm that the DVS-NIR allows sensitive monitoring of solvent treatment effects on the water sorption/water content of the nail *in vitro*. Detailed information on solvent influence on the bulk and bound water uptake of the nail was also elucidated. Future work will investigate the effect of other permeation enhancers using the selected wavelengths. In the longer term the effects of selected solvents on the nail structure and its hydration properties will be elucidated.

### References

- [1] Xiao, P., *et al.*, Opto-Thermal Transient Emission Radiometry (OTTER) to image diffusion in nails *in vivo*. International Journal of Pharmaceutics, 2011. 406(1-2): p. 111-113.
- [2] Brinkmann, I. and C.C. Müller-Goymann, Role of Isopropyl Myristate, Isopropyl Alcohol and a Combination of Both in Hydrocortisone Permeation across the Human Stratum corneum. Skin Pharmacology and Physiology, 2003. 16(6): p. 393-404.

## P5. Biophysical Techniques to Probe *In Vitro* and *In Vivo* Nail Water Content.

Haydar Abghalfor<sup>1</sup>, Diar Mohammed<sup>1</sup>, Jonathan Hadgraft<sup>1</sup>, Majella E. Lane<sup>1</sup>, Perry Xiao<sup>2,3</sup>  
<sup>1</sup>The School of Pharmacy, University of London. <sup>2</sup>London South Bank University, U.K. <sup>3</sup>Biox Ltd., U.K.

The human nail is an excellent barrier to the penetration of most drugs and xenobiotics. Hydration of the nail has a profound effect on both its physical and chemical properties. The degree of hydration of the nail should therefore influence nail permeability and nail barrier function. From the perspective of promoting transungual drug delivery it is therefore of interest to determine nail water content both *in vitro* and *in vivo*. In this study we aimed to firstly determine the utility of three biophysical techniques to characterise nail hydration and secondly to investigate how the various techniques could be correlated. Both *in vitro* and *in vivo* measurements were conducted.

Opto-thermal transient emission radiometry (OTTER) measurements of nail hydration using un-occluded conditions were conducted on five volunteers. Confocal Raman Spectroscopy measurements were taken from six volunteers and nail clippings were also evaluated for their water content. Dynamic Vapor Sorption measurements were taken for nail clippings from three volunteers. All measurements were conducted at ambient temperature.

A good correlation between OTTER and Confocal Raman Spectroscopy results *in vivo* was found. Both techniques showed that the nail water content was ~ 21% for the tested subjects. A good *in vitro* correlation was also found between the *in vitro* data from the DVS study and Confocal Raman experiments where the water content was ~10%.

In conclusion, this investigation demonstrates the application of three different techniques to study nail water content both *in vivo* and *in vitro*. *In vivo* and *in vitro* data were in line with the literature<sup>[1-3]</sup>. Correlations were obtained between Confocal Raman and OTTER techniques, as well as between Confocal Raman and the DVS-drying study. Future work will focus on solvent interaction with the nail plate both *in vitro* and *in vivo*.

### References

- [1] Egawa, M., Y. Ozaki, and M. Takahashi, *In vivo* measurement of water content of the fingernail and its seasonal change. *Skin Research and Technology*, 2006. 12(2): p. 126-132.
- [2] Wessel, S., *et al.*, Hydration of human nails investigated by NIR-FT-Raman spectroscopy. *Biochimica et Biophysica Acta*, 1999: p. 210-216.
- [3] Stern, D.K., *et al.*, Water content and other aspects of brittle versus normal fingernails. *Journal of the American Academy of Dermatology*, 2007. 57(1): p. 31-36.

## P6. Opto-Thermal Transient Emission Radiometry (OTTER) to Image Diffusion in Nails *In Vivo*

P. Xiao<sup>1,2</sup>, X. Zheng<sup>1</sup>, R.E. Imhof<sup>2</sup>, R. Mateus<sup>3</sup>, K. Hirata<sup>3</sup>, W.J. McAuley<sup>3</sup>, J. Hadgraft<sup>3</sup>, M.E. Lane<sup>3</sup>  
<sup>1</sup>London South Bank University, UK, <sup>2</sup>Biox Ltd., U.K., <sup>3</sup>School of Pharmacy, University of London, U.K.

Nail diseases affect many people in their everyday life and are very difficult to treat. Topical treatment is desirable in terms of compliance and reduction of side effects associated with systemic drug delivery. This work describes the first application of Opto-Thermal Transient Emission Radiometry (OTTER) to probe the extent to which solvents permeate the human nail *in vivo* and *in vitro*. OTTER is a high speed infrared remote sensing technique which uses a pulsed Er:YAG laser (2.94 $\mu$ m, 100 ns pulse width, < 4mJ/per pulse) as a heat source to heat up the sample surface, and a fast infrared detector (Mercury Cadmium Tellurium or MCT) to pick up the consequent increase of sample's blackbody radiation. The detection wavelength is 9.5  $\mu$ m and it measures the changes of solvent optical absorption coefficient within a few microns of the nail surface.

Glycerol, decanol and butyl acetate were selected for investigation. Glycerol and butyl acetate are found in a number of commercial nail lacquers and decanol permeation in human nails *in vitro* has previously been reported. The solvents were applied for 5 min to the nail plate of the fingernail *in vivo* and the toenail *in vitro* using a filter pad. The pad was removed and the nail wiped, and the depth profile of the solvents was measured on removal and at 8 and 10 min after removal.

The rate of diffusion of the solvents may be ranked as glycerol>decanol>butyl acetate. Assuming a simple molecular weight dependence of solvent diffusion the findings are consistent with the permeation data reported by other authors. They are also consistent with the possibility of lipid extraction by decanol. The results for butyl acetate suggest some evaporation during the course of the experiment.

These preliminary results demonstrate the potential of OTTER as a tool to investigate fundamental permeation mechanism of solvents across the human nail and the barrier properties of the nail. Comparative experiments with other biophysical techniques e.g. Confocal Raman spectroscopy, Dynamic Vapor Sorption are ongoing.

## P7. Investigation of TBF-HCl Loaded Ethosomes for Ungual Drug Delivery

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The nail plate is most visible part of the nail apparatus, consists of tightly packed dead cells and is highly keratinized. Onychomycosis is a fungal infection of the nail plate and bed. Ethosomes contain phospholipids, alcohol in relatively high concentration and water<sup>(1)</sup>. The objectives of this study were to prepare and characterize ethosomal formulations containing Terbinafine-HCl (TBF-HCl) and investigate release properties of formulations through dialysis bag for further unguinal permeation studies.

Ethosomal formulations were prepared according to the method reported by Touitou<sup>(1)</sup>. Six formulations were prepared based on two concentration of three different kind of lipids (EI-VI). Phospholipids and TBF-HCl were dissolved in ethanol. The aqueous phase was added slowly to the lipid and active substance mixture with constant stirring at 700 rpm. The resulting vesicle suspension was homogenized by sonication. The z-average mean (Z-size) and polydispersity (PI) of the formulations was determined by light scattering spectroscopy, after dilution with appropriate concentration of ethanol:water. The vesicles were visualized using TEM by negative staining method. The entrapment efficiency (EE) was calculated by dialysis method<sup>(2)</sup>. The cumulative amount of TBF-HCl permeated through the dialysis bag was plotted as a function of time. All the data was statistically analyzed by one way analysis of varyans.

The particle size and PI were ranged between 200-600 nm and 0.190-0.250, respectively. High entrapment efficiency (75-85%) was determined due to lipophilic character of TBF-HCl. According to release studies it was shown that 80% of TBF-HCl permeated through the dialysis bag in 3 hours. As a conclusion, ethosomal formulations containing TBF-HCl are promising formulations for transungual drug delivery. Further studies are in progress.

### Rerferences

<sup>(1)</sup> Touitou E., Ethosomes- Novel Vesicular Carriers for Enhanced Delivery: Characterization and Skin Penetration Properties, J. Control. Release, 65, 403-418 (2000).

<sup>(2)</sup> Vejnovic I., "Investigation of different formulation for drug delivery through nail plate" Int.J. Pharm. 386 (2010) 185-194.

## P8. The Use of Raman Spectroscopy in the Search for Ungual Enhancers

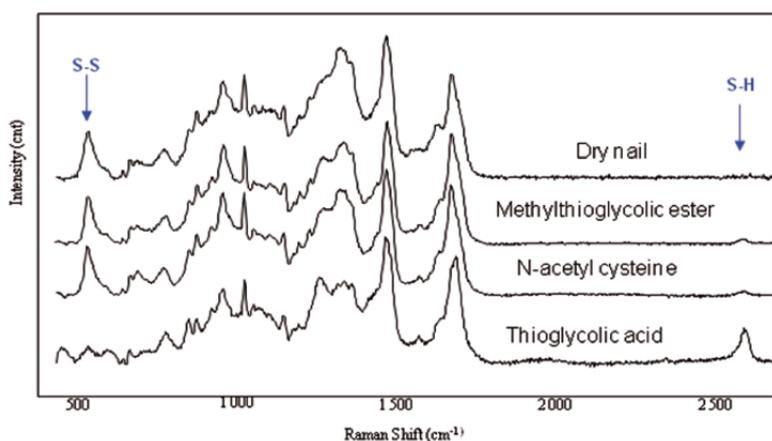
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Topical nail medicines have limited efficacy, partly due to the poor permeability of the nail plate. To overcome the nail plate's poor permeability, a number of chemicals such as n-acetyl cysteine, thioglycolic acid, mercaptoethanol, N-(2-mercapto-propionyl) glycine, Urea Hydrogen peroxide have been investigated as unguinal enhancers. The mechanism of action of many of these enhancers is thought to be the reduction of the disulphide bonds of the nail keratin<sup>(1)</sup>, such that the integrity of the nail plate is decreased which in turn, results in increased nail plate permeability.  
Nail-S-S-Nail + Enhancer → Nail-SH + HS-Nail + modified Enhancer<sup>(1)</sup>.

We hypothesised that Raman spectroscopy might be useful to establish the mode of action of such enhancers. Raman spectroscopy may enable changes in –S-S- and –SH bonds in the nail plate following treatment with an unguinal enhancer to be quantified. Accordingly, if correlations with the extent of such changes and that of unguinal permeation enhancement are found, Raman spectroscopy could be used to rapidly screen chemicals for their potential as unguinal enhancers. To test this hypothesis, a number of chemicals were tested for i) their ability to change the extent of the nail plate's SS and SH bonds (as determined by Raman spectroscopy) and ii) their ability to increase unguinal permeation of a model drug. The figure below shows the Raman spectra of nail plates incubated in some of these chemicals. It can be seen that the ratio of SS to SH bonds changes when the nail is incubated with certain compounds containing thiol groups, as the nail disulphide bonds are reduced and thiol bonds are formed. Permeation experiments are being conducted and correlations between the nail plate permeability and the Raman spectroscopy results are being explored.



## P9. The Influence of the Strength of Adhesion Between an Ungual Topical Film and the Nail Plate on its *In Vivo* Residence Time

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Nail lacquers are a convenient means of delivering drugs for the topical treatment of nail diseases. For sustained drug delivery, the lacquer films should remain in contact with the nail for long durations, e.g. a week. As the *in vivo* experimental investigation of the residence time of nail lacquers is lengthy, *in vitro* investigations that could accurately reflect the *in vivo* situation are desired.

We hypothesised that the *in vivo* residence time is a function of the strength of adhesion between the lacquer film and the nail plate. The aim of the study was therefore to determine correlations, if any, between lacquer film-nail adhesion and *in vivo* residence time using a range of lacquers.

Five colourless cosmetic nail varnishes and, one pharmaceutical nail varnish (Curanail®) were selected for inclusion in the study. The strength of adhesion between lacquer film and the nail plate was measured *in vitro* using 180° angle peel tests, using an Instron, and a high density polyethylene (HDPE) sheet as a model for the nail plate. HDPE was used as its surface energy (36 mJ/m<sup>2</sup>) is similar to that of the nail plate (34 mJ/m<sup>2</sup> [1]), and as adhesion between 2 surfaces is a function of their respective surface energies.

The *in vivo* study was conducted following ethics approval, in 6 volunteers (all females; aged 15-65 years old). In each volunteer, the same nail varnish was applied to all the fingernails by the operator. Subsequently, the percentage of nail varnish film remaining on the different fingernails was visually estimated by the operator every day for the next 2 weeks (or until all the varnish film had come off).

The *in vivo* residence time of the different cosmetic nail varnishes varied greatly, despite similar peak adhesive strengths of four of the five varnishes. In contrast, the low adhesion of the pharmaceutical lacquer Curanail was reflected in its lower residence time.

*In vitro* peel tests do not seem to reflect the *in vivo* residence time of nail varnishes. While the strength of adhesion of the film to the nail plate is important, other factors, such as the film's interactions with water that would occur during daily activities seem to be more important at determining the lacquer film's residence on the nail plate.

### References

[1] S Murdan *et al*, An investigation into the surface energy of human fingernail plates, J. Pharm. Pharmacol., 62 (2010) 1247-1248.

## P10. Optimisation of a Novel Phase Separating Nail Lacquer for the Treatment of Onychomycosis

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Topical treatment of onychomycosis (fungal nail disease) is desirable to avoid the systemic toxicity associated with oral antifungal treatment. Treatment of onychomycosis is lengthy; commonly taking one year to complete and the clinical cure rate from topical treatment is low. Hydrophobic / water insoluble polymers are often used in topical nail lacquers to form a film on the infected nail tissue which prevents the lacquer being removed during bathing or hand washing. However such hydrophobic polymers may not be best suited to delivery of the antifungal drug into the nail. A water soluble polymer based ciclopirox nail lacquer has recently demonstrated improved clinical efficacy over the commercially available product for the treatment of onychomycosis<sup>1</sup>. This nail lacquer was designed for daily overnight application. Amorolfine nail lacquers are typically applied weekly which has considerable benefits in terms of patient convenience and compliance which can improve treatment outcomes. The purpose of this study was to optimise the design of an amorolfine nail lacquer containing two polymers of different hydrophilicities which would phase separate on solvent evaporation, leading to the formation of a film with a protective water insoluble upper layer and a hydrophilic layer next to the nail matrix. This type of system should allow improved drug delivery to the nail, whilst protecting the lacquer from being washed off, allowing it to be used over extended periods of time.

Polymeric films containing hydroxyl propyl methyl cellulose (HPMC) and Eudragit E100 were prepared by solvent casting from solutions containing varying proportions of ethanol and water. Films containing the polymers and amorolfine were also prepared. The films were analysed using differential scanning calorimetry (DSC) and attenuated total reflectance FTIR (ATR-FTIR) spectroscopy. Dissolution experiments were used to examine the waterproof nature of the formulation. These were performed by first forming a drug containing polymeric film on the bottom of the dissolution vessel, exposing the film to water and then sampling the aqueous compartment over time.

The visual appearance and drying times of the films were affected by the proportion of water in the solvent, with lower proportions of water improving the visual appearance and decreasing the drying time. Solvent proportions of 95:5 ethanol to water allowed sufficient concentrations of HPMC to be dissolved and provided optimal film drying times and visual appearance. The DSC results of the combined polymer films indicated the presence of two separate glass transitions corresponding to those of the two polymers, confirming the presence of two separate phases. ATR-FTIR spectroscopy results demonstrated a higher concentration of the more hydrophobic Eudragit E100 in the upper layer of the formed film. In addition, amorolfine appeared at a higher concentration in the lower hydrophilic HPMC rich layer. The dissolution data indicated that the combined polymer film similar to Eudragit E100 reduced the loss of amorolfine into the dissolution media in comparison to HPMC. These data indicate the feasibility of developing a phase separating nail lacquer system containing a drug stabilising hydrophilic layer next the nail tissue with a protective hydrophobic polymer coating.

### References

<sup>1</sup> Baran R, Tosti A, Hartmane I, Altmeyer P, Hercogova J, Koudelkova V, Ruzicka T, Combemale P, Mikazans I. An innovative water-soluble biopolymer improves efficacy of ciclopirox nail lacquer in the management of onychomycosis. *J Eur Acad Dermatol Venereol* 23(7):773-781 (2009).