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The human nail – Barrier characterisation and permeation enhancement

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ABSTRACT

The human nail remains one of the most challenging membranes for formulation scientists to target and for clinicians to heal. Its formidable barrier properties are the primary reason that oral therapy remains the primary approach to manage ungual infections. This article considers the major structural properties underlying the excellent barrier function of the nail, with particular emphasis on the role of biophysical methods in advancing our knowledge of this appendage. Formulations currently available for management of ungual disease are discussed and their therapeutic efficacy is assessed. Finally, experimental strategies to enhance ungual permeation are reviewed and prospects for future developments in the field are considered.

1. Introduction

Topical treatment of skin and nail diseases is desirable in terms of patient acceptability and reduction of side effects associated with systemic drug delivery. This is particularly the case for nail diseases as they are frequently difficult to cure and require long periods of treatment (Rich and Scher, 2005). The nail plate is a highly keratinised tissue, which is characterised by low permeability to diffusing substances, however a number of formulation approaches have proved effective in increasing transungual permeation (Hui et al., 2004). In this review we examine the physiological and morphological properties of the nail which are responsible for its excellent barrier properties and mechanical strength. Major emphasis is given to the biophysical and bioanalytical approaches which have contributed to our knowledge of nail composition and function and which we believe have not yet been exploited to their full potential. The methodologies which have been explored as diagnostic tools of the nail are also considered. Finally, current and future approaches to enhance drug delivery to the nail are discussed.

2. Nail structure and physiology

The human nail plate is made up of layers of flattened keratinised cells that are fused into a dense but somewhat elastic mass. The plate is surrounded on three sides by the periungual grooves, more commonly referred to as nail folds, which are an extension of the epidermis and composed of cornified tissue. A further extension of the epidermis is the nail matrix, a germinative tissue located at the proximal end of each nail plate beneath and behind the plate. Unlike the viable epidermis that generates the stratum corneum, however, the nail matrix does not contain a granular layer (de Berker et al., 2007) and this has a profound influence on the amount of intercellular lipid in the nail plate as discussed later. The hardened cells of the nail plate are derived within and grow distally from the nail matrix at a rate of about 2–3 mm per month. The matrix is partially visible as a slightly opaque convex margin beneath the proximal plate, an area known as the lunula. Macrosopic features of the human nail are shown in Fig. 1. During keratinisation, cells undergo shape and other changes similar to those experienced by the epidermal cells forming the stratum corneum. As a result, the nail plate cells contain no organelles or nuclei and comprise keratin fibrils embedded in a protein matrix.

As a cornified epithelial structure, the chemical composition of the nail plate has many similarities to hair (Baden et al., 1973). Although the majority of the protein in hair and nail is classified as 'hard' trichocyte keratins, both hard and soft keratins have been detected in the nail matrix and both forms of the protein have been found in the nail plate. Keratins 1 and 10 (soft) are found in the matrix together with the hard keratin Ha-1 (de Berker et al., 2000) and several further hard keratins have also been shown to be present in the nail matrix, nail bed and nail plate (Perrin et al., 2004; Perrin, 2007). Intermediate filament-associated proteins and trichohyalin are also found within the nail (Cashman and Sloan, 2010). Sulphur content of the nail amounts

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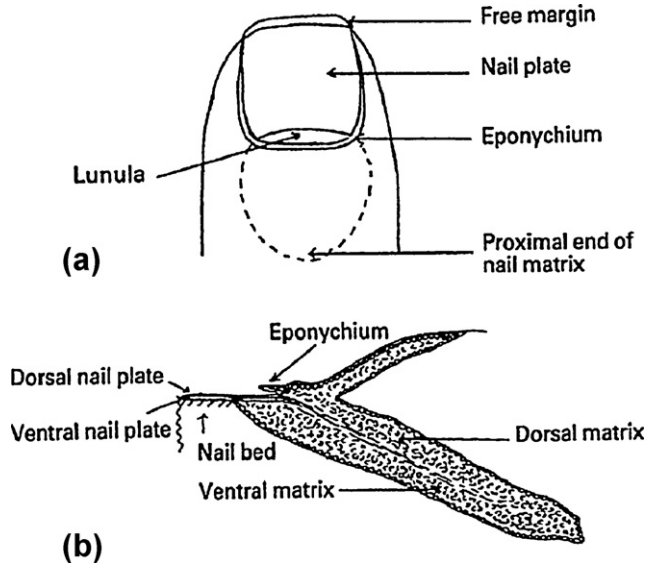

Fig. 1. Features of the human nail plate.

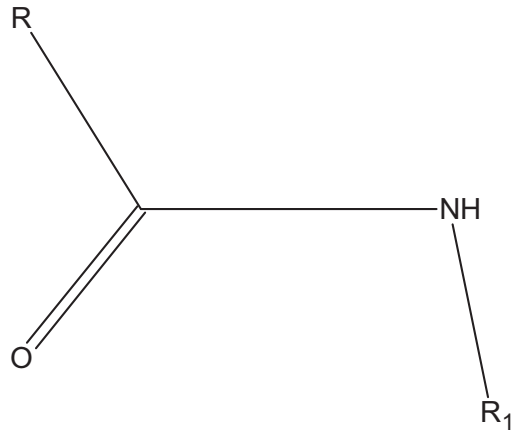

Fig. 2. Peptide bond in proteins.

to about 10% and is mainly present within the cystine disulphide bonds that contribute to nail tensile strength by linking the keratin fibres.

There are three very tightly knit keratinised layers within the nail plate, a hard thin outermost dorsal lamina, a softer yet thicker intermediate lamina and an innermost ventral layer (Runne and Orfanos, 1981). Collectively it appears that the expression of different keratin types in different regions of the nail matrix and nail bed may be responsible for the variable physical characteristics of the nail plate layers (Bragulla and Homberger, 2009; Nogueiras-Nieto et al., 2011).

The nail contains significant amounts of phospholipid, mainly in the dorsal and intermediate layers, that contributes to its flexibility. Glycolic and stearic acids are also found in the nail. The total lipid content of the nail plate is between 0.1% and 1%, which is considerably different to that of the stratum corneum (~10%). The principal plasticiser of the nail is water, the content of which varies widely dependent on prevailing relative humidity (Farran et al., 2008), but it is normally present at around 18%. When the water content is less than 16% the nail plate becomes brittle and when the nail is hydrated to water levels of ~25% it becomes soft. Minerals are also important constituents of nails. Lack of selenium and magnesium can have a profound effect on the health of the nail plate (Bauer and Stevens, 1983; Kien and Ganther, 1983).

3. Biophysical characterisation of the nail and its barrier function

As the nail is a skin appendage many biophysical and bioengineering techniques which were originally developed to study the skin have subsequently been investigated for their utility to probe the nail and its constituents. The types of features probed at a molecular level have generally been the keratin composition, structure and packing, water content and hydration effects. Some techniques have also been used to examine nail elemental composition with proposed applications for the use of this tissue as diagnostic of other underlying conditions. Since much of the literature is concerned with vibrational spectroscopy, namely Infra Red and Raman, these will be considered first.

3.1. Near infra red Raman spectroscopy

Near infrared Fourier transform (NIR-FT) Raman spectroscopy exploits an effect in which a small portion of monochromatic light scattered by a substance has a frequency that is different from that of the incoming beam by an amount equal to the vibration frequency of the chemical bond. Frequency shifts of the scattered light can be analysed and presented as spectra. The bands represent vibrations characteristic for chemical bonds within the molecules of the examined sample. NIR-FT Raman spectroscopy is especially suited to analysis of biological material as it may be used for complex, impure samples and only minute amounts of tissue are required.

The significant Raman vibrations sensitive for protein conformation are those of the peptide bonds and two of several vibrations, designated as Amide I and Amide III are prominent in nail as well as skin, hair and stratum corneum (Fig. 2). The amide I involves mainly a stretching of the C=O bond. The amide III mode is more complex involving a stretch of the C—R and C—N bonds together with a deformation of the CNH angle. The disulfide stretch for proteins occurs in the region 500–550 cm^{-1}. Depending on the conformation of the —C—S—S—C— atoms the following Raman bands may be present: the most common and energetically favourable gauche–gauche–gauche conformation (~510 cm^{-1}), the less stable gauche–gauche–trans (~525 cm^{-1}) and trans–gauche–trans (540 cm^{-1}). The CH stretching vibrations of protein are represented in the region 2800–3000 cm^{-1}. The 2890 cm^{-1} and 2850 cm^{-1} bands originate from the lipids whereas the bands 2980 cm^{-1}, 2930 cm^{-1} (originating from CH_3 asymmetric vibrations) and 2900 cm^{-1} (CH_2 symmetric vibration) are protein specific. A band at 1425–1453 cm^{-1} represents vibrations of $\delta(CH_2)$ scissoring of lipids and $\delta(CH_2)\delta(CH_3)$ of proteins (Gniadecka et al., 1997).

The intensity of bands in the region 1000–1150 cm^{-1} may be used to determine fluidity and intramolecular conformation of lipids. In the crystal state, all chains are in the all-trans conformation represented by the bands 1066 cm^{-1} and 1130 cm^{-1}. Lipid melting causes a transition to a more chaotic gauche conformation characterised by the disappearance of the 1066 cm^{-1} and 1130 cm^{-1} peaks and the emergence of a peak at 1100 cm^{-1}. Further information on lipid structure can be gathered by examining the regions of CH group vibrations at 2850–2890 cm^{-1}. The ratio of the symmetric methylene C—H stretching mode intensities (peak height) I_{2890}/I_{2850} is sensitive to the packing of the acyl chains. An

order parameter for the lateral interaction, S_{lat} has been developed and is defined as:

$$S_{lat} = \frac{I_{CH_2}(\text{sample}) - 0.7}{1.5} \quad (1)$$

where I_{CH_2} is the intensity of the 2890 cm^{-1} band divided by the intensity of the 2850 cm^{-1} band. $S_{lat} = 1$ when a phospholipid is in the liquid state ($I_{CH_2} = 2.2$), and $S_{lat} = 0$ when a phospholipid is in the liquid state ($I_{CH_2} = 0.7$). The parameter reflects the intermolecular structures of lipids and decreases in the following order: lamellar liquid, hexagonal or cubic liquid crystal, micellar solution, and solution in organic solvent (Gaber and Peticolas, 1977).

The intramolecular vibrations of hydrogen bonded water include two OH stretching bands in the 2800–3400 cm^{-1} region and the bending band near 1645 cm^{-1}. The bending band is weaker in intensity than the stretching bands and typically, it is hidden by the amide I vibrations around 1650 cm^{-1}. The symmetric OH stretching is found around 3250 cm^{-1} whereas the Raman component near 3400 cm^{-1} has been assigned to asymmetrical OH stretching. Water molecules non-hydrogen bonded to biomolecules are ordinarily called free water, although they are hydrogen bonded to other water molecules with a tetrahedral structure. This tetrahedron water structure is reflected by a band at ~180 cm^{-1} representing hydrogen bonded stretching.

Gniadecka et al. (1998) used near infrared Fourier transform Raman spectroscopy to investigate skin, hair and nail samples collected from 10 human subjects. The spectral range of the acquired spectra was from 0 to 3500 cm^{-1}. Combining the frequencies of amide I and amide III intensity maxima indicated that the majority of the proteins in the nail were in the α-helix conformation, similar to whole skin, hair and stratum corneum. A strong C—C stretch band at ~935 cm^{-1} which is typical of the α-helix was also present in these spectra. The S—S band for nail tissue was prominent and was located at around 510 cm^{-1} indicating that these bonds are primarily in the gauche–gauche–gauche conformation; however a shoulder at 520 cm^{-1} indicated that some S—S bonds are in the gauche–gauche–trans conformation. The mean wave numbers of the CH_3 asymmetric band for nail compared with whole skin, suggested that, in nail, the protein is highly folded, interacting with its surroundings to only a minimal degree. A band at 1447 cm^{-1} representing scissoring vibrations was also present in nail spectra. For lipids, prominent peaks were observed for nail at 1130 cm^{-1} and 1030 cm^{-1}. This implies that lipid structure is highly ordered in nail. The S_{lat} ratio for the CH group vibrations in the 2850–2890 cm^{-1} region was 1 for nail, indicating that the majority of lipids are in the crystal state, suggested to be a lamellar liquid crystalline form by the authors. This also suggests a low degree of intermolecular interactions between lipids and proteins in nail. The best estimate of total hydrogen bonded water was obtained by calculating I_{2940}/I_{3250} and was reported as 19.82 ± 3.02%. The hydrogen bonded water content for nail was comparable to that for stratum corneum. The 180 cm^{-1} water band did not appear indicating that, in nail, the majority of water molecules are bound to other components and do not form tetrahedron structures.

In a later study by the same group (Wessel et al., 1999), NIR-FT Raman spectroscopy was used to investigate hydration effects on human nails. Raman spectra were obtained both *in vitro* from nail samples and *in vivo*, before and after soaking in water. For *in vitro* studies, measurements were taken before and after hydration of dorsal toenail clippings from 31 volunteers. Samples were immersed in water for 5, 10 and 15 min. For *in vivo* studies, the adherent nail plate and the free distal part were examined in 10 volunteers after soaking in tap water for 15 min. The ratio of the integrated areas under the ν(OH) band of water at 3250 cm^{-1} and the ν(CH) band of proteins at 2932 cm^{-1} was used as an index of the water content of the nails. The low frequency area of the Raman spectrum was analysed to provide information about water structure. Signal-to-noise ratio was used to compare the quality of *in vivo* and *in vitro* spectra.

Water was identified at the bands at 3250 cm^{-1} ν(OH) and the shoulder at 1630 cm^{-1}, which represents the δ(OH) signal. The water uptake for samples from the distal portion of the nail studied increased with increasing hydration time with an apparent saturation after about 10 min. As for the earlier study, the water molecules were mostly protein-bound with no sign of the band at 180 cm^{-1}. A comparison of the protein bands of nail samples before, and after soaking for 15 min in water, indicated changes in a number of bands, but significant effects were only observed at 1620 cm^{-1} possibly because of the underlying water band. The frequency of the band representing the S—S bridges (510 cm^{-1}) of proteins was not influenced by the water content. No major differences were observed between the spectra for the *in vivo* and *in vitro* distal nail measurements. Water uptake was smaller for finger nails *in vivo* (27 ± 6%) compared with toe nails *in vitro* (45 ± 6%). For *in vivo* studies, the adherent nail plate showed a slightly different Raman spectrum in comparison with the distal part. The baseline increased between 800 and 1100 cm^{-1}. The shoulder at 1247 cm^{-1} (which indicates amide III) increased, and this result was ascribed to the underlying collagen structure by the authors. The relative water content of the adherent nail plate (including the underlying tissue) was much higher than that for the free part. Soaking of the adherent nail plate *in vivo* had no apparent effect on the Raman spectra. Overall the results indicate that the nail has a limited water-holding capacity and that the adsorbed water causes changes in protein geometry.

3.2. Near infrared-diffuse reflectance (NIR-DR) spectroscopy

Preliminary studies with NIR-DR on nail were first reported by Sowa et al. (1995).

Egawa et al. (2003) investigated its potential to determine the water content of human nail plates. Spectra were collected from 108 cut nail plates from the free edge of the nail tip from the fingernails of 17 healthy volunteers (22–60 years, mean 41.3; 8 males, 9 females). The cut samples were equilibrated overnight at 26 °C at 44% relative humidity (RH). Samples were then incubated at 25 °C, varying RH from 1 to 99% in at temperature and humidity controlled room for 18 h. NIR-DR spectra were subsequently collected under controlled conditions of temperature and humidity and the thickness of cut nail plates was also recorded. Partial least squares (PLS) regression was applied to the NIR spectra in the 1115–1645 nm region in order to develop calibration models to determine water content in the cut nail plates and *in vivo*. The water holding capacity of cut nail plates was determined by immersing samples in distilled water for up to 60 min and measuring their NIR spectra. NIR-DR spectra were measured *in vivo* for the free edges of fingernails, before and immediately after washing, for three women aged 26–32 years (mean 29.7). The water content of cut nail plates changed from 1.4 wt% to 33.9 wt% in the RH range 1–99%. Water content increased markedly when ambient RH increased above 80%. Nail thickness appeared to have little effect on the amount of water in nails. Raw NIR spectra were dominated by a broad feature in the 1400–1550 nm region. The NIR spectra in the 1400–1450 nm region were also significantly different from those of skin, probably reflecting the differences in free water in the tissues. The authors also observed a broad feature in the 1500–1530 nm regions which they postulated might be due to hydrogen bonded water and keratin. With higher water content, the intensity of a band at 1420 nm, associated with free

water, was observed to increase. At very high RH%, a new peak at 1488 nm was noted and was suggested to reflect changes in the states of hydrogen bonds in cut nail plates under these conditions. Cut nail plates with a water content of about 7–9 wt% before immersion had a water content as high as 24–33 wt% immediately after the end of the immersion period. Results from NIR and gravimetry were in good agreement. *In vivo* water content ranged from ~15% to 21%. The PLS model also confirmed an increase in water content *in vivo* after hand washing. Good correlations for *in vitro* water content were obtained between the NIR approach, Karl Fischer analysis and nuclear magnetic resonance measurements.

The same authors (Egawa et al., 2005) investigated seasonal variation of nail water content. Spectra were collected *in vivo* both in summer and in winter from 15 female subjects (21–46 years, average 27.6), under controlled conditions of temperature and humidity (23.8–25.0 °C, 40–41% RH). The water content of the nail plate *in vivo* was calculated from the NIR spectra using PLS regression analysis. Broad water bands were observed at 1400–1500 nm and around 1910 nm. It was possible to apply NIR-based prediction of water content of the cut nail plate *to in vivo* measurements. The water content of the nail plate varied between subjects but not between the nails of individual subjects. Average water content was significantly ($p < 0.05$) lower in winter than in summer. Significant differences in spectra were not observed between finger and toenails.

3.3. Dynamic vapour sorption near infra red (DVS-NIR)

Dynamic vapour sorption is a gravimetric method for studying the sorption properties of materials under controlled conditions of temperature and humidity. Recently we have evaluated the sorption properties of human nails in a specialised DVS apparatus which is also fitted with a NIR probe (Abdalghafor et al., in press). Nail clippings from 6 volunteers ($n = 6$; 3 males, 3 females) were subjected to a fixed temperature of 32 °C and a range of relative humidity (RH%) values, ranging from 40% to 90%, with a 10% increment. Real time NIR spectra were acquired using a NIR fibre optic probe in order to monitor water bands at 1422 nm (free water) and 1908 nm (bound water). The increase in the RH% (40–90%) was associated with a linear change in mass profile. The NIR spectra also indicated increases in band absorbance (Second derivative spectra) at 1422 nm and 1908 nm Good correlations (Fig. 3) were obtained for the gravimetric changes and band absorbances at 1422 nm ($r^2 = 0.96$) and 1908 nm (0.99).

3.4. Attenuated total reflectance Fourier transform infra red (ATR FTIR)

Sowa et al. (1995) examined nails *in vivo* using ATR FTIR. The presence of lipid on the nail surface was confirmed from the appearance of the lipid ester carbonyl peak at 1745 cm^{-1} and the lipid methylene stretching vibrations at 2924 and 2853 cm^{-1} which are typical of unordered lipid acyl chains. Characteristic protein absorptions were also identified at 1650, 1540 and 1250 cm^{-1}. In a more recent application, ATR FTIR was used to study secondary protein structures in 41 healthy donors (Male/Female: 11/30; 37.7 ± 10.2/37.4 ± 9.2) and 65 patients (Male Female: 19/46; 37.9 ± 7.4/35.0 ± 9.6) with Chronic Fatigue Syndrome (CFS) by Sakudo et al. (2009). Spectra in the region 4000–6000 cm^{-1} at 4 cm^{-1} resolution were obtained. The α helix, β sheet, β turn and random coil contents of nails were estimated from the amide I region of the ATR FTIR spectra after correction for the refractive index of the material and Fourier deconvolution and curve fitting. The secondary protein structure of healthy fingernails was observed to consist of 11.74% α-helices, 37.58% β-sheets, 25.01% β turns and 25.67% random coils. The CFS nail samples contained less α-helix and greater levels of β-sheet which was attributed to reduced levels of normal elements of the nail plate however evidence for this was not provided. Generally, the potential of the technique for nail characterisation is expected to be limited because of the shallow depth of penetration into tissue (Sowa et al., 1995), typically <10 μm.

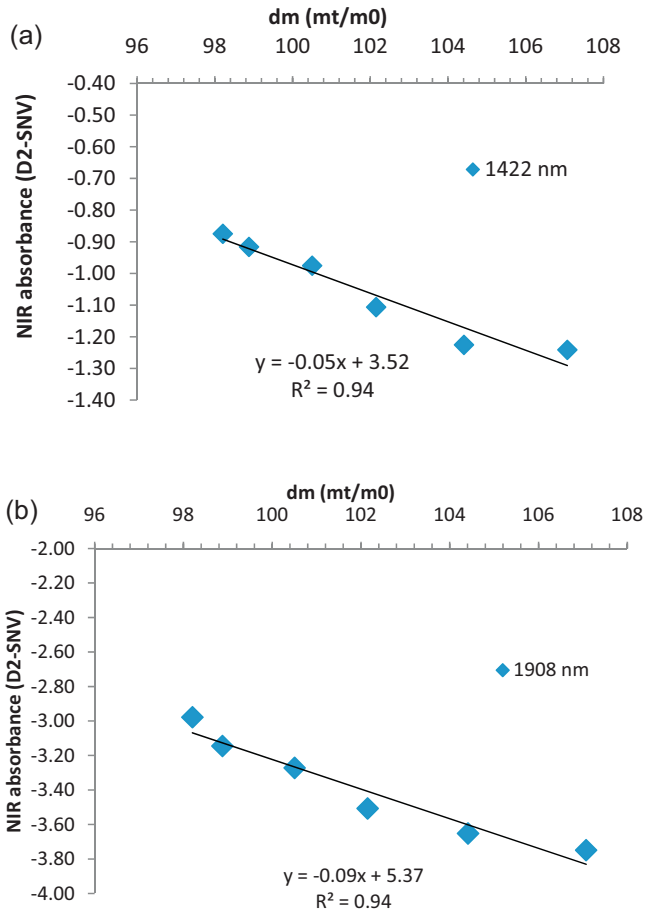

Fig. 3. Correlation between increase in mass change from 40 to 90% RH with band intensity at (a) 1422 nm – free water and (b) 1908 nm – bound water.

3.5. Confocal Raman spectroscopy (CRS)

In vivo CRS combines Raman spectroscopy with Confocal Laser Scanning microscopy (CLSM) to accurately determine the skin depth from which the Raman signal is collected and was pioneered by Caspers et al. (1998, 2001). However, its application to the nail has not been explored. Recently we investigated the application of this technique in the measurement of nail hydration (unpublished data). Healthy human subjects with no history of nail disease or application of cosmetics to the nail were studied. Fingernails were soaked in water for 0, 2.5, 5, 10 and 15 min before the measurements were taken. Fingernails were also hydrated for 2 hrs with water and measurements were collected at time 0, 10, 30 and 60 min. *In vivo* measurements were conducted using a CRS microscope 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. The baseline water content at 0 μm within the nail was ~8% (w/w) (Fig. 4a and b). This value increased with depth to ~21% (w/w) at a depth of 30 μm into the nail, at which it showed a plateau phase (Fig. 4a) Hydrating the

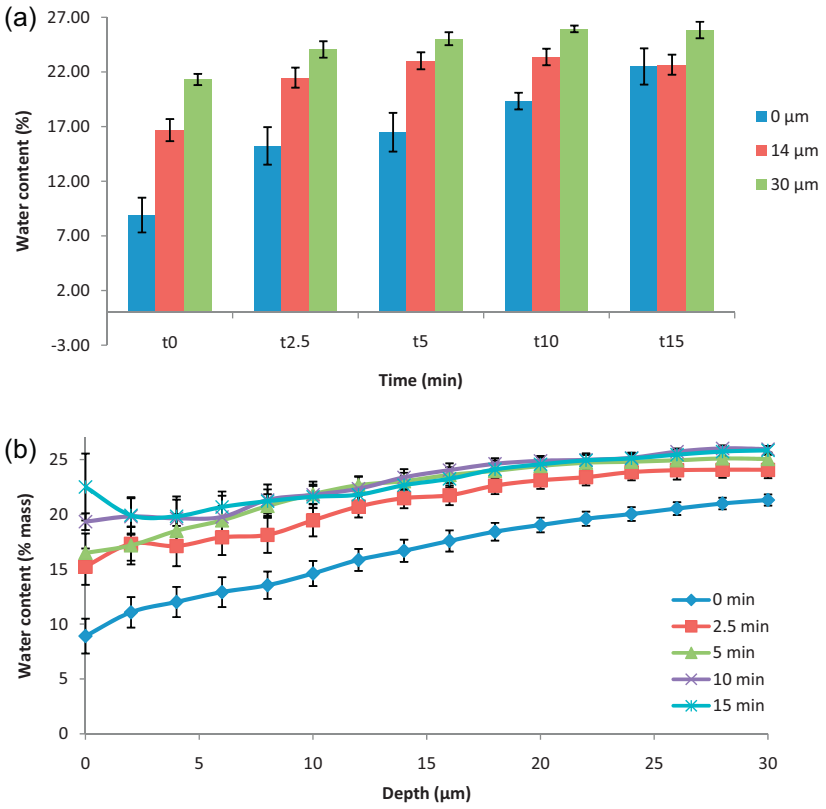

Fig. 4. (a) Water content at 0, 14 and 30 µm after 2.5, 5, 10 and 15 mins soaking in water. (b) Nail water content *in vivo measured* 2 h after hydration for 0, 2.5, 5, 10, 15 min soaking in water ($n = 6$; Mean ± S.D.).

nail for 2.5 min elevated the water content within the nail to ~15% (w/w) at 0 µm and ~24% (w/w) at 30 µm. The greater the hydration time the higher the water content within the nail up to 15 min after which there was no further increase even after 2 h of hydration. The water content within the nail returned back to the baseline 30 min after 2 hrs hydration. The values are in reasonable agreement with the NIR-FT Raman spectroscopy measurements (Gniadecka et al., 1998) and NIR DR values (Egawa et al., 2003) discussed earlier.

3.6. Atomic absorption spectrometry (AAS)

Because of a proposed correlation between systemic levels of trace elements with nail levels, there are a number of AAS reports on nail in the literature. Harrison and Tyree (1971) were the first to report the application of flame atomic absorption to determine five elements (calcium, zinc, magnesium, copper and iron) in fingernail clippings from seven females and ten males. Barnett and Kahn (1972) also reported a method to determine copper levels in fingernail clippings from newborn infants. However, despite early optimism, and many publications on this application, nail sampling has not been developed as a routine non-invasive method to assess mineral status of individuals possibly because of the wide intersubject variation in trace element levels.

3.7. Gamma ray spectrometry

Petushkov et al. (1969) reported the application of high-resolution gamma spectrometry in the determination of trace elements in human nails. Fingernail clippings were obtained from three subjects ranging from 22 to 38 years of age. The Samples were subject to neutron irradiation and gamma-ray spectra were subsequently collected. Quantitative and qualitative determination of sodium bromine, gold zinc and antimony were obtained. As for AAS, this method has not been advanced further.

3.8. Energy dispersive X-ray spectroscopy (EDS)

EDS gives information about the elemental composition of a specimen, in terms of both quantity and distribution. A high-energy beam of charged particles such as electrons or protons (proton induced X ray emission; PIXE), or a beam of X-rays, is focused into the sample being studied in order to stimulate the emission of characteristic X-rays from a specimen. As for the previous two methodologies, this technique gained attention because of its ability to profile trace element amounts, initially in skin and subsequently in nails Roomans et al. (1978) reported the use of EDS to profile Na, K and Cl levels in cystic fibrosis patients and noted elevated levels of these elements in CF patients compared with healthy subjects. Djaldetti et al., 1987 used this technique to examine the elemental content of nail clippings from 13 patients with liver cirrhosis compared with those from 50 healthy subjects. Statistically significant increases in the percentage of Na, Mg and P and a slight, but statistically significant decrease in S and Cl were observed for patients with liver cirrhosis. The percentage of K, Ca, Fe, Cu and Zn did not show any difference from control subjects. Lapatto et al. (1989) used PIXE to measure concentration values for the elements Ca, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, and Pb in finger and toe nail cuttings from 12 healthy individuals. No differences in elemental content in nails from different fingers for individuals were reported, however large differences between individuals were noted. Elemental concentrations in toenails were also observed to be same as those in fingernails even though toenails grow more slowly than fingernails. The data obtained were generally consistent with previous published measurements using this technique.

3.9. Inductively coupled plasma mass spectrometry (ICP-MS)

This technique utilises a gas which contains a sufficient concentration of ions and electrons to make the vapour electrically conductive. The plasma used in an ICP-MS is made by partially ionising argon gas using an electrical current. After the sample is introduced into the plasma, the extreme temperature causes the sample to separate into individual atoms. For coupling to mass spectrometry, the ions from the plasma are extracted through a series of cones into a mass spectrometer. Goullé et al. (2009) have reported its use for multi-elemental analysis of fingernail and toenail clippings collected from healthy volunteers. Li, Be, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Ba, La, Gd, W, Pt, Hg, Tl, Pb, Bi, and U were detected with validated measurements. The authors suggested the application of ICP-MS as a noninvasive technique for detection of industrial, domestic, or environmental metal exposure. Button et al. (2009) used this technique to assess arsenic uptake in toenail clippings and proposed this approach as a biomarker of exposure to environments with elevated arsenic.

3.10. Dielectric spectroscopy

Marzec and Olszewski (2009) investigated dielectric spectroscopy as a tool to discriminate the properties of fingernail samples from healthy and diabetic subjects. The study was conducted in 10 type 2 diabetic patients (50–78 years old) and 12 normal individuals (44–80 years old) comprising the controls. Under controlled conditions of water content (0% and 3%) permittivity of diabetic nails at a constant temperature was significantly lower than for the healthy nails. The authors suggested that the polar side-chains, interchain hydrogen bonds and disulphide bridges of the keratin macromolecule might have been damaged because of intermolecular cross-linkage of keratin, associated with the presence of advanced glycation end products in diabetes.

3.11. Opto-thermal transient emission radiometry (OTTER)

OTTER uses pulsed laser excitation to induce temperature jumps of the order of a few °C in the top few microns of the material under study. These temperature jumps decay on a time scale of microseconds and do not materially increase the average substrate temperature or the rate of diffusion under study. They are observed with a high speed infrared detector sensitive to the heat radiation emitted by the surface (Bindra et al., 1992). For bio-tissue, this radiation is strongest in the mid-infrared 6–13 μm band of wavelengths. The measurement captures the decay dynamics of this transient component of the heat radiation and relates it to the physical properties of the near-surface layers through appropriate mathematical models. In such models, depth-resolution is linked to the time parameter of the transients and chemical specificity to the absorption spectra of the molecules of interest (Imhof et al., 1994). OTTER has been used to measure nail water content, nail water concentration depth profiles, as well as topically applied solvent penetration (Fig. 5) through nail (Xiao et al., 2010, 2011). Combining the water content results with Transonychial water (TOWL) flux, through nail also allows the water diffusion coefficient of nail to be calculated (see Section 3.14).

3.12. Optical coherence tomography (OCT)

OCT is a noninvasive optical imaging technology that provides cross-sectional, tomographic images of tissue *in situ* and in real time (Fujimoto, 2003). In this technique the reflection of infrared

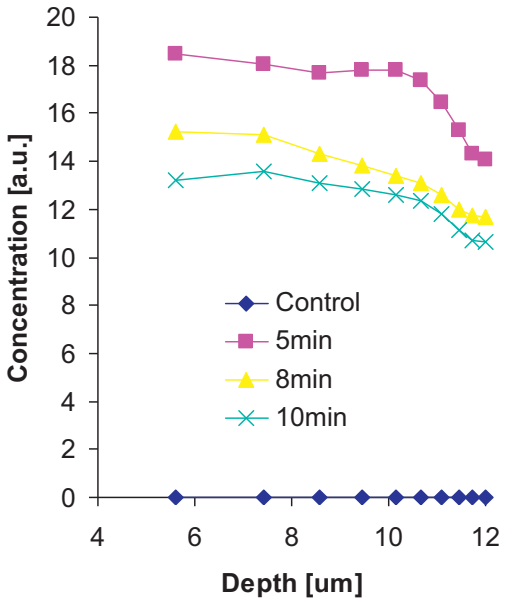

Fig. 5. Glycerol depth profiles in human nail *in vivo* expressed in arbitrary units (Xiao et al., 2011).

light from the tissue of interest is measured and the intensity is imaged as a function of position. It is possible to obtain images of tissue *in situ* with a high axial resolution. A representative image of the nail and proximal tissue is shown in Fig. 6 (unpublished data). Mogensen et al. (2007) investigated nail morphology and thickness in 10 healthy volunteer using OCT images and compared the results with high-frequency ultrasound (HFUS) images and measurements with callipers and polarisation-sensitive (PS) OCT. In standard OCT the nail plate appeared as a layered structure containing a varying number of horizontal homogeneous bands of varying intensity and thickness. PS-OCT images of the nail plate also showed a layered structure. It was possible to measure nail thickness using OCT and subtle changes not detected by HFUS could be discriminated by OCT. The authors suggested that OCT was a promising tool in nail anatomy and thickness measurements. The potential of OCT and PSOCT in differentiation of tumours of the nail unit was also highlighted. More recently Sattler et al. (2011) compared OCT, Confocal laser scanning microscopy (CLSM) and TOWL as tools for study of the nail. Healthy nails were investigated using OCT (Nail plates of 33 healthy subjects, 19–53 years) and CLSM (Measurements conducted on 15 fingernails of 10 healthy individuals, age not given). In a separate study 30 healthy volunteers washed the middle finger for 30 min at 38 °C for two weeks. OCT, TOWL and CLSM measurements were conducted before, and at the end of the washing period. The penetration depth was about 2 mm with a resolution of 10 μm OCT confirmed that nail thickness was significantly higher for the middle finger (Mean value 465.8 μm) of the dominating hand of subjects. As for the earlier OCT study, the band like structure of the healthy nail plate was confirmed. The washing experiment did not result in any significant increase in nail thickness as measured with OCT.

3.13. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy utilises a laser and a pair of pinhole apertures for imaging and optical sectioning of tissue at high resolution. One of the first applications of the technique to study the nail was described by Hongcharu et al. (2000). Both *in vivo* and *in vitro* analyses were conducted on the nails of a patient suffering from onychomycosis. Full thickness nail clippings

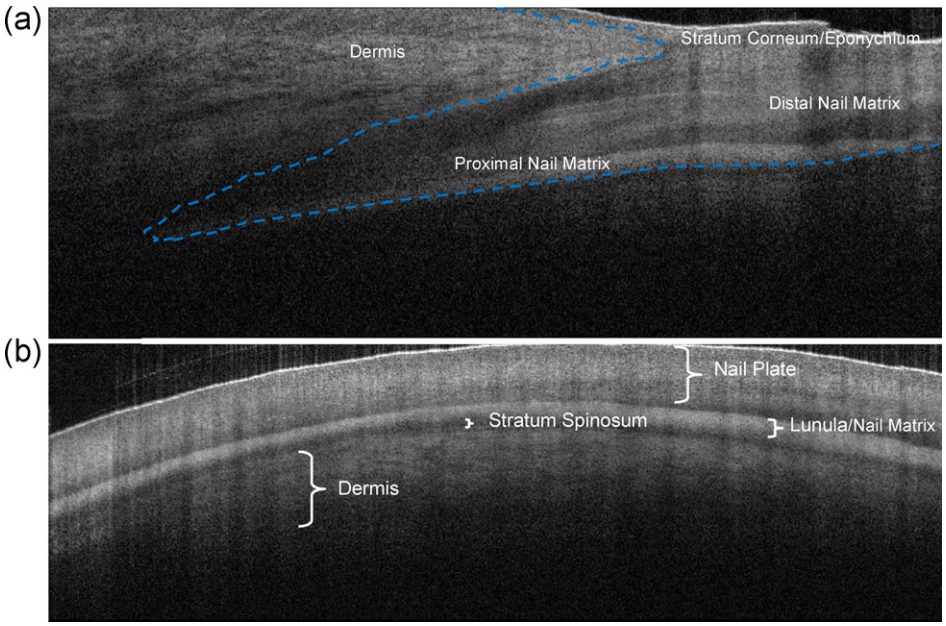

Fig. 6. (a) Representative OCT scan of nail and proximal tissue. (b) OCT scan of finger nail with scanning axis perpendicular to direction of nail growth. VivoSight OCT scanner (Michelson Diagnostics, UK).

were collected and imaged. Virtual sections were obtained *in vivo* by focusing the confocal microscope inside the nail plate. *In vivo* confocal images from just below the surface of the nail plate revealed a network of branched hyphae. The authors hypothesised that CLSM may be a faster and more accurate method of diagnosing onychomycosis compared with fungal culture, conventional microscopy or chemical hydrolysis.

Sattler et al. (2011) utilised CLSM along with other methods to evaluate healthy nails. The major advantage of CLSM compared with OCT was the ability to examine the microscopic structure of the nail as corneocyte borders could be visualised and their integrity examined. The authors achieved a depth of 400–500 μm. Various limitations associated with the method including the need for experienced examiners, difficulty in positioning and holding the nail during imaging and the necessity for an index matching medium to be applied to the CLSM before measurement were also highlighted. Finally, the possibility of diagnosing leukonychia and mycotic infection was also demonstrated with individual images of diseased nails.

3.14. Transonychial water loss (TOWL)

TOWL is a measure of water flux through the nail and is frequently used with other biophysical techniques to characterise nail barrier function or permeability. Jemec et al. (1989) measured TOWL in 21 healthy volunteers with an evaporimeter, to establish the usefulness of this technique and study the influence of sex, age and nail-plate thickness. The median TOWL was typically higher than for transepidermal water loss and it decreased with increasing age of subject. No significant correlation between nail-plate thickness, as measured by ultrasound was found. Krönauer et al. (2001) investigated TOWL values (measured by evaporimetry) in healthy patients ($n = 10$; age 20–51 years, median 29 years) as well as patients with atopic eczema ($n = 18$; age 17–68 years, median 32 years), psoriasis ($n = 25$; age 24–78 years, median 56 years) and onychomycosis ($n = 50$; age 23–79 years, median 54 years). TOWL values were significantly higher ($p < 0.05$) in healthy patients compared with those with atopic eczema and onychomycosis; only patients with nail psoriasis showed significantly lower TOWL compared with healthy subjects.

4. Ungual drug delivery – the present and the future

Both chemical and physical enhancement strategies have been explored with to a view to developing novel and efficacious systems for ungual delivery and these approaches are discussed in this section. For a review of the actual models used to evaluate such experimental techniques we refer the reader to a comprehensive discussion of the subject by Nair et al. (2012).

4.1. Current approaches and clinical evidence

For many years dermatologists believed that topical treatment for anything other than the most superficial infections of the nail plate was futile (Zaias and Serrano, 1989). The nail plate was often viewed as an impenetrable barrier and the only means of treating diseases of the nail unit was by delivering drugs *via* the blood supply to the nail matrix or by removal (surgical avulsion) of the diseased nail prior to topical application of the therapy. The former involved prolonged oral dosing with powerful antifungal agents and other drugs (see, for example, Roberton and Hosking, 1983) and the latter was only considered if only a few nails were involved or oral therapy was contra-indicated (Hettinger and Valinsky, 1991). Many of the oral antifungal agents can achieve mycological and clinical cure rates following greater than 48 weeks of therapy, with terbinafine being superior to other agents including griseofulvin, fluconazole and itraconazole (Cribier and Paul, 2001). However, our knowledge of the nail plate barrier has improved considerably with the result that our ability to rationally develop antifungal and other agents and delivery vehicles specifically for the topical treatment of nail diseases has increased. The dermatologists armamentarium has been supplemented with nail lacquers and other formulations including those containing tioconazole, amorolfine, ciclopirox, clobetasol-17-propionate, calcipotriol and tazarotene. However, it is well known that the true test of any potential therapeutic regimen is the ability to cure the disease and to prevent remission.

The conservative approach is to supplement oral therapy with topical treatment. Nakano et al. (2006) performed a pilot study to assess the safety and efficacy of pulse therapy with oral terbinafine in 66 patients with onychomycosis. Each pulse consisted of oral

terbinafine (500 mg/day) for 1 week followed by a 3-week interval. Topical 1% terbinafine cream was applied daily. Efficacy was assessed 1 year after treatment initiation. There was a complete cure in 51 patients (approximately 77%), marked improvement in five patients, improvement in five patients and slight improvement in one patient. Four patients showed no change. Although they concluded that terbinafine pulse therapy in combination with topical application of terbinafine cream was safe and effective, it was not possible to determine whether the topical applications improved the outcome. An earlier study (Alpsov et al., 1996) using a similar oral treatment regimen but with no supplemental topical therapy reported 74% cure rate, suggesting that topical application was of little additive benefit. However, it is important to appreciate that the application vehicle is very important for achieving successful delivery of the drug into the nail plate and it is possible that the cream used in the Nakano study had not been optimised in this respect. Furthermore, the highly lipophilic nature of terbinafine suggests that it would not penetrate into the nail to any great extent. Perhaps the use of a more innovative delivery vehicle would generate a clearer picture of the effectiveness of dual therapy. For example Ghannoum et al. (2011) have incorporated terbinafine within transfersomes (described by Ghannoum et al. as "carrier vehicles [consisting of] responsive, composite lipid aggregates that are highly deformable and have high surface hydrophilicity") and demonstrated that such vehicles, at an active concentration of 1.5% (w/v), were considerably more potent (with MIC_{50} values against *Trichophyton rubrum* and *Tricophyton mentagrophytes* being 30-fold and 60-fold lower, respectively) than a 1% terbinafine spray *in vitro*.

The usefulness of supplemental topical treatment with oral therapy was also investigated by Rigopoulos et al. (2003) who evaluated a combination of systemic and topical antifungals to improve the cure rates and reduce the duration of systemic treatment for onychomycoses. Itraconazole pulse therapy was combined with amorolfine lacquer and compared with itraconazole alone in the treatment of nail Candida. Ninety patients with Candida of the fingernails were randomised into two treatment groups of 45. Group 1 received itraconazole pulse therapy for 2 months with amorolfine 5% nail lacquer solution application for 6 months, while group 2 received monotherapy with three pulses of itraconazole. Eighty-five patients, with a mean duration of onychomycosis of 11 months, were eventually analysed. After three months of therapy, mycological cure was seen in 32 (74%) of 43 patients in group 1 and in 25 (60%) of 42 patients in group 2. Following 9 months therapy, a global cure was observed in 40 patients (93%) in group 1 and in 34 patients (81%) in group 2. The combination therapy achieved greater mycological cure and increased total cure rate. Although statistical analysis showed no statistically significant difference ($p > 0.1$) between the two treatment groups, the combination of topical amorolfine and oral itraconazole was shown to exhibit considerable synergy.

In a multicentre, randomised, open-label, parallel group study, Baran et al. (2007) randomised patients to receive either topical amorolfine HCl 5% nail lacquer once weekly for 12 months plus terbinafine 250 mg once daily for 3 months (AT group) or terbinafine alone once daily for 3 months (T group). The study over 18 months included a 6-month treatment-free phase subsequent to 12-months active therapy for the AT group and a 15-month treatment-free phase following the 3-months active therapy for the T group. A total of 249 patients were enrolled in the study, 120 in the AT group and 129 in the T group. A significantly higher success rate was observed for patients in the AT group relative to those in the T group at 18 months and both treatment regimens were well tolerated. These results demonstrated that amorolfine nail lacquer in combination with oral terbinafine was more effective in the treatment of toenail onychomycosis that included matrix involvement. On the other hand, a study using a similar protocol to that of Baran et al., 2007 suggested that terbinafine pulse therapy was effective and safe in the treatment of onychomycosis, and that additional therapy with topical ciclopirox olamine (8%) or amorolfine HCl (5%) did not show any significant difference in efficacy in comparison to monotherapy with oral terbinafine (Jaiswal et al., 2007).

Monotherapy with topical treatment has also shown some success. Baran and Coquard (2005) treated 13 onychomycotic patients, aged 25–78 years, with a solution of 1% fluconazole and 20% urea in an ethanol–water mixture, applied once daily. There was complete resolution of the disease in four cases and four patients demonstrated a 90% improvement. Of four patients with onychomycoses in both big toenails, two showed 50% improvement bilaterally and in the remaining two patients there was a 90% improvement in one nail and a 50% improvement in the other. Overall the response to local therapy was appreciable.

Gupta et al. (2000) reviewed the efficacy and safety of 8% ciclopirox nail lacquer in the treatment of onychomycosis. In one study, 223 patients were randomised to treatment and in another, 237 subjects were randomised. The active and placebo formulations were applied daily for 48 weeks and mycologic evaluation was performed every 12 weeks. Data from these pivotal trials demonstrated that ciclopirox nail lacquer was significantly more effective than placebo in the treatment of onychomycosis. At the end of the treatment period, the mycologic cure rate in the first study was 29% and 11% in the ciclopirox and vehicle groups, respectively, and in the second study the cure rate was 36% and 9%, respectively. In non-US studies, mycologic cure rates ranged from 47% to 86%, and the lacquer demonstrated a broad spectrum of activity showing efficacy against Candida species and some nondermatophytes. The authors concluded that the nail lacquer provided a treatment choice with a favourable benefit-to-risk ratio. In subsequent studies, topical ciclopirox efficacy has been confirmed and, more recently, the efficacy of the lacquer in a case of infantile congenital candidal onychomycosis was reported to be excellent (Sardana et al., 2006).

In an attempt to enhance the topical delivery of ciclopirox, Malay et al. (2009) evaluated the effect of physically damaging the dorsal nail plate (debridement) prior to application of the drug. They randomly allocated 55 patients to either nail debridement (27 patients) or debridement plus application of a topical antifungal nail lacquer containing 8% ciclopirox (28 patients). After a median follow-up of 10.5 months, patients in the antifungal nail lacquer group showed statistically significantly improvement over those in the debridement alone group, with an ~77% rate of mycological cure. Although the number of patients was considerably less, the mycological cure rate demonstrated in this study was greater than those shown in Gupta et al. (2000) over a similar time period, suggesting that debridement of the nail plate may have improved drug delivery. Perhaps unsurprisingly, none of the patients in the debridement alone group experienced mycological cure.

As previously pointed out, not all nail diseases are fungal or bacterial infections. Psoriasis can affect the entire nail plate and is a common feature in psoriasis patients. A lacquer formulation containing 8% clobetasol-17-propionate was evaluated for the efficacy and safety (Sanchez Regana et al., 2005). Ten patients, with both nail bed and matrix psoriasis, were treated with the nail lacquer applied once daily for 21 days and subsequently twice weekly for 9 months. There was a reduction of all the nail alterations, including nail pain, within 4 weeks of initiating therapy and response was directly related to the length of treatment. The lacquer was a safe and effective treatment for nail bed and matrix psoriasis. Tazarotene gel has also been evaluated as a therapy for nail psoriasis. In a double-blind, randomised, vehicle-controlled, parallel-group trial, 31 patients with fingernail psoriasis were randomised to receive tazarotene or vehicle gel, which was applied daily for up to 24 weeks to 2 target fingernails, one under occlusion and one unoccluded (Scher et al., 2001). Tazarotene treatment

resulted in a significantly greater reduction in onycholysis (loosening of the nail plate-nail bed connection) in both occluded and nonoccluded nails together with a significantly greater reduction in pitting in occluded nails. The gel was well tolerated. Nail psoriasis was also improved following topical application of calcipotriol and betamethasone dipropionate ointment applied once daily for 12 weeks (Rigopoulos et al., 2009).

Brittle nail syndrome is a common problem and refers to nails that exhibit surface roughness, raggedness, and peeling. Sherber et al. (2011) evaluated the usefulness of tazarotene cream (0.1%) for the treatment of brittle nails. Patients applied tazarotene cream to the nails twice daily for 24 weeks. All participants achieved improvement of the target nails at week 12 and 16 participants (88.9%) at week 24. The study showed that tazarotene was effective at reducing the symptoms of brittle nail syndrome with minimal to no irritation. However, it is difficult to rationalise how tazarotene, a retinoid, would be effective in brittle nail syndrome and it is possible that some other ingredient in the cream formulation was responsible for the beneficial effects. There were, however, no reported placebo data.

4.2. Experimental approaches

The major experimental enhancement approaches either focus on compounds which disrupt the nail barrier or vehicles such as lacquers with improved residence time on the nail.

4.2.1. Chemical enhancement – barrier disruption

Quintanar-Guerrero et al. (1998) investigated the effects of three keratolyics (papain, urea and salicylic acid) on the permeability of three imidazole antimycotics (miconazole nitrate, ketoconazole, itraconazole) through healthy human nails samples mounted in side-by-side diffusion cells. Only the combined effects of papain (15% for 1 day) and salicylic acid (20% for 10 days) were capable of enhancing the permeability of the antimycotics.

Kobayashi et al. (1998) assessed the enhancing effect of urea and sodium salicylate on the transungual delivery of a hydrophilic model drug (5-fluorouracil) using human nail samples in modified side-by side diffusion cells. Aqueous suspensions containing either urea or Na salicylate decreased drug flux compared with suspensions containing no keratolytic. The disulphide reducing agents, acetyl cysteine and mercaptoethanol were also evaluated for their effects on 5-FU and tolnaftate permeation. Suspensions of the drugs were formulated with acetyl cysteine or mercaptoethanol in ethanol:water or ethanol:isopropyl myristate vehicles. The ethanol:water vehicles promoted up to 16 times greater 5 FU flux compared with aqueous suspensions and the ethanol:isopropyl myristate vehicles enhanced 5FU permeation up to 8-fold. The permeation of tolnaftate was also enhanced in the same vehicles but to a much lesser extent.

Using human nail samples in a Franz cell set up, Malhotra and Zatz (2002a,b) screened a range of molecules with known ability to interact with keratin. The compounds studied included mercaptans (N-[2-mercaptopropionyl] glycine, zinc and sodium pyrithone, 8-mercaptomenthone, meso-2,3-dimercapto succinic acid), sodium metabisulphite, keratolytic agents (salicylic acid, urea, guanidine hydrochloride). Gels were prepared containing enhancers either alone, or in combination with each other, in a vehicle that was either aqueous, hydroalcoholic, or one containing dimethylsulfoxide (DMSO). The results indicated that the chemical structure of the modifier is most important in determining its ability to enhance penetration. The best enhancement effect was obtained using N-(2-mercaptopropionyl) glycine in combination with urea. Mercaptan compounds contain sulphydryl groups (SH) and the primary mechanism for their enhancement of nail penetration is therefore reduction of disulfide linkages in the nail keratin matrix. Generally, the barrier integrity of nails was compromised irreversibly after treatment with effective chemical modifiers (Table 1).

Hui et al. (2004) prepared a novel gel formulation of ciclopirox containing urea as well as propylene glycol, ethanol and EDTA and evaluated drug permeation through nail samples and amount of drug localised in nail samples at the end of the experiment. A commercial gel and lacquer formulation were also examined which did not contain any keratolytic. Ciclopirox delivery into and through the nail was significantly greater from the commercial gel, than from either the experimental gel or the nail lacquer ($p < 0.05$).

4.2.2. Chemical enhancement – vehicle effects

Mertin and Lippold (1997) investigated the effect of (i) lipophilic vehicles and (ii) nail lacquers on the *in vitro* permeability of chloramphenicol in human nail plate and bovine hooves. The lacquer vehicles were composed of chloramphenicol, Eudragit RL PO, dibutyl sebacate and methanol and experiments were conducted with varying amounts of chloramphenicol (0.5–20%). Suspensions of chloramphenicol were formulated with Miglyol 812 and octanol. Maximum fluxes from the lacquer formulations were comparable to flux values for aqueous suspensions using hoof membranes ($\sim 6 \times 10^{-6}$ mg cm^{-2} s^{-1}) and the lacquer with the highest drug concentration was significantly better ($p = 0.05$) at promoting drug permeation than the aqueous suspension. Not all formulations were evaluated in human nail plates. These values were also comparable to flux values for the lipophilic vehicles in the bovine hoof model and to flux from octanol in the human nail plate model.

Although ethanol is a widely used penetration enhancer in topical and transdermal delivery, the delivery of 5-FU in menthol:ethanol:water or lactic acid:ethanol:isopropyl myristate vehicles did not enhance drug permeation compared with the aqueous control (Kobayashi et al., 1998).

The ability of a skin penetration enhancer, 2-*n*-nonyl-1,3-dioxolane, to promote transungual delivery of econazole was evaluated by Hui et al. (2003). The test topical lacquerformulation contains 5% (w/w) econazole, Eudragit® RL/PO, ethanol, and 2-*n*-nonyl-1,3-dioxolane (18%, w/w). A commercial control (Econail) has the same formulation with the exception of nonyl-1,3-dioxolane. Ungual permeation was evaluated in human nail samples in a modified diffusion cell set up. The addition of 2-*n*-nonyl-1,3-dioxolane to econazole nail lacquer delivered six times more antifungal drug through human nail than an identical lacquer-drug formulation without enhancer. Concentrations of econazole in the deep nail layer and nail bed were significantly higher in the test group than in the control group. The authors suggested that the penetration enhancer had plasticising effects on the lacquer formulation and this may contribute to its efficacy.

Monti et al. (2005) investigated the transungual permeation of ciclopirox from a water-soluble hydroxylpropyl chitosan-based formulation compared with a commercial water-insoluble nail lacquer (Penlac®). Formulations were evaluated using bovine hoof samples mounted in vertical diffusion cells. The flux values at steady state were of a similar order of magnitude for both vehicles. The percentage of drug permeated was significantly higher ($p < 0.05$) for the water-soluble polymer based formulation and the lag time was also significantly shorter for this formulation compared with the commercial control. The percentage of drug retained in the membranes at the end of the experiment was also comparable for both vehicles. The authors suggested that the chitosan-based formulation might have better adhesion properties to the membrane than the commercial lacquer. More recently, Monti et al. (2010), compared permeation of ciclopirox in bovine hoof membranes with that of amorolfine in the same hydroxypropyl chitosan-based nail lacquer described earlier and with a non-water-soluble reference (Loceryl®). Amorolfine flux from the water soluble formulation was significantly higher ($p < 0.05$) when

Table 1
Chemical agents which disrupt/damage the nail barrier.

Chemical/biochemical classification	Compound	Mechanism of action
Beta hydroxy acid	Salicylic acid, sodium salicylate	Keratolytic
Carbamide	Urea	Protein denaturant
Imine	Guanidine hydrochloride	Protein denaturant
Mercaptans, thiols		
	Dithiothreitol	
	Mercaptoethanol	
	8-mercaptomenthone	
	N-[2-mercaptopropionyl] glycine	
	Meso-2,3-dimercapto succinic acid	
	Sodium pyrithone, zinc pyrithone	
	Thioglycolic acid	
Enzyme	Papain	Protease
Sulphite, bisulphite	Sodium metabisulphite, sodium sulphite	Reduce disulphide links in nail keratin matrix

compared with Loceryl. Ciclopirox was able to permeate hoof membranes more easily compared with amorolfine, which was not explained by the different amounts of actives in the respective formulations.

Hui et al. (2007a) investigated a range of different nail lacquers and a co-solvent vehicle for delivery of a novel oxaborole antifungal to the nail. Lacquers were formulated with a film-forming agent, solvent, and, optionally, a penetration enhancer. Ethanol was the solvent, poly (vinyl methyl ether alt maleic acid monobutyl ester), poly (2-hydroxyethyl methacrylate), poly (vinyl acetate) were the film formers. The co-solvent vehicle consisted of ethanol and propylene glycol. Approximately the same amount of the test compound penetrated the nail plates regardless of the vehicle. The co-solvent vehicle showed a superior permeation capability into and through the normal human nail plate *in vitro* compared with a commercial formulation of ciclopirox (Penlac, ciclopirox 8%).

The same group (Hui et al., 2007b) compared the ungual penetration of pantothenol in a non-lacquer film forming nail formulation (ethanol, acrylates co-polymer, phytantriol) with an aqueous solution formulation. The nail treatment formulation showed better effectiveness on enhancement of transungual panthenol delivery into/through the deeper layer of the human nail plate than the aqueous solution of panthenol.

4.2.3. Physical enhancement

4.2.3.1. Iontophoresis. The most frequently investigated physical enhancement approach for transungual permeation is iontophoresis. For a more detailed review of the theoretical concepts underlying this approach the recent excellent reviews by Shivakumar et al. (2012) and Delgado-Charro are recommended (2012). This section focuses on the actual application of the approach in drug delivery to, and through, the nail as opposed to more fundamental issues concerning the approach.

One of the earliest studies which investigated the application of iontophoresis for ungual drug delivery was reported by James et al. (1986). Prednisolone sodium phosphate (1%) was delivered across the human thumb nail and gave peak plasma levels of about one third of that produced by oral ingestion of 10 mg prednisolone.

Murthy et al. (2007) investigated the delivery of salicylic acid across human nail *in vitro*. The influence of pH, ionic strength, and current density was studied. An ionic strength of 50–100 mM was required for optimal conduction of electric current across nail. The flux enhancement factor (iontophoretic flux/passive flux) also increased with increase in pH due to increased ionisation of SA. The results were promising and substantiated the earlier report of James et al. (1986) suggesting that the use of transungual iontophoresis approach could result in safer and more effective transungual delivery.

Hao and Li (2008) investigated the transungual iontophoretic transport of model neutral permeants mannitol (MA), urea (UR), and positively charged permeant tetraethylammonium ion (TEA) across fully hydrated human nail plates *in vitro.* Iontophoresis enhanced the transport of MA and UR from anode to cathode, but this effect was marginal. The transport of TEA was significantly enhanced by anodal iontophoresis. The same group investigated the combined effects of chemical enhancement and iontophoresis in a later study (Hao et al., 2008). Nails treated with glycolic acid and urea did not show any transport enhancement. Treatment with TGA enhanced passive and iontophoretic transungual transport of MA, UR, and TEA but the effects of TGA on the nail plate were irreversible.

The rapid iontophoretic delivery of a potent antifungal agent, terbinafine from a Tween®-water system and a gel formulation was demonstrated by Nair et al. (2009a) using this approach. The potential of inorganic salts and polyethylene glycols for transungual delivery of terbinafine was also assessed by the same group (Nair et al., 2009b, 2010). More recently, the same group demonstrated that terbinafine delivery *in vitro* to onychomycotic nails, did not differ significantly when compared with normal nails, although the extent of drug permeation and drug load differed between finger and toe nails (Nair et al., 2011).

Amichai et al. have also investigated iontophoresis for delivery of terbinafine across the nail and have also recently reported the first application of iontophoresis to treat onychomycosis in patients (Amichai et al., 2010a,b). An iontophoretic patch at a constant current density of 100 mA cm^{-2} was used to deliver terbinafine in one group of patients and compared with a control group treated with terbinafine. The percentage of patients with healthy toenail growth at the end of treatment was 40% for the iontophoretic patch compared with 11% in patients treated with terbinafine without current. The presence of fungal elements, assessed by microscopy, was significantly lower ($p < 0.05$) in the iontophoretic patch group and the patch was generally well tolerated.

4.2.3.2. Ultrasound, microporation. Ultrasound is acoustic energy in the form of waves having a frequency above approximately 20,000 Hz. A novel ultrasound-mediated drug delivery system has recently been developed for treatment of nail fungal disorders (Abadi and Zderic, 2011) however the actual delivery of antifungal agents has not yet been investigated to date. Ultrasound induces cavitations in the ungual structure and is thus proposed to enhance ungual permeability.

A microcutting device has recently been investigated to deliver terbinafine to subjects with subungual onychomycosis in the toenails (Boker et al., 2007). A row of microconduits was created 5 mm distal to the nail lunula with at least 3 mm between each hole. Control formulations and terbinafine formulations were subsequently

applied twice daily. 64% of subjects showed improvement in clinical assessment following treatment with terbinafine compared with 28% for the control group. However, no information was provided on the extent of enhancement or the extent of perforation of the nail.

5. Conclusions

A diverse number of biophysical and bioengineering techniques have been used by scientists and clinicians to date to study the nail and to gain insight into how its properties may be influenced. The potential of many of these approaches has still not been fully exploited and with the development of more sophisticated approaches for data deconvolution, it should be possible to identify new opportunities for overcoming this excellent barrier. The modest success of current clinical therapies should be a spur to innovation in the field. Conventional formulations are largely lacquer based, suggesting that as for skin, residence time of both active and excipients in or on the nail is critical for efficacy. Experimental approaches which clearly damage the nail must be viewed with caution and are likely to be faced with significant regulatory hurdles. Iontophoresis remains one of the most promising of the physical enhancement strategies for ungual therapy.

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