Expert Opinion

Enhancing the nail permeability of topically applied drugs

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The topical therapy of nail diseases, especially of onychomycosis, and to a smaller extent, of nail psoriasis, is desirable to avoid the side effects associated with their systemic therapy, to increase patient compliance and reduce the cost of treatment. Systemic therapy is however the mainstay of treatment due to the poor permeability of the nail plate to topically applied drugs. For effective topical therapy, ungual drug permeation must be enhanced. This can be achieved by disrupting the nail plate using physical techniques or chemical agents. Alternatively, drug permeation into the intact nail plate may be encouraged, for example, by iontophoresis or by formulating the drug within a vehicle which enables high drug partition out of the vehicle and into the nail plate. The physical techniques (manual and electrical nail abrasion, acid etching, ablation by lasers, microporation, application of low-frequency ultrasound and electric currents) and chemicals (thiols, sulphites, hydrogen peroxide, urea, water, enzymes) that have shown ungual enhancer activity are discussed in this review. Optimal drug formulation, while crucial to ungual drug delivery, is only briefly reviewed due to the limited literature.

Keywords: drug delivery, nail, permeability, ungual

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1. Introduction

In the last decade or so, interest in the topical delivery of drugs to the nail for the treatment of nail disorders, such as fungal infections and nail psoriasis, has increased greatly, as evidenced by the number of publications, including reviews [1-3] and patents, academic researchers and pharmaceutical industries involved in formulating nail products. Such interest led to the first international meeting dedicated solely to topical ungual drug delivery in 2007 [4]. The increased interest is likely due to a combination of factors, such as, the launch (and subsequent economic success) of the topical antifungal nail lacquers Loceryl® (Galderma) in Europe and Penlac® (Dermik Laboratories) in the US, the infancy of the field, and the obvious potential for research and development of new products in this previously neglected area. Topical therapy of nail diseases has obvious advantages, such as elimination of systemic adverse events and of drug interactions and reduced cost of treatment. However, topical therapy has had limited success [5] and accounts for under 20% of the global market for onychomycosis [6]. The limited success of topical therapy is thought to be mainly due to the poor permeability of the nail plate to topically applied drugs. For example, van Hoogdalem calculated the total drug uptake into the nail to be less than 0.2% of the applied dose after twice daily application for 6 weeks [7]. To address the poor nail permeability, a number of physical and chemical techniques are being researched and these are discussed in this review. Firstly, the nail unit, the disease targets and ungual drug permeation are briefly introduced.

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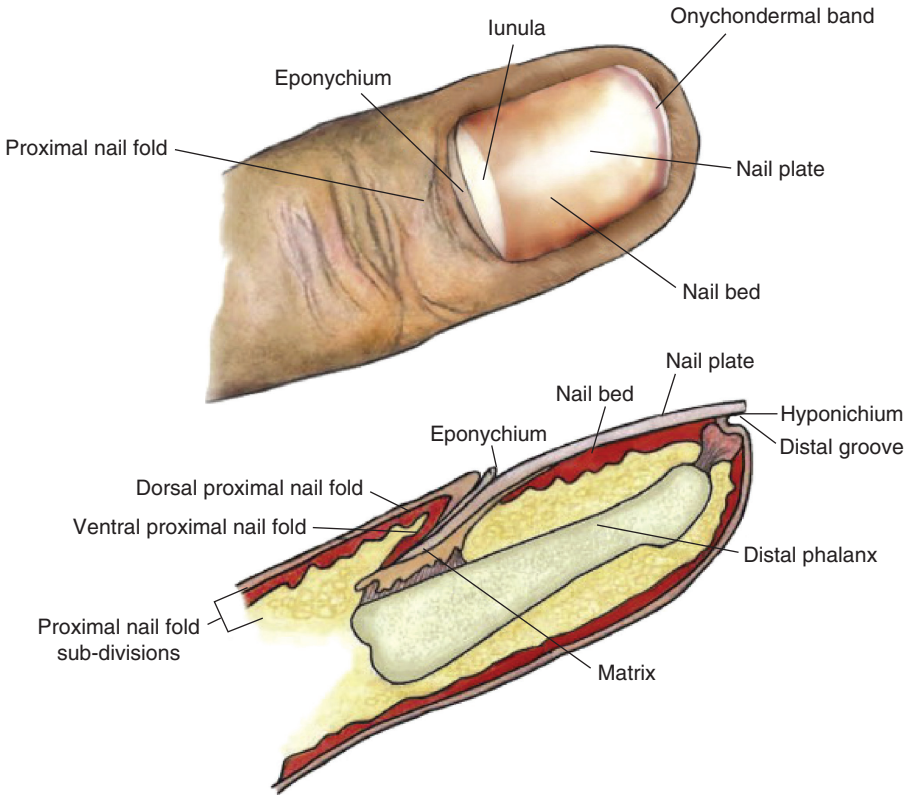

Figure 1. Schematic diagram of the nail unit.
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1.1 The nail unit

The nail apparatus has been described in many publications, including [2,8-12] and will only be briefly described here. The nail unit (schematically shown in **Figure 1**) is composed of the nail plate, nail folds, nail matrix, nail bed, and the hyponychium. The nail matrix forms the nail plate whose growth rate varies considerably among individuals; average values of 3 mm per month (fingernails) and 1 mm per month (toenails) are often used. Thus, on average, a normal fingernail grows out completely in about 6 months while a normal toenail in about 12 – 18 months. The most obvious part of the nail unit – the nail plate – is a thin (0.25 – 0.6 mm), hard, yet slightly elastic, translucent, convex structure and is made up of approximately 80 – 90 layers [13] of dead, keratinized, flattened cells which are tightly bound to one another. The cells at the dorsal surface of the plate overlap (**Figure 2A**), producing a smooth (and poorly permeable) surface, and a cross-section view highlights the plate's compact nature (**Figure 2B**). The nail plate can be divided into two layers: dorsal and ventral, based on differential ultrasound transmission [14,15]. The ventral layer is more plastic, while the dorsal one is hard and brittle. Some authors consider the nail plate to consist of three strata: dorsal, intermediate and ventral, the latter being taken as the few layers of cells which connect the nail plate to the nail bed [16]. On average, the dimensions of the flattened corneocytes in the upper nail layer are 34 × 60 × 2.2 μm, while those in the lower nail layer are thicker, at 40 × 53 × 5.5 μm [13]. Chemically, the nail plate consists mainly of proteins, namely low sulphur keratins, and high sulphur and high glycine/tyrosine proteins. The latter two groups are believed to form an interfilamentous matrix, while the low sulphur keratins form 10 nm filaments [11]. The plate also contains water at 10 – 30%, and very small amounts of lipid at 0.1 – 1%.

1.2 Disease targets for topical therapy

Onychomycosis (fungal infection of the nail plate and/or nail bed) is the main disease target, simply due to the fact that it accounts for the majority of nail disorders [17] affects 10 – 40% of the population [12] and its incidence appears to be rising. The latter is thought to be a result of the growth in susceptible populations such as the elderly and the immunosuppressed, current lifestyle factors such as the use of communal recreational facilities and healthclubs, improved detection, and higher public awareness and desire for treatment [18-21]. Onychomycosis is usually caused by the dermatophytes *Trichophyton rubrum* and *Trichophyton mentagrophytes*, and these infections can be divided into categories depending on where the infection

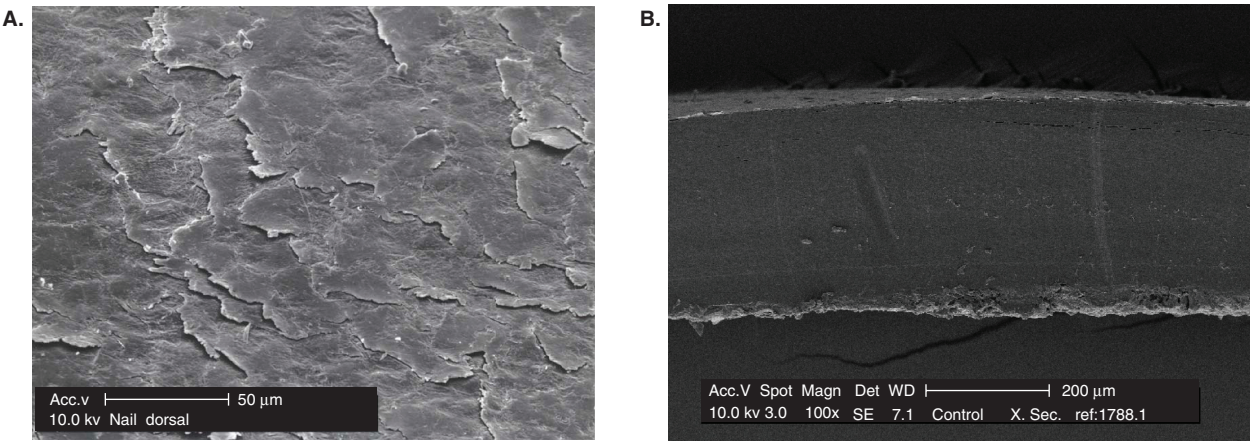

Figure 2. Scanning electron micrograph. A. Dorsal surface of the nail plate. **B.** Cross section of the nail plate.

begins: distal and lateral subungual onychomycosis; superficial white onychomycosis; proximal subungual onychomycosis; and total dystrophic onychomycosis, which is the potential endpoint of all forms of onychomycosis when the entire nail plate and bed are invaded by the fungus. Toenails are affected more often than fingernails [22], and are also more recalcitrant to treatment, needing longer durations (e.g., a year). The pharmacotherapy of onychomycosis, using systemic (e.g., terbinafine, itraconazole, fluconazole) and topical (e.g., amorolfine, ciclopirox, tioconazole) antifungal agents, alone and in topical/systemic combinations, and with mechanical treatments (e.g., surgical/mechanical nail avulsion or debridement) has been reviewed elsewhere and will not be discussed in this paper [23-26], except to say that topical therapy on its own is generally only recommended for mild disease and when only a few nails are affected, due to its limited success rate. Treatment of onychomycosis is known to be challenging; failure to achieve 'disease-free nail' is fairly common with one course of therapy [23], and a high relapse rate (22.2%) has been reported [27]. Such failure of treatment has been ascribed to a possible lack of diagnostic accuracy, incorrect antifungal or delivery method, presence of dormant spores (which are not killed by many antifungals, and therefore represent a reservoir of fungus, which may germinate when antifungal treatment is stopped, so that the infection recurs), concentrated zones of fungi (into which drug diffusion is inadequate) or resistant fungi [28]. The challenge of onychomycosis treatment makes it even more imperative to develop successful topical therapies, which could be used alone or in conjunction with systemic therapy.

Nail psoriasis is the second most important target for the development of topical nail products as it is also fairly common, occurring in 80 – 90% of people with skin psoriasis, which affects between 1 and 3% of most populations, although it can also be found without cutaneous involvement in a smaller number of patients [29,30]. The clinical features depend on which part of the nail unit is affected by psoriasis. These include pitting (due to psoriatic lesion within the nail matrix), 'oil-drop' discolouration and onycholysis (due to lesion within the nail bed; onycholysis more prevalent if hyponychium is also involved), subungual hyperkeratosis (results from the deposition and collection of cells under the nail plate that have not undergone desquamation, for example due to psoriatic activity in the hyponychium) and splinter haemorrhages (a result of trauma). A standardised therapeutic regimen is lacking, although intralesional injection of cortisone into the nail fold, topical application of corticosteroids, vitamin D3 analogs, 5-fluorouracil (5-FU), anthralin, tazarotene, phototherapy and photochemotherapy, systemic administration of immunosuppressants, combination therapies and biological therapies have shown some success [10,31]. However, treatment duration is long and relapse is common, and currently, there are no topical products marketed specifically for nail psoriasis in the UK.

2. Ungual drug permeation following topical application

Following the topical application of a drug formulation onto the nail plate, the drug is expected to partition out of the formulation into the nail plate, diffuse through the latter, and finally, partition into the nail bed. Drug transport into the nail plate is thus expected to be influenced by the physico-chemical properties of the drug molecule (e.g., size, shape, charge, hydrophobicity), the formulation characteristics (e.g., nature of vehicle, pH, drug concentration), nail properties (e.g., hydration, disease state), as well as interactions between the permeating molecule and the keratin network of the nail plate [2]. For investigations into these parameters, excised nail plates, nail clippings and bovine hoof membranes (as a model for the nail plate) have been used.

2.1 Permeant properties

The most important permeant property has been found to be the molecular weight; permeability coefficient decreasing sharply with increase in molecular weight [32,33]. Permeant hydrophilicity/lipophilicity is also thought to be important. However, investigations into the influence of permeants' lipophilicity on their permeability have been hampered by the fact that increasing lipophilicity in a series, e.g., n-alkanols [34] and p-hydroxybenzoic acid esters [35], is also accompanied by increasing permeant molecular weight. While Walters *et al.* concluded that greater permeability of the shorter-chain, less lipophilic alcohols indicated that the nail plate behaves like a concentrated hydrogel [34], Mertin and Lippold concluded that the greater permeability of the shorter chain, less lipophilic p-hydroxybenzoic acid esters, was due to their smaller size, rather than a higher hydrophilicity [35].

Permeant charge (or lack of) seems to be an important parameter for passive diffusion. Irrespective of the nature of the charge, non-ionic species were found to have ~ 10-fold greater permeability than their ionised counterparts [33]. The lower permeability of the charged species was thought to be caused by a small increase in the apparent molecular size due to hydration of the ionic species. Lower permeation of the ionised species of weakly basic or acidic permeants was also reported when the influence of formulation pH and permeant charge on ungual permeation were being investigated ([35,36] as quoted in [1]). The lower permeation was thought to be due to electrostatic repulsion between the charged nail keratin (pK_a ~ 5 [37]) and the like charged permeant. Whether the lower permeability of the charged species is mainly due to electrostatic repulsion or to the small increase in apparent molecular size, as suggested by Kobayashi *et al.* [33], remains to be established. An exception to the lower ungual permeation of the charged species was reported by Walters *et al.* [38], who showed the permeability coefficient of the weakly basic miconazole to be essentially the same from pH 3.1, where the drug is mostly dissociated, to pH 8.2, where the drug is mostly undissociated. The lack of pH and drug charge effect was suggested to be due to the high ionic strength of the drug solutions, such that the influence of Donnan equilibrium was lowered [35]. This remains to be elucidated, as does the influence of permeant shape.

2.2 Nail plate properties

The nail plate's diseased state is expected to have a large influence on nail permeability, but has not been studied sufficiently. Kobayashi *et al.* [33] found similar permeation of 5-FU in healthy and (mildly) fungally infected nail plates; heavily fungally infected nail plates were not used due to their uneven thickness and as the plates 'collapsed' when placed in water. However, the diseased nail plate may be heavily thickened, which increases the distance the drug has to permeate through the nail plate before reaching the nail bed; this would have a negative effect on the success of topical therapy. The presence of dermatophytoma (a dense focus of fungi with thick shortened hyphae) in onychomycosis is thought to reduce the success of oral therapy [39]; the same is likely in topical therapy, as drug diffusion within the dermatophytoma is likely to be altered. Diseased nail plates may also be more 'crumbly', which could increase nail porosity and thereby increase drug permeability. Diseased nail plate can also detach from the nail bed. Such detachment presents a huge barrier to drug movement from formulation to the nail bed if the detachment is surrounded by 'non-detached' nail areas. On the other hand, if onycholysis occurs at the proximal edge of the nail plate, drug formulations can be applied into the detached space, which would facilitate drug delivery to the nail bed. Thus, it can be seen that the direction and extent of the change in nail permeability in the disease state will be dependent on the clinical features of the diseased nail. This could also contribute to the large variation in clinical outcomes of topical drug therapy that is commonly observed.

3. Enhancement of nail plate permeability

Ungual permeation can be enhanced by disrupting the nail plate using physical techniques or chemical agents. Alternatively, the drug permeation into intact nail plate may be encouraged, for example, by iontophoresis or by formulating the drug within a vehicle which enables high drug partition out of the vehicle and into the nail plate. These are discussed below.

3.1 Physical enhancement of ungual drug delivery

3.1.1 Abrasion of the nail plate surface

The simplest method of physically enhancing topical ungual drug delivery is to file the surface of the nail plate using an abrasive. Indeed abrasive sticks are provided in medicinal nail lacquer packs, and the product information leaflets instruct patients to file down infected areas of the nail plate. Filing removes the topmost layer of the nail plate, thus reducing the barrier that drugs have to permeate through to reach the deeper nail layers and was shown *in vitro* to double the permeability coefficient of 5-FU and flurbiprofen through the nail plate [40]. In clinical trials, filing the nail plate prior to the application/re-application of drug-containing formulations was found to be essential for the success of topical treatment [41,42].

More aggressive abrasion of the nail plate surface, using electrical equipment, has also been performed in the clinic. Hargreaves [43] found that nail drilling increased the efficacy of topical antifungal application in onychomycosis accompanied by nail thickening. Dental drills have been used to remove the diseased parts of onychomycotic nail [44,45]. More recently, in a study on the influence of foot care intervention on topical onychomycotic therapy in

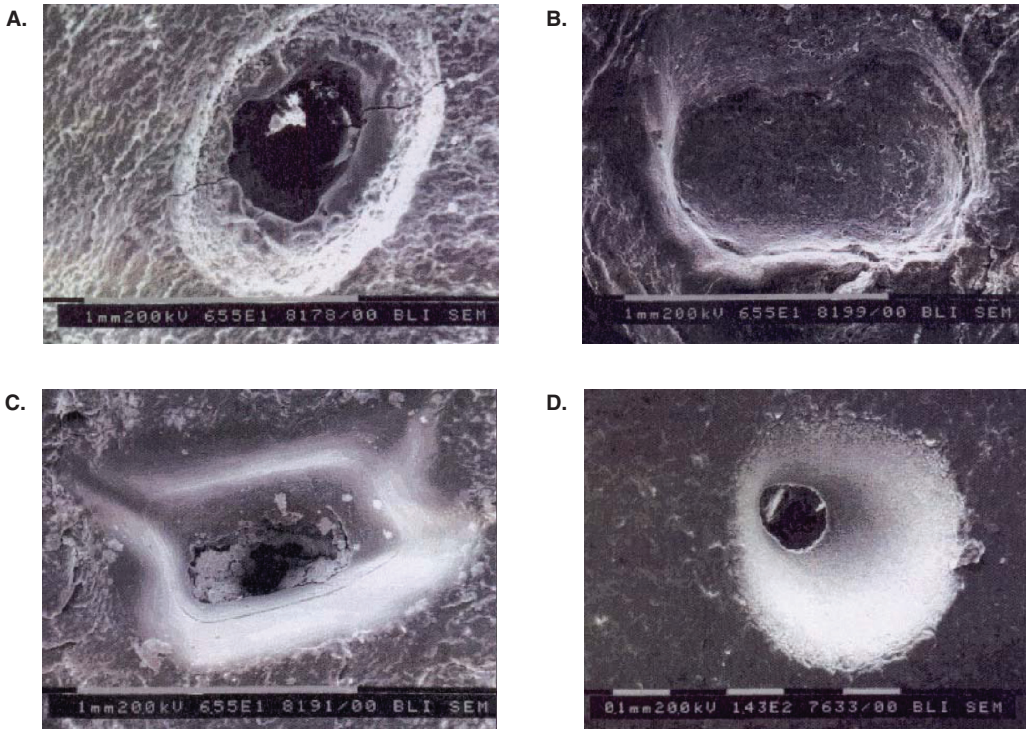

Figure 3. Different morphologies of craters formed on the nail plate. Craters formed by the application of **A.** Ho:YSGG laser, **B.** Er:YAG, **C.** XeCl laser, **D.** ultrashort laser systems.
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patients with diabetes, drilling was performed to reduce nail thickness to 1 – 2 mm in thickened nail areas in patients with distal-lateral subungual onychomycosis [46].

3.1.2 *Etching of the nail surface with acid*

Brief (up to 90 s) application of a 20% tartaric acid solution or a 10% phosphoric acid gel onto the dorsal surface of nail clippings was used *in vitro* to modify the nail plate surface prior to application of drug formulations, such as adhesive polymeric films [47,48]. Atomic force microscopy showed that application of tartaric and phosphoric acids increased the mean surface roughness by 1.3 and 1.7 times, respectively, and thus its surface area. Etching the nail plate surface with phosphoric gel also increased the adhesion of drug-loaded polymeric films onto the nail plate and drug permeation (the latter by six-fold) through the nail. Enhanced adhesion and drug permeation were thought to be due to the increased nail plate surface area which provided a greater opportunity for the polymer chains (in the drug-loaded film) to bond with the nail plate, and for drug diffusion. The latter also benefited from a decreased effective membrane thickness of the etched nail plate.

3.1.3 *Ablation of the nail plate using pulsed lasers*

Neev *et al.* [49] investigated the effects of laser light on nail plates *in vitro* [49]. Following topical application, laser energy would be absorbed by water or protein in the nail plate and the scattered heat would lead to vaporisation (thus, removal) of nail layers. Four laser systems – solid state Er:YAG (λ 2.94 μm, pulse duration 250 μs), a Ho:YSGG (λ 2.08 μm, pulse duration 250 μs), XeCl Excimer (λ 308 nm, pulse duration 15 ns) and an ultrashort pulse laser (λ 1.05 μm, pulse duration 350 fs) – were tested, at beam areas of 1 mm^2, pulse repetition rate of 4 Hz and fluence of 10 – 20 J/cm^2 in all cases, except for the ultrashort laser where the beam area, pulse repetition rate and fluence per pulse were 0.4 mm^2, 10 Hz and 1 – 3 J/cm^2, respectively.

Laser application on the nail plate *in vitro* resulted in the formation of craters whose shape, size and other properties, such as crater wall smoothness, presence of cracks and of melted and resolidified tissue depended on the nature of the laser system (**Figure 3**). For example, rectangular craters approximately 1 mm^2 in size, with clean wall and crater floor surfaces were obtained with XeCl while oval-shaped craters that were ~ 1 mm in length, and conical in shape, dropping to smaller dimensions towards the floor, with walls that were jagged, partially cracked and covered with melted and resolidified tissue were obtained with Ho:YSGG. In addition to the crater morphology, the four laser systems demonstrated different ablation rates (calculated by dividing nail thickness by the

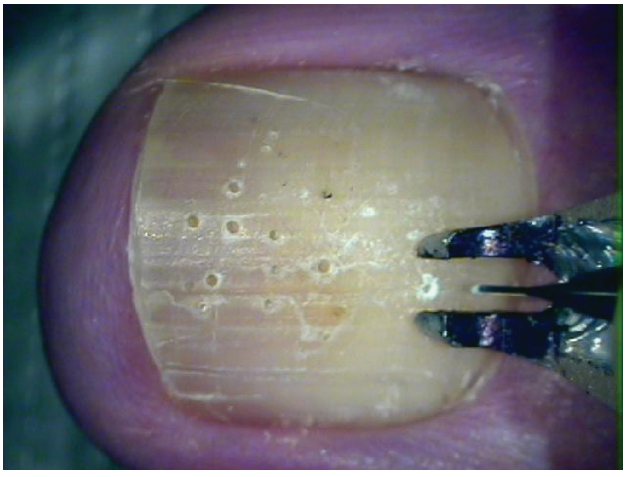

Figure 4. A photograph showing the drilling of individual holes in the nail plate by the PathFormer.
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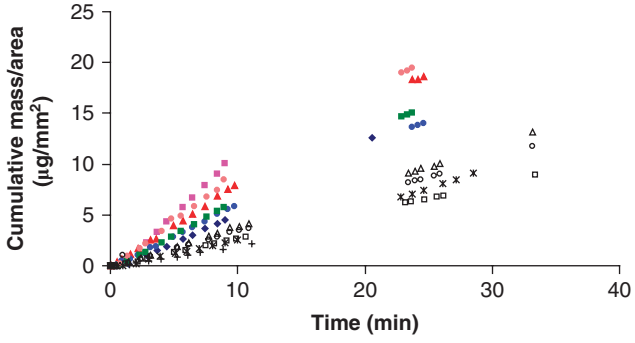

Figure 5. Increased permeation of a model drug metformin through hoof membranes. Membranes pretreated with low-frequency ultrasound (top six curves with solid symbols) compared with control membranes (bottom five curves with empty symbols).

number of pulses needed to perforate the nail plate) and ablation efficiencies (defined as the depth of ablation in µm produced by 1 mJ of energy). The optimal laser system was found to be the ultrashort pulse laser due to its high efficiency, negligible collateral damage, i.e., absence of cracking or thermal damage in craters and minimum mechanical and acoustical impact. While this study showed the promise of lasers to reduce nail plate integrity, the influence of laser on the nail plate *in vivo* and on ungual drug flux remains to be established.

3.1.4 *Microporation of the nail plate*

In a clinical trial to evaluate the efficacy of a terbinafine cream, an FDA-approved hand-held microcutting device – PathFormer (**Figure 4**, Path Scientific, Carlisle, USA), developed to drill a single hole in the nail plate to drain subungual hematomas [50] – was used to make holes in toenails prior to application of the test and placebo creams [51]. The number of holes drilled in each patient's toenail and their depth was not specified, and since nail plate trephination was not itself the subject of the clinical trial, there were no controls for trephination, i.e., patients receiving drug formulations without microporation of the nails. Thus, the extent of enhancement of ungual permeation by trephination is not known.

The depth of the hole created by the device is said to be controlled by electrical resistance; the electrical resistance of the nail decreases upon micro-cutting from 5 MΩ (undrilled) to 10 – 20 kΩ (when the nail bed is reached) as each successive layer of nail tissue is removed [52]. In a study to investigate how deep a hole could be drilled without ill-effects, Ciocon *et al.* [52] showed that the procedure was well tolerated, with respect to pain and pressure felt by the volunteers, when five holes (of diameter 400 µm and depths corresponding to electrical resistance of 90 – 25 kΩ) were drilled in each person's toenail, without penetrating the nail bed. A limitation of the PathFormer is the fact that each hole has to be individually drilled; an array would be more practical, as long as the depth of each hole was controlled.

The same company also developed another technique, which they called microscission, to create microconduits (100 – 500 µm diameter) into the nail plate. A stream of inert gas was used to bombard small areas of the nail plate with microparticles of aluminium oxide, and thereby remove the surface layers of the nail, creating microconduits at the bombarded sites [53]. Disadvantages of this technique included an inability to control the depth of the microconduit, and longer procedure duration, compared with the PathFormer (Carlisle Scientific 3 July 2008, personal communication).

3.1.5 *Application of low-frequency ultrasound*

Application of low-frequency ultrasound has been found to enhance drug permeation through whole nail plates and through bovine hoof membranes (used as model nail plates) [54,55]. Drug permeation through hoof membranes pretreated with low-frequency ultrasound via a liquid coupling medium was roughly doubled, as shown in **Figure 5** [55]. Pretreatment of the nail with ultrasound prior to drug application (in contrast to concomitant application of ultrasound waves and drug) was chosen as it avoids exposure of the drug to potentially damaging ultrasound waves. The enhanced drug permeation suggests ultrasound-induced disruption of the hoof membrane. While the mechanism of membrane disruption has not yet been elucidated, it is possible that inertial cavitation is involved, as has been proposed for low-frequency ultrasound-assisted transdermal drug delivery [56]. When low-frequency ultrasound waves are applied in a liquid, cavitation (formation and collapse of gas bubbles) occurs. Cavitation may be stable

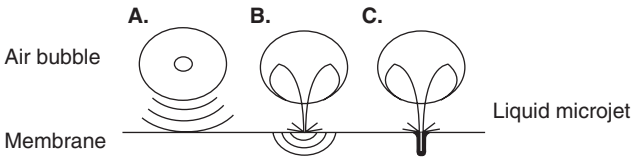

Figure 6. Suggested mechanisms by which low-frequency ultrasound enhances membrane permeability. Collapse of cavitation bubbles in the bulk medium result in shockwaves which impact on the membrane **A**, while bubble collapse near the membrane results in the formation of liquid microjets which impact on **B** and can even penetrate into the membrane **C.**
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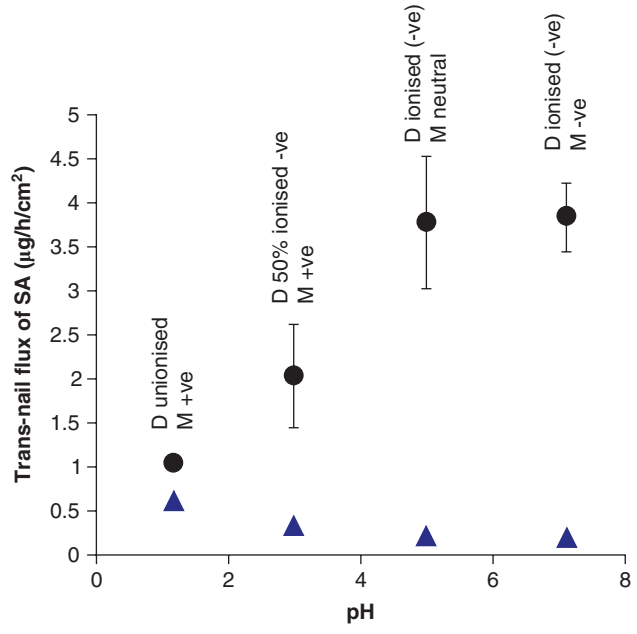

Figure 7. Influence of formulation pH and model drug (D) and nail membrane (M) charge on the passive transport flux (▲) and iontophoretic transport flux (●) of salicylic acid (SA).
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(corresponding to periodic bubble growth and oscillations) or inertial (violent growth and collapse). The violent collapse of a very large number of gas bubbles in the bulk liquid results in shockwaves, which travel through the liquid and impact on any membrane present (**Figure 6A**). The collapse of gas bubbles near the membrane results in asymmetrical pressures and the formation of liquid microjets which impact on and can even penetrate into the membrane (**Figure 6B, C**), resulting in the formation of pits on the membrane surface, which could act as conduits for ungual drug flux.

3.1.6 Application of electric currents

The application of electric currents to enhance ungual drug flux seems to be the most systematically researched physical technique [57-65] and causes large increases in ungual drug flux compared with passive transport. For example, **Figure 7** shows increases in permeant flux by up to 16-fold. Iontophoresis (defined as a method to deliver a compound across a membrane with the assistance of an electric field) has been found to enhance the flux of a number of molecules, including fluorescein, glucose, salicylic acid, tetraethylammonium, mannitol, urea, griseofulvin and terbinafine into and/or through the nail plate. Movement of charged molecules is enhanced mainly by electrophoresis, with a small contribution from electro-osmosis, while movement of uncharged species will mainly be due to electro-osmosis [61].

Ungual iontophoretic flux of salicylic acid through the nail into the receptor medium increased with increasing permeant concentration in the donor solution and with current density (0.1 – 0.5 mA/cm^2 tested) [57]. When drug concentration in the hoof membrane (model nail plate) was measured, the relationship between current density and terbinafine concentration (mg drug/g hoof membrane) was not, however, totally linear when electric current densities up to 1 mA/cm^2 were tested [64]. Murthy *et al.* [57] also showed a significant influence of the buffer ionic strength on iontophoretic flux. Low buffer ionic strength is desired during iontophoresis to reduce competition between the drug and co-ions to carry the electric charge across the membrane. Thus, increasing ionic strengths above 100 mm resulted in an expected decrease in ungual salicylic acid flux. At ionic strengths below 100 mm, a higher flux was expected, but was not observed due to changes in pH of the buffer during iontophoresis.

Donor pH has a major influence on iontophoretic flux, due to its effects on the ionisation of, firstly, the permeant and secondly, the nail plate. The latter, with a pI (isoelectric point) of ~ 5, carries a net positive, negative and neutral charge when it is exposed to a drug solution whose pH is below 5, above 5 and 5, respectively. It is expected, therefore, that for the greatest iontophoretic drug flux, the pH of the donor drug solution should be such that the permeant is fully ionised and the nail plate has an opposite charge to that of the permeant. Such ideal conditions are unlikely to be fully met, as shown in **Figure 7**. The latter also shows that ionisation of the permeant has a greater influence on iontophoretic ungual drug flux than the nail plate's charge.

The nail plate's charge is, however, critical for the electro-osmotically driven transport of uncharged molecules, as shown in **Figure 8** [58]. An increase in donor pH resulted in increased anodal iontophoretic flux and a decrease in cathodal flux of glucose. At a donor solution pH < 5, the nail plate is positively charged, and hence more receptive to the flux of anions. Migration of the latter into the nail coupled with solvent (and glucose) flow would therefore occur if the cathode is placed in the donor solution, i.e., cathodal iontophoresis would enhance drug flux at pH < 5. Conversely, at donor solution pH > 5, the nail plate would

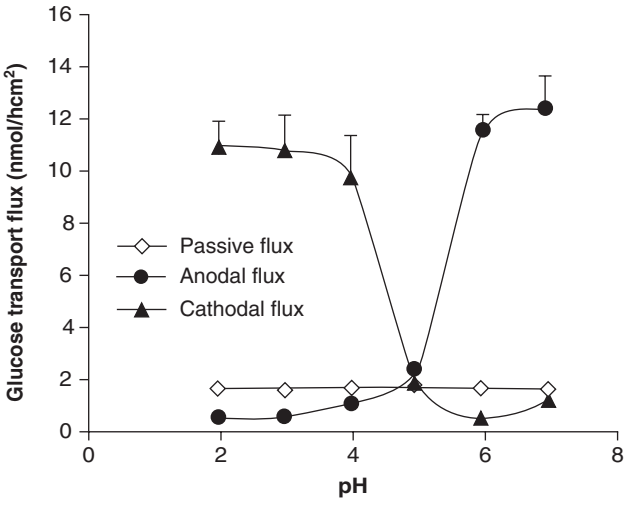

Figure 8. pH-dependent electro-osmotically driven ungual flux of the uncharged glucose by anodal and cathodal iontophoresis at current density of 0.5 mA/cm^2.
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be negatively charged, more receptive to cations, which should be repelled by the anode, i.e., anodal iontophoresis for enhanced drug flux at pH > 5.

Iontophoresis is different from the techniques described above; application of electric current enhances ungual drug delivery by influencing the movement of the permeant into and through the nail rather than by reducing the integrity of the nail plate. Indeed at the low current densities (< 0.5 mA/cm^2) employed, no electrically induced change in the nail plate's barrier properties were observed [61].

3.2 Chemical enhancement of ungual drug delivery

As mentioned in Section 2, the nail plate contains lipid at 0.1-1%. The very low lipid levels explain why transdermal enhancers, many of which act by fluidising skin lipid layers, have generally been unsuccessful as ungual enhancers. One exception is the enhanced permeation of econazole into the deeper nail layers by the transdermal enhancer dimethyl sulfoxide (DMSO) [66]. However the extremely high concentration of DMSO used (99% of the formulation) shows that such a formulation would not be practical due to the irritant effects of DMSO [67].

Chemical enhancement of the nail plate's permeability has therefore focused on breaking the chemical and physical bonds responsible for the stability of nail plate keratin, and the disulphide, peptide, hydrogen and polar bonds were identified as potential targets [68]. Of these, the disulphide bond has received the most attention, due to its critical role in protein stability. Cleavage of the disulphide bond has mainly been achieved by its reduction, though oxidation has also been attempted. The chemical agents may be applied to the nail plate prior to or concomitantly with drug application. Enhancer activity has been determined mainly by measuring ungual drug flux and drug uptake within the nail plate using Franz diffusion cells. In certain cases, nail plates have been incubated in drug solutions, such that both the dorsal and ventral surfaces were exposed to the drug and enhancer, after which drug uptake in the nail plate was measured. Obviously, exposure of both surfaces would result in a much greater drug uptake, especially due to the greater permeability of the ventral nail surface [40]. The ungual enhancers are discussed in more detail in the following sections and are compiled in **Table 1**. It is important to remember that due to the use of widely different experimental protocols – such as, enhancer concentrations, enhancer application prior to or concomitantly with drug, drug nature and concentration, experimental durations, drug uptake into nail or permeation into the receptor medium, and Franz diffusion cell set-up or nail incubation in drug/enhancer solutions – the chemicals should not be ranked with respect to enhancer activity from this compilation.

3.2.1 Cleavage of nail disulphide bond by reducing agents

3.2.1.1 Thiols

A range of reducing agents have been tested, and thiols (–SH containing compounds) have shown the greatest promise, although not all SH-containing compounds tested have demonstrated ungual enhancer effect [69]. Thiol enhancers are thought to reduce nail keratin as follows [3]:

$$\text{Nail-S-S-Nail} + \text{R-SH} \leftrightarrow 2\ \text{Nail-SH} + \text{R-S-S-R}$$

where R-SH represents a thiol. Examples of such ungual enhancers include *N*-acetylcysteine, mercaptoethanol, thioglycolic acid, *N*-(2-mercaptopropionyl) glycine (MPG) [68-72]. Increasing the concentration of MPG and *N*-acetyl cysteine was found to increase ungual drug flux [69,71] and enhancer action was found to be fairly durable and poorly reversible [71]. Once disulphide bonds have been cleaved, they are unlikely to be reformed in the dead nail plate. Thus, nail plates may be pretreated with the enhancer prior to drug application (rather than being present in the drug formulation). Pretreatment with the enhancer would solve drug-enhancer incompatibility issues, if any, and may enable higher drug and enhancer concentrations to be used. However, a potential disadvantage of separate enhancer and drug application is a less simple drug administration protocol which may reduce patient compliance.

3.2.1.2 Sulphites

Incubation of proteins or peptides containing disulfide bonds with sodium sulfite is known to cleave the disulphide bond to produce thiols and thiosulfates according to the reaction below [73]:

$$\text{R-S-S-R} + \text{Na}_2\text{SO}_3 \rightarrow \text{R-S-H} + \text{R-S-SO}_3\text{H}$$

Thus, it was hypothesised that incubation of nail plates with sodium sulfite could reduce the nail plate's barrier

Table 1. Enhancement of ungual drug permeation by chemicals.

Chemical enhancer (concentration)	Permeant [ref.]	Increase in ungual permeation compared with control (i.e., in the absence of enhancer)	Experimental setup
N-acetylcysteine (5%)	Itraconazole [3]	49 × increase in drug content of nail	Nails immersed in drug solution
N-acetylcysteine (15%)	Oxiconazole [7]	2 × increase in the mean ungual drug uptake, although this was not statistically significant. The mean residence time of oxiconazole in the middle layer of the nail plate was increased from 4.2 to 5.5 weeks	Drug was applied twice daily on nail plates of humansfor 6 weeks
N-acetylcysteine (3%)	5-Fluorouracil [71]	13 × increase in flux from an aqueous formulation; 7 × increase from a lipidic formulation	Diffusion cells
	Tolnaftate [71]	Drug measurable in presence of enhancer, but not in its absence	Diffusion cells
Mercaptoethanol (3%)	5-Fluorouracil [71]	16 × increase in flux from an aqueous formulation; 8 × increase in flux from a lipidic formulation	Diffusion cells
	Tolnaftate [71]	Drug measurable in presence of enhancer, but not in its absence	Diffusion cells
Thioglycolic acid (5%)	Caffeine [72]	3.8 × increase in flux	Diffusion cells
	Mannitol [72]	2. × increase in mannitol concentration in receptor medium	Diffusion cells
Cysteine	Mannitol [72]	1.7 × increase in mannitol concentration in receptor medium	Diffusion cells
N-(2-mercaptopropionyl) glycine (MPG, 10%)	Water [69]	2.5 × increase in flux	Diffusion cells
Hydrogen peroxide (35%)	Mannitol [72]	3.2 × increase in mannitol concentration in receptor medium	Diffusion cells
Urea hydrogen peroxide (MedNail)	Terbinafine [75]	18 × increase in flux	Diffusion cells
Sodium sulphite	5.6-Carboxyfluorescein [unpublished]	2 × increase in permeation through nail clipping	Diffusion cells
Keratinase (3%)	Metformin [84]	2.3 × increase in flux*	Diffusion cells

*Hoof membrane used as model nail plate.

properties and enhance ungual permeation. Indeed the penetration of 5,6-carboxyfluorescein (used as a model drug) through nail clippings from a healthy volunteer was found to be significantly enhanced in the presence of sodium sulfite [74]. Permeant penetration was increased both when sodium sulphite was used to pretreat nail plates prior to permeant application and when it was present in the permeant donor solution (P Bubba Bello and S Murdan, 2008, unpublished results). Such enhancer effect of sulphite conflicts with findings by Malhotra and Zatz [69], who reported a lack of enhancer activity of sodium meta-bisulfite which, when combined with water, is converted into sodium hydrogen sulphite (also a reducing agent). Further investigations are ongoing in our laboratory.

3.2.2 Cleavage of nail disulphide bond by the oxidising agent hydrogen peroxide

Much less attention has been given to the oxidation of the disulphide bond as a means of bond cleavage to increase nail permeability. Hydrogen peroxide was the only example found, used on its own or when complexed with urea, in the form of urea hydrogen peroxide [72,75]. Pretreating nails with hydrogen peroxide on its own (35% wt in water) for 20 h increased mannitol permeation three-fold following a 120 h permeant application [72]. Urea hydrogen peroxide (a hydrogen bonded stable and convenient adduct) is part of the MedNail® (MedPharm) technology, which consists of pretreating nails with the reducing agent thioglycolic acid, followed by the oxidising agent

UHP [75]. Nail pretreatment with MedNail increased the ungual flux of terbinafine 18-fold and increased fungal kill by Penlac and Loceryl; the mechanism by which MedNail increased antifungal permeation through the nail is thought to be the reduction and oxidation of the disulphide bonds in the nail plate [75].

3.2.3 Urea

In addition to its role in MedNail technology mentioned above, urea has been widely investigated due to its keratolytic activity. It hydrates and softens nail plates [71] and damages the plate surface [76], but has mostly been found to be unable to enhance ungual drug flux *through* the nail on its own [69,71,76]. In fact, it has been found to reduce ungual flux of 5-FU [71], although it had no effect on water flux [69]. The reduction in the flux of 5-FU was assigned to changes in pH of the solution upon inclusion of urea (from pH 4.7 to 7.2), which resulted in ionisation of the acidic 5-FU (pK_a = 8.0, 13.0) into negatively charged species and repulsion between the like-charged drug and the nail (which also carries negative charges above pH 5 due to its pK_a of ~ 5) [71]. An exception to urea's inability to act as an ungual enhancer was reported by Sun *et al.* [3], who demonstrated increased (20-fold compared with control) itraconazole (pK_a = 3.7) concentration *in* the nail when the latter was incubated in a drug solution containing 10% urea. The discrepancy between this report showing a positive influence of urea and a neutral or negative influence could partly be due to the different pK_a of the drugs (which influences drug ionisation and charge), the different experimental setups (diffusion cell where only the dorsal nail surface is exposed to drug/urea or a setup where the whole nail is incubated in a drug/urea formulation) and endpoints investigated (drug flux through nail or drug concentration in the nail).

When used in combination with other ungual enhancers, urea has a synergistic influence on ungual drug permeation [3,69]. For example, itraconazole concentration in nail plates in the presence of both *N*-acetyl cysteine and urea was found to be 94 times higher than that of the control (no enhancer), compared with 20 times in the presence of urea only and 49 times in the presence of *N*-acetyl cysteine only [3]. Interestingly, the beneficial influence of combining urea with proven ungual enhancers was observed even when urea did not enhance ungual drug permeation on its own; ungual flux of water being increased by 3.5-fold in the presence of urea and MPG, compared with an increase of 2.5-fold in the presence of MPG only [69]. Urea is a known keratolytic agent and is thought to act by unfolding, thus solubilising and/or denaturing keratin. Protein unfolding by urea (probably via interaction with their hydrogen bonds) may facilitate the cleavage of the disulphide linkages by ungual enhancers such as acetyl-cysteine, and thereby increase the disruption to the nail plate barrier and subsequent drug permeation. Thus, urea has been included in nail lacquers [77], although most often, the role of urea in the topical treatment of nail diseases has been to chemically avulse diseased nail plate, and in such instances, urea has been used at high concentrations, e.g., 40% [78,79].

3.2.4 Water

Water seems to have a facilitating role in ungual permeation of certain (but not all) molecules, and the relationship between nail hydration and ungual drug permeation does not seem to be universal. The permeability coefficient of C2 – C10 n-alknaols (but not methanol) from a saline solution through the nail was five times greater than from neat alcohols [34]. The beneficial effect of water in the donor medium was confirmed when its replacement with an organic solvent (isopropanol or dimethyl sulfoxide) reduced the permeability of hexanol (but not of methanol) in a concentration-dependent fashion [80]. Kobayashi *et al.* [71] showed higher flux of the hydrophilic 5-FU from aqueous (compared with lipophilic) drug-saturated formulations containing the enhancers acetyl cysteine and mercaptoethanol. In contrast, Mertin and Lippold [81] showed similar flux of chloramphenicol from aqueous and lipophilic vehicles containing the solute at maximal thermodynamic activities.

Nail hydration by water (and conversely its dehydration by certain solvents) has been suggested as a possible reason for the greater drug flux from certain vehicles. Indeed, an increase in nail plate hydration (by nail incubation in environments of increasing relative humidities) was found to increase the permeation ketoconazole by three-fold and the diffusivity of water by more than 400-fold, when relative humidity was increased from 15 to 100% [82,83]. In addition, Mertin and Lippold [81] hypothesised (and showed) similar drug fluxes from drug-saturated aqueous and lipophilic vehicles as long as the nail plate remained hydrated in all cases. Total nail hydration in all cases was assumed (but not measured) by the fact that the nail plate's ventral surface was in contact with an aqueous receptor medium in the Franz diffusion cell.

A swollen nail plate could be assumed to allow easier and greater diffusion of drug molecules. Indeed, a correlation between the extents of nail swelling and of ungual permeation enhancement was found for thioglycolic acid, cysteine and hydrogen peroxide, and nail swelling was proposed as a convenient preformulation screen for the selection of ungual enhancers [72]. Kobayashi *et al.* [71] also found a correlation between increasing flux of 5-FU and increasing nail weight in the presence of increasing concentrations of the enhancer *N*-acetyl cysteine. However, increased nail swelling and enhanced drug flux do not always occur together, as shown for resorcinol [72] and urea [69], which caused nail swelling, but no increase in drug permeation. In addition, enhanced tolnaftate flux was observed from a lipophilic vehicle which did not cause significant nail swelling [71]. Thus, it appears that increased

nail hydration by a formulation cannot always be taken as an indication of increased ungual drug flux.

3.2.5 *Enzymes*

Keratinolytic enzymes were expected to hydrolyse nail keratins, thereby reducing the barrier properties of the nail plate and subsequently enhancing ungual drug permeation [84]. Indeed, the enzyme profoundly affected the surface of human nails (**Figure 9A**, **B**). The enzyme seemed to act on the intercellular matrix which holds nail corneocytes together, such that corneocytes on the dorsal surface of the nail separated from one another and 'lifted off' the nail plate (**Figure 9A**). In addition, the surface of the corneocytes was corroded (**Figure 9B**), implying enzyme action on the interfilamentous matrix. In the presence of the enzyme, drug flux through hoof membranes (used as model nail plate), and the permeability and partition coefficients were more than doubled [84].

Papain – an endopeptidase enzyme that contains a highly reactive sulfhydryl group – has also shown some promise as an ungual enhancer [76]. Incubation of nail in an enzyme solution (15% w/v) for 1 day, followed by soaking in salicylic acid solution (20% w/v) for 10 days enabled the permeation of the antimycotic agents, miconazole, ketoconazole and itraconazole (compared with unmeasurable drug levels without the enzyme treatment). The aggressive pretreatment regime had fractured the surface of the nail plates, as revealed by scanning electron microscopy, and possibly created pathways into the nail for drug penetration.

3.3 Optimal drug formulation

The formulation of a drug into a suitable topical preparation is critical to the success of topical therapy. The topical drug formulation should be easy to use, remain in contact with nail plates over long periods without being wiped/washed off to reduce application frequency and thereby increase patient compliance. More importantly, the formulation should contain the drug in a form that encourages its release and diffusion into the nail plate. A high drug thermodynamic activity in the formulation is expected to achieve a high gradient, and increased drug diffusion. In addition, formulations that hydrate nail plates may be desirable, due to the facilitating role of water on the diffusion of certain permeants, as discussed above. In certain cases, a 'solvent-drag' effect may be able to carry a permeant from a topically applied formulation into the nail as an organic solvent itself traverses into the tissue. Thus, a number of different preparations have been formulated for ungual delivery, including paints, solutions, gels, ointments, pastes, lacquers, extruded films and patches [85-93]. Judicious choice of the formulation excipients is obviously critical for ungual drug flux. For example, in an *in vitro* study, inclusion of 2-n-nonyl-1,3-dioxolane in an econazole lacquer resulted in a 200-fold increase in drug mass in the support bed under the nail plate; such enhancer effect was thought to be due to the plasticising effect of 2-n-nonyl-1,3-dioxolane on the lacquer film, which had a positive influence on film adhesion to the nail plate, which in turn allowed greater drug permeation [93]. Similarly, permeation of ciclopirox from a water-soluble hydroxypropyl chitosan film into bovine hoof membranes (model nail plate) was found to occur with a shorter lag time compared with the commercial ciclopirox lacquer that forms a water-insoluble film based on a vinyl resin [92]. Greater adhesion of the hydroxypropyl chitosan film to the keratin substrate was thought to be responsible for the faster transfer of the drug from the vehicle into the hoof membrane [92].

Of all the drug formulations, the lacquer seems to be the most commercially favoured, for its many advantages such as long residence on nail, aesthetically pleasing appearance and patient familiarity with product. Lacquers also reduce trans-onycheal water loss [94,95], which could lead to increased hydration of the nail plate [94], which could in turn increase drug diffusivity in the nail [96]. In the treatment of onychomycosis, increased hydration of the nail plate by an occlusive lacquer could also induce the germination of drug-resistant fungal spores into drug-susceptible hyphae, such that all fungi could be eradicated from the nail by the antifungal agent [97]. Pharmaceutical nail lacquers generally consist of solvents, film-forming polymers, resins that increase the adhesion of the film to the nail plate, plasticisers that contribute to the flexibility and durability of the film, in addition to the drug. Following application to the nail plate, the solvent evaporates, leaving a drug-loaded water-insoluble film adhered to the nail plate. Amorolfine and ciclopirox lacquers are commercially available and have been evaluated in comparative studies [98], in combination with oral antifungals, in special populations such as people with diabetes [99], and in pharmacoeconomic analyses [100]. Lacquer formulations of terbinafine [101], fluconazole [77], urea [77], econazole [93,102], and clobetasol for psoriasis [103] have been tested, and a large number of lacquer formulations have been patented, including aqueous formulations and those containing vitamins, colourants, drug combinations, and so on [104].

4. Conclusions

A range of physical techniques and chemicals have been investigated with the aim of enhancing the (normally low) ungual permeation of topically applied drugs. Some of the physical techniques, such as iontophoresis, have been adopted from the transdermal drug delivery field, while attempts at using chemical transdermal enhancers have generally failed in the ungual field. Attempts to identify chemical ungual enhancers have yielded only a few potential candidates so far, and much more work is needed in this field. In addition to the search for suitable physical and chemical means of enhancing drug flux into the nail,

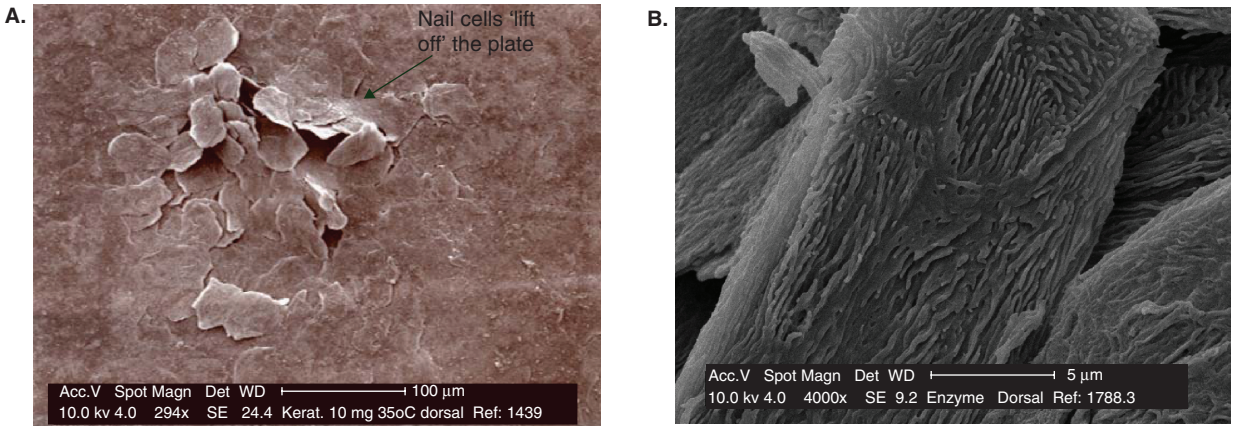

Figure 9. Effects of a keratinase solution. A. Incubation of nail clippings in a keratinase solution resulted in nail corneocytes 'lifting off' the nail plate, showing enzyme action on the intercellular cement. **B.** Keratinase seems to 'corrode' the surface of individual corneocytes, possibly due to action on the interfilamentous matrix.
Reproduced from [84] with kind permission of Elsevier Ltd.

optimal drug formulation is critical in maximising ungual drug flux.

5. Expert opinion

The ungual drug delivery field is young, few commercial products have been specifically developed for the topical treatment of nail disorders, the factors that influence ungual drug permeation are still being elucidated and there are quite a few contradictory reports in the literature, possibly due to incomplete elucidation of the factors and mechanisms involved. It is an exciting field and despite the scant literature on the basic science, a number of pharmaceutical companies are developing new products, mainly for onychomycosis. Mostly, existing antifungal agents are being formulated for topical application to the nail, with/without enhancers, and there is, to the author's knowledge, only one report of a new antifungal agent – AN2690 – developed specifically for the treatment of onychomycosis [105,106]. Now that the permeant properties which influence ungual drug penetration are being elucidated, there is obviously the possibility of designing new potent antifungal agents, specifically for ungual permeation.

Enhancers (physical or chemical) are expected to play a critical role in drug formulation, and it is surprising that the existing products – Loceryl and Penlac – do not contain any chemical enhancers. This could be due to the fact that during the development of these products there were few known ungual enhancers. The number of chemical ungual enhancers is still small today and many more need to be identified. From **Table 1**, it can be seen that some of the ungual enhancers cause modest improvements in ungual drug permeation. Considering the low nail permeability, much more potent ungual enhancers are needed, keeping in mind skin irritancy and patient safety. It is also possible that chemical ungual enhancers could be drug specific, in a similar fashion to transdermal enhancers. Any such drug specificity would increase the need for a wider range of proven ungual enhancers, and this needs further investigation.

Chemical enhancers have a number of advantages over most of the physical enhancers, for example, they can be incorporated in the drug formulation, rather than application prior to drug application, which would simplify topical drug administration. Chemical application is also easy, in contrast to some of the physical techniques, such as nail drilling and microporation, which require skilled operators and may not be very practical. The whole nail plate or affected areas may be easily exposed to chemical enhancers, thus increasing their activity, compared with certain physical enhancers, such as lasers, whose application is spatially focused. Finally, chemical enhancers are likely to be much cheaper than most of the physical enhancers. On the other hand, the physical enhancer iontophoresis seems to be more potent compared with the chemicals identified thus far, and it is likely that a combination of iontophoresis and chemical enhancers will further increase ungual drug delivery. The other physical enhancers, such as microporation and lasers, have shown that holes can be made into the nail plate, but the enhancement of ungual drug flux has not been reported. Microporation and laser application have the advantage that their application could be targeted to areas of the nail plate where drug permeability is lowest, for example, in dermatophytomas or in onycholytic pockets within the nail plates. Optimisation of low-frequency ultrasound application is also needed and could result in higher drug permeation than reported so far. What influence the physical and chemical treatments have on the disease itself, for example, on growing and resting fungal cells, remains to be investigated.

Optimal drug formulation is key to drug delivery, however the literature on drug formulation for ungual delivery is minimal. While innumerable papers have been published on the evaluation of the two commercial nail lacquers in clinical trials, the literature on their formulation and physico-chemical properties is negligible. This is obviously for commercial reasons. However, more basic, publicly funded, publishable research is needed to enable a systematic, rather than empirical, approach to drug formulation and avoid some of the duplication that is undoubtedly happening in product-driven research. Basic research is also needed on the science of other drug carriers such as gels and nail patches, which may solve some of the problems associated with nail lacquers, for example, the potential of drug precipitation in the lacquer film upon solvent evaporation.

Following topical application of the antifungal formulations, drug concentrations in the nail plate in excess of the drug's minimum inhibitory concentration (MIC) have often been measured and are quoted as an indication of the potential antifungal efficacy of those formulations. It must be remembered, however, that the MIC of antifungal agents in infected nail plate/bed is likely to be very different to the MICs determined in standard microbiological assays in liquid media. Attempts to determine more relevant antifungal MIC, by using powdered nail plate for fungal growth, revealed much higher MIC values [107,108]. Measurement of antifungal kill *in vitro*, following drug permeation through nail plates from topically applied formulations, gives a slightly more relevant indication of formulation potency, though correlation between drug concentrations in the nail plate and in agar gel (simulating the nail bed) and antifungal inhibition in the agar gel was found [109]. These *in vitro* models are, however, still far from the situation *in vivo*. A 'plea to bridge the gap between antifungals and the management of onychomycosis' has been made [110]. It is possible that routine measurement of drug concentrations in the affected and healthy parts of the nail plate during clinical trials, and investigations into correlations with formulation efficacy, may enable the determination of more relevant MICs of the antifungal agents.

The mantra is basic research, basic research, basic research…

Declaration of interest

The authors state no conflicts of interests and have received no payment in the preparation of this manuscript.

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