ABSTRACT

Common nail diseases are onychomycosis and psoriasis. The absorption of drugs into the nail unit, following topical application to the nail plate, is highly desirable to treat nail disorders, such as onychomycosis (fungal infections of the nail). Nail permeability is however quite low and limits topical therapy to early/mild disease states. In this paper, the recent research into tansungual drug delivery is reviewed. The factors, which affect drug uptake and permeation through the nail plate such as solute molecular size, hydrophilicity/hydrophobicity, charge, and the nature of the vehicle, are then discussed, followed by ways of enhancing drug transport into and through the nail plate. The major task in treating the nail disorders such as nail psoriasis and Onychomycosis is to deliver and maintain consistent therapeutically effective concentration of drugs in the deeper nail strata during the course of treatment. This requires large doses and frequent administration of drugs. Systemic administration of antifungal and antipsoriatic drugs is always associated with severe side effects and potential drug interaction risks. Topical monotherapy is considered “less successful” in treating Onychomycosis due to poor trans-nail bioavailability of drugs. The major reasons for poor trans-nail absorption includes unfavorable physicochemical properties of the drugs, lack of formulations that can overcome the barrier properties of the nail plate, short residence time of topical formulations and extensive binding of drug to the nail keratin. All these factors necessitate the development of effective drug delivery methods which can rapidly drive therapeutically effective quantity of drugs across the nail plate.

Key words: Transungual, Onychomycosis and psoriasis.
1. INTRODUCTION
The human nail is an important organ of human body. It protects the delicate tips of fingers and toes against trauma, enhances the sensation of fine touch and allows one to pick up and manipulate objects. The nail is also used for scratching and grooming, as a cosmetic organ and sometimes, to communicate one’s social status. [1] The nail plate is the most visible part of the nail apparatus, consists of tightly packed dead cells and is highly keratinized. It is also very variable among individuals. The plates can be small, large, wide, narrow, hard, smooth, ridged, thin, etc.

The disorders of the nail unit range from conditions such as pigmentation in heavy smokers, to painful and debilitating states where the nail unit can be dystrophied, hypertrophied, inflamed, infected etc. Such conditions affect patients physically as well as socially and psychologically and can seriously affect the quality of life. Many nail diseases are notoriously difficult to cure, need a long duration of treatment and relapse is common. Oral therapy has the inherent disadvantages of systemic adverse effects and drug interactions while topical therapy is limited by the low permeability of the nail plates. The importance of nail permeability to topical therapeutics has been realized, primarily in the treatment of onychomycosis, which affects approximately 19% of the population. [2] Topical therapy is highly desirable due to its localized effects, which results in minimal adverse systemic events and possibly improved adherence. Recent advances in topical transungual delivery have led to the development of antifungal nail lacquers. However, the effectiveness of topical therapies is limited by minimal drug permeability through the nail plate. [3] Current research on nail permeation focuses on altering the nail plate barrier by means of chemical treatments and penetration enhancers. [4, 5] Physical and mechanical methods are also under examination.

Common nail diseases are onychomycosis and psoriasis. Onychomycosis is a fungal infection which occurs in the elderly rather than in children. [6] It is responsible for approximately 50% of all nail disorders. [7] The most frequently reported symptoms are discoloration, thickening, and deformity of the toenails. [8] Treatment options for this persistent disease include oral, topical, mechanical, and chemical therapies or a combination of these modalities. Orally applied antifungal drugs are the most effective agents available to treat onychomycosis, among them terbinafine is the first choice. Griseofulvin, which was the first oral antifungal drug approved by the US Food and Drug Administration or ketoconazole
from the Azole group are currently not used much. [9] However, oral systemic antifungal therapy is limited by its toxicity, drug interactions, contraindications, high cost of medication, increased microbe resistance, a long duration of treatment, and relapse is very common. Topical therapies, on the other hand, have difficulties, too. The active drug from the applied formulation has to permeate and overcome highly restrictive barrier properties of the human nail. [10] On the market there are nail lacquers and nail solutions available for topical treatment of onychomycosis. After application, the solvent from the lacquer formulation evaporates leaving an occlusive film on the nail in which the drug concentration is higher than in the original formulation. This increases the diffusion gradient and permeation through dense keratinized nail plate occurs. [11] For the use of nail solutions, patients are advised to apply the formulation not only on the nail surface, but on the surrounding skin as well, usually by using the provided brush.

1.1 Human nail
The chemical composition of the human nail differs significantly from other body membranes. The plate, composed of keratin molecules with many disulphide linkages and low associated lipid levels, does not resemble any other body membrane in its barrier properties – it behaves more like a hydrogel than a lipophilic membrane. Drug transport into the nail plate is influenced by: physicochemical properties of a drug molecule (size, shape, charge, and hydrophobicity), formulation characteristics (nature of the vehicle and drug concentration), presence of permeation enhancers, nail properties (thickness and hydration), and interactions between the permeant and the keratin network of the nail plate. The chemical composition and some experimental evidence indicate that the aqueous pathway plays the dominant role in drug penetration through the nail. Furthermore, water is the principle nail plasticizer. Once hydrated, the nail becomes more elastic and possibly more permeable to topically applied substances. However, the effects of hydration on nail permeation requires elucidation.
The nail apparatus, schematically shown in Fig.1, is composed of the nail folds, nail matrix, nail bed and the hyponychium, which together form the nail plate. [12] The nail plate, produced mainly by the matrix, emerges via the proximal nail fold and is held in place by the lateral nail folds. It overlays the nail bed and detaches from the latter at the hyponychium (skin under the free edge of the plate). The nail plate is a thin (0.25–0.6 mm), hard, yet slightly elastic, translucent, convex structure and is made up of approximately 25 layers of dead, keratinised, flattened cells which are tightly bound to one another via numerous intercellular links, membrane-coating granules and desmosomes. Chemically, the nail plate consists mainly of the fibrous proteins, keratins, 80% of which is of the ‘hard’ hair-type keratin, the remainder comprising the ‘soft’ skin-type keratin. [13] The keratin fibres are oriented into three layers, which are associated with the dorsal, intermediate and ventral nail layers. The hair-like keratin filaments are only present in the intermediate nail layer and are oriented perpendicular to the growth axis, while the skin-type keratin filaments are found in the dorsal and the ventral layers and are oriented in two privileged directions, transverse and perpendicular to the growth axis. [14] The keratin fibres are thought to be held together by globular, cystine-rich proteins whose disulphide links act as glue. [15] The plate also contains water at 10–30%, water content is directly related to the relative humidity and is important for nail elasticity and flexibility. In contrast, the nail plate contains small amounts of lipid, between 0.1 and 1.0%, most of which is organised into bilayers oriented parallel to the nail surface and is concentrated in the ventral and dorsal nail layers. The nail plate is a fairly strong structure. Its hardness and mechanical rigidity is thought to be due to the sandwich orientation of the keratin fibres, the presence of globular proteins that provide the ‘glue’ to hold keratin fibres together, adhesiveness of nail cells to one another, physical and chemical stability of the nail proteins (conferred by the stable disulphide links), the design of the plate (which is curved in both transverse and longitudinal axes) and its water content. The nail plate is formed by the nail matrix which is a highly proliferative epidermal tissue. It is also called the root of the nail, lies underneath the proximal nail fold and its distal portion is often visible through the transparent nail plate as a white, semi lunar area, called the lunula. Cell division of the matrix results in the continuous formation of the nail plate, which grows throughout life. Growth rate is highly variable among individuals; average values of 3 mm per month (fingernails) and 1 mm per month (toenails) are used when treating nails.
2. Factors which influence drug transport into and through the nail plate

2.1 Molecular size of diffusing molecule
As expected, molecular size has an inverse relationship with penetration into the nail plate. The larger the molecular size, the harder it is for molecules to diffuse through the keratin network and lower the drug permeation. [16] It demonstrated the decreasing permeability coefficients through human nail plate and through bovine hoof membrane with increasing molecular size of a series of alkyl nicotinates. Movement of larger solutes through the ‘pores’ in the keratin fibre network is obviously more difficult than the movement of smaller molecules. The authors suggested that the nail plate have a denser network of keratin fibres. A higher concentration of keratin fibres would result in greater chain–chain interactions, smaller ‘pores’, overlapping of ‘pores’, thus a more tortuous path for a diffusing molecule with consequently lowered permeation. The slope of the nail plate curve is twice as steep as that of the hoof membrane. This means that the nail plate is twice as sensitive to changes in the size of a diffusing molecule compared to the hoof membrane. Again, the denser network of the nail keratin was thought to be the reason. A denser network means there would be fewer pores whose size could accommodate the larger diffusing molecules.

2.2 Hydrophobicity/lipophilicity of diffusing molecule
A study was conducted on the permeation of a series of homologous alcohols (C1–C12), diluted in saline, through avulsed human nail plates. Increasing the chain length from one carbon to eight carbon atoms resulted in a decrease in permeability coefficient, after which, increasing chain length (to C12) resulted in increased permeability coefficient. Increasing lipophilicity of the diffusing alcohol molecule reduces the permeability coefficient until a certain point after which further increase in lipophilicity results in increased permeation. The nail plate seems to be a hydrophilic structure when the permeation of the lower alcohols (C8) is considered. The authors concluded that the nail plate behaves like a concentrated hydrogel. The permeation of neat alcohols follows a similar trend. However, except for methanol, the permeability coefficient of neat alcohols (absence of water) was approximately five times smaller than the permeability coefficient of diluted alcohols. The authors suggest that this indicate a facilitating role of water towards the diffusion of the alcohol molecules. It is possible that when an aqueous formulation is used, nails swell as water is taken up into the nail plates. Consequently, the keratin network expands, which leads to the formation of larger pores through which diffusing molecules can permeate more easily. The increase in permeation of the higher alcohols (C10 and C12) with increasing lipophilicity was suggested
to occur through a lipiddic pathway. Despite the low content of lipid (up to 1% of the total weight) in the nail plate, this lipid pathway seems to be important for the passage of very hydrophobic substances. Indeed, extraction of the nail lipid by incubating the nail plates in chloroform/methanol mixture for 24 h reduced the permeation of decanol and dodecanol even though the permeation of water, methanol, ethanol and butanol were increased. [17]

2.3 Nature of vehicle

Nature of vehicle also plays an important role on the transport of drug through nail plate. Water hydrates the nail plate which consequently swells. Considering the nail plate to be a hydrogel, swelling results in increased distance between the keratin fibres, larger pores through which permeating molecules can diffuse and hence, increased permeation of the molecules. Replacing water with a non-polar solvent, which does not hydrate the nail, is therefore expected to reduce drug permeation into the nail plate. This was indeed demonstrated by, who reported that the addition of a non-polar co-solvent such as DMSO and isopropanol decreased the nail permeation of hexanol through human nail plate. Increasing concentration of the co-solvent results in decreasing permeability coefficient of hexanol. In other words, as the amount of water in the medium decreases, permeability co-efficient of hexanol through the nail plate decreases. It is not known whether the nails soaked in water/co-solvent mixtures swelled to a lesser extent (or de-swelled) compared to nails soaked in water alone or whether there was a correlation between co-solvent concentration and nail swelling and between nail swelling and permeability coefficient of hexanol. [18]

2.4 pH of vehicle and solute charge

The pH of aqueous formulations affect the ionisation of weakly acidic/basic drugs, which in turn influences the drug's hydrophilicity/hydrophobicity, solubility in the drug formulation, solubility in the nail plate and its interactions with the keratin matrix. There have been conflicting reports in the literature on the influence of pH. Walters et al. [17] studied the permeation of the weakly basic drug, miconazole, through hydrated human nail plate. The pH of the miconazole donor solution was varied from 3.1 (where the drug is mostly dissociated) to 8.2 (drug is mostly undissociated). The permeability coefficient of the drug was found to be essentially the same at all pH studied i.e. there was no effect of pH and of drug charge on its permeability coefficient. In other studies, pH of the medium was found to have a distinct effect on drug permeation. Soong (1991), investigated the permeation of benzoic acid through the nail plate at different pH. The donor cells contained saturated solutions of the permeate
and pH of the receptor phase matched that of the donor phase. It was found that as the pH of the medium was increased from 2.0 to 8.5, the permeability coefficient of benzoic acid decreased by 95.5% and the lag time increased. In this study, the uncharged molecules (at pH 2.0) permeated through the nail plate to a greater extent compared to the charged species. [19]

3. METHODS TO ENHANCE NAIL PENETRATION

Physical, chemical and mechanical methods have been used to decrease the nail barrier. Within each of these broad categories, many techniques exist to enhance penetration. Mechanical modes of penetration enhancement are typically straightforward, and have the most in vivo experience associated with them. In contrast, many of the chemical and physical methods discussed are still in the in vitro stages of development; laboratory studies are currently examining these techniques using human nail samples. The goal of topical therapy for onychomycosis is drug penetration into deep nail strataums at amounts above the minimal inhibitory concentration (MIC). Effective penetration remains challenging as the nail is believed by some to be composed of approximately 25 layers of tightly bound keratinized cells, 100-fold thicker than the stratum corneum. Furthermore, De Berker et al. have observed increase in toe nail thickness along the nail. Mean nail plate thickness increased progressively along the entire length of the nail ranging between 590µm and 1080µm. [20] While there is disagreement on the exact thickness of the nail there is consensus that the nail structure is difficult to penetrate. In addition, poor permeability and prolonged transport lag time contribute to disappointing topical efficacy in nail diseases. [21] Chemical and physical modes of penetration enhancement may improve topical efficacy. There are two main factors to consider: physicochemical properties of the drug (polar compounds are more permeable) and binding of the drug to keratin within the nail. Binding to keratin reduces availability of the active (free) drug, weakens the concentration gradient, and limits deep penetration. [22]

3.1 Mechanical methods to enhance nail penetration

 Mechanical methods including nail abrasion and nail avulsion have been used by dermatologists and podiatrists for many years with varying results. Additionally, they are invasive and potentially painful. Thus, current research focuses on less invasive chemical and physical modes of nail penetration enhancement.

3.1.1 Nail abrasion- Nail abrasion involves sanding of the nail plate to reduce thickness or destroy it completely. Sandpaper number 150 or 180 can be utilized, depending on required
intensity. Sanding must be done on nail edges and should not cause discomfort. An efficient instrument for this procedure is a high-speed (350,000 rpm) sanding hand piece additionally; dentist’s drills have been used to make small holes in the nail plate, enhancing topical medication penetration. [23] Nail abrasion thins the nail plate, decreasing the fungal mass of onychomycosis, and exposing the infected nail bed. In doing so, it may enhance the action of antifungal nail lacquer. The procedure may be repeated for optimal efficacy. [24]

3.1.2 Nail avulsion - Total nail avulsion and partial nail avulsion involve surgical removal of the entire nail plate or partial removal of the affected nail plate, and under local anesthesia. Keratolytic agents such as urea and salicylic acid soften the nail plate for avulsion. Urea or a combination of urea and salicylic acid has been used for nonsurgical avulsion (chemical avulsion) in clinical studies, prior to topical treatment of Onychomycosis. [25] Nail abrasion, using sandpaper nail files, prior to antifungal nail lacquer treatment may decrease the critical fungal mass and aid penetration.

3.2 Chemical methods to enhance nail penetration
Studies examining the efficacy of chemical compounds with Transungual penetration properties are currently underway. As would be expected, skin penetration enhancers do not usually have the same effect on nails. Thus far, only a few chemicals which enhance drug penetration into the nail plate have been described.

3.2.1 N-acetyl-l-cysteine and mercaptan compounds - Kobayashi et al. demonstrated that N-acetyl-l-cysteine and 2- mercaptoethanol, in combination, enhanced permeability of the antifungal drug tolnaftate into nail samples. [4] They suggested that these compounds may be generally useful in enhancing drug permeation across the nail plate. Hoogdalem et al. evaluated the penetration-enhancing properties of N-acetyl-l-cysteine with the antifungal drug oxiconazole in vivo. N-acetyl-l-cysteine promoted oxiconazole retention in upper nail layers. [26] Malhotra and Zatz screened nail penetration enhancers, including: mercaptan compounds, sulfites, bisulfites, keratolytic agents and surfactants in vitro. N-(2-mercaptopropionyl) glycine, demonstrated superior penetration enhancement to all other compounds, urea acted synergistically to increase nail permeation to the greatest extent. [27] However, post-treatment barrier integrity studies demonstrated that changes induced in the nail keratin matrix by these effective chemical modifiers were irreversible. It is believed that these enhancers act by breaking disulphide bonds, which are responsible for nail integrity thus producing structural changes in the nail plate.
3.2.2 2-n-nonyl-1,3-dioxolane- Hui et al. have showed that 2-n-nonyl-1,3-dioxolane (SEPA®) enhances penetration of econazole (from a lacquer formulation) into the human nail. They demonstrated that econazole penetrates the nail six times more effectively in a lacquer containing 2-n-nonyl-1, 3-dioxolane than in an identical lacquer. [28]

3.2.3 Keratolytic enhancers

Guerrero et al. described the effect of keratolytic agents (papain, urea, and salicylic acid) on the permeability of three imidazole antifungal drugs (miconazole, ketoconazole, and itraconazole). [29] In the absence of keratolytic agents, no transungual antifungal permeation was detected over a period of 60 days. Despite these findings, it is likely that the spectrophotometric method of analysis was insufficiently sensitive to accurately measure drug concentrations. Permeation of these agents did not improve by pre-treatment with 20% salicylic acid (for 10 days) and the addition of 40% urea to the donor solution. However, pre-treatment with both 15% papain (for 1 day) followed by 20% salicylic acid (for 10 days), enhanced antimycotic permeation. Presence of ethanol (as a co-solvent) did not promote flux. Although ethanol is an effective skin permeation enhancer, it does not have a similar effect on the nail. Ethanol acts on the SC by altering intercellular lipids; however, the lipid content of the nail comprises just 0.15–0.76% of its total weight. The authors proposed that aggressive pre-treatment (with papain and salicylic acid) produced pore formation in the nail matrix, allowing for effective drug permeation which was supported by the SEM images they obtained.

3.2.4 Enzymes

Due to an abundance of keratin filaments, keratinic tissues like the SC, are effectively hydrolyzed by keratinase. [30] Mohorćic et al. hypothesized that keratinolytic enzymes may hydrolyze nail keratins, thereby weakening the nail barrier and enhancing transungual drug permeation. This group conducted permeation studies using modified franz diffusion cells and metformin hydrochloride as a model drug and found keratinase to markedly enhance drug permeation through bovine hoof membranes. [31] In another study, human nail clippings were incubated in keratinase for 48 h, and subsequently examined with scanning electron microscopy. Keratinase clearly disrupted the nail plate, acting on both the intercellular matrix that holds the cells of the nail plate together and the dorsal nail corneocytes by corroding their surface.
3.3 Physical methods to enhance nail penetration-
Physical permeation enhancement may be superior to chemical methods in delivering hydrophilic and macromolecular agents. We discuss several physical enhancement methods, both established and experimental. [32]

3.3.1 Iontophoresis
Iontophoresis involves delivery of a compound across a membrane using an electric field (electromotive force). The principle has been applied clinically for cutaneous anesthesia, hyperhidrosis management, antibiotic penetration, and herpes simplex treatment. Currently both LidoSite® (lidocaine HCl/epinephrine topical iontophoretic patch) and GlucoWatch® (iontophoretic measurement of glucose in diabetics) are FDA approved. Iontophoresis has been used for various applications different from transdermal ophthalmic, dental, orthopaedic, etc. Drug diffusion through the hydrated keratin of a nail may be enhanced by iontophoresis. [33]

3.3.2 Etching
“Etching” results from surface-modifying chemical (e.g. phosphoric acid) exposure, resulting in formation of profuse microporosities. These microporosities increase wettability and surface area, and decrease contact angle; they provide an ideal surface for bonding material. Presence of microporosities improves “interpenetration and bonding of a polymeric delivery system and facilitation of interdiffusion of a therapeutic agent”. [34] Once a nail plate has been “etched,” a sustained-release, hydrophilic, polymer film drug delivery system may be applied. Bioadhesion, “a phenomenon related to the ability of biological or synthetic material to adhere to biological substrate,” must be considered; improved bioadhesion results in superior application of a transungual bioadhesive drug delivery system.

3.3.3 Carbon dioxide laser
CO2 laser may result in positive, but unpredictable, results. One method involves avulsion of the affected nail portion followed by laser treatment at 5000W/cm² (power density). Thus, underlying tissue is exposed to direct laser therapy. Another method involves penetrating the nail plate with CO2 laser beam. This method is followed with daily topical antifungal treatment, penetrating laser-induced puncture holes. The first method is preferred. In Nine onychomycosis patients treated with CO2 laser, complete resolution and healing occurred in 6, with 7 reporting mild or no pain; average healing time was 21 days. Another trial of 50 patients demonstrated good or excellent results in 70% (35/50). [35]
3.3.4 Hydration and occlusion
Hydration may increase the pore size of nail matrix, enhancing transungual penetration. Additionally, hydrated nails are more elastic and permeable. Iontophoresis studies have utilized this property to further enhance penetration. Solution pH and ionic strength have demonstrated no significant effect on nail hydration. Diffusivity of water and other materials (i.e. drugs) increases as human skin becomes more hydrated. Human stratum corneum retains up to \( \sim 300\% \) of its weight in water. [36] When SC is saturated, diffusivity increases several-fold. In contrast, Gunt et al. demonstrated that nail hydration capacity occurs at \( \sim 25\% \), only twice its normal water content of 10–15\%. Hence, unlike SC, transungual diffusivity does not dramatically increase with relative humidity (RH). Nonetheless, hydration still has a pronounced effect on drug penetration in the region of high water content (RH > 80\%). [37]

CONCLUSION
The permeability of the compact, highly keratinized nail plate to topically applied drugs is poor and drug uptake into the nail apparatus is extremely low. Topical therapy is worth pursuing however, as local action is required in many nail disorders. A review of the literature has revealed that research aimed at enhancing ungual drug uptake following topical application may be divided into three approaches: (i) understanding the physico-chemical factors that influence drug permeation into the nail plate; (ii) the use of chemical enhancers which cause alterations in the nail plate, thus assisting drug permeation; and (iii) the use of drug-containing nail lacquers which are brushed onto nail plates and which act as a drug depot from which drug can be continuously released into the nail. The field of ungual drug delivery following topical application is relatively young and more research in this field is needed to resolve the conflicting reports on the physico-chemical parameters that influence ungual drug permeation and to find and characterise new penetration enhancers and delivery vehicles. It might be possible to include enhancers within nail lacquers and to formulate water-based nail lacquers, which may hydrate the nail plate and thus assist drug permeation into the nail.
REFERENCES


