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REVIEW ARTICLE

CHALLENGES AND NOVEL APPROACHES FOR THE TREATMENT OF ONYCHOMYCOSIS

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ABSTRACT

Onychomycosis is a fungal disease of nail and approximately half of the patients of nail disorders suffers from it. The current review focuses on human nail, disease, factors affecting drug penetration for understanding the challenges in drug delivery, as well as, current and novel approaches for the treatment of onychomycosis. Human nail composed of compressed keratinized cell act as major barrier for drug penetration for treatment of damaged nail. Variety of factors affecting drug penetration through human nail like molecular weight, partition coefficient, affinity to keratin, pKa and ionization, type of vehicle, use of penetration enhancers, etc are to be taken into consideration while design and development of topical formulations. Apart from oral treatments, mechanical approaches as nail abrasion, physical approaches like iontophoresis, etching, laser, ultrasound have also been explored for penetration enhancement. Formulation scientists are working towards development of novel nano-formulations like nanoparticles, SLN, NLC, liposomes, spanlastics, nano-emulsions, etc. Lastly, the review focuses on several attempts for drug penetration studies using human nail or bovine hoof membrane.

Keywords: Onychomycosis, Nail drug delivery, Transungual formulations

INTRODUCTION

Human Nail

The human nail **(fig.1)** is comprised of bunch of keratinized cells as approximately 25 flattened dense dead layers which are tightly bound to one another, and having average thickness of about 0.5 to 1 mm and the growth rate of 2 to 3 mm per month of nail matrix in

single direction [1]. Structurally, the human nail is hard, translucent and having the convex structure, which contains alpha keratin in the fibrous form linked with cystine disulphide bonds (about 3.2% sulfar on dry basis) to provide tensile strength [2]. Nail plate can be further subclassified as dorsal, intermediate and ventral layer with ratio of 3:5:2 thick, whereby an intermediate plate layer exhibit softness and flexibility. Human nail is considered as most resistant barrier for penetration of any external material including drug molecules; and hence it become difficult to treat when affected adversely.

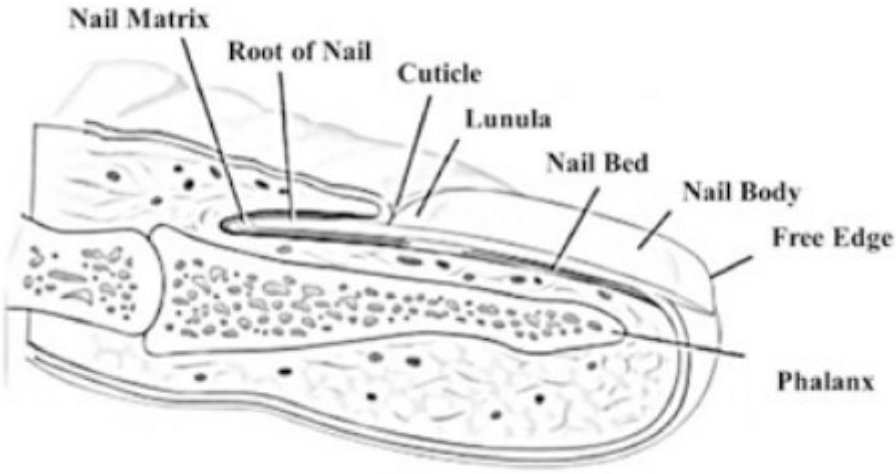

Figure 1: Anatomy of Normal Human Nail

Nail Diseases

Human nail is susceptible to wide range of disease or disorders as shown in **Table 1**, ranging from discolouration as yellow, white or red, pigmentations, brown colour bands, brittle nails, ingrowing nails, thicken nail plates, as well as infection [2]. Few of the disorder may be an adverse effect of systemic drugs as well as few reflects the diagnostic hint for other major ailment in body. Other major disease nail psoriasis is an inflammatory auto-immune disease which is observed in more than 80% of the patients having skin psoriasis [3].

Table 1 : Types of nail disorder with possible causative reason

Type of Nail Disorder	Possible causative reason
Nail Pitting	Psoriasis
Absent Part	Anonychia congenita
Cuticle invasion	Lichen pianos
Pigmentation & Ridging	Monilla
Distal Onycholysis	Tinea
Spoon Nails	Iron deficiency Anaemia
Discolored & Inverted Edges	Ectodermal dysplasia
Clubbing	Hypoxia, Malignancy or Toxins
Bitten Nails (Short)	Anxiety
Splinter Haemorrhage	Bac. Endocarditis
Yellow	Bronchiectasis, Lymphoma & Edema
Half & Half	Hepatic Necrosis
Ridging	Rheumatoid arthritis
Longitudinal Brown lines	Addisons's, Breast cancer & Melanoma
White Nails	Anemia
Red Nails	SLE, Polycythemia
Horizontal White & Pink Bands	Nephrotic Syndrome
Brittle Nails	Hypothyroidism

Onychomycosis

Onychomycosis, also known as tinea unguium, is one of the major nail infections which occurs at skin beneath the nail bed that comprises about 50% of the nail diseases, and it accounts for about one fifth of the population may develop it during life span [4]. In majority of the cases, the patient ignores it considering the cosmetic changes due to age or nature of work. If left untreated on-time, may cause discomfort as well as spread of infection to nearby tissues. Onychomycosis is originated from combining two Greek words “onyx” a nail having “mykes” a fungal infection. Onychomycosis development is observed due to microbial infections namely (i) Dermatophytes like Trichophyton rubrum as well as mentagrophytes; (ii) the Non-dermatophyte fungi like species from Acremonium, Alternaria, Aspergillus, Fusarium, Scytalidium, etc. as well as (iii) the Yeast like Candida albicans; among all, it has been observed that more than 90% were caused by Trichophyton family alone.

Onychomycosis can be further divided in to five different subtypes [5] by expert as

per the location and spread of disease. The more prevalent type of onychomycosis is Distal Lateral Subungual Onychomycosis (DLSO/DSO), causing nail bed and nail plate underside invasion. The Proximal White Subungual Onychomycosis (PWSO/PSO) is comparatively rare type and more observed in immunocompromised patients, whereby the fungal infection of proximal nail occurs through the cuticle which results as a white or yellow colour change of nail, keeping distal in ordinary condition. White superficial onychomycosis (SWO/WSO) is also a less common sub-type, shows opaque or white colour spots on the external nail plate getting amalgamate and spread over a period of time. Candida onychomycosis (CO) is observed in patients having the long-term muco-cutaneous yeast infection by *C. krusei, C. tropicalis* and *C. parapsilosis*. Lastly, most severe condition whereby the nail plate becomes thick and dystrophic is known as Total dystrophic Onychomycosis (TDO) as late-stage nail disease.

Poor hygiene conditions and/or weakened immunity are the obvious causes of nail disorder; however, the prevalence has been also observed with older age, improper cutting of nails, chronic exposure to pathogenic fungi, repeat trauma in the nail region as well as in few cases concurrent disease like diabetes; and the immunocompromised patients may observe the recurrence.

FACTORS AFFECTING NAIL PERMEATION

For the treatment of nail disorders, the drug must penetrate into the most resistant barrier of dead thick keratinized cell layer of nail plate. Traditionally, it has been observed that softening of nail through pre-treatment with aqueous solutions helps to improvise skin penetration to some extent; however, few chemical treatments have also resulted in loosening of the nail plate leading to complete detachment of nail causing traumatic condition for patient. Several drug related factors must be taken into consideration for design and development of treatment protocol.

Molecular Weight of Drug

The obvious relationship of molecular weight of drug for penetration through any membrane is inverse relation – i.e. larger molecules are difficult to penetrate; and the same in true for nail plate also. Moreover, the nail is hardest tissue of body as most resistant for penetration into or through the nail plate. Few researchers have proposed the “free volume theory of diffusion” for establishing the relationship as dependence of molecular weight on permeability to determine diffusion coefficient [6-8]. Studies revealed that hydration has increased the nail porosity, facilitated permeability of drug as reduction of resistance to diffuse by performing the comparative studies of contact with aqueous media against fully hydrated nail. Based on pharmacodynamic outcomes, it

can be correlated that drug with the molecular weight less than 350 gm per mole have exhibited desirable penetration of anti-fungal drug in onychomycosis treatment.

Partitioning of Drug Molecule

The partition coefficient (i.e. Log P) of molecule for octanol vs water has been an indicative of lipophilicity and predicts the permeation across membrane for majority of the biological membranes. However, for nail plate permeation, the studies revealed the plot of log of partition vs log of permeation resulted in zero value of slop indicating that the permeation is not dependent on lipophilicity of drug molecule. Moreover, the hydration studies have revealed that permeation is positively affected by increasing hydrophilicity of membrane, which suggest that unlike other biological membranes which are treated lipidic bilayer, the nail plate should be considered as hydrophilic hydrogel layer.

Affinity to Keratin

The keratin protein consists of major nail composition, and binding or affinity of drug to keratin is expected to significantly affect the drug performance in nail drug delivery. Binding data for drug to plasma protein are available for majority of drugs; however, very few have studied the drug binding to keratin matrix [9]. Few researchers have studied the drug binding of commonly used drug and penetration enhancers for nail treatment with either defatted keratinized powder with different drug concentration as in-vitro study, as well as, ex-vivo or in-vivo study with human nail. Study discovered that keratin binding is not only reducing drug permeability but also reduces anti-fungal activity of drug, demanding the higher concentration of drug to maintain similar activity. However, further it has been observed that keratin binding is reversible process leading to reduced activity in initial duration and providing prolonged drug action as per depletion of concentration as a function of time.

pKa & Ionization of Drug Molecule

Above factors suggested hydrophilic vehicle for nail drug delivery, leading to consideration of pka and degree of ionization of drug molecule in vehicle and its effect on site of action [10]. Uncharged molecules as well as drug at higher concentration exhibits higher permeation than charged molecules. The keratin in human nail exhibits iso-electric point between 4.0 to 5.0, i.e. it carries positive charge on nail for vehicle having pH below isoelectic point and negative charge for higher pH vehicle. The proof of concept observed by few researchers as study on miconazole, benzoic acid, pyridine, etc exhibited significant impact on diffusion and permeability by change in vehicle pH [11].

Type of Vehicle

The discussion propagates towards use of hydrophilic vehicle for nail delivery; however, it has several bottlenecks in

topical formulations as fast drain-out during routine washing or wiping and comparatively lesser adherent to nail plate. Hydrophilic solvents cause swelling of keratin fibrous to create pores and increases drug diffusion. Several studies conducted whereby comparison of use of alcohols, co-solvents, DMSO, etc which exhibited that change in solvent was not affecting the nail barrier, the drug permeation is independent of type of vehicle. It has also reported that lipophilic material and alcohol as cosolvent has reduced permeation, which may be due to deswelling of keratin fibres.

Use of Chemicals as Penetration Enhancer

The nail barrier resistance can be reduced by targeting the stability of keratin, which can be compromised by chemical attack on polar bonds, disulphides bonds, hydrogen linkages and peptide linkages in keratin structure. Urea and salicylic acid have been widely explored as nail softening agents, whereby urea act as keratolytic agent and salicylic acid is considered to dissolve the intracellular cement like glue structure [12]. However, there are several negative reports indicated absence of penetration enhancement or reduction i.e. 5-flourouracil even though softening of nail observed, which may be due to change in other factors like pH of vehicle. Sulfhydryl group containing molecules can cause disulphide cleavage like mercaptoethanol, cysteine, acetyl-cysteine, etc. Synergistic improvement in penetration have been observed by simultaneous use of urea with acetyl-cysteine.

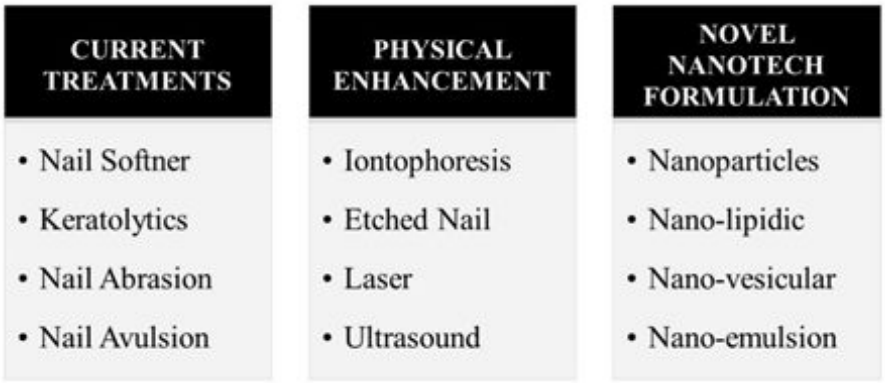

Figure 2: Treatment Approaches for Onychomycosis

CURRENT APPROACHES

Conventional Approaches

Multiple approaches for treatment of nail disorder explored includes oral delivery, physical removal of nail, use of nail-softner and penetration enhancers for topical drug delivery, etc [13,14]. Historically, the surgical removal of damaged nail for allowing fresh growth of new nail was considered as first line of treatment option; however, the panic trauma to patient needs a high level of pain threshold and currently it is considered as out-dated approach. Oral administration of drugs is still in practice by few of the prescribers, which may deliver small fraction of drug to desired site of action and may result in unwanted adverse reaction. Currently, topical administration of drug along with use nail softner &/or penetration enhancer are being practised; however, the desired outcome is yet to achieve, which indicated the need of novel approaches to increase penetration enhancement with minimal adverse effect and higher patient compliance.

Mechanical Approaches

Nail abrasion technique involves the application of rough sand paper to abrase carefully the top surface of nail plate (mainly on the edges) with appropriate intensity as per the pain threshold of patient before the application of topical formulation [15]. This technique reduces the nail thickness, removes the fungal infection mass from the surface and exposes the infected nail bed. Use of drill has also employed to make hole in nail plate for direct delivery of topical medication to lower surfaces. Total or partial nail avulsion involves surgical removal of entire nail or affected area using local anaesthesia.

PHYSICAL APPROACHES

Iontophoresis

Delivery of active moiety by creating an electromotive force across the membrane is the key principle of iontophoresis technique, which has been widely explored for transdermal, ocular, dental, orthopaedic, etc fields with an aim of penetration enhancement compared to conventional topical delivery. Multiple variation possible as per type of membrane, active moiety as permeant, solvent vehicle and additives, which may lead to interaction between electric field and ionic charge of molecule, creation of flow pathway, pore induction, etc. Researchers have explored iontophoresis for transungual drug delivery for several molecules and reported as multi-fold improvement in penetration compared to passive diffusion [10, 16-18]. The studies have varied the pH of solvent, ionic strength, buffer concentration, current density, etc and reported their significant impact on permeation.

Pre-treatment as Etching

The damaged nail is etched by applying phosphoric acid solution or gel as surface modifying agent to create micropores on

dorsal surface as pre-treatment process before topical application of conventional formulation [19]. Researchers have supported the significant change in etched nail as increase in roughness factor compared to untreated nail using advance microscopic techniques, as well as multi-fold rise in permeation of conventional topical formulation. Prolong bioadhesion of sustained release film was observed as added advantage due to higher roughness to increase the therapeutic outcome.

Laser and Photodynamic Therapy

The laser exposure to damage nail with or without presence of carbon dioxide have been explored by researchers. Daily application of topical drug on laser treated nail have shown rapid healing of disease with mild of no pain. The targeted delivery to affected nail area by avoiding peripheral tissue damage with high efficacy and minimal discomfort are the key merit of laser treatment [20]; however, high cost, need of sophisticated equipment, and long-term photo ageing would be bottlenecks of same. In the similar approach, light source is impacted on the fungal infected area by applying photosensitizer, which in-turn generate oxygen species locally to destroy fungus growth. The technique offers high efficacy and sensitivity by avoid drug interactions; but may also cause pain, burning and erythema.

Ultrasound

Phonophoresis is a non-invasive technique which employs application of ultrasound to create temporary micropores to enhance topical drug delivery. It has been a proven technique for transcellular delivery in joints, muscles and nerves; and hence recommended for nail delivery by several researcher groups; however, no detailed documented proof of concept for ungual delivery available.

NOVEL NANOTECH-BASED FORMULATION

Nanoparticles

In past few decades, nanoparticles have been explored for many drugs having solubility &/or permeability, as it offers many advantages faster penetration due to nano-scale and solubility improvement due to higher surface area. Several techniques have been employed by scientists as top-down approaches (reducing micro-scale particles to nano-scale) as well as bottom-up approaches (precipitation, spray drying, emulsion solvent evaporation, salting-out, etc). Matrixing of drug in to polymeric nanoparticles offers advantage of sustained release. Researchers across the globe have attempted nanoparticles [21-23] of anti-fungal drugs and delivered as suspension or embedded into gel formulation. Study revealed that in-depth penetration was observed with nanoparticles for 1 week of treatment compared to conventional. Nanoparticles of pullulan exhibited lesser irritation and proven safe for prolong treatment.

Lipidic Nano-Formulations

Lipidic nano-formulations have gain a lot of attention due to various merits offered like ease of formulation, biocompatibility, biodegradation, improved stability (photo-degradation and oxidation) of drug by engulfing into lipid matrix, etc. Although, the hydrophilic formulations recommended for nail delivery, researchers have explored using lipidic nano-formulations along with other physical or chemical approaches of penetration enhancement and showcased the improved result against conventional formulations. Solid lipid nanoparticles (SLNs) and its extended version Nanostructured lipidic carriers (NLCs) have been formulated for antifungal drugs for nail delivery and co-administered with urea as penetration enhancer. Formulation scientists observed that water dissipation has been reduced because of solid lipid matrix, which indicated that the occlusivity of SLN was higher comparted to that of NLC [24].

Nanovesicular Formulations

Liposomes as unilamellar or multilamellar vesicular systems designed majorly using phospholipids and cholesterol as bilayers, which allows amphillic systems for hydrophilic drug as well as hydrophobic drug encapsulation. Ethosomes (i.e. use of ethanol in liposomes) and liposomal poloxamer gel formulation have been studied and found superior for nail penetration. Flexible nanovesicular systems also known as transferosomes and spanlastics formulations using surface active agents like tween and span have proved almost double penetration than that of marketed preparation [25,26]. In another study, the co-administration of keratolytic agents have showcased improved therapeutic compared to marketed formulation.

Nanoemulsion

The microemulsion or nanoemulsion formulated with blend of surfactant and co-surfactants or co-solvents to enclose the hydrophobic drug containing oily globules at nano-scale in hydrophilic vehicle. Improved solubility, stability and penetration enhancement of nanoemulsion have been proven superior to that of lesser steady liposomal formulations [27-29]. Additionally, improvement in antimycotic actions were also reported due to drug solubilization in nanoglobules. Formulation scientists have further embedded into gel as nanoemulgel [30] to improve retention for better therapeutic outcomes.

CHARACTERIZATION OF TRANSUNGUAL FORMULATION

The physicochemical characterization of the transungual formulations like nail lacquer, solutions, gel, cream, ointment, spray, film, emulsion, etc would be as per standard protocols. For nano-based formulations the size specific characterizations shall include particle size distribution, zeta potential, use of advanced microscopic techniques like SEM, TEM, AFM, etc. The antifungal

activity of the drug can be tested using growth inhibition studies in well-planned protocol in micro-biotech labs. Recent advancement in the field is TurChub® cells developed by MedPharm (UK) as modified franz diffusion cell containing human nail as barrier for drug delivery against reservoir compartment containing fungus grown in agar media [31]. The major area of focus for performance of formulation as nail delivery is to ensure penetration of drug, which can be characterised by in-vitro, ex-vivo or in-vivo evaluation using animal or human nail.

Nail Penetration Study

It is always desirable to characterized formulation on human nail plates for getting real analysis; however, it is difficult to manage the human nails in large quantity as comparative and repeat studies, as well as, nail clippings are generally hard, curve and small in size that are difficult to handle in experimental set-up to get high level of accuracy of method [32]. Alternatively, the scientists are using the bovine hoof [33] as biomimicking nail plate membrane to overcome above issues of human nail, but the differences swelling capacity, density of keratin layers, complexity of network, resistance to drug penetration, etc. are to be taken into consideration. Studies have proven that human nail are more resistant than bovine hoof membrane and researchers have also established the mathematical relationship for same to extrapolate the findings [8]. The attempts were also made to develop the biomimicking human nail using human hair keratin as film with adequate resistance. Majority of the characterizations employs use of modified franz diffusion cell whereby drug is expected to be travelled from formulation to pass through nail plate membrane barrier hold in diffusion cell, and drug concentration were measured in reservoir compartment at regular interval ranging from hours to days [34]. Modification to technique also includes pretreatment as hydration by soaking human nail, use of chemical or physical techniques for penetration enhancement; as well as use of cotton ball beneath the nail plate to keep it continuously hydrating.

CONCLUSION

Nail diseases are common yet not addressed much for innovations in novel formulation, novel treatments and detailed clinical investigations, due to limited drug suitable for penetration to most resistant barrier as nail plates. Detailed investigation of human nail properties, factors affecting nail penetration, drug suitability study, etc may open the newer avenue for young and budding researchers. The use of nanomaterials in the formulation may raise a hazard-related concern; however, for nail delivery, the risk is very low due to comparatively less vascularisation than that of nasal, mucosal, ocular, etc. A topical formulation for treatments of onychomycosis will have less stringent regulatory requirements compared to oral delivery. Employing novel physical,

chemical and formulation approaches may be offer promising outcomes; however, establishing standardized protocol for characterization and in-vitro in-vivo correlation are future requirements.

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