

UNDERSTANDING OUR NATURAL NAIL – ANTIFUNGAL AGENTS

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ABSTRACT

The body normally hosts a variety of microorganisms, including bacteria and fungi. Some are useful and others may cause infections. Fungi can live on the dead tissues of the hairs, nails. Continuous exposure of nail to warm, moist environments usually develops nail infection. Nails are the hard and durable epidermal appendages structurally horny in nature. Nail plate is responsible for the penetration of the drug across it. There are number of formulations with antifungal agents viz. gels, creams and oral antifungals for the treatment of transungual infections. Among these medicated nail lacquers is a new concept in treating nail infections. These are effective as monotherapy in treatment of superficial, distal and subungual diseases. The main purpose of these preparations is to protect the nail plate and enhance beauty of nails. These preparations are generally used in fungal diseases. This system avoids oral toxicity of anti-fungal drugs. The main challenge associated with developing nail lacquers for nail disorders is to deliver the active concentration to the site of infection which is often under nail. The effectiveness of topical therapies is limited due to minimal drug permeability through the nail plate. Nail permeability is quite low and limits topical therapy to early/mild disease states such as Onychomycosis, Leuconychia, Onychogrypos and Onychotrophia etc. Penetration of topical antifungal through the nail plate requires a vehicle that is specially formulated for transungual delivery. Recent focus is emphasizing on development of a promising antifungal treatment in the form of nail lacquer owing to its beneficial advantages.

Keywords: Antifungal, Nail, Nail lacquer, Onychomycosis, Psoriasis, Transungual.

INTRODUCTION

Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of the pharmacological or other effect of the drug to the surface of the skin or within the skin. The nail is a horny structure. Nail plate is responsible for penetration of drug across it. As it is hard enough the penetration becomes difficult, only a fraction of topical drug penetrates across it. Hence the effective therapeutic concentration is not achieved. The nail plate may appear abnormal as a result of decreased growth.¹

Onychomycosis (Tineaunguium) is a fungal infection of the nail bed or nail plate. Among superficial infections, Onychomycosis is the most difficult to manage and eradicate and it tends to recur. These diseases can be cured by achieving desired therapeutic concentration of drug by nail drug delivery system. In order to successfully deliver active pharmaceutical ingredients (APIs) across the nail it is necessary to consider the anatomy and physiology of barriers. To obtain the right amount of drug to the right place at the right time more effectively, newer drug delivery approaches could be utilized to maximize the effectiveness of the API.² The benefits include first pass avoidance, convenience non-invasiveness, drug targeting to the site of action, elimination of systemic adverse events and drug interactions, increased patient compliance, possibly reduced cost of treatment and sustained release. Medicated nail lacquers are formulations that are used for transungual drug delivery system for the drugs that exhibit poor oral bioavailability, for maximal antifungal efficacy and to avoid oral toxicity of fungal drugs. Topical nail preparations like lacquers, varnish, enamel etc. are generally used to imparting colour and luster to nail.²

The film on the nail surface acts as a drug depot that permits optimized and sustained diffusion across the nail and leads to continuous penetration of active principle to high tissue concentration required for the efficacy for the treatment for Onychomycosis.^{1,2,3} Oral treatment has the limited use of some of the more potent antifungal drugs such as itraconazole and ketoconazole. The nail plate is too thick and too dense for drugs to penetrate. Although nail is similar to stratum corneum of the skin in that it is derived from epidermis, it is mainly composed of hard keratin (highly disulfide linked) and approximately hundred folds thicker than stratum corneum. The permeability of the drug can be enhanced in order to deliver sufficient amount of drug. The

permeation related properties of the nail differ from those observed in stratum corneum, primarily in three aspects:

1. The total lipid content of the nail is much less than the lipid content of stratum corneum.
2. The nail has high sulphur content (cysteine) in its hard keratin domain than stratum corneum.
3. Under the average condition the nail contains much less water than the stratum corneum.⁴

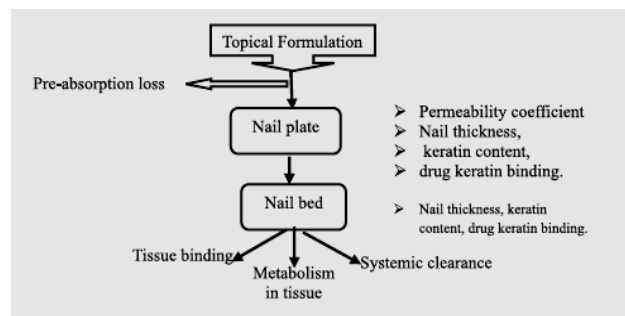


Fig.1: The fate of the drug following topical application to the nail plate.

ANATOMY OF NAIL

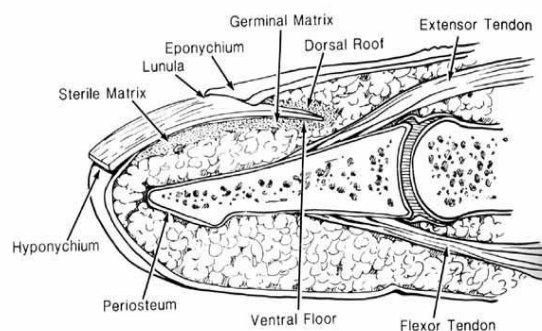


Fig.2: Schematic structure of nail

The nails are composed of flat, horny scales which form protective covering for the distal of the finger & toes.⁵ Each nail consist of following parts –




- **Matrix (matrix unguis, keratogenous membrane, nail matrix, onychostroma)⁶:** It is the tissue (or germinal matrix) upon which the nail rests⁷, the part of the nail bed that extends beneath the nail root and contains nerves, lymph and blood vessels⁸. The matrix is responsible for the production of the cells that become the nail plate. The width and thickness of the nail plate is determined by the size, length, and thickness of the matrix. The shape of the fingertip itself determines if the nail plate is flat, arched, or hooked.⁹
- **Lunula("the moon"):** It is the visible part of the matrix, the whitish crescent-shaped base of the visible nail.¹⁰The lunula is largest in the thumb and often absent in the little finger.
- **Nail bed:** It is the skin beneath the nail plate.¹⁰It is composed of two types of tissues.
 1. The deeper dermis - the living tissue fixed to the bone which contains capillaries and glands.¹¹
 2. The superficial epidermis - the layer just beneath the nail plate which moves forward with the plate.
- **Nail sinus (sinus unguis):** It is the deep furrow into which the nail root is inserted.⁷
- **Nail root (radix unguis):** It is the part of nail situated in the nail sinus⁷. It originates from the actively growing tissue below, the matrix.⁸
- **Nail plate (corpus unguis)⁷:** It is the actual nail, translucent keratin protein made of amino acids. In the nail it forms a strong flexible material made of several layers of dead, flattened cells.⁹ The plate appears pink because of the underlying capillaries.¹¹
- **Free margin (margo liber):** It is the anterior margin of the nail plate corresponding to the abrasive or cutting edge of the nail.⁷





- **Hyponychium ("quick")¹²:** It is the epithelium located beneath the nail plate at the junction between the free edge and the skin of the fingertip. It forms a seal that protects the nail bed.⁸
- **Onychodermal band:** It is the seal between the nail plate and hyponychium. It can be recognized by its glassy, greyish color (in fair-skinned people).⁹
- **Eponychium:** It is the small band of epithelium that extends from the posterior nail wall onto the base of the nail.⁷It is called the "proximal fold" or "cuticle". Together, the eponychium and cuticle form a protective seal. The cuticle on the nail plate is dead cells but the eponychium is living cells.¹³
- **Perionyx:** It is the projecting edge of the eponychium covering the proximal strip of the lunula.⁷
- **Nail wall (vallum unguis):** It is the cutaneous fold overlapping the sides and proximal end of the nail.
- **Lateral margin (margolateralis):** It is lying beneath the nail wall on the sides of the nail and the nail groove.⁷
- **Paronychium:** It is the border tissue around the nail¹⁴ and paronychia is an infection in this area.

Functions & Growth

A healthy nail protects the distal phalanx, the fingertip, and the surrounding soft tissues from injuries. It also serves to enhance precise delicate movements of the distal digits through counter-pressure exerted on the pulp of the finger.¹⁵The nail acts as a counterforce when the end of the finger touches an object, thereby enhancing the sensitivity of the fingertip.¹⁶ The growing part of the nail is the part still under the skin at the nail's proximal end under the epidermis, which is the only living part of a nail. In mammals, the length and growth rate of nails is related to the length of the terminal phalanges. In humans, the nail of the index finger grows faster than that of the little finger and fingernails grow up to four times faster than toe nails.¹⁷

Table1: Diseases of nail¹

Disorders	Description of Disorder	Treatment available	Images of disorders
Green-nail syndrome	It is caused by Pseudomonas, a harmless infection, usually of 1 or 2 nails.	Patients should soak their affected nails with 1% acetic acid solution or alcohol diluted 1:4 with water twice a day for 10 min and avoids trauma and excess moisture. Frequent clipping increases the response to treatment.	
Onychogryphosis	It is a nail dystrophy; most often the big toe becomes thickened and curved.	Treatment consists of trimming the deformed nails.	
Subungual hematoma and nail bed trauma	It occurs when blood becomes trapped between the nail plate and nail bed, usually as a result of trauma. It causes significant pain, eventual separation and temporary loss of the nail plate.	If the injury is acute, nail trephination (e.g., creating a hole in the nail plate using a cautery device, 18-gauge needle, or red-hot paperclip) can help relieve pain by draining accumulated blood.	

Onychomycosis	It is a fungal infection of the nail plate, nail bed. Both toe nails are 10 times more commonly infected than fingernail.	Topical antifungal nail lacquer containing Ciclopirox 8% or Amorolfine 5% is used as an adjunct with oral drugs like terbinafina and itraconazole.	
Psoriasis	Irregular pits and oil spots on nail.	Corticosteroids, salicylic acid, calcipotriol or Tazarotene may be given alone or in combination.	
Onychotillomania	Patients pick at and self-mutilate their nails which can lead to parallel transverse grooves and ridges.	---	
Pincer nail deformity	It is a transverse over-curvature of the nail plate.	----	

MAJOR CHALLENGES

- The nail plate is thicker and harder because of the stable disulphide bonds which restrict drug penetration. Potential penetration enhancers can be used to permeate formulations inside the nail barrier.¹⁸
- It is essential to consider the physicochemical properties of the drug molecule, formulation characteristics, possible interactions between the drug and keratin and possible penetration enhancer when designing topical nail formulations.¹⁹
- In oral antifungal therapy, liver function tests have to be performed regularly. Such therapies are costly and hindered by poor patient compliance. Thus topical therapy remains the treatment of choice.¹⁹

Factors which influence drug transport through the nail plate

- Molecular size of compound/diffusing species:** It plays a major role in determining the permeability of compounds through the nail. The logarithm of the permeability coefficient decreases as the molecular weight increases. Thus for optimal unguinal permeation, drug molecules must be of small size and carry no electric charge on them.
- Degree of ionization:** The nail plate is less permeable to ionic compounds than non-charged equivalents.
- Nail plate hydration:** The permeation of ketoconazole through excised human nails under different relative humidities (RH) from

15 to 100% showed a 3-fold improvement in the delivery of the radio labeled drug.

d) **Presence of an intact dorsal layer:** Very thin dorsal layer with its overlapping cells represents the greatest barrier to the drug penetration across the nail plate. If this layer is partially or totally removed by debridement or chemical etching with 30-40% phosphoric acid or use of keratinolytic enzymes, then drug permeability increases.

e) **Binding of the drug to keratin and other nail constituents:** Keratin have a PI of around 5 and is positively and negatively charged at pH below and above this res., it therefore may bind or repel molecules depending on their charge. This may be part of the reason for the lower nail permeability of ionic compounds.

f) **Formulation effects:** pH affects the degree of ionization of weak acids and bases which decreases their permeability through the nail plate. The nature of the solvent will also affect nail hydration. It affects their solubility in formulations, their ability to partition into the nail plate and their interactions with keratin. Theoretically, aqueous based formulations should provide the best delivery. Lacquers facilitate delivery by drying to form a depot of drug and assist its hydration by reducing transonychia water loss.²⁰

g) **Nail thickness and presence of disease:** The thicker the nail the more difficult it will be for drugs to reach the nail bed.

Enhancement of nail penetration

Nail penetration can be enhanced by following methods:

1. Mechanical method.
2. Chemical method.
3. Physical method.

Mechanical modes of penetration enhancement are straight forward and have the most in vivo experience associated with them. The goal of topical therapy for Onychomycosis is drug penetration into deep nail stratum at amounts above the minimal inhibitory concentration (MIC).

There are two main factors to consider:

1. Physicochemical properties of the drug.
2. Binding of the drug to keratin within the nail.²¹⁻²⁵

Mechanical methods

They are invasive and potentially painful.

a) Nail avulsion

Removal of the entire nail plate or partial removal of the affected nail plate is done surgically by total nail avulsion and partial nail avulsion and under local anesthesia. Keratolytic agents like urea and salicylic acid soften the nail plate for avulsion. Urea or combinations of urea and salicylic acid have been used for nonsurgical avulsion (chemical avulsion) prior to topical treatment of Onychomycosis.²⁶

b) Nail abrasion

It involves sanding of the nail plate to reduce thickness or destroy it completely prior to lacquer treatment to decrease the critical fungal mass. Sandpaper number of 150 or 180 and instrument of high-speed (350,000 rpm) sanding hand piece can be utilized. Additionally, dentist's drills have been used to make small holes in the nail plate, facilitating topical medication penetration. The procedure may be repeated for optimal efficacy.²⁷

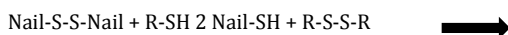
Chemical methods:

a) Keratolytic Enhancers or nail softening agents

In the absence of keratolytic agents such as papain, urea and salicylic acid no transungual antifungal permeation was detected over a time period. Pre-treatment with the use of both 15% papain (for 1 day) followed by 20% salicylic acid (for 10 days) enhanced antimycotic permeation.²⁸

b) Compounds containing sulfhydryl groups

Compounds which contain sulfhydryl (SH) groups such as acetyl cysteine, cysteine, mercapto ethanol can reduce, thus cleave the disulphide bonds in nail proteins which are responsible for nail integrity, as shown in the reaction sequence below:



R represents a sulfhydryl-containing compound.

The structural changes were irreversible.^{29,30}

c) Keratinolytic enzymes:

Keratinase may hydrolyze nail keratins, thereby weakening the nail barrier and enhancing transungual drug permeation. It act on both the intercellular matrix that holds the cells of the nail plate together and the dorsal nail corneocytes by corroding their surface.³¹

d) 2-n-nonyl-1, 3-dioxolane

Penetration of econazole into the human nail has been achieved by the use of 2-n-nonyl-1, 3-dioxolane. Studies reported that Econazole penetrates the nail six times more effectively in a lacquer containing 2-n-nonyl-1, 3-dioxolane than in an identical lacquer without enhancer.

Econazole concentration in the deep nail layer was 14,000 times greater than the Minimum Inhibitory Concentration necessary to inhibit fungal growth.³²

Physical methods

Physical permeation enhancement may be superior to chemical methods in delivering hydrophilic and macromolecular agents.

a) Carbon dioxide laser

CO₂ laser may result in positive but unpredictable results. Two methods were suggested so far;

- i. One method involves avulsion of the affected nail portion followed by laser treatment at 5000W/cm².
- ii. Second method involves penetrating the nail plate with CO₂ laser beam and followed by topical antifungal treatment, penetrating laser-induced puncture holes. The first method is preferred.³³

b) Hydration and occlusion

Hydrated nails are more elastic and permeable and may increase the pore size of nail matrix, enhancing transungual penetration.³⁴

c) Electroporation

The application of an electric pulse of about 100–1,000 V/cm creates transient aqueous pores in the lipid bilayers making the solute particles permeable through it.³⁵

d) Micro needle

It involves using arrays of microscopic needles to open pores in the SC directly to the skin capillaries. It has the advantage of being too short to stimulate the pain fibers, thus facilitating drug permeation.³⁵

e) Etching

It is done by exposing the nail with surface-modifying chemical (e.g. phosphoric acid). It results in the formation of profuse micro porosity. These micro porosities increase wettability and surface area and decrease contact angle.

They provide an ideal surface for bonding material. Additionally presence of micro porosities improves "interpenetration and bonding of a polymeric delivery system and facilitation of inter diffusion of a therapeutic agent".³⁶

f) Iontophoresis

It involves the application of electric field for the delivery of a compound across a membrane. Drug diffusion through the hydrated keratin of a nail may be enhanced by iontophoresis.

Factors that contribute to this enhancement include electro repulsion/electrophoresis, electro osmosis and permeabilization/electroporation. Iontophoretic trans-nail flux improved with higher SA concentrations (up to 2 mg/ml), higher current density (up to 0.5mA/cm²), higher buffer ionic strength (optimal strength at 50–100 mM), and higher pH.

Increased transungual glucose and Griseofulvin flux with higher pH (pH > 5) in anodal iontophoresis. pH dependent transport due to cathodal iontophoresis followed the opposite trend (i.e. lower pH correlated with increased flux). Griseofulvin transport was enhanced ≈8-fold with iontophoresis.³⁷

g) Phonophoresis

It may result in improved penetration by the application of ultrasound waves through the SC transcellularly via increased pore size.³⁸ It has been used to enhance percutaneous penetration to joints, muscle, and nerves.

Advantages (and iontophoresis) include enhanced drug penetration, strict control of penetration rates, rapid termination of drug delivery and lack of immune sensitization.

h) Ultraviolet light

Heat and/or ultraviolet (UV) light are used to treat onychomycosis³⁹ and subsequently treating with topical antifungal therapy.

i) Photodynamic therapy of onychomycosis with aminolevulinic acid

Photodynamic therapy (PDT) is based on the combination of a sensitizing drug and a visible light used together for destruction of cells. PDT with aminolevulinic acid (ALA) is used in oncological field. It is used for onychomycosis. This would negate the need for prolonged topical or systemic treatment regimens, with their associated poor success rates and potential for drug resistance, side effects, drug–drug interactions, and increased morbidity.⁴⁰

j) Use of nail clippings as a model of nail penetration

Nail clippings have been previously used as a model for the human nail plate. It is easier to obtain nail clippings from healthy volunteers and use them for in vitro testing. It might not be the best model for nail studies.⁴¹



Fig.3: Nail support and incubation system: (a) Teflon one chamber diffusion cell components, (b) Teflon one-chamber diffusion cell top view, and (c) Teflon one chamber diffusion cell side view.

Table 2: Developed formulations for nail disorders.⁴²

Sl. No	Name of the product	Name of Drug	Uses/Indications	Name of Company
1	Eco-Nail nail lacquer	5% econazole +18% SEPA nail lacquer	Promotes the release of econazole from dried lacquer film, creating a large chemical gradient at the lacquer nail interface, to drive econazole into the deep nail plate. SEPA acts as a percutaneous penetration enhancer.	MacroChem Corporation
2	Loceryl nail film	Antifungal drug, Amorolfine	A non-water-soluble film of Amorolfine formed on the nail plate, and this film remains in place for 1 week. It contains a high concentration of drug and forms a depot from which the drug is delivered and allows the drug to permeate the nail plate.	Galderma Australia Pty Ltd
3	Umecta nail film	Urea 40%	Psoriatic nails, brittle and thick nails, and Calluses	JSJ Pharmaceuticals
4	Tazorac 0.1% gel	Tazarotene	Used in the Treatment of Fingernail Psoriasis	Allergan Inc.
5	Zalain nail patch	Sertaconazol Nitrate	Once-a-week nail patch for treatment of onychomycosis&onychodystrophy	Labtec
6	Penlac nail lacquer	Ciclopirox topical solution	A broad-spectrum antifungal medication that also has antibacterial and anti-inflammatory properties	Dermik Laboratories Inc.

EVALUATION PARAMETERS

1. Nonvolatile content

Pre-determined weight of sample is taken in a glass petridish. Samples are spread evenly with the help of tared wire. The dish is placed in the oven at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 1 hr. After 1 hr. the petridish is removed, cooled and weighed. The difference in weight of sample after drying is determined.

2. Drying time

A film of sample is applied on a glass petridish with the help of brush. The time to form a dry-to-touch film is noted using a stopwatch.

3. Smoothness of flow

The sample is poured to approximately 1.5 inches and spread on a glass plate and made to rise vertically.

4. Gloss

Gloss of the film is visually seen, comparing it with a standard marketed nail lacquer.

5. In vitro studies

Diffusion studies are performed using artificial membrane (Gelman Laboratory) of pore size $0.2 \mu\text{m}$. The membrane is soaked for 1 h in solvent system A (phosphate buffer, pH 7.4; and methanol, AR grade, in the ratio of 4:1), and the receptor compartment is filled with solvent A. Test vehicle equivalent to $200 \mu\text{g}$ is applied evenly on the surface of the membrane. The prepared membrane is mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly is maintained at 37°C , and the speed of stirring is kept constant (600 rpm) for 7 h. The 2 mL aliquot of drug sample is taken after a time interval of 1 h and replaced by the fresh solvent A. The experiment is performed in triplicate.

6. In vitro transungual permeation studies

Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, are soaked in distilled water for 24 h.

Membranes of about 1-mm thickness are then cut from the distal part of hooves. In vitro permeation studies are carried out by using Franz diffusion cell (respective volume, 25 mL), the hoof membrane is placed carefully on the cell, and the surface area available for permeation was 1.23 cm². Then the test vehicle equivalent to 200 µg is applied evenly on the surface of the nail membrane. The receptor compartment is filled with solvent A (phosphate buffer, pH 7.4; and methanol, AR grade, in the ratio of 4:1), and the whole assembly is maintained at 37°C with constant stirring (600 rpm) for 30 h. The 2-mL aliquot of drug sample is taken after a time interval of 1 h and is replaced by the fresh solvent A.^{43,44}

RECENT ADVANCES IN NAIL DELIVERY

Apart from the traditional formulations like nail lacquers, nail varnish, and nail patches recent technologies are introduced in the development of more efficient drug delivery.

a) Electro chemotherapy

This therapy is developed as an active method to deliver the drugs across the nail plate which in turn to increase the success rate of topical monotherapy and decrease the duration of treatment of nail disorders.

b) Mesosclissioning technology

It creates a micro-conduit, fully open pathways of 300-500 microns in diameter through the nail without sensation. These pathways are used to deliver drugs across the membrane (in vivo human experiments have shown full anaesthesia occurs within 3 minutes through micro conduits). In nails, micro conduits reduce the painful pressure of subungual hematoma (black toe) and could serve as a prophylactic to prevent such pressure build-up in runner's nails.



Fig.4: Mesosclissioning technology

c) Nanopatch nail fungus

Electrochemistry and targeted drug delivery are used Nanopatch fungus AC/DC to actively push antifungal drugs right through the nail cuticle to the actual location of the fungus growth. This would be the first treatment option to directly target nail fungus at its source of growth.⁴²

CONCLUSION

Topical delivery of systemic therapeutics offers benefits but presents a greater technical challenge. Drug transport into the nail plate can be done by filing the nail plate before topical application of drug formulations as well as by the use of chemical enhancers. The permeability of the highly keratinized nail plate to topically applied drugs is poor and drug uptake into the nail apparatus is extremely low. Enhancing the unguinal drug uptake following topical application may be divided into three approaches: first understanding the physico-chemical factors that influence drug permeation into the nail plate; second the use of chemical enhancers which cause alterations in the nail plate, thus enhancing drug permeation; and third the use of drug-containing nail lacquers which are brushed onto nail plates and which act as a drug depot. The nail plate behaves like a concentrated hydrogel to permeating molecules and diffusion of drug through the nail plate. Thus, for optimal unguinal permeation and uptake, drug molecules must be of small size and be uncharged. The review would help to design and develop novel drug

delivery system. This new approach could be beneficial to overcome the problems associated with oral antifungal agents.

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