INVASOMES: A NOVEL APPROACH FOR TREATMENT OF FUNGAL INFECTIONS

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Abstract

Topical drug administration is a localised drug delivery strategy that can be used anywhere in the body via ophthalmic, rectal, vaginal, and cutaneous channels. The skin is one of the most easily accessible organs on the human body for topical administration and serves as the primary channel of the topical medication delivery system. Fungus causes a variety of skin illnesses. An antifungal drug is a fungicide that is used to treat mycoses such as athlete's foot, ringworm, and candidiasis. Antifungals function by leveraging differences between mammalian and fungal cells to eliminate the fungal organism without causing harm to the host. Antifungal medicines are used to treat fungal infections. However, it has a limited bioavailability (55%) due to its low water solubility and first pass impact. Also. Many medications rely on the stratum corneum, the skin's outer layer, as a skin penetration barrier. Several strategies have been developed to circumvent this barrier, including the use of vehicles and nanocarriers to increase medication penetration. Recently, several types of nanocarriers have been devised to improve the dermal and transdermal administration of drugs like 'invasome'. This study provides a concise summary of fungal infections, invasome as a novel drug delivery method, formulation & evaluation of invasome, and marketed invasome formulations for treatment of various diseases.

Keywords: Fungal infections, Nanocarries, Novel drug delivery system, invasome.

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Introduction

Fungal infections are becoming increasingly common, posing a significant challenge to healthcare practitioners. This rise is directly related to the expanding population of immune compromised people as a result of medical practise changes such as the usage of intensive chemotherapy and immunosuppressive medicines. Most fungi are widespread, successfully reproducing in their natural surroundings without the use of human or animal substrates. In humans, however, some species are adventitious pathogens, causing superficial, subcutaneous, or systemic infection (Rex *et al*., 1998). The majority of fungi that cause systemic (or deep-seated) infection do so through direct inhalation into the lung or invasion of a wound site. Others, such as Candida albicans, are commensal occupants of the gastrointestinal tract and skin that can grow and move into the systemic circulation under certain situations, such as when introduced into the body via medical devices such as vascular catheters (Wisplinghoff *et al*., 2004; Dixon *et al*., 1996).

The current rise in invasive fungal infections is due to changes in disease management, which include the use of powerful immunosuppressive agents, multiple antibiotics, organ support procedures such as mechanical ventilation, hemodialysis, and venovenous hemofiltration, and parental hyperalimentation. These pharmacological and surgical breakthroughs, together with the use of more powerful antineoplastic therapy and the transplantation of persons with preexisting cardiac, renal, and hepatic disease processes, have altered the frequency and approach to fungal infections (Fleming and Walsh, 2002). In particular, as a result of these discoveries and therapeutic triumphs, the population at risk for fungal infections has grown significantly. Systemic candidiasis was recognised as a significant medical concern in the early 1980s. The mortality rate related with candidiasis rose gradually until 1988, when it peaked at 0.6 per 100,000 people (Vallabhaneni *et al*., 2016; Benedict *et al*., 2022).

Fungal infections have significant direct costs. Candida infections have a global cost that is 2.5 times that of Aspergillus infections. When the global cost is adjusted for the number of people affected, the individual cost for those with Aspergillus infection is 2-3 times higher than for those with candidiasis (Hay *et al*., 2014).

Transmission of fungal infection

Fungi found in soil, air, and water, as well as on plants, animals, and people, cause fungal illnesses. Fungal infections are spread to healthy people by coming into touch with fungal spores. It is spread through contact with infected soil, water, or air. Fungal transmission can also occur from person to person; however, in the case of skin diseases, the chances of person to person transfer are higher. Fungal infections are usually not significant, but they can be serious or even fatal in immune compromised patients, such as those suffering from AIDS.

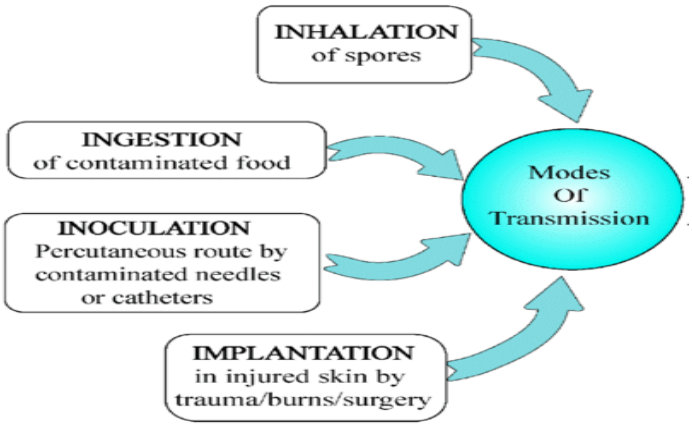

Figure 1: Mode of transmission of fungal disease

The ability to use keratin as a nutritional supply is shared by all dermatophytes. Keratins are structural proteins present not just on animal and human hosts, but also in the environment, as keratinous structures are regularly lost as part of the body' renewal process. Depending on their preferred niche, dermatophyte species are classified as geophilic, zoophilic, or anthropophilic; interestingly, clinical infection tends to be more severe, driven by both fungus-driven host tissue destruction and overt inflammation, if dermatophytes colonise a human or animal to which they are not adapted. For example, many zoophilic dermatophytes, such as Microsporium canis and Trichophytum benhamiae, can be found on asymptomatic animal

hosts while causing inflammatory reactions in humans. This shows that these fungi's particular adaptation to natural host species results in commensal behaviour, whereas in untypical hosts, the immune response and possibly altered fungal behaviour drive inflammation, resulting in clinical illness (Hube *et al*., 2015).

Immune response against fungal infections

Innate response

The innate response to fungi is designed to be as efficient as possible, and it also induces various adaptive immune system responses. Physical barriers, such as the skin and mucosal epithelial surfaces in areas of the body that are frequently exposed to environmental organisms, such as the mouth, upper airways, gastrointestinal and genitourinary tract, are the first lines of defence. The epithelium also plays a crucial role in actively distinguishing commensal fungus, such as Candida albicans, which can be pathogenic or nonpathogenic (Borghi et al., *2014*; Cutler et al., *2007*).

Adaptive response

T-cells must be activated once the innate immune system has been stimulated for effective clearance and the formation of protective immunity against fungus. As a result, the majority of invasive fungal infections arise in the presence of T-cell depletion. Cytokines generated by APCs such as DCs and macrophages are essential for the differentiation of CD4+ T-cells (T-helper cells) (Hamad, 2011).

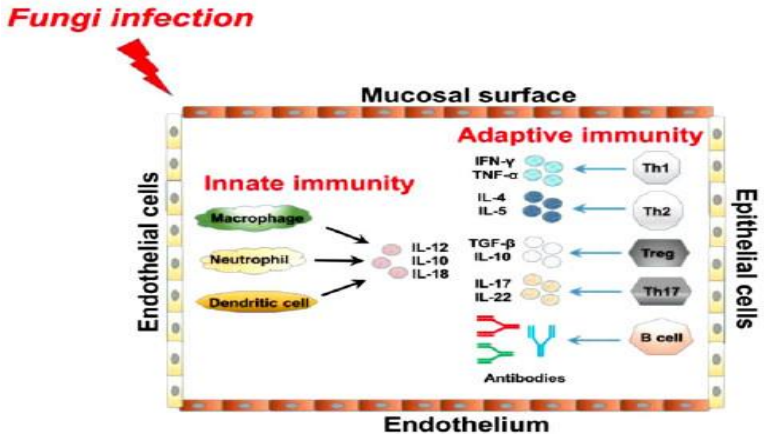

Figure 2: Immune responses in fungal infection

Current treatments available

The ability to target the infection site, lower risk of systemic adverse effects, increased treatment success, and high patient compliance are all advantages of topical treatment of fungal infections. A variety of topical antifungal medicines have been used to treat a variety of dermatological skin infections. Polyenes, azoles, and allylamine / benzylamine are the three primary groups of topical antifungals. These antifungal drugs are currently available in traditional dose forms such as creams, gels, lotions, and sprays. Drug penetration through the target tissue determines the efficiency of topical antifungal treatment. As a result, effective medication concentration levels in the skin must be determined. When antifungals are given topically, the drug components must pass through the stratum corneum, the outermost layer of the skin, to reach the lower levels, notably the viable epidermis (Meis *et al*., 2001; Spitzer & Robbins, 2017).

Topical drug delivery system for treating fungal infection

For thousands of years, people have applied ointments and lotions on their skin for cosmetic and therapeutic purposes. Currently, more than one-third of medications in clinical trials are connected to distribution into or through the skin. In terms of ease of application and patient accessibility, skin delivery is an ideal route of drug administration for achieving local (dermal) or systemic effects (Transdermal delivery).

Transdermal drug delivery systems are self-contained discrete dose forms that, when applied to intact skin, convey the drug to the systemic circulation at a controlled rate. A new drug delivery system must include a transdermal drug delivery system. To circumvent the limitations of traditional drug delivery methods, innovative drug delivery methods such as TDD have been used. Transdermal therapy systems are intended to enable controlled continuous drug administration to the systemic circulation via the skin. Novel drug

delivery technologies must be developed in order to enhance the number of medications supplied via transdermal route. Physical means such as iontophoresis, sonophoresis, micro needles, and so on, as well as chemical means such as penetration enhancers and biochemical means using liposomes, niosomes, invasomes, transferosomes, and ethosomes, have been reported to improve drug permeability through the stratum corneum (Mathur Devi, 2017; Gupta and Agrawal,2012).

Invasomes

A new family of vesicles known as Invasomes is helping to improve the transdermal penetration of active pharmaceutical substances. The structure of these vesicles contains phospholipids, ethanol, and different terpenes or terpene combinations. These components had excellent transdermal penetration properties. Composition Invasomes are soft liposomal vesicles that contain small amounts of ethanol and various terpenes or terpene combinations and may act as carriers with increased skin penetration. Phospholipids and a trace of alcohol are present, as are terpenoids (such as citral, limonene, and cineole), water, and a trace of ethanol (e.g., 3-3.3 percent by volume). Terpenoids (such as citral and eugenol), water, and ethanol are also present. Terpenes (C5H8) have a broad formula for improving the absorption of hydrophilic and hydrophobic medicines. Terpenes, which are found in essential oils, are often utilised penetration enhancers. Terpenes are less irritating to the skin when administered in modest doses. Terpenes are also regarded as safe by the FDA (Haque and Talukder, 2018).

Composition of Invasome

Phospholipids

In phospholipids, water-loving hydrophobic acyl chains are connected to the alcohol. Distinctions in head groups, aliphatic chains, and alcohols allowed a diverse range of phospholipids to survive. As a result, the changed phospholipid sources benefit the phospholipid classes. Natural and synthetic phospholipids, such as PEGylated Phospholipids, are used in a variety of formulations, including those for skin care products. Even hydrogenated phosphatidylcholine has been described as a way for forming nanovesicles (Li *et al*., 2015; Lakshmi *et al*., 2014).

Ethanol

To improve permeability, ethanol can be utilised. Vesicles in nano-vascular systems have a major influence due to their specific size, zeta potential, entrapment efficacy, and skin permeability. According to numerous studies, the size and entrapment efficacy of vesicles decrease as ethanol concentration increases. The vesicles dissolve as the ethanol concentration rises. Increased ethanol levels reduce membrane thickness and consequently vesicular volume. Ether penetrates hydrocarbon chains and changes the net charge of vesicles, resulting in smaller average vesicles. Ethanol can also improve the fluidity of nanovesicles. Ethanol disrupts the densely packed structure of SC lipids, causing them to split. Because ethanol influences the structure of keratinize or lipophilic domains, it can lower the transition temperature of lipids. In comparison to liposomal nanovesicles, ethanol-based nanovesicles have a softer and less rigid structure. Because of their negative surface charge and electrostatic repulsion, ethanol nanovesicles may be more stable in storage (Curic *et al*., 2018)

Terpenes

Terpenes or terpene mixtures in very low doses have also been proven to be penetration enhancers (also known as sorption boosters or accelerants) in transdermal drug delivery systems, allowing them to penetrate the skin and reduce barrier resistance. Terpenes pose little risk of skin irritation, hence they are classed as "Generally Recognised As Safe" (GRAS).Terpenes' capacity to permeate the skin is influenced by their solubility, the breakdown of lipid and protein layers, and the loss of skin micro-ingredients (Babaie *et al*., 2020).

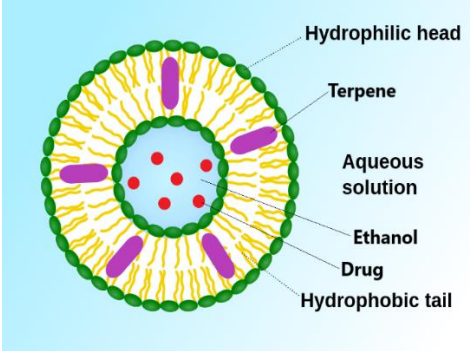

Figure 3: Structure of invasome

Methods for preparing invasome

Mechanical Dispersion Method

In an ethanolic phospholipid solution, a drug and a terpene or terpene combination are dissolved. To

create a clear solution, the mixture is vortexed for 5 minutes and then sonicated for 5 minutes. A syringe is used to add phosphate buffer saline (PBS) (pH: 7.4) to the solution, which is constantly vortexed. The vortexing is maintained for another 5 minutes to achieve the final invasomal preparation (Lakshmi *et al*., 2013).

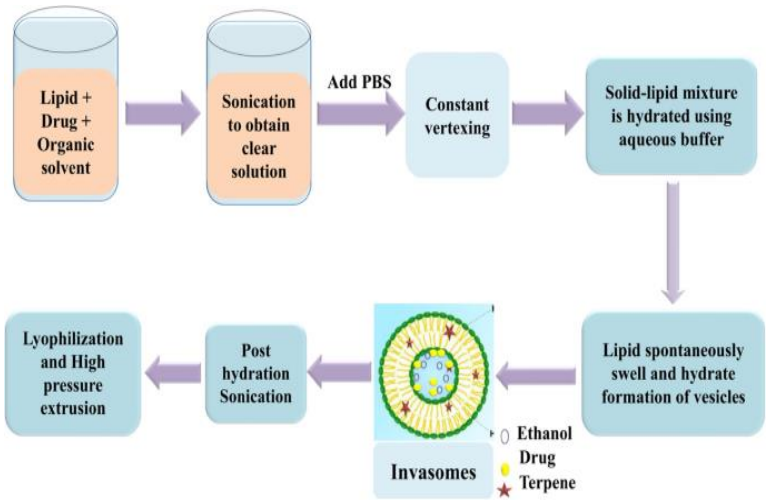

Figure 4: Mechnical dispersion method

Thin Film Hydration Method

The traditional film process can also be used to prepare invasomes. Phospholipids in ethanol are dissolved in a 2:1 v/v mixture of methanol and chloroform. The rotary flash evaporator is used to dry this mixture to a thin film by gradually lowering the pressure from 500 to 1 mbar at 50°C. The film is vacuumed (1 mbar) for 2 hours at room temperature before being flushed with nitrogen. The film is then hydrated for 30 minutes at the lipid phase transition with a mixture of phosphate buffer (pH: 7.4; PBS) containing ethanol and terpenes, or it is hydrated with PBS (pH: 7.4) and after cooling to room temperature, ethanol and a single terpene or a terpene mixture is added to obtain Invasomes (Pandey & Srivastava, 2021).

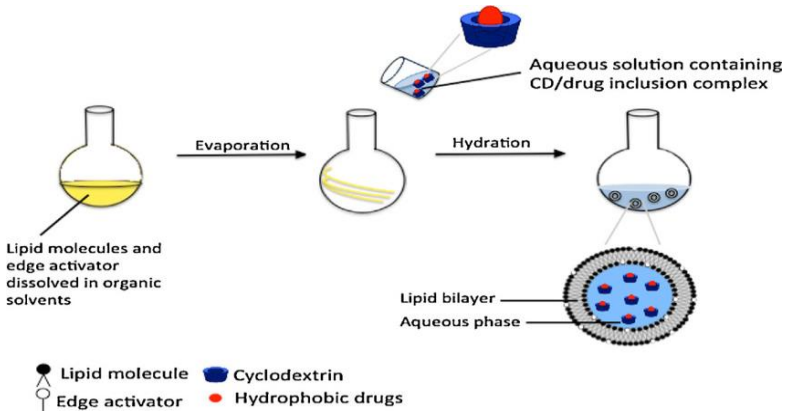

Figure 5: Thin film hydration

Penetration Mechanism of Invasomes

Terpenes and ethanol in invasomes distort the vesicles, damage the SC bilayer skeleton, and act as penetration enhancers, boosting invasome permeability. According to Dragicevic-Curic et al., during invasome penetration, one part of the vesicle disintegrates and releases its components, including terpenes, phospholipid segments, and single phospholipid molecules, which increase penetration and fluidize the SC lipids (Honeywell & Nguyen, 2002). Smaller invasome vesicles that do not dissolve pass through the SC unharmed. Following penetration, undamaged invasomes may reach the inner sections of the SC via the follicular transport pathway or the SC's intercellular region's tiny hydrophilic channels. Honeywell-Nguyen et al. discovered that smaller intact invasomes can penetrate deeper into the SC via channel-like regions. This was derived from the discovery of flexible vesicles of varying sizes in the deeper layer of the SC and skin surface vesicles. A proportion of invasomes disintegrate when they enter the SC, but smaller vesicles and flexible invasomes pass through the deeper layers intact

(Zellmer et al., 1995; Dayan & Touitou, 2000).

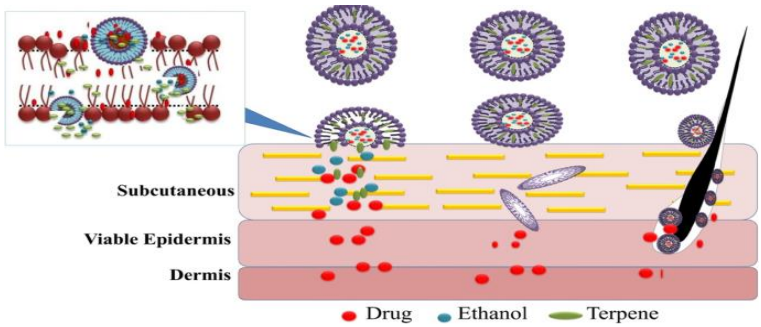

Figure 6: Penetration mechanism of invasome

Evaluation of invasome

Entrapment Efficiency:

The ultracentrifugation method was used to focus capture productivity. To separate the unentrapped medication, 1ml of invasomal detailing was transferred to Ephendroff tubes and centrifuged at 15000 rpm, 4°C for 15 minutes in two cycles. The reasonable percentage was used to guarantee free medication. The rate of entangled is governed by the amount of free drug in the formula (Afreen & Shailaja, 2019).

Entrapment Efficiency (%)

$= total\ drug - free\ drug total\ drug \times 100$

Surface Morphology:

Surface morphology was concentrated by putting a drop of planning on a clear glass slide, air drying it, covering it with gold using a spray coater (Polaron E5100, Watford, UK), and viewing it under scanning electron microscopy (Verma *et al.*, 2022).

Drug content

An UV spectrophotometer can be used to determine the drug content of invasomes. A modified high performance liquid chromatographic method can also be used to quantify this (Jain *et al*., 2021).

Stability studies

The stability of vesicles can be measured by analysing their size and structure over time. DLS measures mean size, and TEM detects structural changes. The invasomal suspensions of temoporfin and finasteride have both been observed to be stable at 4°C.

Vesicular size:

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) can be used to visualize. Dynamic Light Scattering (DLS) and photon relationship spectroscopy can be used to alter the invasomes' vesicle size and zeta potential molecule size (Kalpana & Lakshmi, 2013).

Ex Vivo Permeation Studies:

The Franz dissemination cell was used to control the penetration of invading details. The cell's persuasive surface zone was 2.0 cm2 with a receptor volume of 20ml. The skin was placed on the receptor compartment, with the layer corneum side facing the contributor compartment. A top was placed on the highest point of the dissemination cell. The contributor compartment was used with invasomal planning, and the receptor medium was 20 cc of pH 7.4 phosphate cushion saline held at 37oC. To maintain a sink condition, aliquot sums were withdrawn and replaced with new media. A UV spectrophotometer was used to assess the results of the tests (Chen *et al*., 2011).

Marketed formulations of invasome

Drug	**Tested condition**	**References**
Carboxyfluorescein and temoporfin	Skin penetration and deposition	Chen *et al*., 2011
Ferulic acid	Skin delivery capability	Chen *et al*., 2010
Finasteride	Permeation through skin	Prasanthi &, Lakshmi,2013
Temoporfin	Subcutaneously implanted tumours	Nina *et al*., 2008
Carboxyfluorescein and calcein	Skin permeation ability	Ntimenou *et al*.,2012
PCA	Percutaneous penetration	Haag *et al*., 2011
Dapsone	Antibiotic and anti-inflammatory	El-Nabarawi *et al*., 2018
Ketoconazole	Topical Fungal Treatment	Gupta *et al*., 2022
Clotrimazole	Fungal treatment	Verma & Pal,2022

Ocimum basilicum	Acne treatment	Han,2018
Itraconazole	Onychomycosis	Hoda *et al*., 2021
5,10,15,20-tetra(*m*-hydroxyphenyl)chlorin (mTHPC)	Infected dental root canals	Ossmann *et al*., 2015
Thymol	Antibacterial activity	Kaltschmidt *et al*., 2020
Propranolol hydrochloride	Contraceptive gel	Teaima *et al*., 2022
Terbinafine (TBF-IN)	Treatment of onychomycosis.	Gupta *et al*., 2023
Dexketoprofen trometamol	For inhibiting oxidative stress	Soliman *et al*., 2023
Curcumin	Skin diseases	Kumar *et al*., 2022
Carvacrol	repellency activities of carvacrol against tick species	Gamal *et al*., 2023
Fenticonazole nitrate	Ocular fungal infection	Albash *et al*., 2021
Buprenorphine and Bupivacaine	Analgesic effect	El-Zaafarany & Nasr,2021

Conclusion

Invasomes, as a fluidic system, have been studied and found to be useful in treating skin problems. Preclinical investigations on several actives for dermal applications have completely established the targeted efficacy. Exploration of invasomes for delivery of biological macromolecules is one of the topics vying for attention. Scale-up, acute and chronic toxicity, and clinical complexities must all be solved before these technologies can be commercialised.

Invasome formulation could be a viable technique for delivering medications through the skin and improving skin permeability. Several pharmaceutical active components and chemical compounds are extremely potent, but their therapeutic activity is limited. Because of their extraordinary and customizable features, they can be targeted using invasomes as a new carrier. Invasomes have made a successful entry into Furthermore, the success of the invasome dosage form is dependent on penetration rate, ability to transport actives to the targeted region, and minimal toxicity, among other factors.

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