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Review

Vesicular nanocarrier based treatment of skin fungal infections: Potential and emerging trends in nanoscale pharmacotherapy

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ABSTRACT

Occurrence of skin fungal infections is increasing nowadays and their presence is more prominent in patients suffering from immunocompromised diseases like AIDS. Skin fungal infections are a major cause of visits by patients to dermatology clinics. Although, a large number of antifungal agents are available for treatment of skin fungal infections, but, their toxic profile and physicochemical characteristics reduce therapeutic outcome. When these antifungal agents are delivered topically using conventional formulations like creams and gels, they may cause various side effects like redness, burning, and swelling at the site of application. Therefore, various vesicular formulations (phospholipid based or non phospholipid based) have been explored by pharmaceutical scientists to treat skin fungal infections topically. Vesicular formulation explored for the purpose are liposomes, ethosomes, transfersomes, transethosomes, niosomes, spanlastics, oleic acid vesicles, and nanoparticles. These formulations show various advantages like bioavailability enhancement of bioactives, high skin permeation power, no side effects at application site, dosing frequency reduction, and sustained drug release. Therefore, in the present article, we have discussed about the utility of various vesicular nanocarrier systems to treat skin fungal infections.

1. Introduction

Fungi are parasitic microorganisms which can affect the skin and mucous membrane along with generation of systemic infections of various internal organs [1]. Fungal infections of skin or mucous membrane, in majority, promote visits of victims to dermatologists [2]. It has been reported that 20%–25% of human population show presence of skin fungal infections [3]. Incidences of occurrence of skin fungal

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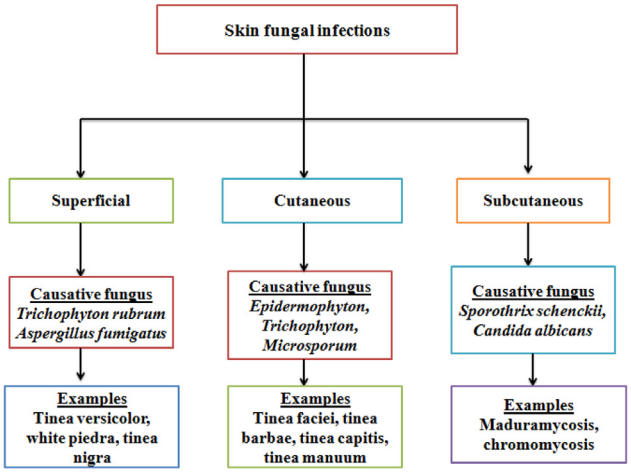

Fig. 1 – Classification of skin fungal infections depending upon the depth of penetration of parasitic fungus into the skin.

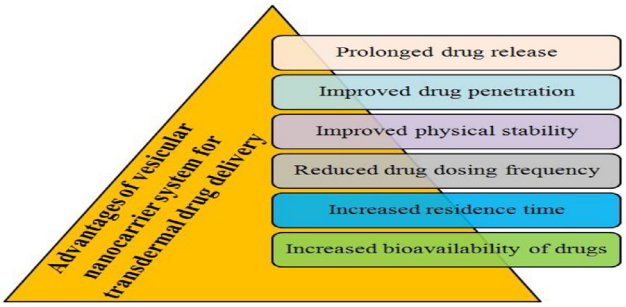

Fig. 2 – Advantages of vesicular nanocarriers over conventional delivery systems for transdermal delivery.

infection are very high in immunocompromised patients [4]. Skin fungal infections are categorized into superficial, cutaneous, and subcutaneous depending upon the level of tissue invasion [5]. When attack of invading fungi is limited to outermost skin layers only then generated infection is called superficial fungal infection. Tinea versicolor, white piedra, and tinea nigra are examples of superficial fungal infections. Superficial fungal infection leads to increase in the skin pH along with mild scaling, redness, and inflammation at the invading site. The barrier nature of skin becomes poor in such a state [6]. Invasion of parasitic fungus into deeper epidermal skin layer develop cutaneous fungal infection. This infection is also known as dermatomycoses and it may have involvement of skin appendages like nails and hairs [7]. Dermatomycoses can also instigate cellular immune response developing pathological variations in patients [8]. Various fungi generating dermatomycoses come under three genera, namely *Epidermophyton*, *Trichophyton*, and *Microsporum*. Tinea faciei, tinea barbae, tinea capitis, and tinea manuum are the examples of cutaneous fungal infections [9]. Furthermore, extension of fungal infection to dermal or subcutaneous region results subcutaneous fungal infection. It is caused by fungi namely *Sporothrix schenckii* and *Candida albicans* [10]. This fungal infection is characterized by either ulcerated or infiltrated nodular lesions in the infected areas [11]. Maduramycosis and chromomycosis are other examples of subcutaneous fungal infections [12]. Fig. 1 gives a brief overview of skin fungal infections.

2. Conventional strategies for treatment of skin fungal infections

Conventionally, skin fungal infections are treated with creams, gels and lotions containing free antifungal agents [13]. Topically delivered antifungal agents show local action, therefore, they exhibit less toxic effects compared to oral antifungal agents [14]. Topical formulations used for treatment of skin fungal infections may be fungicidal or fungistatic depending upon therapeutic nature of incorporated antifungal drugs [15]. Chances of drug-drug interactions are negligible in the case of topical formulations which are more common in orally administered antifungal drug molecules [16]. However, topical antifungal formulations like creams, gels, and lotions may show redness of skin, erythema, stinging, and burning sensation as side effects [17].

Poor skin penetration of hydrophilic antifungal drugs and high dosing frequency of conventional antifungal formulations reduce their effectiveness against skin fungal pathogens [18]. For the topical delivery, an antifungal drug must have some specified properties and lipophilic nature is most important amongst them [19]. Lipophilic drugs show excellent skin penetration upto underlying skin layer, however, their release rates should be controlled to obtain sufficient local concentrations and prolonged pharmacological effects [20]. Molecular weight of antifungal drug affects its topical delivery and it becomes more prominent for the delivery of antifungal drugs like amphotericin B whose molecular weight exceeds 500 daltons (Da) [21]. Therefore, several nanocarrier systems have been investigated by pharmaceutical scientists to fulfil these criteria and considerations for topical delivery of antifungal drugs [22]. Nanocarriers can make their way easily to hair follicles and they may show accumulation between corneocytes, merging with lipidic layer, and high intermingling with lipids present in the skin [23]. Advantages of vesicular nanocarriers over conventional delivery systems for transdermal delivery are explained in Fig. 2. Nanocarriers also have the capability to sustain the drug release, which reduce the side effects and dosing frequency of antifungal drugs [24].

So, in the present review our major aim was to explore the utility of various vesicular nanocarrier systems for effective treatment of skin fungal infections.

3. Novel vesicular nanocarrier systems in the treatment of skin fungal infections

3.1. *Phospholipid-based vesicular nanocarriers*

3.1.1. *Liposomes*

Liposomes represent firstly used phospholipid based nanocarrier systems for drug delivery, which were described during 1980s [25]. Structurally, liposomes are bilayered vesicular systems having an aqueous core along with one or several con-

centric phospholipid membranes [24]. Fig. 3 describes various phospholipid based nanocarrier systems used for topical delivery of antifungal drugs [26]. Due to their unique structural characteristics, liposomes have the capability to deliver both hydrophilic and lipophilic bioactive molecules [27].

Other advantages of liposomes are high drug loading, toxicity reduction, improved stability and bioavailability along with higher biocompatibility [28]. Cholesterol is also added into the liposomal system to enhance rigidity of bilayer, improve vesicles stability, and to sustain the release of encapsulated material [29]. The topical drug delivery route is quite effective as it generates high drug concentration in local area reducing dosing frequency along with elimination of side effects of drugs. Topical delivery also reduces cost of therapy and improve patient compliance because of ease of application and removal of formulation [30]. Liposomes show effective penetration upto stratum corneum, which is a site of invasion of parasitic fungus [22]. Mechanism of transdermal delivery through various vesicular nanocarrier systems is explained in Fig. 4 [31]. Sudhakar et al. evaluated terbinafine HCl loaded liposomes dispersed in gum karaya gel for *ex-vivo* drug retention in rat skin. Developed liposomes showed approximately 70% entrapment of terbinafine HCl and prolonged retention of drug in rat skin compared to plain gum karaya gel containing free terbinafine HCl upto 24 h [32].

Furthermore, a comparative assessment between propylene glycol liposomes and conventional liposomes loaded with miconazole nitrate was carried out by Elmoslemany et al. for transdermal delivery. Propylene glycol liposomes loaded with miconazole nitrate showed minimum inhibitory concentration (MIC) value of 1.46 µg/ml against *Candida albicans* which was low compared to the MIC value of conventional liposomes (2.93 µg/ml). Propylene glycol (PG) liposomes also showed high skin retention and skin permeation of miconazole nitrate compared to conventional liposomes and miconazole nitrate (MN) suspension in human skin (Fig. 5) [33].

Agarwal and Katare performed an evaluation of liposomes prepared from two different phospholipids namely phosphatidyl choline saturated 97.3% content (PCS) and phosphatidylcholine unsaturated 98.0% content (PCU) for topical delivery of miconazole nitrate. Liposomes developed from both phospholipids showed high stability and good colloidal characteristics. However, PCS based liposomes loaded with miconazole nitrate showed higher skin retention of miconazole nitrate compared PCU based liposomes *in-vitro* in mouse skin [34]. Table 1 gives an overview of liposomes as effective nanocarriers for treatment of skin fungal infections.

3.1.2. *Ethosomes*

Ethosomes are nanocarrier systems which are structurally soft and having high ethanol content, phospholipids, and water in them [38]. Ethosomes may contain 2%–5% content of phospholipids and 20%–40% concentration of ethanol [39]. Skin penetration capacity of ethosomes is higher compared to liposomes due to capability of ethanol to fluidize various intercellular lipids present in the stratum corneum of skin [40]. It has been reported that as the amount of ethanol increases size of ethosomes decreases by keeping concentration of phospho-

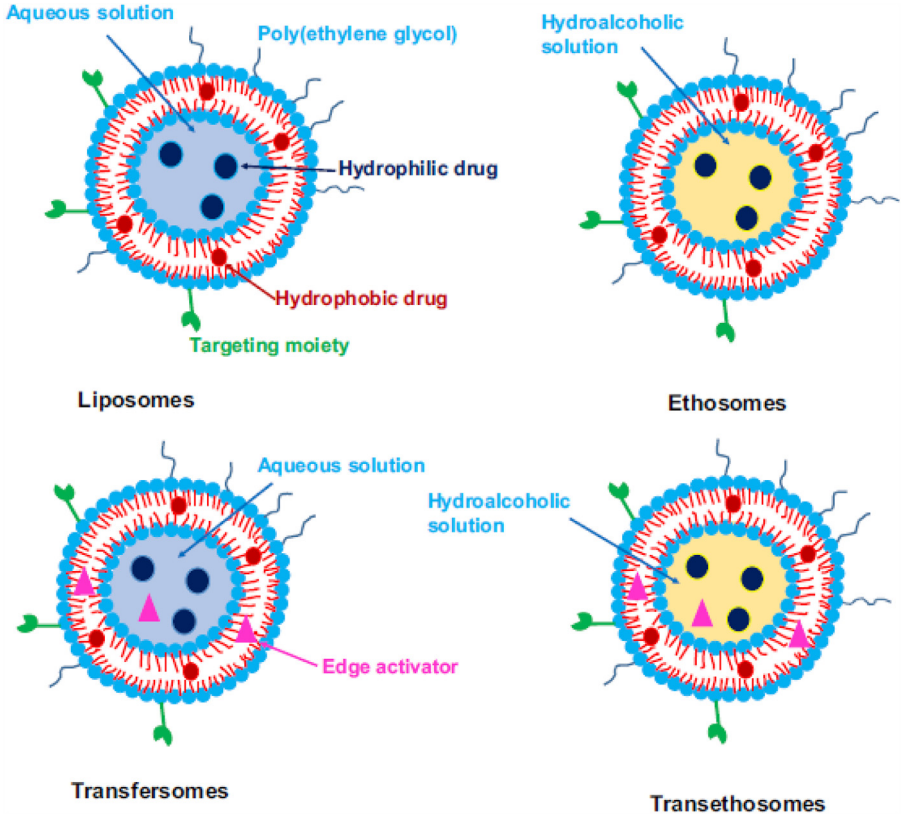

Fig. 3 – Different phospholipid-based vesicles used in drug delivery (Reproduced with permission from reference [26]) Copy right 2017, Elsevier.

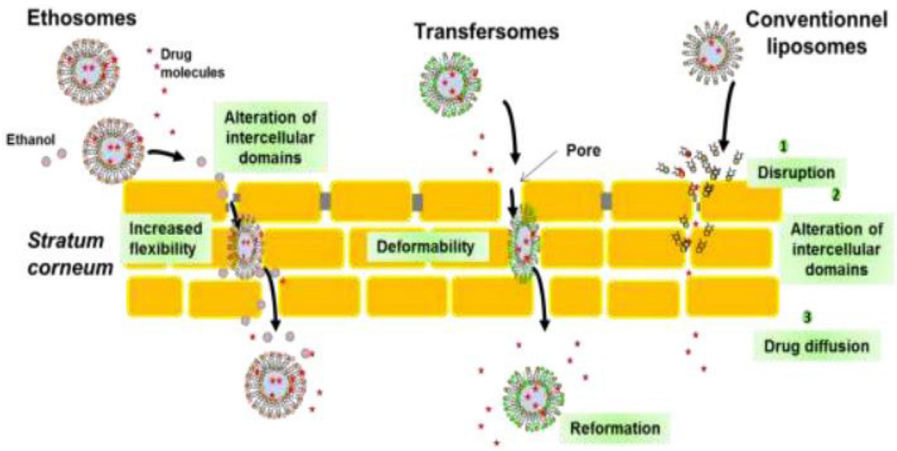

Fig. 4 – Schematic representation of the main permeation mechanisms of lipid-based vesicles (Reproduced with permission from reference [31]) Copy right 2018, Elsevier.

Table 1 – Role of liposomes in effective elimination of skin fungal infections.

Composition of liposomes	Drug	Entrapment/size /zeta potential	Animal model/Route of Administration	Key findings	Ref.
1,2-Dipalmitoyl-sn-glycero-3-phosphocholine, Oligolysines (Lys-5 and Lys-7)	Fluconazole	67.28% ± 15.86%/ 61.15 ± 4.25 nm/ + 3 mV	Not available (NA)	Oligolysines incorporation in a liposomal formulation produced structural variations in fluconazole loaded liposomes along with their size reduction, promoting their *in-vitro* skin retention effect	[35]
Soya lecithin, Cholesterol	Keto conazole	74.05%/ 5.64 ± 0.014 µm/ NA	NA	Liposomal gel showed extended the drug release upto 24 h along with higher *in-vitro* skin deposition compared to the marketed gel of the same drug	[36]
Soya phosphatidyl choline, Cholesterol	Keto conazole	54.41% ± 0.19%/ 0.86 µm/ NA	NA	Developed liposomes showed 34.96% ± 0.86% drug release after 12 h and were found stable at the 25 °C temperature for two months indicating their effectiveness in antifungal treatment	[37]

lipid constant [41]. Presence of ethanol in ethosome also provides a negative charge to its surface enhancing its colloidal stability [42]. However, ethosomes show high leakage of hydrophilic/ionized drugs compared to liposomes due to disruption of close packing of phospholipid bilayer by the presence of high ethanol amount [43]. Bhalaria et al. investigated fluconazole loaded ethosomes for treatment of cutaneous candidiasis in eight patients for a period of one month. Ethosomal gel containing fluconazole showed 50%–70% reduction in skin lesions in patients, which was very high compared to liposomes (30%–60%) and commercial fluconazole cream (25%–30%) [44]. Later on, econazole nitrate loaded ethosomes were compared with liposomes loaded with the same for the treatment of deep fungal infection by Verma and Pathak in gel form. Ethosomal gel showed 2 fold higher diffusion of the drug in the albino rat skin compared to liposomal gel after 12 h of application. Results of CLSM (confocal laser scanning microscopy) studies revealed accumulation of econazole nitrate loaded ethosomes in the stratum basale layer of animal skin (Fig. 6) [45]. Maheshwari et al. carried out a comparative assessment between ethosomes and ultradeformable liposomes loaded

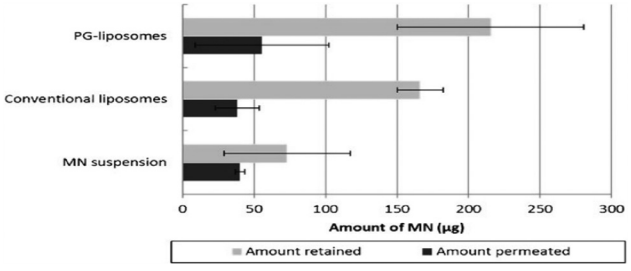

Fig. 5 – MN retained in and permeated through skin after 24 h, determined at 32 °C, using human skin in Franz diffusion cells under non-occlusive conditions (Reproduced with permission from reference [33]) Copy right 2012, Springer Nature.

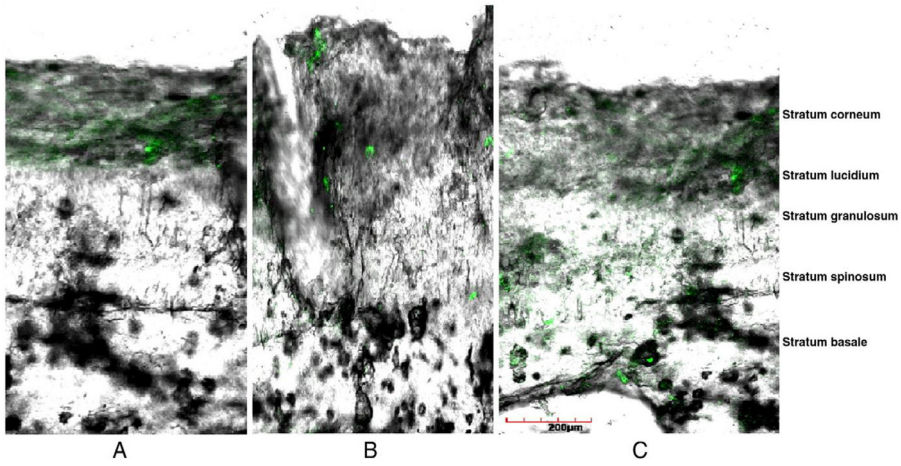

Fig. 6 – Confocal laser scanning microscopy. (A) CLSM image of control gel; (B) CLSM image of liposomal gel showing less penetration of drug; (C) CLSM image of ethosomal gel showing penetration of drug as far as the last layer (stratum basale) of epidermis (Reproduced with permission from reference [45]) Copy right 2012, Elsevier.

with clotrimazole for the treatment of cutaneous candidiasis. Drug loaded ethosomes showed higher *in vitro* antifungal activity against *Candida albicans* by showing 34.6 mm zone of inhibition compared to ultradeformable liposomes which showed 29.6 mm inhibition zone. Results of Fourier-transform infrared spectroscopy revealed higher *in vitro* skin penetration of ethosomes compared to ultradeformable liposomes [46].

Furthermore, voriconazole loaded ethosomes were investigated by Faisal et al. for effective skin deposition. Developed ethosomal formulation showed six fold more *ex vivo* drug permeation in the rat abdominal skin compared to hydroethanolic solution of voriconazole [47]. Ethosomes having concentration of ethanol more than 30% cause excessive release of entrapped material and irritation of skin [48]. Therefore, Akhtar and Pathak developed Cavamax W7 composite ethosomes to minimize these harmful effects of high ethanol concentration in vesicles by lowering its amount in vesicles. Cavamax W7 is a permeation enhancer and it shows synergistic effect on ethanol's skin penetration power. Developed Cavamax W7 composite ethosomes showed high stability and *ex vivo* skin permeation, antifungal activity against *Candida albicans* and *Aspergillus niger* compared to conventional ethosomes [49].

3.1.3. *Transfersomes*

Conventional liposomes have poor penetration through the skin, which can be improved by modifying their bilayer composition [50]. Liposomes were firstly modified by Cevc and Blume by the addition of edge activators to liposomal composition and resulted modified liposomes were called 'deformable liposomes', 'elastic liposomes', or 'transfersomes' [51]. Various examples of edge activators used in transfersomes are sodium deoxycholate, sodium cholate, dipotassium glycyrrhizinate, Tween 80 , Tween 60, Tween 20, Span 80, Span 65, and Span 60 [52]. Transfersomes show enhanced deformability due to the weakening of their lipid bilayers because of edge activators [53]. Transfersomes have higher skin penetration compared to conventional liposomes due to their higher deformability and they can easily cross through the pores having diameter 5–10 times less compared to their own diameter [54]. Pandit et al. investigated topical antifungal efficacy of transfersomes loaded with miconazole nitrate in Sprague-Dawley rats. Developed modified liposomes showed high *in vivo* antifungal activity and reduced toxicity compared to conventional liposomes and free drug solution [55]. Aggarwal and Goindi carried out evaluation of transfersomes loaded with griseofulvin in guinea pigs for eradication of *Microsporum canis* induced dermatophytosis. Optimized transfersomal formulation showed better skin retention and permeation compared to conventional liposomes. Histopathological analysis revealed complete eradication of fungal spores from guinea pig skin within 10 d treatment by using griseofulvin loaded transfersomes (Fig. 7) [56].

Recently, transfersomes loaded with amphotericin B were developed by Perez et al. and were evaluated for *in vitro* antifungal activity and human skin permeation. They reported maximum deformability in transfersomes using Tween 80 as an edge activator. *In vitro* sensitivity of clinical isolates of *Candida albicans* was very high towards transfersomes loaded with amphotericin B compared to mammal cells. Transfersomes showed forty times better accumulation in human skin compared to a marketed liposomal formulation of amphotericin B (AmBisome) [57].

3.1.4. *Transethosomes*

Transethosomes are highly advanced vesicular nanocarrier systems which encompass the advantages of transfersomes and ethosomes. Their composition is exactly similar to ethosomes additionally having the presence of a penetration enhancer or an edge activator [58]. Song et al. evaluated voriconazole loaded transethosomes for *in vivo* skin deposition of drug in mice. Transethosomes (TEL) showed increased *in vivo* skin deposition of voriconazole in the dermis and epidermis area compared to other nanocarriers like deformable liposomes (DL), conventional liposomes (CL), ethosomes (EL), and polyethylene glycol drug solution (PG) (Fig. 8) [59]. Table 2 gives a brief summary of research work done on phospholipid based nanocarriers other than liposomes for transdermal de-

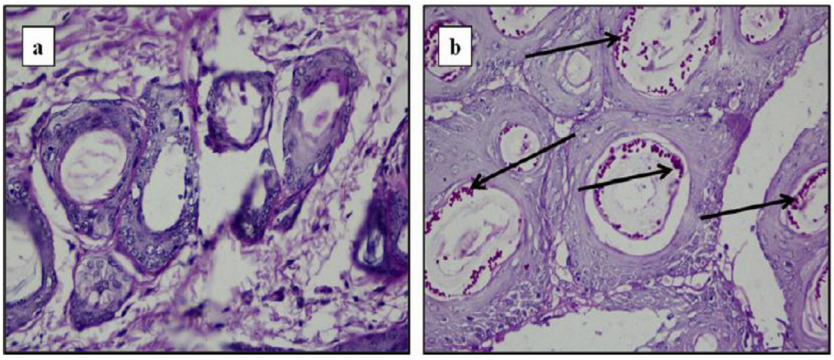

Fig. 7 – Histopathology of skin of guinea pig infected with M. *canis* after treatment with (A) test formulation (griseofulvin loaded transfersomes) showing complete absence of fungal elements (B) placebo, arrows show presence of spored hyphae in hair follicles ($n = 5$) (Reproduced with permission from reference [56]. Copy right 2012, Elsevier.)

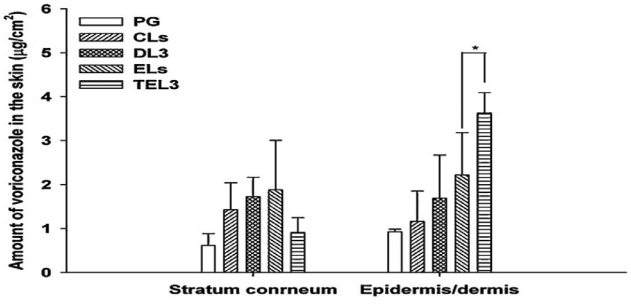

Fig. 8 – Amount of voriconazole retained in skin at the of *in-vivo* skin deposition studies after applying lipid vesicles or control PG solution. Each value is the mean ± S.D. (n = 4) (*P < 0.05 vs ELs) (Reproduced with permission from reference [59]. Copy right 2012, Elsevier.)

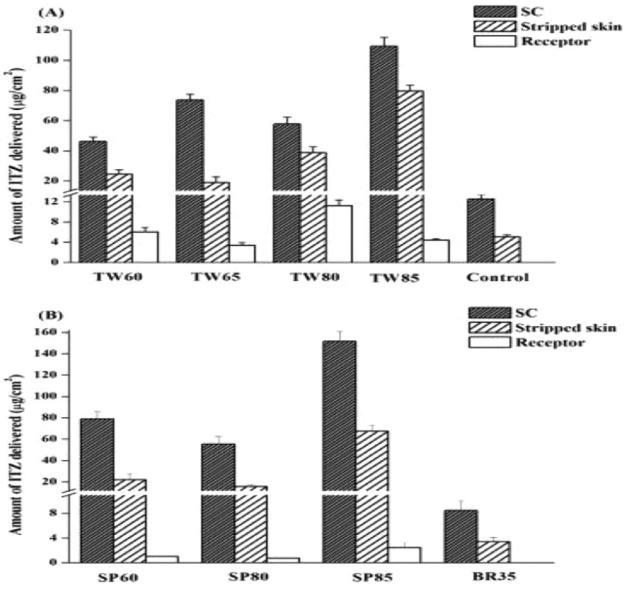

Fig. 9 – The Amount of ITZ delivered into the stratum corneum (SC), Stripped skin and the receptor fluid after 6 h of incubation with rat skin after applying different type of niosomes(Reproduced with permission from reference [70]) Copy right 2015, Elsevier.

livery of antifungal drugs. Overview of various advantages and disadvantages of phospholipid based nanocarrier systems is explained in Table 3.

3.2. *Non phospholipid-based vesicular nanocarriers*

3.2.1. *Niosomes*

Niosomes are bilayered vesicular systems which are made up of single alkyl chain non-ionic surfactants [60]. Handjani-Vila et al. gave first description of niosomes in 1979 [61]. Structurally, they have a hydrophilic head of surfactant oriented towards the exterior and interior of bilayer while, hydrophilic tail endorsed inside the bilayer [62]. Therefore, niosomes are capable of encapsulating both hydrophilic or lipophilic drugs [63]. Cholesterol is also added in the production of niosomes to enhance the rigidity of bilayer and reduction of premature drug leakage [64]. Various characteristics like low production cost, higher chemical stability, high loading capacity, and regular conditions storage make them efficient carries than liposomes [65]. Characteristics of niosomes can be easily modified by varying their composition and preparation methods [66]. Niosomes are suitable to deliver various therapeutic agents through various routes like topical, oral, and parenteral [67]. Examples of surfactants which are used for niosome production are Tweens, Spans, polyglycerol alkyl ethers, polyoxy- ethylene alkyl ethers, Brij, and ester-linked surfactants [68]. Kassem et al. carried out a comparative assessment between niosomal gel loaded with griseofulvin, and liposomal gel loaded with same for topical treatment of tinea corporis. These formulations were investigated clinically in sixteen patients for about three week treatment period. Niosomal gel showed approximately 80% cure rate, which was very high compared to liposomal gel (50%) [69]. Later on, Alomrani et al. prepared niosomes loaded with itraconazole (ITZ) using different non ionic surfactants for transdermal delivery. A comparison was done using Span surfactants [like Span 60 (SP60), Span 80 (SP80), and Span 85 (SP85)], Tween surfactants

Table 2 – Phospholipid based vesicles other than liposomes as effective nanocarrier for treatment of skin fungal infections.

Vesicular carrier/ Composition	Drug	Entrapment/size /zeta potential	Animal model/Route of Administration	Key findings	Ref.
Ethosomes/ Soya phosphatidyl choline, ethanol	Fluconazole	82.68%/ 144 ± 6.8 nm/ NA	Human/ topical	Ethosomal gel containing fluconazole showed 50% - 70% reduction in skin lesions in patients, which was very high compared to liposomes (30% - 60%) and commercial fluconazole cream (25% - 30%)	[44]
Ethosomes/ Soya phosphatidyl choline, ethanol	Econazole nitrate	81.1% ± 0.13%/ 202.8 ± 5.10 nm/ –75.1 ± 0.21 mV	Albino rats/ topical	CLSM (confocal laser scanning microscopy) studies revealed accumulation of econazole nitrate loaded ethosomes in the stratum basale layer of animal skin	[45]
Ethosomes/ Soybean phosphatidyl choline, ethanol	Clotrimazole	68.7% ± 1.4%/ 132 ± 9.5 nm/ NA	Sprague–Dawley rats/ topical	Fourier-transform infrared spectroscopy revealed higher *in vitro* skin penetration of ethosomes compared to ultradeformable liposomes	[46]
Ethosomes/ Soybean phosphatidyl choline, ethanol	Voriconazole	46.5% ± 2.1%/ 423.67 ± 26.64 nm/ −18.20 ± 0.30 mV	NA	Ethosomal formulation showed six fold more *ex-vivo* drug permeation in the rat abdominal skin compared to hydroethanolic solution of voriconazole	[47]
Ethosomes/ Soya lecithin, Cavamax, propylene glycol	Clotrimazole	98.42% ± 0.15%/ 202.8 ± 4.8 nm/ 83.6 ± 0.9 mV	NA	Cavamax W7 composite ethosomes showed high stability and *ex-vivo* skin permeation, antifungal activity against *Candida albicans* and *Aspergillus niger* compared to conventional ethosomes	[49]
Transfersomes/ Soya phosphatidyl choline, sodium deoxycholate, Tween-80, Span-60, Span-80, cholesterol	Miconazole nitrate	91.3% ± 1.20%/ 182 ± 8.53 nm/ NA	Sprague-Dawley rats/ topical	Ultraflexible liposomes loaded with miconazole nitrate showed high *in-vivo* antifungal activity and reduced toxicity compared to conventional liposomes and free drug solution	[55]
Transfersomes/ Phospholipon® 90 G, Span 85, Cholesterol	Griseofulvin	63.44% ± 0.45%/ 284.6 nm/ – 22.0 ± 3.68 mV	Guinea pig/ topical	Histopathological analysis revealed complete eradication of fungal spores from guinea pig skin within 10 d treatment by using griseofulvin loaded transfersomes	[56]
Transfersomes/ Soybean Phosphatidyl choline, Sodium cholate, Tween 80, Cholesterol	Ampho tericin B	NA/ 98 ± 8 nm/ -1 ± 0.2 mV	NA	Transfersomes showed forty times better accumulation in human skin compared to a marketed liposomal formulation of amphotericin B (AmBisome)	[57]
Transethosomes/ Soybean Phosphatidyl choline, ethanol, sodium taurocholate, Tween 80	Voriconazole	96.6% ± 2.7%/ 191.9 ± 41.5 nm / – 6.9 ± 0.6 mV	Mice (HanLim Animal, Korea)/ Topical	Transethosomes (TEL) showed increased *in-vivo* skin deposition of voriconazole in the dermis and epidermis area compared to other nanocarriers like deformable liposomes (DL), conventional liposomes (CL), ethosomes (EL), and polyethylene glycol drug solution (PG)	[59]

[like Tween 60 (TW60), Tween (TW80), and Tween 85 (TW85)], and Brij 35 (BR35). Niosomes prepared from Span 85 and Tween 85 showed maximum skin penetration, however, skin permeation power of Tween 85 niosomes was higher compared to Span 85 niosomes and Brij 35 niosomes (Fig. 9) [70].

A comparative assessment between niosomal formulation loaded with terbinafine hydrochloride and its marketed cream was carried out by Sathali and Rajalakshmi *in-vitro* against pathological fungus *Aspergillus niger*. Niosomes containing encapsulated drug (purified niosomes) and niosomes containing encapsulated drug and free drug both were dispersed into gel base and their antifungal effects were compared with marketed formulation. A zone of inhibition was found in the order - niosomal gel containing encapsulated drug and free drug both > niosomal gel containing encapsulated drug only > marketed cream formulation > gel containing pure drug [71]. A brief overview of niosomes for treatment of skin fungal infection is given in Table 4.

3.2.2. *Spanlastics*

Spanlastics are novel vesicular carriers, which are also termed as 'modified niosomes' as they contain edge activator in niosomal composition. Spanlastics usually contain Spans alon-with presence of edge activators like Tweens and many others [76]. Kakkar and Kaur firstly developed spanlastics loaded with ketoconazole using Span 60 as surfactant and

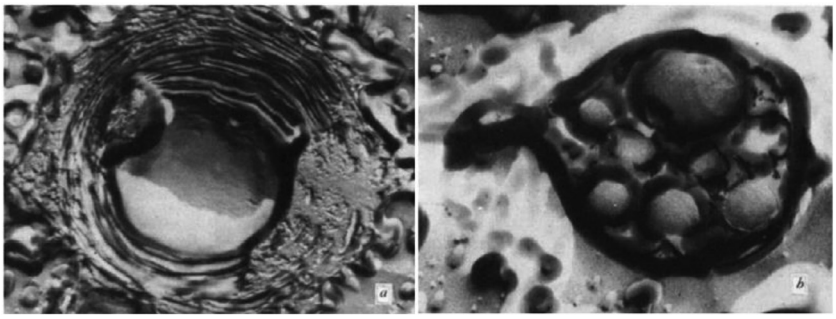

Fig. 10 – Electron micrographs of freeze-etched oleic acid spheres (x 41 650). (A) A central aqueous region surrounded by concentric membranes- the flat pitted area was cut by the microtome. (B) Smaller spheres enclosed by a common envelope (Reproduced with permission from reference [80]. Copy right 1973, Nature.)

Table 3 – Advantages and disadvantages of phospholipid based nanocarrier systems.

Vesicular nanocarrier	Advantages	Disadvantages	References
Liposomes	Improved drug stability Available in various size range Reduced drug toxicity Prolonged drug release	Drug leakage Scale-up difficulty Dose dumping Sterilization problems	[22, 28, 29, 30]
Ethosomes	High skin permeation compared to liposomes Non-toxic raw materials Smaller size compared to liposomes	Chances of coalescence Poor yield problems Less stability	[41, 42]
Transfersomes	Higher penetration compared to liposomes and ethosomes Systemic and topical delivery	Expensive Difficult manufacturing	[53, 54]
Transethosomes	Higher penetration compared to ethosomes Higher stability compared to ethosomes	Scale-up problems Expensive	[58]

Table 4 – Niosomes as effective nanocarrier for treatment of skin fungal infections.

Composition of Niosomes	Drug	Entrapment/size /zeta potential	Animal model/Route of Administration	Key findings	Ref.
Span 60, Cholesterol, Ethanol	Itraconazole	89.67 ± 1.85%/ 16.02 ± 1.35 μm/ NA	NA	Itraconazole loaded niosomes showed high *in vitro* skin permeation and a larger zone of inhibition against *Candida albicans* compared to a commercially available topical formulation of itraconazole	[72]
Span 40, Span 60, Tween 60, Cholesterol	Keto conazole	69.39 ± 0.94%/ 5.94 ± 2.14 μm/ NA	NA	Niosomes having Span 60 and cholesterol in the ratio 1 : 0.2 loaded with drug showed a prolonged effect than formulation containing free ketoconazole	[73]
Span 60, cholesterol, stearic acid	Nystatin	80.25%/ 189 ± 0.55 nm/ −30.55 ± 0.28 mV	Albino rabbits/ Topical	Niosomal gel showed two fold increased deposition of nystatin in porcine skin and less irritation in rabbits on topical application compared to conventional gel of nystatin	[74]
Span 80, cholesterol	Econazole	98%/ 0.050 μm/ NA	NA	Niosomes containing Cholesterol and Span 80 in the ratio 1 : 4 showed maximum drug entrapment and extended the drug release upto 24 h indicating their efficacy to treat skin fungal infections	[75]

Table 5 – Role of various non-phospholipid based vesicular carriers in treatment of skin fungal infections.

Vesicular carrier/ Composition	Drug	Entrapment/size /zeta potential	Animal model/Route of Administration	Key findings	Ref.
Spanlastics/ Span 60, Span 65, Tween 80, sodium deoxycholate	Terbinafine hydro chloride	79.09% ± 1.46%/ 1512.5 ± 192 nm/ − 42.35 ± 0.212 mV	NA	Confocal laser scanning microscopy (CLSM) revealed efficient *ex vivo* nail permeation of optimized spanlastic formulation	[78]
Oleic acid vesicles/ Oleic acid, methanol	Fluconazole	44.11% ± 1.13%/ 527 ± 15 nm/ NA	Guinea pigs/ Topical	Confocal microscopic studies revealed accumulation of drug loaded oleic acid vesicles in the lower epidermis area of skin after topical application indicating their effectiveness in the localized drug delivery	[81]
Oleic acid vesicles/ Oleic acid, methanol	Clotrimazole	49.5% ± 1.0%/ 455 ± 22 nm/ −22.45 ± 0.25 mV	Guinea pigs/ Topical	*In vivo* study revealed capability of drug loaded oleic acid vesicles to release clotrimazole upto 5 d after the time of application	[82]

Tween 80 as an edge activator for ocular drug delivery [77]. Elsherif et al. developed and evaluated spanlastics loaded with terbinafine hydrochloride for treatment of nail fungal infection namely onychomycosis. Study was carried out by using Span 60 and Span 65 as surfactants while Tween 80 and sodium deoxycholate as edge activators. Spanlastics formulated with Span 65 as surfactant and sodium deoxycholate as edge activator showed maximum drug entrapment, smaller size and good colloidal properties therefore, categorized as optimized formulation. Confocal laser scanning microscopy (CLSM) revealed efficient *ex vivo* nail permeation of optimized spanlastic formulation [78].

3.2.3. *Oleic acid vesicles*

Fatty acids are amphiphilic molecules consisting of a carbon atom chain behaving as polar part while terminal carboxylic group behaving as non polar part. The presence of double bonds in structure of fatty acid governs whether they are saturated or unsaturated [79]. Gebicki and Hicks firstly reported ability of unsaturated fatty acid like oleic acid to form vesicles [80]. Fig. 10 gives structural elucidation of spherical vesicles prepared by using oleic acid.

Zakir et al. evaluated oleic acid vesicles loaded with fluconazole for efficient transdermal drug delivery. Developed vesicles showed maximum drug entrapment, acceptable size, and good colloidal properties at 7 : 3 oleic acid to drug ratio. Results of *ex-vivo* skin permeation and confocal microscopic studies revealed accumulation of drug loaded oleic acid vesicles in the lower epidermis area of skin after topical application indicating their effectiveness in the localized drug delivery [81]. Later on, clotrimazole loaded oleic acid were investigated by Verma et al. for cutaneous candidiasis treatment in guinea pigs. Developed oleic acid vesicles showed high skin permeation along with good skin retention in animal skin. *In-vivo* study revealed capability of drug loaded oleic acid vesicles to release clotrimazole upto 5 d after the time of application [82]. A brief summary of various non phospholipid based vesicular nanocarriers for skin delivery of various antifungal drugs is given in Table 5. Table 6 describes advantages and disadvantages of various non phospholipid based nanocarrier systems.

3.3. *Nanoparticles*

Various types of nanoparticles explored for transdermal delivery of antifungal drugs are polymeric nanoparticles (NPs), solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) [83]. Polymeric nanoparticles may be present either in particulate dispersion form or solid powder form having size range 10–1000 nm. Nanoparticle matrix may have drug in entrapping, encapsulated, or dissolved form [84]. Various examples of natural polymers used for NPs preparation are gelatin, albumin, chitosan, and alginate. Synthetic polymers used for NPs preparation may be biodegradable [poly(lactide-coglycolide) (PLGA), poly(ε-caprolactone)] and non-biodegradable [poly(methyl methacrylate), polystyrene and polyacrylates] [85]. Kumar et al. evaluated clotrimazole loaded PLGA microparticles in guinea pigs for successful eradication of cutaneous candidiasis. Gel containing drug loaded PLGA microsphere showed penetration upto 50 µm in the dermis of animal skin along with better antifungal efficacy after 4 d of application [86]. Solid lipid nanoparticles (SLNs) are spherical structures having solid lipid core which may solubilize non polar drug molecules [87]. Examples of various solid lipids implemented for the preparation of SLNs are glycerol bahenate, tristearin, glycerol monostearate, stearic acid, and cetyl palmitate [88]. SLNs manufacturing involves the use of physiological lipids and less use of organic solvent which make them efficient carrier for transdermal drug delivery [89]. Bhalekar et al. performed *ex-vivo* skin permeation studies of miconazole nitrate loaded SLNs prepared using solid lipid Compritol 888 ATO (glycerol bahenate). Developed SLNs formulation showed better *ex-vivo* accumulation of drug in the skin along with better skin targeting effect compared to the marketed gel [90]. Furthermore, glycerol palmitostearate (Precirol ATO 5) based SLNs loaded with econazole nitrate were evaluated by Sanna et al. *in vivo* by using five healthy living subjects (females). SLNs

Table 6 – Advantages and disadvantages of non phospholipid based nanocarrier systems.

Vesicular nanocarrier	Advantages	Disadvantages	References
Niosomes	Cheap compared to liposomes	Poor drug loading	[65, 66]
	High stability compared to liposomes Reduced toxicity due presence of non ionic surfactant	Special manufacturing equipments required	
Spanlastics	High skin permeation compared to niosomes	Expensive	[76, 78]
Oleic acid vesicles	Cheap compared to niosomes and spanlastics High penetration power compared to niosomes	Stability issues	[82]

Table 7 – List of patents regarding the use of vesicular nanocarriers for treatment of skin fungal infections.

Title of patent	Brief description	Inventors	Patent number	Ref.
Topical liposomes compositions for delivering hydrophobic drugs and methods preparing same	This invention describes a method of loading amphotericin B in liposomes and their role in the treatment of cutaneous candidiasis and cutaneous leishmaniasis	Mahmoud Reza Jaafari, Ali Khamesipour	US20150147382 A1	[96]
Allylamine-containing liposomes	This patent deals with a method of preparation of terbinafine encapsulating liposomes and their utility to treat fungal infections through topical delivery	David Bodmer, Thomas Kissel, Friedrich Richter, Harry Tiemessen	US6623753 B1	[97]
Topical terbinafine formulations and methods of administering same for the treatment of fungal infections	This invention describes about the utility of niosomes loaded with terbinafine for effective removal of skin fungal infections	Gregor Cvec, Ulrich Vierl	US7820720 B2	[98]
Terbinafine compositions for onychomycosis treatment	This patent explains a method of preparation of nanoethosomes containing 60% (w/w) ethanol and loading of terbinafine into them for onychomycosis treatment	Elka Touitou	WO2010086723 A1	[99]
Design of terbinafine hydrochloride loaded liposome included pullulan film system for ungual treatment of onychomycosis	This invention describes the development method of terbinafine hydrochloride containing liposomes and their efficacy to treat onychomycosis when delivered in the form of the pullulan film system	Kevser Ozgen Ozer, Sakine Tuncay Tanriverdi	WO2014209246 A1	[100]

revealed better diffusion of drug in lower epidermal skin layers after 180 min of application compared to marketed gel in living subjects [91]. Nanostructure lipid carriers (NLCs) are nanocarriers consisting of the lipid matrix embedded with special nanostructures [92]. NLCs produced using high pressure homogenization technique may be in lipid particle dispersion form having 60%–80% solid content [93]. Gupta and Vyas evaluated fluconazole loaded NLCs *in vivo* for effective eradication of cutaneous candidiasis in albino rats. Drug loaded NLCs showed 3.3 fold higher skin retention, better skin targeting effect, and high therapeutic efficacy compared to drug loaded SLNs [94]. Furthermore, econazole nitrate loaded thermodynamically stable NLCs were developed by Keshri and Pathak by the use of central composite design for transdermal drug delivery. Results of confocal microscopy revealed penetration of drug loaded NLCs upto stratum basale layer of animal skin. The developed formulation showed a minor change in particles size and zeta potential during 90 d stability analysis [95]. So, after reviewing literature, it can be considered that nanoparticles may be a good alternative to improve transdermal antifungal therapy.

4. Intellectual property rights (IPR) related to use of various vesicular nanocarriers for treatment of skin fungal infections

Vesicular nanocarriers show high therapeutic potential in the treatment of skin fungal infections. These novel carriers can be considered as an effective alternative to currently available marketed products to treat skin fungal infections. Therefore, pharmaceutical scientists have filed various patents regarding the use of various vesicular nanocarriers for treatment of skin fungal infections. Table 7 gives an overview of various patents granted regarding the use of vesicular nanocarriers in treatment of skin fungal infections.

5. Limitations and challenges in the use of vesicular nanocarriers for treatment of skin fungal infections

Vesicular nanocarrier systems are very effective to treat skin fungal infections due to their capability to modify the deliv-

ery of bioactive molecules to different skin layers and target diseased portion of the skin. Vesicular nanocarriers show high penetration of drug molecules in the skin through various mechanisms like fusion, absorption, and lipid exchange in skin layers. But, the excessive skin penetration enhancement may become a double edged sword because after heavy penetration, drug molecules may reach in blood circulation which can be harmful for localized treatment of skin fungal infections. Therefore, serious concerns regarding this must be taken by pharmaceutical scientists. Many other factors like safety profile, clinical efficacy, scale up techniques, and the fate of vesicular nanocarriers in transdermal therapeutics are still challenging for the scientists. Successful future research will help to answer these challenges and develop an ideal model of vesicular nanocarrier for effective transdermal antifungal therapeutics.

6. Conclusions

Significant increase in mortality rate has been observed in patients from last one decade due to fungal infections whether topical or systemic. There are several effective antifungal drugs available, but their therapeutic efficacy is limited due to unfavorable physicochemical characteristics and high toxicity profiles. Vesicular nanocarriers have capability to minimize these drawbacks of antifungal drugs due to their unique properties like high biocompatibility, ease of surface modification, and smaller size. Vesicular nanocarriers may be very effective to treat invasive skin fungal associated with immunosuppressive disease like AIDS as they show controlled drug release which do not activate the immune system of the patient. Beside all this, vesicular nanocarriers may improve stability and targeting effect of antifungal drugs to infected tissues along with the enhancement of their solubility and antifungal efficacy. Clinical evaluation of vesicular carriers is still a challenge because amphotericin B is the only antifungal drug which has been clinically evaluated in liposomal form and available in the market therefore, their presence in pharmaceutical market will be governed by successful clinical evaluation.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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