Carriers Containing Phospholipid Soft Vesicles

Subjects: Dermatology | Pharmacology & Pharmacy
Contributor: Elka Touitou

Topical drug delivery has many advantages over other ways of administration, having increased patient compliance, avoiding the first-pass effect following oral drug administration or not requesting multiple doses administration. However, the skin barrier prevents the access of the applied drug, affecting its therapeutic activity. Carriers containing phospholipid soft vesicles are a new approach to enhance drug delivery into the skin and to improve the treatment outcome. These vesicles contain molecules that have the property to fluidize the phospholipid bilayers generating the soft vesicle and allowing it to penetrate into the deep skin layers. Ethosomes, glycerosomes and transethosomes are soft vesicles containing ethanol, glycerol or a mixture of ethanol and a surfactant, respectively.

Keywords: soft phospholipid vesicle ; skin disorders ; ethosomes ; glycerosomes ; transethosomes ; skin infection ; skin inflammation ; skin cancer

1. Treatment of Acne Vulgaris

Acne vulgaris is one of the commonest skin disorders which mainly affect adolescents. Clinical symptoms of this condition include comedones, seborrhoea, erythematous papules and pustules. Nodules, deep pustules, and ultimate scarring are symptoms of severe acne. The pathogenesis of acne involves four mechanisms: follicular hyperkeratinization, *Propionibacterium acne* (*P. acne*) colonization, increased sebum production and local inflammation [1][2][3].

Owing to a better understanding of the pathogenesis of acne, new therapeutic modalities have been designed. Treatment of acne vulgaris is based of systemic or topical administration of therapy agents. Retinoids, antibiotics, benzoyl peroxide, salicylic acid and azelaic acid are the most prescribed topical medications for acne treatment [4]. These compounds are conventionally administrated in solution, gel or lotion-based formulations.

Touitou et al. [5] investigated an ethosomal gel containing clindamycin and salicylic acid for the treatment of acne vulgaris in a randomized double-blind clinical study of 8-weeks on 40 patients. The results indicated that in patients with mild to moderate symptoms, a significant reduction in the clinical signs including the number of inflammatory, non-inflammatory and total lesions was observed following the 8-weeks treatment (**Figure 1**). More than 70% of the participants reported partial or complete improvement. In addition, more than 80% of the patients, who underwent previous treatment with 1% clindamycin lotion, 5–10% benzoyl peroxide gels, 5% benzoyl peroxide–2% erythromycin gel, reported a better tolerability of the ethosomal gel with less burning, pruritus, erythema, and photosensitivity reactions.

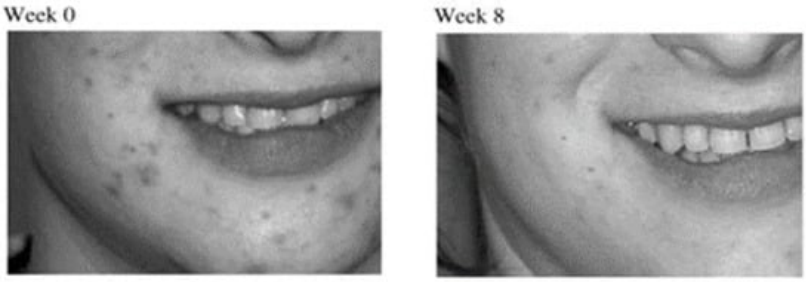

Figure 1. Photographs of a patient with moderate acne treated with clindamycin phosphate and salicylic acid ethosomal gel for 8 weeks at the baseline (week 0) and at the end of the study (week 8) (Reprinted with permission from ref. [5]. Copyright 2008 Touitou, E., et al.).

This superior effect of the ethosomal system is mainly attributed by the ethanol high concentration which fluidizes the bilayers of the phospholipid vesicles and the lipid membranes in the SC of the treated skin. Upon topical administration of

ethosomes, a dual mechanism of penetration enhancement takes place; the soft ethosome with fluid bilayer penetrates easily through the disrupted SC bilayers and reaches deeper into the skin where releases its drug content [6].

Another work describing the use of azelaic acid ethosomal systems for the treatment of acne vulgaris was published by Esposito et al. [7]. Ethosomal and liposomal dispersions of azelaic acid incorporated in Carbopol© 934P gel were designed and investigated. The active ingredient used in this work is a saturated natural dicarboxylic acid with activity against microbial colonization in the pilosebaceous unit in the skin, inflammation of the perifollicular area, sebum production and excretion and keratinization of the follicular channel. Diffusion studies through synthetic membranes indicated a rapid release rate of azelaic acid from the ethosomal systems containing higher ethanol concentration. For example, ethosomal dispersions containing 20 and 40% ethanol yielded release rates of 87.78 and 119.96 $\mu g \cdot cm^{-2} \cdot min^{-0.5}$, respectively. These values were up to 2-times higher than those achieved with the liposomal dispersion which yielded a release rate of 59.63 $\mu g \cdot cm^{-2} \cdot min^{-0.5}$. The authors also reported that the gel systems exhibited slower release rates yet maintained a higher release from ethosome versus liposome.

Yu et al. [8] designed ethosomes loaded with the natural anti-inflammatory molecule, cryptotanshinone (CPT) and incorporated them in a Carbomer 974 gel. The formulations were investigated for acne management as compared to conventional hydroethanolic gels. The anti-acne activity and skin irritation of the gel were evaluated in the oleic acid-induced acne rabbit model. Following treatment with CPT loaded ethosomes, the affected skin recovered and returned to its normal structure. No evidence of inflammation in the pilosebaceous unit or keratoplasia in the follicles and SC were observed. Lymphatic cell infiltration in the dermis was not observed. The epidermis had a similar thickness to that of the normal skin. This was in contrary to the outcomes of conventional gel treatment where keratoplasia and inflammation were present in the pilosebaceous follicles and SC.

Ansari et al. [9] incorporated the anti-inflammatory molecule karanjin in an ethosomal gel to enhance its topical delivery and activity against acne. The in vitro experiment using rat skin and Franz diffusion cells indicated a 2.4 higher deposition of the molecules in the animal skin than that of the solution. In addition, their formulation was found to be non-irritant to the skin as shown by Draize score which tested erythema, edema and scar formation. The anti-acne effect was also evident with the ethosomal treatment with a significant decrease in the number and size of the sebaceous gland units in dermis, as shown by the histopathologic examination. Furthermore, substantial anti-inflammatory effects in the carrageenan-induced edema in the rat paw were evident with inhibition of rat paw edema by 66.66 and 70.37% upon application of the ethosomal system and a clindamycin containing marketed formulation (0.5% gel), respectively.

The studies on acne vulgaris and other medical applications for carriers containing drugs incorporated in phospholipid soft vesicles are presented in **Table 1**.

Table 1. Skin disorders treated with active molecules incorporated in carriers containing phospholipid soft vesicles.

Treated Disorder	Investigated Vesicular Carrier	Study	Ref.
Acne Vulgaris	Clindamycin and salicylic acid ethosomal system	Clinical study on the reduction of acne vulgaris and skin tolerability of the formulation	[5]
	Azelaic acid ethosomal system	Diffusion study through synthetic membrane	[7]
	Cryptotanshinone ethosomal system	In vitro skin permeation and skin deposition; in vivo anti-acne activity on rabbits	[8]
	Karanjin ethosomal system	In vitro skin premeation study on excised rats skin; in vivo skin irritation study on rats; in vivo anti-inflammatory and anti- acne studies on rats	[9]
Viral infections	Acyclovir ethosomal system	Two-armed double-blind clinical study on subjects with recurrent herpes labialis	[10]
	Acyclovir ethosomal system	Antiviral activity against HSV-1 by plaque reduction assay in monolayer cultures of Vero cells	[11] [12]
	9-[(2-hydroxyethoxy) methyl]guanine ethosomal system	Antiviral activity against HSV-1 by plaque reduction assay in monolayer cultures of Vero cells	[12]
	Ethosomal system of the essential oil of *Melissa officinalis* L.	In vitro activity against HSV type 1 in mammalian cells	[13]

Treated Disorder	Investigated Vesicular Carrier	Study	Ref.
Bacterial infections	Bacitracin ethosomal system	Intracellular penetration and localization in fibroblasts (3T3); in vitro deposition and permeation through human cadaver skin	[14]
	Erythromycin ethosomal system	In vivo activity in mice model of deep dermal *S. aureus* infection	[15]
	Psoralen ethosomal system	Photodynamic therapy in biofilms formed in Petri dishes	[16]
Fungal infections	Voriconazole transethosomal system	In vitro skin permeation and deposition studies through mice skin; in vivo deposition study on mice	[17]
	Griseofulvin ethosomal system	In vitro permeation and deposition study on new-born pig skin	[18]
	Clove oil ethosomal system	Ex vivo permeation studies on rat skin; antifungal activity in cup plate test against *Candida albicans*	[19]
	Voriconazole ethosomal system	In vitro anti-fungal activity against *Asperigillus flavus* colonies. In vitro skin deposition and permeation through abdominal rat skin	[20]
	Econazole nitrate transethosomal system	Ex-vivo skin permeation and retention studies followed by in vitro antifungal activity *against C. albicans* fungus	[21]
Skin inflammation	Diclofenac ethosomal system	In vitro permeation study on rat skin; in vivo anti-inflammatory activity in carrageenan-induced rat paw edema model	[22] [23]
	Ammonium glycyrrhizinate ethosomal system	In vitro permeation through human skin; clinical study to evaluate the anti-inflammatory activity in volunteers with methyl nicotinate erythema	[24]
	Matrine ethosomal system	In vitro percutaneous permeation study on rat skin; in vivo anti-inflammatory activity in rat measured by reflection spectrophotometery	[25]
	Apigenin ethosomal system	In vitro and in vivo deposition study on rat skin; evaluation of the reduction of cyclooxygenase-2 levels in mouse with skin inflammation	[26]
	Crocin ethosomal system	Evaluation of the anti-inflammatory activity on healthy volunteers.	[27]
	Diclofenac ethosomal system and Diclofenac transethosomal system	In vitro permeation and deposition studies on rat skin	[28]
	Diclofenac glycerosoomal system	In vitro penetration and permeation studies on new-born pig skin	[29] [30] [31]
	Paeoniflorin glycerosomal system	In vitro permeation experiments through excised rat abdominal skin; in vivo deposition in rat synovium	[32]
	Achillea millefolium L. extract ethosomal system	In vitro permeation strudy through fresh rat skin	[33]
Psoriasis	Psoralen ethosomal system	In vitro permeation and penetration study using Franz diffusion cells and excised rat skin	[34]
	Methotrexate and Salicylic acid ethosomal system	In vitro retention and permeation study on pig ear skin; in vivo anti-psoriatic activity in mice model with imiquimod-induced psoriasis	[35]
	Anthralin ethosomal system	Preparation, comparative evaluation and clinical assessment in psoriatic patients	[36]
	Curcumin ethosomal system surface-modified with glycyrrhetinic acid-D-α-tocopherol acid polyethylene glycol succinate	In vitro anti-inflmmatory effect on interleukin-6-induced oxidative stress cell model; in vivo anti-psoriatic activity in mice model with imiquimod-induced psoriasis	[37]

Treated Disorder	Investigated Vesicular Carrier	Study	Ref.
Skin cancer	5- Fluorouracil ethosomal system containing -decyl methyl sulfoxide (Tumorep)	In vitro anti-tumor effect on five cell lines; in vivo anti-tumor effect in mice model of skin cancer	[38]
	Paclitaxel ethosomal system	In vitro permeation study on human SC; in vitro antiproliferative effect in squamous carcinoma cells	[39]
	Fe-chlorophyllin transethosomal system	In vitro skin permeation and deposition studies through mice skin; in vivo evaluation of the anti-cancer effect in mice	[40]
Skin injury (wound healing)	Plumbagin glycerosomal system	Ex vivo permeation study on rats skin	[41]
	Curcumin ethosomal system	In vivo wound healing effect in rats	[42]
	Curcumin-propylene glycol liposomal system	In vivo wound repair effect is rats with burned skin	[43]
	Thymosinβ-4(Tβ-4) ethosomal system	In vitro drug release study on mice skin; in vivo pharmacokinetic and skin irritation studies on mice	[44]
Skin pigmentation disorders	Linoleic acid ethosomal system	In vitro percutaneous permeation through human stratum corneum and viable epidermis membrane	[45]
	Methoxsalen ethosomal system	Ex vivo release studies and photo- toxicity after exposure to UV light	[46]
Hair loss	Minoxidil ethosomal system Minoxidil glycerosomal system	In vitro penetration and permeation through abdominal nude mice skin	[6][47] [48]
	Ethosomal systems of plant extracts	In vivo effect on hair growth in rats with testosterone induced alopecia	[49]
Skin aging	Vitamin E ethosomal system	In vitro permeation studies through skin and cultured fibroblasts	[50]
	Curcumin ethosomal system	Clinical trial evaluating skin viscoelasticity, total deformation, biological elasticity and sagginess	[51]
	Rosmarinic acid ethosomal system	Ex vivo permeation studies using Franz diffusion cells and mice skin; ex vivo antioxidant activity	[52]

2. Treatment of Viral Skin Infections

A significant percentage of skin diseases are caused by viral infections. Clinical manifestation of these disorders mainly include skin rash and lesions in the mucosal membrane, pain and discomfort. Herpes simplex (HSV) is the commonest cause of these disorders. The virus belongs to the herpes viridae family and has 2 types; type 1 and type 2. HSV-1, also known as herpes labialis, is the primary cause of cold sores, while HSV-2 leads to genital herpes [53]. Topical acyclovir is the first-line treatment of HSV infections. Other treatments include topical pencyclovir and orally administrated valacyclovir and famciclovir. Acyclovir selectively inhibits viral DNA polymerase, preventing further elongation of the nucleic acid chain and stopping the virus replication. Since the introduction of this antiviral agent, nearly 2 decades ago, its topical administration has been extensively investigated for the treatment of Herpes simplex. Unlike oral acyclovir treatments, topical therapy allows administration of smaller amounts of the drug, which is applied directly to the infected target site. Using this route, the dug systemic adverse effects can be reduced. Ointments and creams containing acyclovir at a concentration of 5%, applied every 3 h are the conventional topical dosage forms [54]. However, some clinical studies indicated that these products are of minor clinical effectiveness [55][56][57][58]. This could be due to non-sufficient penetration of the molecule into the basal epidermis, where the virus replicates. To answer the need of an improved treatment, acyclovir has been formulated using skin penetration enhancing carriers containing soft vesicles.

The first clinical evaluation of such a system was published by Touitou's group [10]. A double-blind, crossover 2-armed, randomized study was conducted on 40 subjects with recurrent herpes labialis. The study aimed to test the antiviral efficacy 5% acyclovir ethosomal system compared to the commercial cream (Zovirax®) and to ethosomal carrier not containing the drug. The reported results of the parallel part of the study indicated that the time for lesions crusting in the group treated with ethosomal system was 1.6 vs. 4.3 and 4.8 days for the commercial product and the empty vehicle, respectively. Lesions crusting time of 1.8 and 3.5 days for ethosomal system and the commercial cream, respectively, were obtained in the crossover section. Full crusts recovery was reported 4.2, 5.9 days after treatment with acyclovir ethosomal system and the Zovirax® cream, respectively (**Figure 2**). Regarding other clinical parameters (such as the

proportion of abortive lesions) an improvement was stated in 30% of episodes treated with ethosomes, compared to 10% of episodes treated with Zovirax.

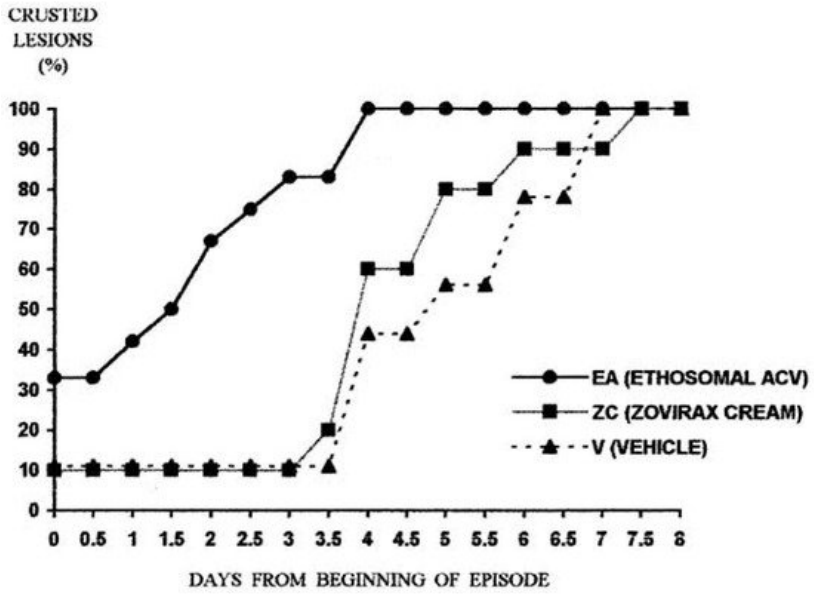

Figure 2. Days to crust formation: parallel arm. Day 0: 33% of lesions aborted in EA subgroup, 10% in ZC subgroup, 11% in V subgroup. Day 3: 80% of lesions crusted in EA subgroup, 10% in ZC subgroup, 11% in V subgroup. Day 4: 100% of lesions crusted in EA subgroup, 60% in ZC subgroup, 44% in V subgroup. Time to crusting of all lesions: 4 days in EA subgroup, 7 days in ZC subgroup, 7.5 days in V subgroup (Reprinted with permission from ref. [10]. Copyright 1999 Horwitz, E., et al.).

This improved antiviral effect of acyclovir when applied topically in ethosome can be explained by the ability of the carrier loaded with the drug to target the virus inside the cells. First, the ethosomal system containing a high ethanol concentration disrupts and fluidizes the cellular membrane which allows the soft vesicle to fuse and release its drug content inside the cell. This hypothesized mechanism of action was supported by the enhanced intracellular delivery of the amphipathic probe 4-(4-diethylamino) styryl-*N*-methylpyridinium iodide (D-289), the lipophilic probe rhodamine red (RR) and the fluorescent phosphatidylcholine (PC*) into fibroblasts, as examined in vitro by confocal laser scanning microscopy (CLSM). As an example, the measured RR fluorescence intensities by CLSM were 150, 40 and 20 arbitrary unit (A.U.) for the probe delivered in ethosomes, hydroethanolic solution and liposomes, respectively (**Figure 3**) [59].

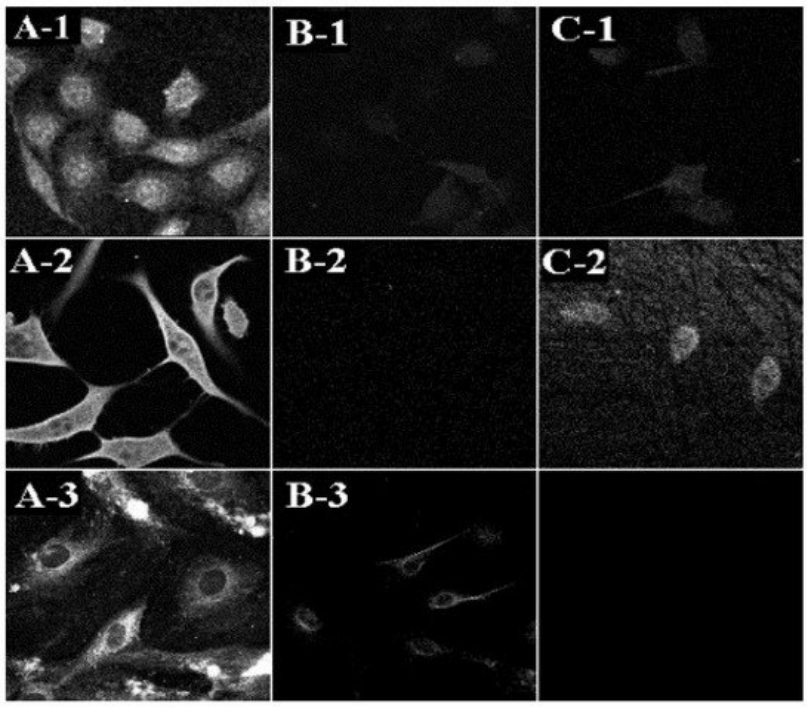

Figure 3. CLS micrographs showing intracellular fluorescence in fibroblasts following delivery of various fluorescent probes by ethosomes and control systems. Fluorescent probes (D289: **A1**–**C1**, RR: **A2**–**C2** or PC*: **A3**, **B3**) were applied to 3T3 fibroblasts for 10 min from various systems as follows: ethosomes: **A1**–**A3**; liposomes: **B1**–**B3**; hydroethanolic solution: **C1**, **C2** (Reprinted with permission from ref. [59]. Copyright 2001 Touitou, E., et al.).

Recently, glycerosomes, carriers containing soft phospholipid vesicles owing to the presence of glycerol, have been investigated for treatment of HSV-1. Vanti et al. [13] incorporated the essential oil of Melissa officinalis, in these carriers as a strategy to increase their stability and enhance their antiviral activity against HSV type 1. The results of the in vitro experiment on mammalian cells indicated that glycerosomes containing the oil were efficient in inhibiting HSV type 1 infection, without producing cytotoxic effects.

3. Treatment of Bacterial Skin Infections

Skin infections such as cellulitis, erysipelas and trauma- and wound-related infections are among the most frequent conditions demanding acute ambulatory care. *Staphylococcus aureus* is the major cause of such conditions. Inappropriate treatment may lead to severe complications and hospital admission. In some cases, these infections may spread to other body parts causing serious morbidity and mortality.

Topical administration of antibiotics from conventional topical dosage forms is associated with limited penetration into the deep skin layer leading to poor prognosis and insufficient treatment efficiency. Oral or parenteral antibiotics at high doses of macrolides, β-lactams or other antibacterial agents are usually used to control such cases. However, systemic antibiotic treatment may increase the incidence of side effects and allergic reactions which may affect the patient convenience [60][61][62][63].

Topical administration of anti-bacterial and antibiotic agents from carriers containing soft vesicles has been considered as a safe and promising approach for the treatment of deep skin infections. This is owing to the ability of these carriers to carry the antibiotic into deep skin strata for eradication of bacterial infections. Godin and Touitou in a CLSM study showed that FITC-bacitracin administrated in ethosomes to rats, penetrated to the deep layers of the skin through the inter-corneocyte pathway in the SC. This efficient delivery was not observed when the molecule was applied in a hydroethanolic solution or in liposomes. These data emphasize the important role of the soft ethosomal vesicles in the delivery mechanism [14].

Touitou and Godin [15] investigated in vitro and in vivo an ethosomal erythromycin system to treat deep skin and soft tissue bacterial infections following topical drug application. In vitro susceptibility studies on the bacterial strains: *S. aureus* ATCC29213, *B. subtilis* ATCC6633, and *S. aureus*, showed that the ethosomal system yielded significantly larger inhibition zones and lower erythromycin minimum inhibition concentration (MIC) in comparison with hydroethanolic solution of the drug. Erythromycin ethosomal system applied topically to Swiss Albino ICR (CD-1) mice with *S. aureus* ATCC29213 infection, completely cured the infection. On the other hand, application of the antibiotic from a hydroethanolic solution was not efficient in inhibiting the infection. Histopathological examination indicated that animals treated with the hydroethanolic solution developed deep dermal and subcutaneous abscesses with necrotic destroyed skin and dense infiltrates of neutrophils and macrophages.

Bagchi et al. tested the antibacterial effect of ethosomes containing psoralen on cultures of *Escherichia coli*, *Staphylococcus aureus* and on bacterial biofilms. The cultures were exposed to UVA radiation for 30 min. As reported by the authors, application of psoralen from ethosomes reduced significantly the bacterial survival by ~70%. Destruction of the bacterial adherence in the biofilm was also observed. It was suggested the fluidizing effect of ethosome enhanced the permeation of into the bacterial cell. Such promising approach may open a new strategy for the treatment of multi-drug-resistant bacterial-induced skin diseases [16].

4. Treatment of Fungal Skin Infections

Fungal infections of the skin and nails account for a large percentage of the skin diseases. These infections vary in the severity from being superficial and potentially self-limited infections, such as the common dermatophyte infections, to severe conditions with dissemination or local invasion into deeper tissue. Many of the fungi affecting the skin can also cause nail infections called Onychomycosis. Topical antifungals creams of griseofulvin, terbinafine, fluconazole and itraconazole for several weeks are the most common treatment in the cases of fungal infections [64]

Ethosomal systems containing antifungal agents such as griseofulvin (GRF), clove oil and voriconazole were investigated. GRF, an antibiotic with activity against a wide spectrum of fungal infections, was incorporated in ethosomes by Marto et al. The ability of the carrier to deliver the compound deeply into/through newborn pig skin was investigated in vitro. The reported results indicated that almost 40% of the drug was found in the SC at the end of the experiment. These findings were confirmed by the fluorescence assay with Nile red-loaded ethosomes which evidenced its retention in this layer. The

in vitro antifungal activity of GRF in ethosomes, tested against *T. rubrum* ATCC 28188, indicated marked fungicidal effect. On the other hand, empty ethosomes did not exhibit any antifungal activity [18].

In another study, the antifungal activity of clove oil ethosomes incorporated in a carbopol 974 gel was studied by Shetty et al. The ex vivo drug permeation into albino rat skin showed that a cumulative drug release of 83.11 ± 0.54% after 12 h application. The antifungal activity against *Candida albicans* measured in studies carried out using the cup plate method revealed a very significant ($p < 0.01$) antifungal activity for the ethosomal composition relative to pure clove oil. The authors suggest that the above described formulation could be promising in the topical delivery of clove oil for the treatment of fungal infections [19].

In a recent study, a modified ethosomal system containing the penetration enhancer, cinnamaldehyde, was investigated for dermal targeting delivery of terbinafine [65]. This system significantly improved the targeting efficiency leading to a drug deposition of around 18.5% in the deep skin layers compared to only 5.6% for a commercial Lamisil® cream. The dermal targeting effect was further confirmed by visualization of rhodamine-labeled system across SC into the epidermis and the dermis layers of hairless rats. The skin irritation tendency, also evaluated in vivo on rabbits using the Draize scoring method, which tests erythema, edema and scar formation, indicated that sub-chronic application of the system for 7 days is safe and nonirritating. The antifungal activity of this system was proved in vitro on *Candida albicans* strains by minimal inhibitory concentration (MIC) assay. The reported results indicated that lower drug doses are required for efficient treatment of skin fungal infections as compared to DMSO solution of the drug. As suggested by the authors, this approach may improve the patient compliance and decrease the incidence and severity of side effects leading to better therapeutic outcome.

5. Treatment of Skin Inflammation

Skin inflammation is a major skin disorder requiring medical intervention. It is a complex process occurring in the body in answer to tissue damage. This condition can be provoked by pathogens, noxious mechanical and chemical agents and as an autoimmune response. Common symptoms of inflammation are redness, swelling, itching, heat, and pain [66]. Atopic dermatitis, or atopic eczema, is a common chronic inflammatory skin disease [67]. Treatment of atopic dermatitis comprises topical administration of glucocorticosteroids and immunosuppressants, such as tacrolimus and pimecrolimus. Microbial colonization and superinfection may induce disease exacerbation and can justify additional antimicrobial treatment [68]. Local treatment with these agents administrated to the skin from conventional carriers is not always effective.

Paolino et al. [24] evaluated the anti-inflammatory effect of the ethosomal system of ammonium glycyrrhizinate in a clinical study on twelve healthy volunteers. The anti-inflammatory activity and the tolerability of the system were evaluated using the non-invasive reflectance spectrophotometry method in healthy volunteers with methyl nicotinate induced skin erythema. An ethosomal formulation containing 0.3% *w/v* ammonium glycyrrhizinate was administrated to the participants affected area and compared to the administration of hydroethanolic and aqueous solutions of the active molecule. The measured erythema index (ΔEI) was 29.6% for the ethosomal system versus 62.7 and 60.7% for the comparative ethanolic and aqueous solutions, respectively.

In another work, ethosomal compositions of matrine, triptolide, apigenin and crocin were designed and tested in vivo on animal models of skin inflammation. The effect of the carrier on the percutaneous permeation of the compound in vitro and its anti-inflammatory activity in vivo in rat skin were studied. A rapid and effective anti-inflammatory effect of the tested active molecules incorporated in the ethosomal carrier were reported [25][26][27][69].

Recently, Andleeb et al. [33] incorporated in ethosomes containing ethanol and propylene glycol and in an ethosomal gel the antioxidant extract of *Achillea millefolium* L. In vitro drug release study using Franz diffusion cells and fresh rat skin indicated cumulative peremeated compound amounts of 78.6, 79.8, 30 and 28.7% from the ethosomal formulation, the ethosomal gel, a hydroethanolic extract and a conventional gel, respectivley.

References

1. Gollnick, H.P.; Zouboulis, C.C.; Akamatsu, H.; Kurokawa, I.; Schulte, A. Pathogenesis and pathognesis-related treatme nt of acne. J. Dermatol. 1991, 18, 489–499.
2. Leyden, J.J. New understanding of the pathogenesis of acne. J. Am. Acad. Dermatol. 1995, 32, 515–525.

3. Gollnick, H.P.; Krautheim, A. Topical treatment in acne: Current status and future aspects. Dermatology 2003, 206, 29–36.

4. Strauss, J.S.; Krowchuk, D.P.; Leyden, J.J.; Lucky, A.W.; Shalita, A.R.; Siegfried, E.C.; Thiboutot, D.M.; Van Voorhees, A.S.; Beutner, K.A.; Sieck, C.K.; et al. American Academy of Dermatology/American Academy of Dermatology Association. Guidelines of care for acne vulgaris management. J. Am. Acad. Dermatol. 2007, 56, 651–663.

5. Touitou, E.; Godin, B.; Shumilov, M.; Bishouty, N.; Ainbinder, D.; Shouval, R.; Ingber, A.; Leibovici, V. Efficacy and tolerability of clindamycin phosphate and salicylic acid gel in the treatment of mild to moderate acne vulgaris. J. Eur. Acad. Dermatol. Venereol. 2008, 22, 629–631.

6. Touitou, E.; Dayan, N.; Bergelson, L.; Godin, B.; Eliaz, M. Ethosomes- novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. J. Control. Release 2000, 65, 403–418.

7. Esposito, E.; Menegatti, E.; Cortesi, R. Ethosomes and liposomes as topical vehicles for azelaic acid: A preformulation study. Int. J. Cosmet. Sci. 2004, 55, 253–264.

8. Yu, Z.; Lv, H.; Han, G.; Ma, K. Ethosomes loaded with cryptotanshinone for acne treatment through topical gel formulation. PLoS ONE 2016, 11, e0159967.

9. Ansari, S.A.; Qadir, A.; Warsi, M.H.; Mujeeb, M.; Aqil, M.; Mir, S.R.; Sharma, S. Ethosomes-based gel formulation of karanjin for treatment of acne vulgaris: In vitro investigations and preclinical assessment. 3 Biotech 2021, 11, 1–14.

10. Horwitz, E.; Pisanty, S.; Czerninski, R.; Helser, M.; Eliav, E.; Touitou, E. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 1999, 87, 700–705.

11. Cortesi, R.; Ravani, L.A.; Zaid, A.N.A.; Menegatti, E.A.; Romagnoli, R.A.; Drechsler, M.A.; Esposito, E.A. Ethosomes for the delivery of anti-HSV-1 molecules: Preparation, characterization and in vitro activity. Pharmazie 2010, 65, 743–749.

12. Cortesi, R.; Ravani, L.; Menegatti, E.; Drechsler, M.; Esposito, E. Colloidal dispersions for the delivery of acyclovir: A comparative study. Indian J. Pharm. Sci. 2011, 73, 687–693.

13. Vanti, G.; Ntallis, S.G.; Panagiotidis, C.A.; Dourdouni, V.; Patsoura, C.; Bergonzi, M.C.; Lazari, D.; Bilia, A.R. Glycerosome of Melissa officinalis L. Essential Oil for Effective Anti-HSV Type 1. Molecules 2020, 25, 3111.

14. Godin, B.; Touitou, E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. J. Control. Release 2004, 94, 365–379.

15. Godin, B.; Touitou, E. Erythromycin ethosomal systems: Physicochemical characterization and enhanced antibacterial activity. Curr. Drug Deliv. 2005, 2, 269–275.

16. Bagchi, D.; Dutta, S.; Singh, P.; Chaudhuri, S.; Pal, S.K. Essential dynamics of an effective phototherapeutic drug in a nanoscopic delivery vehicle: Psoralen in ethosomes for biofilm treatment. ACS Omega 2017, 2, 1850–1857.

17. Song, C.K.; Balakrishnan, P.; Shim, C.K.; Chung, S.J.; Chong, S.; Kim, D.D. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: Characterization and in vitro/in vivo evaluation. Colloids Surf. B Biointerfaces 2012, 92, 299–304.

18. Marto, J.; Vitor, C.; Guerreiro, A.; Severino, C.; Eleutério, C.; Ascenso, A.; Simões, S. Ethosomes for enhanced skin delivery of griseofulvin. Colloids Surf. B Biointerfaces 2016, 146, 616–623.

19. Shetty, S.; Jose, J.; Kumar, L.; Charyulu, R.N. Novel ethosomal gel of clove oil for the treatment of cutaneous candidiasis. J. Cosmet. Dermatol. 2018, 18, 862–869.

20. Faisal, W.; Soliman, G.M.; Hamdan, A.M. Enhanced skin deposition and delivery of voriconazole using ethosomal preparations. J. Liposome Res. 2018, 28, 14–21.

21. Verma, S.; Utreja, P. Transethosomes of Econazole Nitrate for Transdermal Delivery: Development, In-vitro Characterization, and Ex-vivo Assessment. Pharm. Nanotechnol. 2018, 6, 171–179.

22. Touitou, E. Compositions for Applying Active Substances to or through the Skin. U.S. Patent 5,716,638, 10 February 1998.

23. Jain, S.; Patel, N.; Madan, P.; Lin, S. Formulation and rheological evaluation of ethosome-loaded carbopol hydrogel for transdermal application. Drug Dev. Ind. Pharm. 2016, 42, 1315–1324.

24. Paolino, D.; Lucania, G.; Mardente, D.; Alhaique, F.; Fresta, M. Ethosomes for skin delivery of ammonium glycyrrhizinate: In vitro percutaneous permeation through human skin and in vivo anti inflammatory activity on human volunteers. J. Control. Release 2005, 106, 99–110.

25. Zhaowu, Z.; Xiaoli, W.; Yangde, Z.; Nianfeng, L. Preparation of matrine ethosome, its percutaneous permeation in vitro and anti- inflammatory activity in vivo in rats. J. Liposome Res. 2009, 19, 155–162.

26. Shen, L.N.; Zhang, Y.T.; Wang, Q.; Xu, L.; Feng, N.P. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. Int. J. Pharm. 2014, 460, 280–288.

27. Esposito, E.; Drechsler, M.; Huang, N.; Pavoni, G.; Cortesi, R.; Santonocito, D.; Puglia, C. Ethosomes and organogels for cutaneous administration of crocin. Biomed. Microdevices 2016, 18, 108.

28. Ghanbarzadeh, S.; Arami, S. Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes. BioMed Res. Int. 2013, 2013, 616810.

29. Manca, M.L.; Zaru, M.; Manconi, M.; Lai, F.; Valenti, D.; Sinico, C.; Fadda, A.M. Glycerosomes: A new tool for effective dermal and transdermal drug delivery. Int. J. Pharm. 2013, 455, 66–74.

30. Manca, M.L.; Cencetti, C.; Matricardi, P.; Castangia, I.; Zaru, M.; Sales, O.D.; Nacher, A.; Valenti, D.; Maccioni, A.M.; Fadda, A.M.; et al. Glycerosomes: Use of hydrogenated soy phosphatidylcholine mixture and its effect on vesicle features and diclofenac skin penetration. Int. J. Pharm. 2016, 511, 198–204.

31. Manca, M.L.; Manconi, M.; Zaru, M.; Valenti, D.; Peris, J.E.; Matricardi, P.; Maccioni, A.M.; Fadda, A.M. Glycerosomes: Investigation of role of 1, 2-dimyristoyl-sn-glycero-3-phosphatidycholine (DMPC) on the assembling and skin delivery performances. Int. J. Pharm. 2017, 532, 401–407.

32. Zhang, K.; Zhang, Y.; Li, Z.; Li, N.; Feng, N. Essential oil-mediated glycerosomes increase transdermal paeoniflorin delivery: Optimization, characterization, and evaluation in vitro and in vivo. Int. J. Nanomed. 2017, 12, 3521–3532.

33. Andleeb, M.; Shoaib Khan, H.M.; Daniyal, M. Development, Characterization and Stability Evaluation of Topical Gel Loaded with Ethosomes Containing Achillea millefolium L. Extract. Front. Pharmacol. 2021, 12, 336.

34. Zhang, Y.T.; Shen, L.N.; Wu, Z.H.; Zhao, J.H.; Feng, N.P. Comparison of ethosomes and liposomes for skin delivery of psoralen for psoriasis therapy. Int. J. Pharm. 2014, 471, 449–452.

35. Chandra, A.; Aggarwal, G.; Manchanda, S.; Narula, A. Development of topical gel of methotrexate incorporated ethosomes and salicylic acid for the treatment of psoriasis. Pharm. Nanotechnol. 2019, 7, 362–374.

36. Fathalla, D.; Youssef, E.M.K.; Soliman, G.M. Liposomal and Ethosomal Gels for the Topical Delivery of Anthralin: Preparation, Comparative Evaluation and Clinical Assessment in Psoriatic Patients. Pharmaceutics 2020, 12, 446.

37. Guo, T.; Lu, J.; Fan, Y.; Zhang, Y.; Yin, S.; Sha, X.; Feng, N. TPGS assists the percutaneous administration of curcumin and glycyrrhetinic acid coloaded functionalized ethosomes for the synergistic treatment of psoriasis. Int. J. Pharm. 2021, 604, 120762.

38. Ainbinder, D.; Touitou, E. A new approach for skin tumor treatment: From delivery system characterization to in vivo evaluation. Drug Deliv. Trans. Res. 2011, 1, 53–65.

39. Paolino, D.; Celia, C.; Trapasso, E.; Cilurzo, F.; Fresta, M. Paclitaxel-loaded ethosomes®: Potential treatment of squamous cell carcinoma, a malignant transformation of actinic keratoses. Eur. J. Pharm. Biopharm. 2012, 81, 102–112.

40. Rady, M.; Gomaa, I.; Afifi, N.; Abdel-Kader, M. Dermal delivery of Fe-chlorophyllin via ultradeformable nanovesicles for photodynamic therapy in melanoma animal model. Int. J. Pharm. 2018, 548, 480–490.

41. Md, S.; Alhakamy, N.A.; Aldawsari, H.M.; Husain, M.; Khan, N.; Alfaleh, M.A.; Asfour, H.Z.; Riadi, Y.; Bilgrami, A.L.; Akhter, M.H. Plumbagin-Loaded Glycerosome Gel as Topical Delivery System for Skin Cancer Therapy. Polymers 2021, 13, 923.

42. Partoazar, A.; Kianvash, N.; Darvishi, M.H.; Nasoohi, S.; Rezayat, S.M.; Bahador, A. Ethosomal curcumin promoted wound healing and reduced bacterial flora in second degree burn in rat. Drug Res. 2016, 66, 660–665.

43. Kianvash, N.; Bahador, A.; Pourhajibagher, M.; Ghafari, H.; Nikoui, V.; Rezayat, S.M.; Dehpour, A.R.; Partoazar, A. Evaluation of propylene glycol nanoliposomes containing curcumin on burn wound model in rat: Biocompatibility, wound healing, and anti-bacterial effects. Drug Deliv. Transl. Res. 2017, 7, 654–663.

44. Fu, X.; Shi, Y.; Wang, H.; Zhao, X.; Sun, Q.; Huang, Y.; Qi, T.; Lin, G. Ethosomal Gel for Improving Transdermal Delivery of Thymosin β-4. Int. J. Nanomed. 2019, 14, 9275–9284.

45. Celia, C.; Cilurzo, F.; Trapasso, E.; Cosco, D.; Fresta, M.; Paolino, D. Ethosomes® and transfersomes® containing linoleic acid: Physicochemical and technological features of topical drug delivery carriers for the potential treatment of melasma disorders. Biomed. Microdevices 2012, 14, 119–130.

46. Garg, B.J.; Garg, N.K.; Beg, S.; Singh, B.; Katare, O.P. Nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: Formulation optimization, in vitro evaluation and preclinical assessment. J. Drug Target 2016, 24, 233–246.

47. Meidan, V.M.; Touitou, E. Treatments for Androgenetic Alopecia and Alopecia Areata. Drugs 2001, 61, 53–69.

48. Rani, D.; Sharma, V.; Manchanda, R.; Chaurasia, H. Formulation, Design and Optimization of Glycerosomes for Topical Delivery of Minoxidil. Res. J. Pharmacol. Technol. 2021, 14, 2367–2374.

49. Madhunithya, E.; Venkatesh, G.; Shyamala, G.; Manjari, V.; Ramesh, S.; Karuppaiah, A.; Sankar, V. Development of et hosome comprising combined herbal extracts and its effect on hair growth. Adv. Tradit. Med. 2021, 21, 131–141.
50. Touitou, E.; Godin, B. Skin nonpenetrating sunscreens for cosmetic and pharmaceutical formulations. Clin. Dermatol. 2 008, 26, 375–379.
51. Jeswani, G.; Saraf, S. Topical Delivery of Curcuma longa extract loaded nanosized ethosomes to combat facial wrinkle s. J. Pharm. Drug Deliv. Res. 2014, 3, 1–8.
52. Yücel, Ç.; Şeker Karatoprak, G.; Değim, İ.T. Anti-aging formulation of rosmarinic acid-loaded ethosomes and liposome s. J. Microencapsul. 2019, 36, 180–191.
53. Ramdass, P.; Mullick, S.; Farber, H.F. Viral skin diseases. Prim. Care Clin. Off. 2015, 42, 517–567.
54. Brady, R.C.; Bernstein, D.I. Treatment of herpes simplex virus infections. Antivir. Res. 2004, 61, 73–81.
55. Spruance, S.L.; Crumpacker, C.S. Topical 5% acyclovir in polyethylene-glycol for herpes simplex labialis: Antiviral effect without clinical benefit. Am. J. Med. 1982, 73, 315–319.
56. Raborn, G.W.; McGaw, W.T.; Grace, M.; Houle, L. Herpes labialis treatment with acyclovir 5% ointment. J. Can. Dent. A ssoc. 1989, 55, 135–137.
57. Fiddian, A.P.; Ivanyi, L. Topical acyclovir in the management of herpes labialis. Br. J. Dermatol. 1983, 109, 321–326.
58. Raborn, G.W.; McGaw, W.T.; Grace, M.; Percy, J.; Samuels, S. Herpes labialis treatment with acyclovir 5% modified aq ueous cream: A double blind randomized trial. Oral Surg. Oral Med. Oral Pathol. 1989, 67, 676–679.
59. Touitou, E.; Godin, B.; Dayan, N.; Weiss, C.; Piliponsky, A.; Levi-Schaffer, F. Intracellular delivery mediated by an ethos omal carrier. Biomaterials 2001, 22, 3053–3059.
60. Naimi, T.S.; LeDell, K.H.; Como-Sabetti, K. Comparison of community- and health care-associated methicillin-resistant Staphylococcus aureus infection. JAMA 2003, 290, 2976–2984.
61. Fung, H.B.; Chang, J.Y.; Kuczynski, S. A practical guide to the treatment of complicated skin and soft tissue infections. Drugs 2003, 63, 1459–1480.
62. O'Dell, M.L. Skin and wound infections: An overview. Am. Fam. Physician 1998, 57, 2424–2432.
63. Nichols, R.L. Optimal treatment of complicated skin and skin structure infections. J. Antimicrob. Chemother. 1999, 44, 1 9–23.
64. Eldridge, M.L.; Chambers, C.J.; Sharon, V.R.; Thompson, G.R., III. Fungal infections of the skin and nail: New treatmen t options. Expert Rev. Anti Infect. Ther. 2014, 12, 1389–1405.
65. Zhang, L.; Li, X.; Zhu, S.; Zhang, T.; Maimaiti, A.; Ding, M.; Shi, S. Dermal Targeting Delivery of Terbinafine Hydrochlori de Using Novel Multi-Ethosomes: A New Approach to Fungal Infection Treatment. Coatings 2020, 10, 304.
66. Ferrero-Miliani, L.; Nielsen, O.H.; Andersen, P.S.; Girardin, S.E. Chronic inflammation: Importance of NOD2 and NALP 3 in interleukin-1beta generation. Clin. Exp. Immunol. 2007, 147, 227–235.
67. Kim, J.; Kim, B.E.; Leung, D.Y. Pathophysiology of atopic dermatitis: Clinical implications. Allergy Asthma Proc. 2019, 4 0, 84.
68. Eichenfield, L.F.; Tom, W.L.; Berger, T.G.; Krol, A.; Paller, A.S.; Schwarzenberger, K.; Bergman, J.N.; Chamlin, S.L.; Co hen, D.E.; Cooper, K.D.; et al. Guidelines of care for the management of atopic dermatitis: Section 2. Management and treatment of atopic dermatitis with topical therapies. J. Am. Acad. Dermatol. 2014, 71, 116–132.
69. Chen, J.G.; Liu, Y.F.; Gao, T.W. Preparation and anti-inflammatory activity of triptolide ethosomes in an erythema mode l. J. Liposome Res. 2010, 20, 297–303.