Dermal drug delivery with ethosomes: therapeutic potential

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There is an increasing need to target drugs to the deeper layers of the skin to achieve efficient dermal therapy. There are many patient populations of various ages waiting for new and advanced treatments, including noninvasive therapy for deep and resistant bacterial and viral skin infections, regional therapy for rheumatoid arthritis and more efficient treatment of pilosebaceous structure-related disorders. Thus, ethosomes, innovative carriers capable of overcoming the skin barrier, could provide a solution for delivering drugs into the deep skin strata for successful dermal therapy. The basic characteristics of the ethosome, as well as the mechanism by which this carrier facilitates absorption of drugs in various layers of the skin, are presented, and results with ethosomal formulations designed for new and improved dermal and regional therapies both at experimental and clinical levels are discussed. The efficiency of ethosomal delivery systems containing acyclovir, clindamycin, minoxidil, cannabidiol and erythromycin for dermal therapies in *ex vivo* and *in vivo* studies is reviewed. New and advanced therapies proposed with ethosomal drugs could highly increase drug-treatment efficiency and patient convenience.

Keywords: acne vulgaris, antibiotics, antivirals, cannabinoids, deep skin infections, dermatological agents, ethosomes, HSV-1, inflammation

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Ethosome characteristics & mechanism of action

For many dermatological drugs, the deep layers of the skin, viable epidermis and dermis, are the target tissues of action. However, the therapeutic efficacy of topically applied drugs is often far from that required, owing to the resistance of the stratum corneum (SC), the outermost layer of the epidermis, to their transport into and across the skin. The barrier characteristics of SC can be explained by its composition and organization. The SC is composed of approximately 70% protein, 15% lipid and 15% water [1–3]. The SC can be described as a 'brick and mortar' structure, where the intercellular lipids usually present in their crystalline phase represent the only continuous region (the 'mortar'). The main components of the intercellular lipids are ceramids, free fatty acids, cholesterol and cholesteryl esters. It is noteworthy that no phospholipids are present in the SC, a characteristic feature that distinguishes it from other biological membranes [3–6].

A penetrant molecule, when applied on the skin surface, has three potential routes of entry to the deep skin tissue: through the transcellular and intercellular routes in the SC, or through the transappendageal route. However, the main path for the permeation of the majority of molecules is commonly believed to be the tortuous intercellular route [1–3,7].

While small molecules with medium lipophilicity are able to permeate through the SC barrier unassisted, for most therapeutic agents the required concentrations in the deep skin layers can usually be achieved only by using an efficient permeation enhancement technique [3,8]. In other words, safe carriers with adequate skin penetration enhancement properties are needed. This paper reviews the ethosomal carrier, which can provide an answer to these needs for many drugs with various physicochemical properties and pharmacological actions.

Ethosomes are innovative carriers that meet the essential criteria for efficient and safe administration of lipophilic or hydrophilic drugs [9,10,101]. Ethosomes differ from liposomes or other lipid vesicles in their composition, structure, mechanism of action and delivery properties. In contrast to liposomes, the ethosomal carrier, based on phospholipid soft vesicles in a hydroethanolic environment, can deliver molecules into the various layers of the skin or transdermally [9]. The existence of vesicles in the ethosomal systems and their size and structure were observed by various methods, including ^{31}P-nuclear magnetic resonance, dynamic light scattering, transmission electron microscopy and scanning electron microscopy [9]. Ethosomal systems contain unilamellar or multilamellar lipid vesicles between 30 nm to several microns in diameter, and their structure and size can be

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modulated by the system composition [9–12,101]. Multilamellar ethosomes are characterized by a fingerprint-like structure, with phospholipid bilayers throughout the vesicle.

One of the unique features of ethosomes is their ability to efficiently encapsulate molecules of various physicochemical properties. The presence of ethanol, together with the high vesicle lamellarity, allow for efficient entrapment of hydrophilic, lipophilic and amphiphilic molecules [9,11–13,101]. For example, ultracentrifugation studies demonstrated that the encapsulation efficiency of ethosomes could be as high as 99 and 83% in the case of the lipophilic molecules α-tocopherol and minoxidil, respectively [9,13]. This is unlike the problematic entrapment of lipophilic compounds in liposomes, which is restricted by a limited number of phospholipid bilayers surrounding the aqueous core.

To investigate the properties of ethosomes that enable them to efficiently promote drug delivery across the SC barrier of the skin, the transition temperature (Tm) of vesicular lipids and free-energy measurements of the vesicle bilayers were assessed by differential scanning calorimetry and fluorescence anisotropy. According to the difference of 20–35°C between Tm values of ethosomes and classic liposomes containing the same phospholipids and no ethanol, as measured by differential scanning calorimetry, phospholipid bilayers in ethosomes are in a more fluid state [9,10–12]. These results are supported by fluorescent anisotropy measurements of 9-antrivinyl labeled analogue of phosphatidylcholine, where a 20% lower value was measured in comparison with liposomes [9]. The above data suggest that, compared with liposomes, ethosomes are much less rigid and possess a 'soft' structure, which could be related to the fluidizing effect of ethanol on the phospholipid bilayers of the vesicle. An additional essential effect of ethanol present in this system is the disturbance of SC lipids organization. The proposed mechanism of skin permeation enhancement by ethosomal systems involves a number of processes. First is the dual fluidizing effect of ethanol on the vesicle phospholipids, as well as on the SC lipids. This step is followed by the transport of the soft ethosomal vesicle between the disturbed SC lipid bilayers. Furthermore, ethosomes penetrating the fluidized SC bilayers, generating a pathway across the SC lipids, fuse with cell membranes within the deep layers of the skin, where they release their contents [9]. Data from a number of studies show that the follicular route is another penetration pathway that can be involved in delivery with ethosomes [12,14].

Numerous studies have shown that ethosomes are safe to the skin and dermal cells and stable for at least 2 years [9–11,15]. The ingredients composing the ethosomal systems have generally been recognized as safe (GRAS status). The safety of ethosomal systems was assessed in *in vitro* and *in vivo* studies, as summarized in

Box 1. Safety studies carried out with ethosomes.

In vitro *studies on cultured cells [10,15]*

- Ethosomal carriers were not toxic to 3T3 fibroblasts and the cultured cells kept their viability as assessed in a live/dead viability/cytotoxicity viability test.

Studies in animals [9,101]

- No acute skin irritation in rabbits was observed following a single-dose, 48-h, occlusive application of patches containing the ethosomal systems.
- No erythema was generated following cumulative 14-day repeated ethosomal patch in rabbits.

Studies in humans [16]

- No signs of erythema following 12-, 24- or 48-h application were reported in tolerability experiments with ethosomes on healthy volunteers using reflectance spectrophotometry.
- No significant variations in erythema index were measured between skin areas treated with ethosomes compared with saline.

Data from clinical trials [17,19]

- No adverse skin reactions were associated with the treatment in clinical trials with ethosomal preparations.

Postmarketing information

- No reported adverse reaction with marketed ethosomal formulations (SupraVir cream).

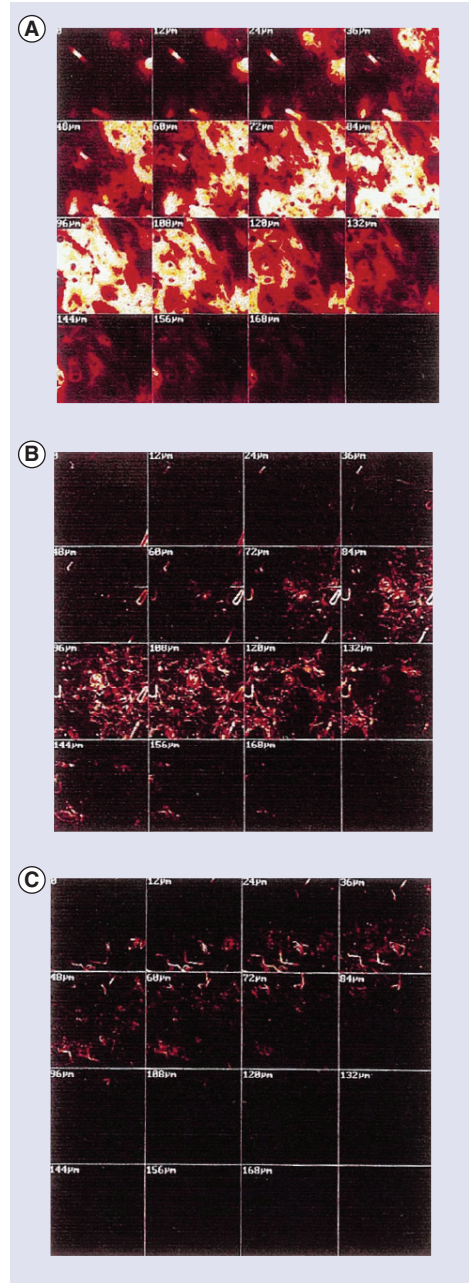
Box 1. Ethosomes versus control systems were examined for their skin tolerability in a study carried out on healthy volunteers, using reflectance spectrophotometry as a noninvasive technique [16]. The authors reported no signs of erythema following 12-, 24- or 48-h application of ethosomes containing 2% phospholipid and 45% ethanol. Moreover, no significant difference in erythema index was measured between skin areas treated with ethosomes compared with saline.

Feasibility of enhanced drug skin penetration from ethosomes, tested in *in vitro* studies

Challenging therapeutic agents were formulated with ethosomes to enhance their dermal and percutaneous absorption. Among these were cationic and anionic drugs, highly lipophilic and hydrophilic molecules, polypeptides and proteins.

In a number of *in vitro* studies designed to assess the enhanced delivery properties of the ethosomal carrier versus other control systems (hydroethanolic solution and liposomes), the penetration of fluorescent probes with different physicochemical characteristics was monitored by confocal laser scanning microscopy (CLSM) [9,11,15]. The evaluated molecules were calcein, a water-soluble compound, the lipophilic phospholipid probe rhodamine red dihexadecanoyl glycerophosphoethanolamine (RR) and the amphiphilic cationic probe 4-(4-diethylamino) styryl-*N*-methylpyridinium iodide. When applied on the skin from the ethosomal carrier, all three probes were delivered deep into the skin layers, exhibiting high fluorescent intensity. For example, CLS micrographs presented in **Figure 1**, obtained after 8-h application of RR from ethosomes, hydroalcoholic solution and liposomes to nude mice skin, illustrate that delivery from ethosomes resulted in the highest intensity of fluorescence up to a depth of 150 µm, while liposomes remained essentially on the surface of the skin and did not carry the probe to the deep strata, and the hydroalcoholic solution containing the same concentration of ethanol as ethosomes resulted in a very low fluorescence intensity [9]. The results obtained in these experiments with RR, an indicator of lipid fusion, which does not usually cross lipid bilayers, suggest that ethosomes traversed to the deep skin strata, in contrast to liposomes, which remained confined on the superficial SC layers.

Figure 1. *In vitro* skin penetration of Rhodamine red dihexadecanoyl glycerophosphoethanolamine.

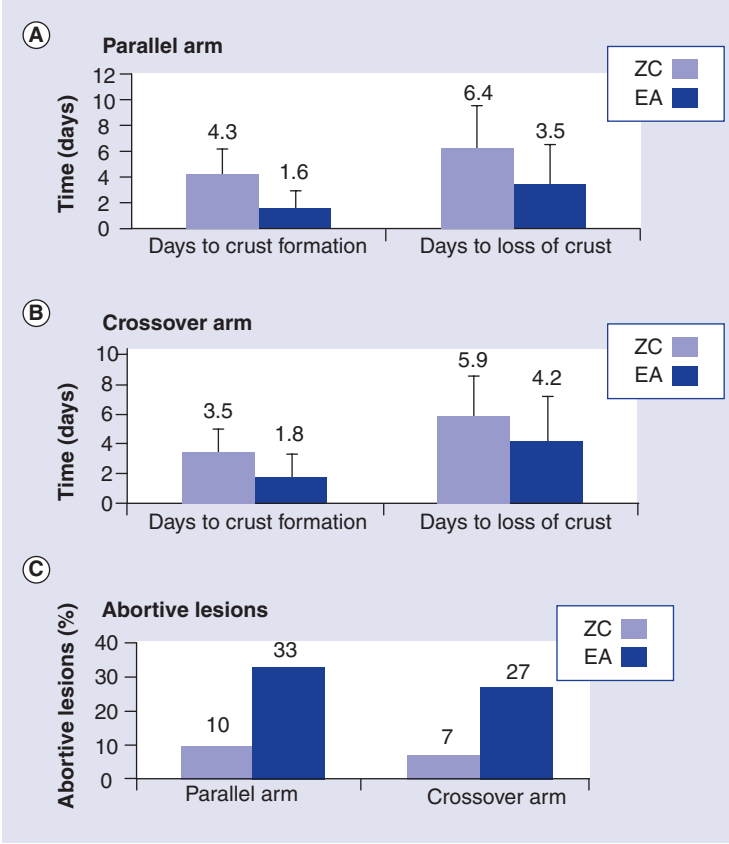


Evaluated systems: **(A)** ethosomes, **(B)** hydroethanolic solution or **(C)** liposomes. The systems containing Rhodamine red dihexadecanoyl glycerophosphoethanolamine were applied nonocclusively to the back skin of 8-week male nude mice. At the end of the experiment, the skin was excised and analyzed by confocal laser scanning microscopy. The square sections in each micrograph represent the optical slices of increasing skin depths. White represents the highest fluorescent intensity. Reproduced with permission from Elsevier [9].

In a recent CLSM study, the efficiency of ethosomes coloaded with two probes, RR and fluorescent polypeptide antibiotic bacitracin, to deliver their contents to deep layers of the dermatomed human skin (200 µm) was examined [12]. Since both probes were delivered from ethosomes into the deepest skin layers, it was suggested that ethosomes penetrated into the skin together with the drug. Again, liposomes were unsuccessful in transporting the polypeptide drug beyond the upper skin.

Data obtained in the above and other *in vitro* experiments carried out versus relevant control systems indicated that ethosomes were a much more efficient delivery carrier than ethanol, aqueous ethanolic solutions, micellar ethanolic phospholipid solutions or liposomes [9–16,101].

Figure 2. Parameters assessed in a two-armed, randomized, double-blind clinical study in recurrent herpes labialis patients with two formulations containing 5% acyclovir: ethosomal acyclovir versus Zovirax® cream.



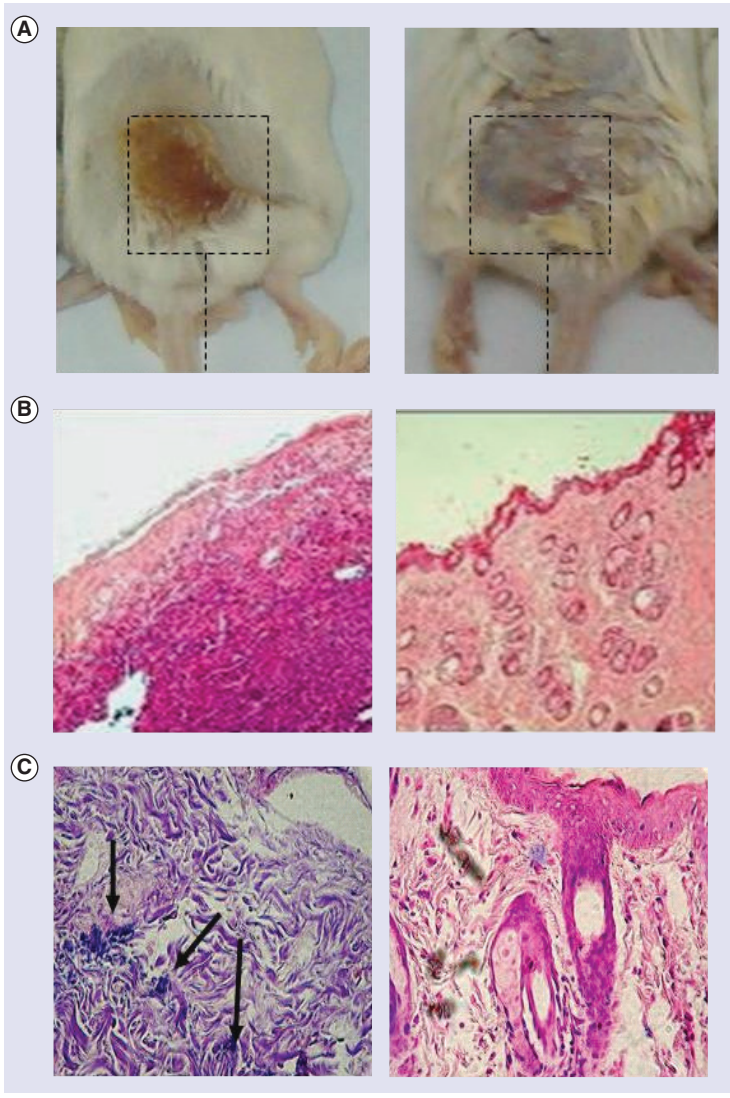
Data obtained in **(A)** parallel arm and **(B)** in crossover arm; **(C)** Percentage of abortive lesions.
EA: Ethosomal acyclovir; ZC: Zovirax cream.
Adapted from [27] with permission from John Wiley & Sons.

Efficiency & safety of ethosomal systems in clinical trials

It is common knowledge that insufficient penetration of drugs into the deep skin strata hampers efficient therapy by a topical formulation. The capability of ethosomal acyclovir to manage labial herpetic infection caused by herpes simplex virus (HSV)-1 was evaluated in a two-armed, double-blind, randomized clinical study. In this work, 5% acyclovir ethosomal cream (EA) was compared with a 5% acyclovir cream (Zovirax® cream [ZC]). The measured parameters were the proportion of abortive lesions not progressive beyond the papular phase, time to crust development and time to loss of crust (healing time). Compared with ZC, the ethosomal preparation significantly improved all evaluated clinical parameters in both parallel and crossover study arms, as shown in Figure 2. The proportions of abortive lesions measured in the parallel and crossover arms were 33 and 27% for herpetic patients treated with EA and only 10 and 7% for those treated with ZC. The healing time in the parallel arm was 3.5 days for EA versus 6.4 days for ZC [17]. Previous studies suggested that a main reason for a nonefficient topical therapy with acyclovir lies in a nonadequate penetration of this hydrophilic molecule into the basal epidermis, where HSV-1 is replicated [18]. The data obtained in the clinical trial described above suggest that ethosomes were able to transport acyclovir to the skin at therapeutically significant levels, causing a more efficient and prompt cure of the condition [17]. These results point toward potential new therapies in which other antiviral drugs can be incorporated in ethosomal carriers. Based on the results of the study with EA, 5% acyclovir ethosomal cream was developed and marketed in Israel (SupraVir, Trima). Data on SupraVir cream indicate that the formulation and the drug have a long shelf-life, with no stability problems.

Recently, a clinical study on ethosomal clindamycin preparation was carried out on 40 patients with mild-to-moderate acne vulgaris. The results of this 8-week randomized, double-blind, parallel-group, placebo-controlled trial show that treatment with clindamycin ethosomal gel significantly reduced the number of inflammatory, noninflammatory and total lesions. Likewise, 71% of the participants reported partial to complete improvement of the condition [19]. It should be emphasized that these results were obtained in only 8 weeks of treatment, compared with at least 12 weeks of application, as reported in previous studies, demonstrating the efficiency of clindamycin topical administration [20,21].

Figure 3. *In vivo* healing of deep dermal infection by ethosomal erythromycin: preclinical study.



Mice intradermally injected with 0.1 cc × 10^8 (10^7) cfu/mice *Staphylococcus aureus ATCC 29213* and treated with externally applied hydroethanolic solution of erythromycin (left) and ethosomal erythromycin applied on the skin (right). In both cases, the treatment started 72 h after challenge and lasted for 4 days. **(A)** Clinical picture; **(B)** Histological skin sections stained with hematoxilin and eosin; **(C)** Gram-stained histological skin sections (arrows point to *S. aureus* colonies within the tissue).
Reproduced with permission from Bentham Publication [10].

No severe or moderate adverse skin reactions were associated with the active treatment. It is also noteworthy that 14 out of 17 participants with a history of previous topical treatment (1% clindamycin lotion, 5–10% benzoyl peroxide gels or 5% benzoyl peroxide – 2% erythromycin gel) reported that the ethosomal gel was much better tolerated and caused less erythema, burning, pruritis and photosensitivity reactions compared with prior commercial topical medications [19]. The treatment efficiency and lack of important side effects, as well as the excellent tolerability, show that clindamycin ethosomes may offer a valuable new therapy for acne.

In vivo studies for new & advanced therapies

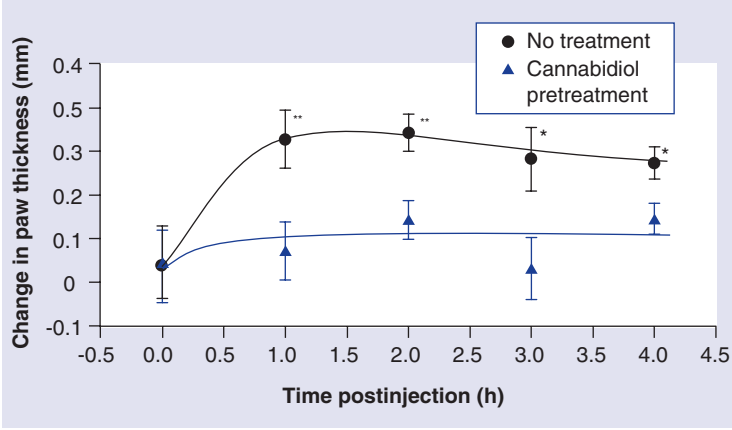
Deep skin microbial infections present a therapeutic challenge, owing to a nonsufficient penetration of antibiotics into the skin and subdermal tissues, where the pathogens are located. Thus, important bacterial skin infections are routinely treated by systemic antiinfective agents [22]. With the goal of finding a new treatment type for deep dermal and subdermal infections, erythromycin ethosomal formulations were characterized and tested *in vivo*, in an animal model for deep skin staphylococcal infection [23]. In this work, the efficiency of ethosomal erythromycin was compared with parenteral drug administration. It was found that treatment with ethosomal erythromycin applied to the infected site was as effective as the systemically injected drug. The wounds were efficiently cured by ethosomal erythromycin; and animals treated by skin application of the ethosomal erythromycin preparation did not demonstrate dermatonecroses. The animals fully recovered from the infection and regrew hair on their backs by the end of the 10-day treatment. Erythromycin ethosomes were further tested by live/dead viability/cytotoxicity assay, and found to be nontoxic to dermal 3T3 cultured fibroblasts [10].

In additional experiments, ethosomal erythromycin and erythromycin hydroethanolic solution, both applied to the skin, were tested for eradication of bacteria located in the deep dermal strata [10]. *Staphylococcus aureus* count immediately prior to antibiotic therapy was 1.5×10^7 cfu/g tissue. Following treatment with ethosomal erythromycin for 7 days, no bacteria were isolated from the inoculation sites. By contrast, bacterial counts of 0.90×10^7 and 1.06×10^7 cfu/g tissue were assayed on day 7 in the wounds of untreated mice and those receiving topical hydroethanolic erythromycin solution, respectively. The histopathologic examination of the animals in the above control groups, as presented in **Figure 3**, revealed necrosis, destroyed skin structures and a dense infiltrate of neutrophils and macrophages within the abscess, mostly situated in the deep skin layer [10,23].

Data with ethosomal erythromycin has an important clinical value, suggesting the possibility to substitute systemic antibiotic administration with topical ethosomal drug application. This new therapeutic approach could decrease systemic drug exposure, with associated adverse reactions, thus improving patient compliance.

A novel and challenging anti-inflammatory regional therapy was proposed by using ethosomes for cannabidiol ($_{log}$Kp: ~8), a potent anti-inflammatory agent produced by *Cannabis sativa* [24]. This highly lipophilic cannabionoid molecule presents a great challenge for dermal and transdermal delivery, as during the transport process it accumulates within the SC lipids, with no further clearance to the hydrophilic skin strata (viable epidermis and dermis). Application of ethosomal cannabidiol to the skin of nude mice resulted in significant accumulation of the drug in the skin (110.1 µg/cm^2) and in the underlying muscle (11.5 µg/g muscle). The anti-inflammatory effect of cannabidiol ethosomal systems was evaluated in immunocompromised mice in a carrageenan-induced aseptic paw inflammation model. **Figure 4** shows that the inflammation was prevented by ethosomal cannabidiol and the pharmacodynamic profiles between cannabidiol-treated and untreated animals were significantly different at all times during the experiment [24]. The results presented above demonstrate the ability of ethosomes to not only enhance the drug partitioning into the SC, but also to permit its clearance into the hydrophilic environment of deeper skin layers. Later, the enhancing properties of the ethosomal carrier for skin delivery of the anti-inflammatory agents ammonium glycyrrhizinate and ketotifen were confirmed by other research groups [16,25].

Figure 4. *In vivo* anti-inflammatory effect of ethosomal cannabidiol.



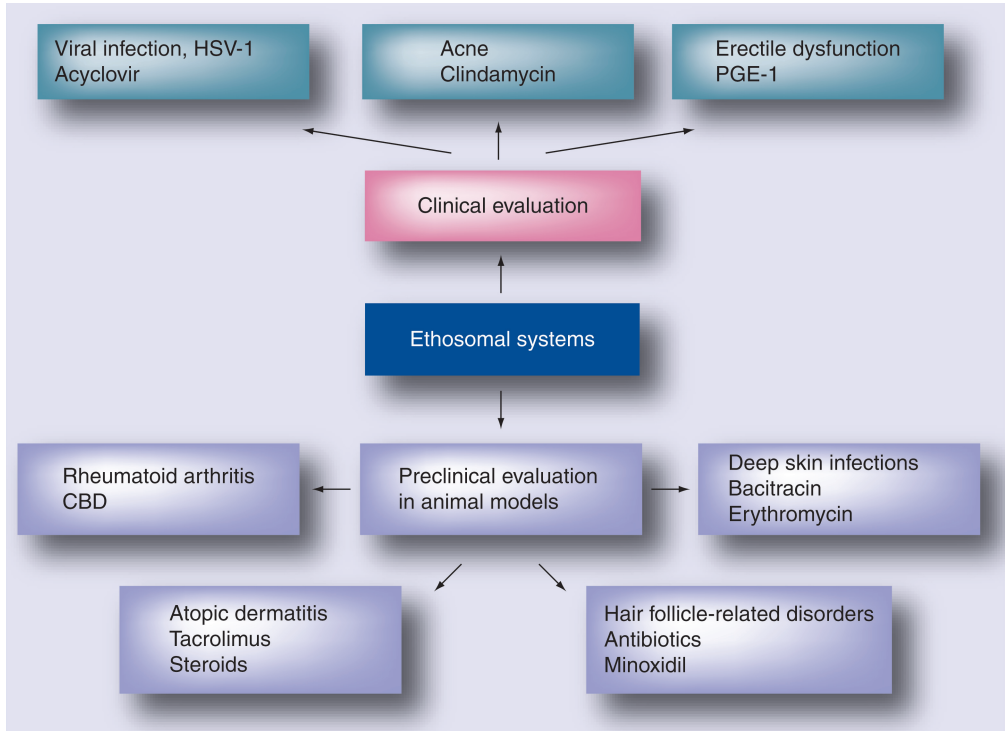
Anti-inflammatory effect of cannabidiol transdermal ethosomal patch, applied 19 h prior to the injection, is compared with no pretreatment. Change (mean ± SEM) in the thickness of carrageenan-injected and saline-injected paws of the same mouse at different time points postinjection.
*$p < 0.05$.
**$p < 0.01$.
Adapted from [24] with permission from Elsevier.

Disorders of follicular skin units, such as alopecia androgenata, seborrhea and acne, affect many patients of various ages. Several studies demonstrated that ethosomes could also be used as efficient delivery carriers of drugs to pilosebaceous structures. In one of these works, minoxidil, a lipophilic drug for hair-loss therapy, was targeted to the hair units using ethosomes. The quantity of minoxidil that accumulated in skin when applied in ethosomes was two-, five- and seven-times higher than from ethanolic phospholipid dispersion, pure ethanol and hydroethanolic control solutions, respectively [9]. The ethosomal system containing 0.5% minoxidil and 50 µCi titrated drug was further evaluated by quantitative skin autoradiography for localization of the drug into the pilosebaceous units *in vivo* in hairless rats. The results of this study revealed that five-times greater amounts of ^{3}H-minoxidil were delivered to pilosebaceous units from ethosomes compared with classic liposomes (22,000 vs 4500 pmol/g tissue, respectively; $p < 0.005$) [15,26–28]. Targeting the hair follicles by using ethosomal minoxidil formulations could provide a feasible improved therapy for alopecia androgenata. Enhanced delivery of active agents to skin shafts could improve treatment efficiency for therapies directed at correcting pilosebaceous disorders.

Conclusion

There is a critical need for more efficient means of administering dermatological agents in order to improve therapy of various conditions. There are many patient populations of various ages waiting for new and better treatments, including noninvasive therapy for deep and resistant skin infections, regional therapy for rheumatoid arthritis and more efficient treatment of pilosebaceous structure-related disorders. Studies reviewed in this article show that ethosomes were efficient for delivery of a variety of dermatological therapeutic agents, including challenging molecules such as cannabinoids and peptides, *in vitro* and in animals and humans (**Figure 5**). Owing to their composition, ethosomes could be used as carriers to deliver both oil- and water-soluble

Figure 5. Summary of studies carried out with ethosomal systems designed for dermal and regional therapies: evaluation in clinical trials and preclinical animal models.



HSV: Herpes simplex virus; PG: Prostaglandin.

active agents across the SC lipids to the deep layers of the skin. Substantial research has been carried out to characterize this novel noninvasive carrier for improved current therapies. New therapies proposed with ethosomal drugs, such as treatment of deep skin infections by means of skin application of ethosomal antibiotics and delivery of antirheumatic drugs to the inflammation site, could greatly increase drug treatment efficiency and patient convenience. Lack of toxicity, good stability and uncomplicated manufacturing make ethosomal carriers a valuable tool for novel and more efficient dermal applications. Another possible benefit from these new therapies could be reduced treatment costs.

Future perspective

To successfully eliminate dermatological disorders, there is a great need for therapeutic carriers to bring drugs to the site of their action in the deep layers of the skin. The goal of future therapy should not only be restricted to costly new medications, but should also include strategies to improve the curative potential of currently used drugs. In addition, many bioengineered and biotechnological therapeutic molecules require potent enhancement techniques for their efficient administration to the deep skin strata or across the skin. Ethosomes could offer efficient delivery for many old and new molecules, including challenging biotechnological agents, through the skin barrier and cellular membranes. Furthermore, by means of these carriers, new noninvasive therapies could be designed to meet unmet needs. These include improved treatments of deep skin microbial and viral infections and skin cancers, and new antirheumatic products. The enhanced pharmacodynamic profile of ethosomal drugs, together with their uncomplicated preparation and good stability, make these agents an excellent choice for the administration of various natural biological, biotechnological and chemical drug compounds for therapeutic use.

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Executive summary

- Therapeutic efficacy of topically applied drugs is often not achieved, due to the resistance of the stratum corneum to their transport into the deep skin strata.
- Ethosomes are proposed as safe and efficient carriers for a wide range of therapeutic agents aiming at new and advanced dermal therapies.
- Future research directions with ethosomes include delivery of biotechnological active agents, noninvasive treatment of skin cancers, noninvasive antirheumatic therapies and skin immunization.
- The goal of future therapy should not only be restricted to costly new medications, but also to create strategies for improving the curative potential of currently used drugs.

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