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Ethosomal drug delivery systems for skin cancer: Mechanistic insights, formulation strategies, and translational challenges

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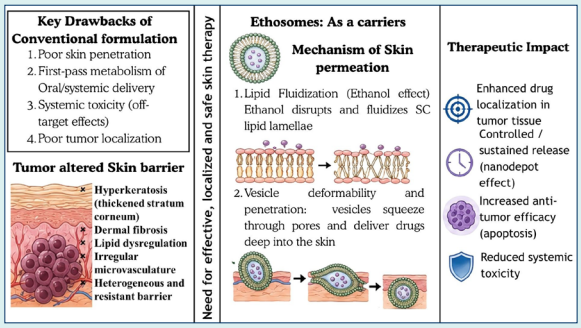
Abstract

Introduction: Skin cancer is one of the most prevalent malignancies worldwide, with incidence increasing by approximately 2–3% annually due to prolonged ultraviolet radiation exposure, genetic predisposition, and an ageing population. Conventional treatments, including oral, injectable, and topical therapies, are often limited by poor skin permeability, first-pass metabolism, systemic toxicity, and inadequate tumor targeting.



Methods: This review systematically examines ethosomal drug delivery systems, focusing on their composition, mechanism of skin permeation, and formulation strategies. Key aspects such as phospholipid composition, ethanol concentration (20–45% w/w), preparation techniques, and interaction with tumor-altered skin barriers are critically analyzed.

Results: Ethosomes, as deformable lipid vesicles, enhance transdermal drug delivery by fluidizing stratum corneum lipid lamellae, enabling deeper dermal penetration. Studies demonstrate improved drug localization, controlled release, and enhanced therapeutic efficacy in various skin cancers, including melanoma, basal cell carcinoma, and squamous cell carcinoma. Applications in chemotherapy, photodynamic therapy, and immunotherapy show promising preclinical outcomes.

Conclusion: Despite significant advancements and encouraging preclinical results, challenges such as formulation stability, scalability, regulatory considerations, and long-term safety must be addressed before ethosomal systems can achieve successful clinical translation in skin cancer therapy.

Introduction

Skin cancer is among the most commonly diagnosed cancers worldwide, with incidence rising by approximately 2–3% every year. This rising trend is due to the prolonged ultraviolet (UV) exposure, possible genetic susceptibility and demographic shifts toward an aging population.[1-5] Although early detection improves survival, major challenges in skin cancer treatment include local disease

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Research Highlights

What is the current knowledge?

- Conventional skin cancer therapies face limitations like poor permeability, systemic toxicity, and inadequate tumor targeting.
- Ethosomes are deformable lipid vesicles known to enhance transdermal drug delivery and dermal penetration.
- Preclinical studies report improved drug localization, controlled release, and enhanced therapeutic efficacy.

What is new here?

- Provides comprehensive insights into ethosomal mechanisms and tumor-modified skin barrier interactions.
- Critically evaluates formulation strategies, including composition optimization and preparation techniques.
- Highlights key translational challenges such as stability, scalability, safety, and regulatory considerations.

control, recurrence prevention, restoration of skin function, and cosmesis.[6,7]

Current treatment approaches are based on the tumor subtype, stage of cancer, and location of the tumor. Approved treatments include surgical removal of the tumor, radiotherapy, photodynamic therapy, and systemic therapy.[8,9] Each treatment approach has its own merits along with certain limitations. Surgical treatment is an invasive therapy that may fail to eliminate microscopic disease, the possibility of damage to normal tissues, limited patient tolerance to surgery and anesthesia, and cosmetic or functional changes of the skin. Radiation therapy is limited by permanent or irreversible skin changes in the irradiated area, patient inconvenience (the need to undergo radiation multiple times), and the risk of recurrence. Systemic therapy is constrained by off-target effects on normal tissue, substantial toxicity, and poor suitability for localized disease.[10] Overall, these limitations highlight the gap between therapeutic efficacy and acceptable safety in skin cancer treatment.[11,12]

Topical intervention is one of the fundamental pillars of dermatological oncology, and 5-fluorouracil (5-FU) and imiquimod as accepted therapeutic benchmarks. Their clinical efficacy, however, is commonly compromised by the deep semi-permeable impedance of the skin barrier.[13] The stratum corneum (SC) is the rate-limiting bottleneck which consists of a highly ordered paracrystalline lipid matrix and a desiccated environment and provides significant resistance to the translocation of ionized and macromolecular species. This inherent permeability is not an unchanging property but a dynamic equilibrium of the interaction of drug ionization at physiological pH, vehicle/skin partitioning dynamics and stoichiometric compatibility between therapeutic payload and host lipids.[14,15] More importantly, within the framework of cutaneous malignancy, another distortion of the delivery environment is a set of tumor-related pathological changes. The appearance of hyperkeratosis, dermal fibrosis, and localized lipid dysregulation, aggravated by an irregular and unsystematic microvascular architecture, makes the skin a heterogeneous and unpredictable barrier. These structural aberrations not only hamper transport but also radically change the flux dynamics of conventional topicals, cause a more complex carrier-mediated method to reach therapeutic levels in the deeper neoplastic layers.[16-18]

This, in the end, implies that there is a necessity to develop more novel drug-delivery systems to address this obstacle. Several nanovesicular carriers, including liposomes, niosomes, transfersomes, and ethosomes, have been investigated for this purpose. The principal advantages of these vesicular systems are its biocompatibility, bilayer system, the possibility of loading hydrophilic and hydrophobic drugs, surface modification, protection of drug degradation, and the capability to increase the skin permeability.[16-18] Among them, conventional liposomes, transfersomes, and niosomes have been most widely examined for topical delivery. Although each provides differing levels of improvements in the dermal drug delivery systems, they remain limited by functionality by restrictions on membrane rigidity, surfactant dependence, or permeation of the SC by diffusion.[19]

Ethosomes are fundamentally different from these other vesicular systems in both composition and mode of action. They are highly deformable lipid vesicles composed of phospholipids, a substantial proportion of ethanol, and water, and are specifically designed to overcome the barrier properties of the SC.[20,21] Ethosomes promote penetration into the deeper skin layers by simultaneously modulating SC lipid organization and conferring vesicle deformability. Beyond enhanced permeation, ethosomal systems also support localized drug delivery within the epidermis and dermis, an attribute particularly relevant to topical anticancer therapy. Consequently, ethosomes are increasingly recognized as an attractive vehicle for cutaneous drug delivery.[22,23]

The use of ethosomal systems in the topical treatment of skin cancer has received growing attention in recent years. While several reviews have addressed ethosomal systems for transdermal delivery, most have focused on general formulation approaches and broad therapeutic applications. Limited attention, however, has been given to the impact of tumor-induced changes in skin structure on drug delivery performance, and how these alterations influence the behavior of ethosomal carriers remains insufficiently explored. The present review therefore focuses on the interaction between tumor-altered skin barriers and ethosomal delivery, together with the key formulation considerations and challenges associated with clinical translation in skin cancer therapy.

Ethosomes: An overview

Mechanism

This enhanced permeability of ethosomes is based on a synergistic, biophysical push-pull mechanism that combines transient destabilization of SC with the deformability of high magnitude of ethanol-solvated bilayers. They have

an elastic shell (compared to their liposomal counterparts) to provide a dual-track benefit: a deep-tissue penetration of nanocarriers and a long tumor nidus residence time of antineoplastic cargo (Fig. 1).

This is mostly a thermodynamic one. The high ethanol fractions are powerful chemical attenuators, since when intercalated in the SC lamellae that contains high concentrations of ceramides, the ethanol in effect depresses the lipid transition temperature (T_m). This physical alteration to a liquid-crystalline phase alters the highly ordered intercellular matrix, which dilates the tortuous diffusion routes among the corneocytes without impairment of the long-term structural integrity of the skin.[24]

This fluidization is particularly important in skin cancer, where the hyperkeratotic and fibrotic environment renders the barrier highly resistant to conventional topical delivery. The internal structure of the vesicle is similarly dynamic. Incorporation of ethanol into the phospholipid bilayer disrupts acyl chain packing and reduces the bilayer bending modulus, enabling extensive yet reversible morphological deformation. This deformability allows ethosomes to traverse intercellular pores several times smaller than the vesicle's own hydrodynamic diameter, driven by the transdermal osmotic gradient. As a result, ethosomes escape the superficial SC entrapment that limits conventional liposomes and reach the viable epidermis and papillary dermis, where they can directly interact with tumor cells (Fig. 2).[22]

Once ethosomes reach the deeper dermal layers, they exhibit a bimodal release profile that depends on the lipid-to-solvent stoichiometry of the vesicle. They may either fuse with native skin lipids to produce a rapid burst release, or persist as intradermal nanodepots that release the payload slowly through transmembrane diffusion. This depot effect is particularly valuable in the heterogeneous and inflammatory tumor microenvironment. By localizing chemotherapeutic and immunomodulatory payloads at the epidermal-dermal junction, ethosomes promote efficient uptake by malignant keratinocytes while limiting systemic absorption and the associated off-target effects. The net outcome is enhanced apoptotic activity and superior tumor volume reduction compared with non-vesicular formulations.[26,27] These results are subsequently reinforced by transmission electron microscopy analyses which show the penetration and intracellular localization of ethosomal vesicles through the skin in a progressive manner through the various skin layers (Fig. 2).[25]

For most topical and transdermal applications, ethosomes mainly act as carriers that enhance drug penetration through the SC and enable drug localization within the epidermal and dermal layers. Drug delivery in such systems is generally associated with vesicle interaction or fusion with skin lipids and cellular membranes, rather than classical endocytic uptake pathways. Therefore, intracellular processes such as endosomal escape are not considered a critical factor for the therapeutic performance of small-molecule ethosomal formulations, and this aspect has not been widely explored in the existing literature. However, in the case of macromolecular therapeutics such as siRNA, mRNA, peptides, or vaccine antigens, where intracellular delivery is required for activity, endosomal escape may become important and should be investigated in future studies.

Comparison with other lipid carriers

Vesicular drug delivery systems differ significantly in structural mechanics, barrier interaction and translational maturity. A comparative study of liposomes, transfersomes, ethosomes, and niosomes demonstrates that the purposeful molecular structure determines transdermal flux and clinical performance in oncological dermatology (Table 1).[28]

Liposomes were the first lipid-based vesicular platform and consist of phospholipid bilayers typically rigidified by

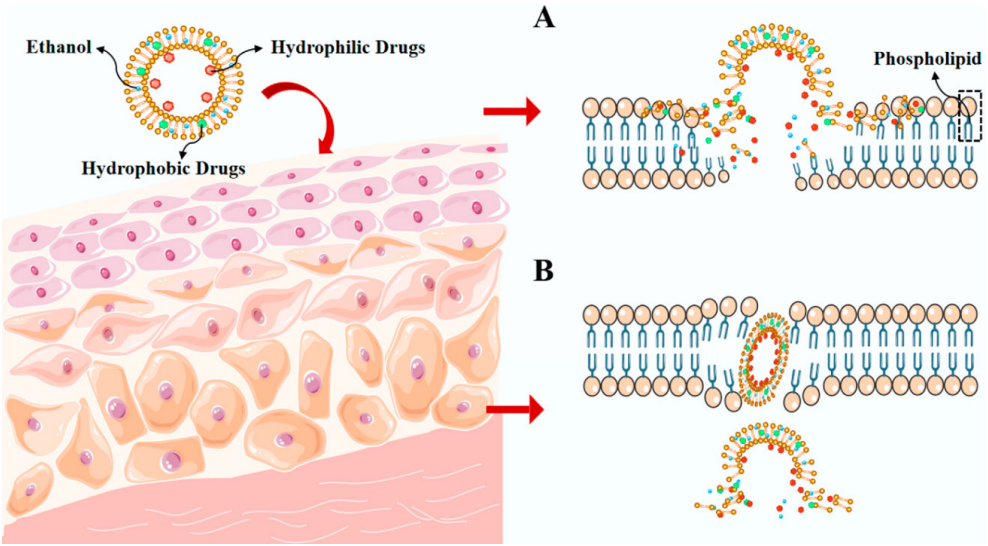

Fig. 1. Schematic representation of ethosomal structure and dual-pathway skin permeation mechanism via lipid bilayer fluidization (Pathway A) and vesicle fusion (Pathway B). Adapted from Zhan et al,[23] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/)

Table 1. Comparative analysis of liposomes, transfersomes, ethosomes, and niosomes based on key physicochemical, biopharmaceutical, and translational parameters

Parameter	Liposomes	Transfersomes	Ethosomes	Niosomes
Average particle size	100–1000 nm	< 300 nm (highly deformable)	50–400 nm	100–500 nm
Skin penetration efficiency	Low to moderate (limited by rigidity)	High (elastic vesicles enhance penetration)	Very high (ethanol-mediated fluidization)	Moderate
Drug loading capacity	Moderate	Moderate to high	High	High
Physicochemical stability	Prone to oxidation, fusion, and drug leakage; poor storage stability	Reduced stability in semisolid systems; surfactant-induced destabilization possible	Ethanol induces negative zeta potential, reducing aggregation; risk of ethanol evaporation during storage	More chemically stable; non-ionic surfactants improve robustness
Cost / Scalability	High (costly phospholipids; oxidation-sensitive)	Moderate–high (specialized surfactants; scale-up challenges)	Moderate (ethanol is inexpensive, but processing and packaging add cost)	Low (non-ionic surfactants are economical and scalable)
Key safety considerations	Generally biocompatible and well tolerated	Possible irritation and cytotoxicity due to surfactants	Generally well tolerated; possible irritation at high ethanol levels	Generally safe; limited toxicity concerns
Translational status (skin-related)	Several approved products (e.g., Doxil®, AmBisome®); dermal/cosmeceutical use	Limited clinical translation; example: Flexiseq®; mostly preclinical in oncology	Emerging clinical and preclinical use; products such as Nanominox®, Cellutight EF®	Mostly preclinical; some cosmetic and drug delivery applications

cholesterol. This architectural rigidity restricts transport to simple diffusion or lipid exchange and confines the vesicles largely to the superficial SC. As a consequence, drug delivery to the viable epidermis is suboptimal, which limits their performance in the localized treatment of skin cancer.[28] Although there are established systemic successes such as AmBisome®, DaunoXome®, and Doxil®, they could not overcome the epidermal barrier and this is the main clinical bottleneck of topical application.[29]

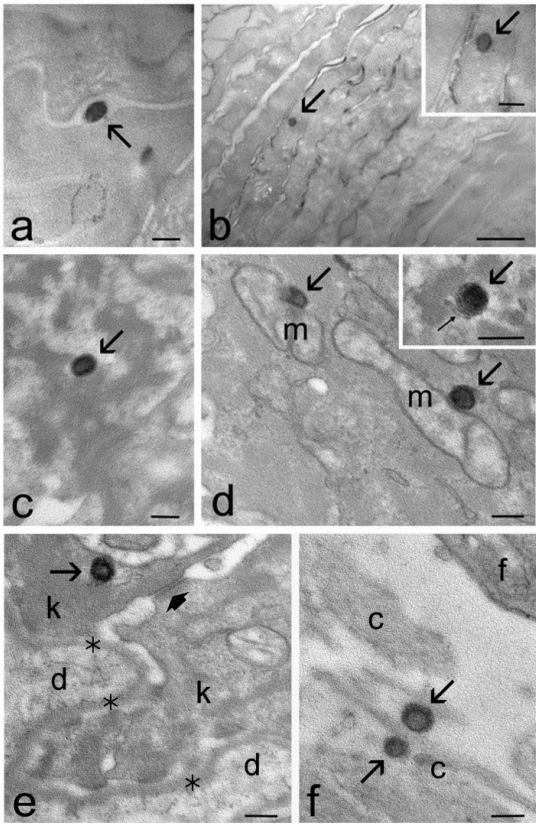

Fig. 2. TEM micrographs showing ethosomes (ET) penetration across skin layers: (a) intercellular space of stratum corneum; (b) internalized within a corneocyte; (c) cytoplasm of stratum granulosum keratinocyte; (d) association with mitochondria and smooth endoplasmic reticulum; (e) stratum basale keratinocyte with intact desmosome and basal lamina; (f) extracellular matrix of upper papillary dermis. Adapted from Esposito et al,[25] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Transfersomes were designed to overcome this rigidity by adding edge-activating surfactants (e.g., sodium deoxycholate) in the bilayer. These surfactants act as molecular destabilizers, conferring extreme membrane elasticity that allows the vesicles to deform and pass through constricted intercellular gaps. This improved flux, however, comes at a cost: high surfactant concentrations may cause local cytotoxicity and reduce stability in semisolid formulations. Although marketed products such as Flexiseq® demonstrate the clinical feasibility of elastic vesicles, these safety and stability limitations have restricted their broader adoption in dermato-oncology.[30]

Ethosomes, often described as third-generation lipid carriers, exploit a high ethanol content to deliver a concurrent push–pull effect: ethanol fluidizes the SC lipids while simultaneously reducing the bending modulus of the ethosomal bilayer itself. This dual fluidization promotes deep dermal penetration and supports high drug loading, allowing both hydrophilic and lipophilic molecules to partition into their respective vesicle compartments. Although the formulation of poorly soluble drugs in hydroethanolic phases remains technically challenging, the clinical adoption of ethosomal platforms in marketed products such as Nanominox®, Cellutight EF®, and Decorin Cream® demonstrates a solid transition toward these high-flux delivery systems.[20,31]

Niosomes are economical, chemically stable vesicles composed of non-ionic surfactants (e.g., Span or Tween) and cholesterol. Although more flexible than conventional liposomes, they remain considerably less deformable than ethosomes and transfersomes. Niosomes typically rely on diffusion-driven skin permeation, which is moderate, but they offer better biocompatibility than highly surfactant-loaded elastic vesicles. Despite these tolerability advantages,

their clinical impact in anticancer therapy has so far remained largely confined to preclinical and experimental modeling.[32,33]

Tumor skin barrier: Implications for ethosomal design

SC in cutaneous malignancy is not merely a thicker extension of normal skin. it is structurally and biochemically changed in a manner that has a direct influence on the behavior of the vesicular carriers at the tissue interface. These changes are crucial for the rational design of ethosomal formulation as well as background context. Hyperkeratosis, caused by the unsynchronized growth of the keratinocytes, squashes intercellular lipid lamella and worsens the channels through which the vesicles can move. Ethosomes loaded with 5-aminolevulinic acid (5-ALA) delivered via ethosomes gels have shown enhanced permeation specifically through hypertrophic scar tissue a hyperkeratotic analog confirming the utility of high-ethanol ethosomal systems in thickened, fibrotic skin barrier.[34]

This places greater demand on vesicle deformability compared to healthy skin models. Ethosomes having larger ethanol concentrations in the 35-45%w/w region should prove to be more effective with hyperkeratotic tumor skin but the cancer specific permeation data to prove this is also limited. The dermal fibrosis is tumour-related and limits interstitial diffusivity below the epidermis. Ethosomes that release the drug gradually by forming nanodepots instead of bursting fusogenically may allow maintenance of a concentration gradient by this fibrotic matrix better than fast-release formulations. Bleomycin sulphate-loaded cationic ethosomes demonstrated sustained ex vivo skin retention in tumor skin models, supporting the hypothesis that nanodepots-forming ethosomes maintain a sustained concentration gradient against fibrotic resistance better than burst-release conventional formulations.[35]

Keratinocytes in cancerous cells exhibit distorted ceramide synthesis and disintegration of sphingolipid structure. This lipid disarrangement can potentially reduce the energy barrier of ethosomal bilayer fusion with SC lipids that may increase permeation, but the extent of this effect differs depending on the tumor subtype and disease stage introducing an unpredictability to healthy skin models. The weak acidic extracellular tumor microenvironment (pH 6.5-7.0) changes the ionization states of drugs, and changes the behavior of surfaces of ethosomal vesicles. Stimuli-responsive nanocarriers including ethosomes exploit tumor-specific lipid disarrangement and acidic pH to achieve spatiotemporally controlled release, as the altered ceramide composition of malignant SC lowers the thermodynamic energy barrier for vesicle-bilayer fusion.[5]

In charged formulations, especially with the addition of dicetyl phosphate or stearyl amine this local pH change can have substantial implications on interactions with vesicles and drug release rates at the tumor site a parameter that is not much considered in typical optimization of formulations. Lastly, the leaky tumor microvasculature may promote systemic uptake of the drug that diffuses into the dermis and reduces the local residence time. This may be counteracted in part by adding a small cholesterol fraction to stabilize the bilayer after permeation, but the cost of deformability has to be weighed. Taken together, these factors suggest that ethosomal formulations developed using normal skin models may not reliably predict performance in tumor tissue. The use of more disease-relevant model like 3D tumor-skin model and patient-derived ex vivo tissue should be increased in the preclinical phase to provide more meaningful performance information to the preclinical phase.[5,36]

Formulation strategies for ethosomal systems

Key formulation components

Accurate stoichiometric ratios of components in ethosomal systems influence vesicle sphericity, membrane elasticity, and entrapment efficiency. These parameters not only influence the physical stability of the dispersion, but also determinants of the thermodynamic activity of the carrier and subsequent interaction with the SC. The usual concentrations and the mechanistic roles of these essential components are depicted in Table 2.[22,37]

The structure of the ethosomal systems is defined by ethanol, which is usually optimized in the 10-50% w/w. Rather than acting as a passive solvent, ethanol serves as a primary bilayer fluidizer, which intercalates between phospholipid acyl chains to decrease the phase transition temperature (Tm). This molecular perturbation allows the necessary hyper-deformability to negotiate the tortuous routes of SC.[19] Ethanol at the skin-vesicle interface causes a temporary condition of disorder in the SC lipid matrix through partial extraction and enhanced chain mobility, which effectively lowers barrier impedance. Although raising the ethanol content would tend to reduce the hydrodynamic diameter and improve the colloidal stability due to the more negative zeta potential, concentrations above 50% may leads to the bilayer dissolution and formation of the micellar phase-coexistence.[22]

The vesicle backbone is provided by the phospholipids and usually 0.5-5% w/w. The selection of phosphatidylcholine as a crystalline form of the compound (e.g. Lipoid® S100, Phospholipon® 90H) is due to the acyl chain saturation. Increasing lipid concentration increases bilayer volume of the bilayer and hence the entrapment effectiveness (EE%), the concentration also arouses the increment in the size and dispersion viscosity of the vesicles. The type of lipid used is also critical: unsaturated phospholipids create smaller and less rigid vesicles preferring to forms of without large and stable flux and saturated lipids being rigidized slow-release profile.[23]

Cholesterol is also used to increase the cohesion of the bilayer to avoid the undue release of payload. However, its inclusion requires balance; its rigorizing action can work against the ethanol induced flexibility, which can prevent the passage of constricted intercellular apertures.[38]

Co-solvents (Propylene Glycol, Glycerol) are incorporated at 5-20% w/w that regulate the evaporation rate and the skin level of hydration of a vehicle. Specifically,

Table 2. Typical component ranges and functions in ethosomal formulations

Component	Typical concentration range	Primary function(s)
Ethanol	10–50% w/w	Fluidizes bilayers, imparts deformability, disrupts SC lipids to enhance permeation
Phospholipids (SPC, EPC, Lipoid variants)	0.5–5% w/w	Vesicle bilayer formation, drug entrapment, size and stability control
Cholesterol	0–3% w/w (up to 30 mol% of lipid)	Membrane stabilizer, reduces leakage, modulates vesicle rigidity
Co-solvents (Propylene Glycol, Glycerol, IPA)	5–20% w/w	Enhance drug solubility, skin hydration, fusion inhibition
Penetration enhancers (DMSO, oleic acid, L-menthol, bile salts)	≤ 1–5% w/w	Disrupt SC lipid packing, enhance drug flux, transiently open pathways
Charge modifiers (Dicetyl phosphate, Stearyl amine)	~0.1–1% w/w	Adjust surface charge, improve colloidal stability and skin interaction
Hydration medium (Water/buffer)	q.s. to 100%	Solvent/hydration for vesicle formation, control size and homogeneity
Stabilizers and additives (PEG, antioxidants)	0.01–0.1% (antioxidants), variable for others	Bilayer protection, vesicle stabilization, shelf-life extension

propylene glycol is utilized as a humectant and fusion inhibitor, which inhibits vesicle coalescence as well as prolongs the topical residence time by blocking excessive concentrations of the bilayer intrinsic elasticity.[39]

The performance of ethosomal systems is highly dependent on the drug properties. Lipophilic moieties can easily be trapped in the swelled lipid vesicles and can also survive high-ethanol conditions. On the other hand, in hydrophilic payloads, a lower ethanol-to-water ratio is necessary to ensure that the aqueous core is not subjected to damage and osmotic collapse is prevented. Such sensitivity requires a optimization approach like Design of Experiments (DoE) so that the performance can be replicated during clinical scale-up.[31]

Methods of ethosomes preparation

A schematic representation of the commonly used ethosomes preparation methods is shown in Fig. 3.

Cold method for preparation

The cold method is the simplest and most widely employed technique for ethosomes formulation and can be conducted under nitrogen if necessary. Originally described by Touitou in 1996, this method involves preparing the organic and aqueous phases separately. In the formation of binary ethosomes, classical formulations consisting primarily of phospholipids and ethanol, sometimes combined with co-solvents such as propylene glycol phospholipids are dispersed (not dissolved) in ethanol or solvent mixtures at room temperature or about 30°C to create the organic phase.[19] For transethosomes, an advanced ethosomes variant, surfactants or penetration enhancers are included alongside phospholipids and ethanol to further improve vesicle flexibility and skin permeation. The aqueous phase consists of water, buffer, or saline. The aqueous phase is slowly added into the organic phase at a controlled rate (e.g., 175–200 µL/min), either dropwise or by syringe pump, under stirring (700–2,000 rpm) to achieve homogeneous mixing. This process is continued for 5 to 30 minutes, resulting in the formation of ethosomal suspensions. The active ingredient is dissolved in either the organic or aqueous phase depending on its physicochemical properties.[30]

Advantages of these methods are the 1) Simple, cost-effective and easy to scale up, 2) Compatible with thermolabile drugs due to mild processing conditions, 3) Allows incorporation of multiple penetration enhancers and surfactants, particularly in transethosomal systems. While demerits are the 1) Vesicle size and homogeneity are highly sensitive to mixing speed and addition rate, 2) Requires prolonged stirring to achieve uniform dispersion, and 3) Susceptible to batch-to-batch variability without stringent process control.

Hot method for preparation

Ethosomal self-assembly is guided by the so-called hot method wherein phase synchronization is achieved by thermally elevating the temperature of ethosomal phospholipid constituents, in a manner that keeps the phospholipid constituents constantly above their respective gel to liquid crystalline transition temperature (T_m). Within this particular structure, not only are the phospholipids dissolved, but the phospholipids are also strategically distributed throughout an aqueous solution that is in equilibrium at 40°C; furthermore, the ethanol counterpart is also pre-heated at the same thermal set-point to eliminate the development of temperature gradients, which tend to cause the aggregation of heterogeneous lipids. This thermal equilibrium is essential. High shear agitation of the aqueous dispersion with the ethanolic phase then subsequently introduces a spontaneous molecular reorganization into highly elastic bilayers. Attached to the vesicles is a payload that can be either sequestered to the lipophilic or hydrophilic phase depending on the inherent lipophilicity or hydrophilicity of the drugs to ensure that drug partitioning and vesicle closure occur simultaneously. This accuracy in thermal control reduces the polydispersity index (PDI) to levels that are hardly attainable by ambient-temperature protocols. As a result, the hot method implies a solvent-free route to clinical-reproducible results, in essence bridging the divide between bench-top chemistry and large-scale manufacturing.[22] Merit of Hot method for preparation liposomes are the reduced viscosity of both phases improves mixing efficiency, often yields smaller vesicles with narrower size distribution and may enhance drug entrapment efficiency for temperature-stable compounds. Although major disadvantages are a unsuitable for thermolabile drugs due to heat exposure, higher energy

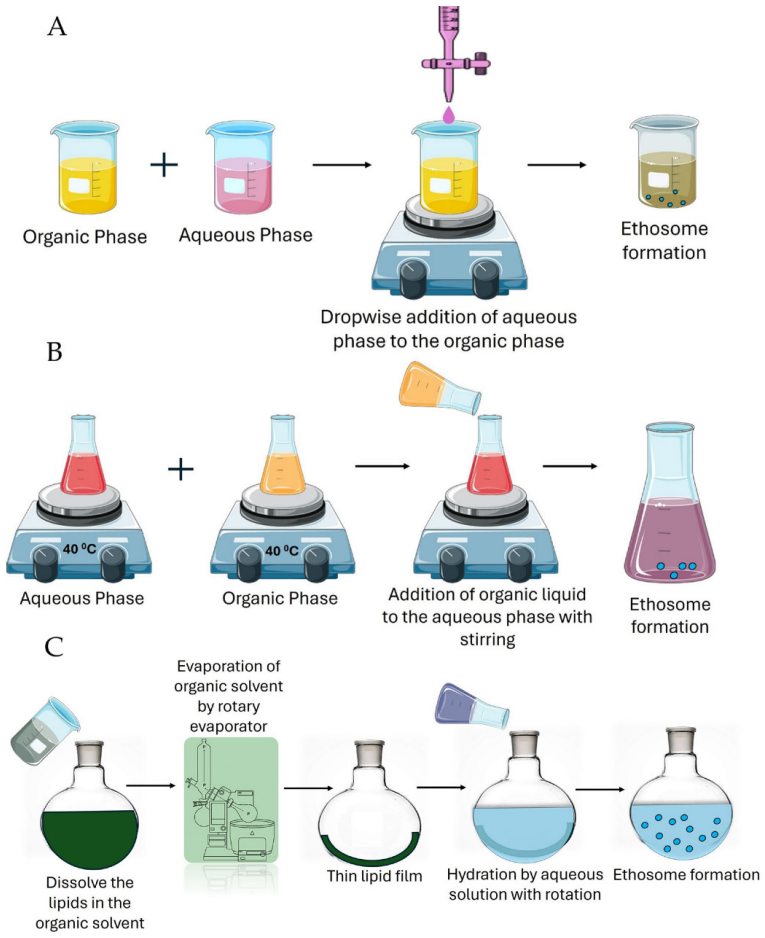

Fig. 3. Schematic representation of ethosomes preparation methods: (A) cold method, (B) hot method, and (C) thin-film method. Adapted from Almuqbil and Aldhubiab,[22] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

consumption compared with the cold method and requires precise temperature control to prevent phospholipid degradation or vesicle instability.

The ethanol injection method followed by sonication for preparation

In ethanol injection method followed by sonication method phospholipids are dissolved in ethanol to form the organic phase, which is further injected to the aqueous phase at a constant injection rate of say 200 µL min^{-1} with the help of a syringe pump under constant stirring. Increased rate of exchange of solvents and spontaneous assembly of vesicles are facilitated by controlled injection. After phase mixing, the dispersion is sonicated briefly (about 5 min) with a probe to decrease the size of the vesicles, decrease the size distribution, and enhance colloidal uniformity. This is a dependable approach of generating nanoscale ethosomes with high physical stability, and a high drug encapsulation efficiency when optimized properly.[23,40]

The ethanol injection sonication method gives precise control over vesicle size and size distribution. Sonication step reduces the vesicle size and may also contribute to better encapsulation efficiency. Compared to other preparation approaches, this method is relatively faster at the laboratory scale. Despite these practical benefits, this method carries certain limitations that cannot be overlooked. Uncontrolled sonication energy may degrade thermolabile or sensitive drug molecules and cause leakage from the vesicle core. Scaling up sonication to industrial batch sizes remains a significant technical hurdle, as consistent energy delivery across larger volumes is difficult to maintain. Additionally, the equipment requirements and operational complexity are considerably higher than those associated with the cold or hot preparation methods.

From a translational perspective, these limitations highlight the need for careful process optimization and alternative scale-up strategies, such as controlled homogenization techniques. Addressing these challenges will be essential for improving reproducibility and enabling clinical application of ethosomal formulations.

Characterization techniques for ethosomes

Comprehensive physicochemical characterization of ethosomal formulations is crucial to evaluate their quality, stability, and potential performance. These characterization techniques focus on measuring physical and chemical properties that influence formulation behaviour and efficacy.

Physical characterization

Vesicle particle size

The size of ethosomes should be optimal as its increases, transdermal penetration, stability and the release kinetics of the drug will be lowered. dynamic light scattering (DLS), or photon correlation spectroscopy, is widely used

to determine vesicle size and the PDI. Generally, the size of ethosomes varies from 50 nm to 400 nm. A lower PDI value (ideally < 0.3) correlates with a tight and uniform size distribution and is favourable for reproducibility and stability of formulations. Controlling the size of the particles becomes an important factor in topical and transdermal drug delivery systems since smaller vesicles are able to deeply penetrate the skin layers. Supplementing DLS, nanoparticle tracking analysis (NTA) provides real-time visualization and size distribution profiling of individual particles in suspension, enabling more accurate enumeration and differentiation of heterogeneous populations.[41, 42]

Zeta potential

The zeta potential is a parameter indicating the surface charge of vesicles and the physical stability of colloidal dispersions. It is quantified by means of electrophoretic light scattering techniques. A high absolute zeta potential value (generally > ± 30 mV) of the ethosomal formulations ensured adequate electrostatic repulsion among vesicles, avoiding flocculation or aggregation over time. The net surface charge is determined by the presence of ethanol and phospholipids in ethosomes, which, in addition to affecting the physical stability, plays a role in skin permeation properties. Zeta potential monitoring aids in forecasting the shelf life and physiological behaviour of vesicles.[42]

Morphology and structural analysis

Ethosomal vesicles morphological characterization is necessary to ensure vesicle architecture and structural integrity. TEM, scanning electron microscopy (SEM), and atomic force microscopy (AFM) are regularly used to determine the size and shape of vesicles, lamellarity and bilayer organization. The methods allow validation of nanoscale dimensions and contrasting between uni- and multilamellar structures and structural perturbations caused by the formulation variables, processing conditions, or instability during storage.[42]

Although traditional TEM and SEM have been popular in the visualization of vesicles, the dehydration and staining of samples may generate artifacts that mask the structure of the sample. Cryogenic transmission electron microscopy (Cryo-TEM) eliminates all these limitations by maintaining ethosomes in a hydrated vitrified form, with the resultant ability to directly observe vesicle morphology, lamellarity, and size distribution without much structural distortion or nanometer-scale resolution (Fig. 4).[25]

Elasticity and deformability

Deformability of vesicles is a functional feature that characterizes ethosomes and is a major factor that determines their ability to pass through the small intercellular pores of the SC. Extrusion-based assays are a common way of measuring elasticity, where the vesicles are forced through membranes of specified pore size at a controlled pressure. Higher deformability is a property of vesicles that indicates the ability to express reversible changes in shape without breaking, and is strongly associated with increased skin permeability relative to rigid liposomal systems. Therefore, elasticity is an important design parameter in optimization of ethosomal formulations to be used in efficient dermal drug delivery.[43]

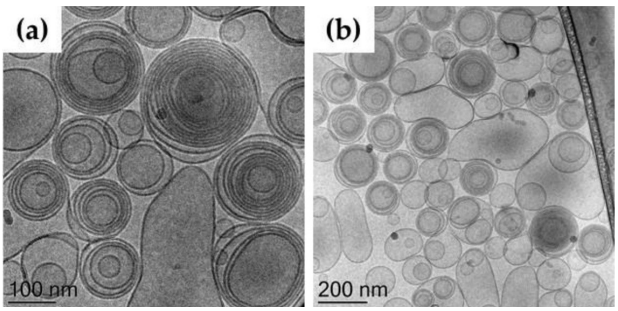

Fig. 4. TEM micrographs of ethosomal (ET) vesicles. (a, b) Spherical, predominantly multilamellar vesicular structures with characteristic concentric bilayer lamellarity, alongside occasional larger unilamellar vesicles. Scale bars: 100 nm (a) and 200 nm (b). Adapted from Esposito et al,[25] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Rheological properties

Ethosomes incorporated in a gel or semisolid state require the rheological assessment of the viscosity and flow behavior. The rheological characteristics of formulations through the spreadability, skin retention and kinetics of drug release directly influence patient acceptability and outcomes.[44]

Chemical characterization

Drug loading and entrapment efficiency

The loading capacity and entrapment efficiency are major parameters to assess the efficacy of ethosomal carriers. % Proportional to the amount of unentrapped drug from the vesicle-entrapped fraction, is determined by the amount of unentrapped drug, which can be separated from the vesicle-entrapped portion by means of ultracentrifugation, dialysis, or gel filtration. Spectrophotometric or chromatographic methods (UV-Vis or HPLC) are typically used to perform quantification. High entrapment efficiency of drug in vesicles means that drug is well incorporated into vesicles, which, gives us an idea about the incorporation of drug into vesicles mainly will depend on the solubility of drug, lipid concentration and ethanol content. Contrarily, drug loading affects dose uniformity and therapeutic results by indicating the quantity of drug per unit weight of vesicles.[26,45]

In vitro drug release

These *in vitro* release studies are performed using diffusion devices like the Franz diffusion cells for release profile studies of the drug from ethosomal formulations. A semi-permeable membrane (dialysis membrane or a synthetic cellulose acetate membrane) is placed in between the donor and receptor compartments.[46] The ethosomal formulation is added to the donor compartment and the receptor compartment is filled with an appropriate medium (e.g. phosphate buffer saline), held at physiological temperature (32–37°C), with continuous agitation. To calculate the total amount of medication released, samples are taken out at periodic times and subjected to spectroscopic analysis. Understanding drug diffusion behavior, release

kinetics (such as zero-order, first-order, Higuchi, or Korsmeyer–Peppas models), and possible ethosome-induced bioavailability enhancement all depend on these investigations. It is recognized by regulatory authorities as an important quality and performance evaluation tool for topical and transdermal formulations.[26,47]

Molecular interaction and thermal analysis

Thermal and spectroscopic studies play a central role in deciphering interactions between drugs and lipids and the organisation of the bilayers in ethosomal systems. Differential scanning calorimetry (DSC) is a typical technique to investigate the phase behavior of lipids, as well as to monitor changes in thermal transitions that result when ethanol or a drug is incorporated or loaded. The difference in melting temperature and transition enthalpy is an indication of the different levels of interaction between drugs and the bilayer and was indirectly indicated by variations in bilayer fluidity, lipid packing, and stability of vesicles.[40]

Fourier transform infrared spectroscopy (FTIR) is a complementary technique, which thermoanalysis fails to determine the interactions at the molecular level between drugs, phospholipids, and ethanol by observing variations in spectral bands. FTIR finds application especially in confirming structural compatibility and non-covalent interactions without chemical degradation and thus aiding formulation stability investigation.[40]

Although not used as much, nuclear magnetic resonance (NMR) spectroscopy provides detailed information on molecular surroundings and dynamic organization of ethosomal membranes. Applications of NMR have been applied to the interactions of ethanol and lipids in the bilayer, and the partitioning behavior of drugs in the bilayer, which helps provide mechanistic insight into the structure and functional properties of vesicles.[47,48]

Confocal laser scanning microscopy (CLSM) is a versatile interface between physicochemical characterization and biological functionality. CSLM can be used to observe ethosomal penetration depth, spatial distribution and transport pathways in ex vivo or in vivo models of the skin by tracing fluorescently labeled vesicles and therefore connecting vesicle properties to the behavior of transdermal delivery.[49]

Ethosomal drug delivery for skin cancer

Role of ethosomes in transdermal and localized drug delivery

Ethosomes facilitate the delivery of drugs with specific physicochemical characteristics, which locally therapies of the skin can use to particular advantage (Table 3, Fig. 5). Instead of playing the role of penetration enhancers, ethosomal vesicles serve as intradermal drug depots, which provide vesicular integrity in the viable epidermis and dermis and facilitate long-term local drug release.[43]

This localized retention is particularly applicable in cutaneous malignancies, where there is need to have prolonged intratumoral retention of the drug but where the systemic drug levels ought to be low. Ethosomal systems decrease systemic drug delivery by delivering the drug into the skin, decrease first-pass metabolism, and decrease the chance of off-target toxicity. These characteristics facilitate non-invasive dosing schemes that are controlled and enhance tolerance to treatment especially in treatments that are repeated or those necessitating long-term use.[58]

Table 3. List of drugs used in ethosomal formulations for skin cancer

Active compound	Formulation technique	Therapeutic use	Delivery system	Notable outcomes	Reference
Mitoxantrone	Thin film dispersion	Anti-melanoma	Ethosomal gel	Increased calreticulin membrane translocation in B16 cells vs. Mitoxantrone solutions	36
Vismodegib	Hot processing method	Basal cell carcinoma	Binary ethosomal gel	Enhanced anti-tumor activity (greater papilloma reduction) compared to oral and ethosomal formulations	50
Berberine chloride & Evodiamine	Single-step injection	Melanoma	Ethosomes	Higher inhibitory effects on B16 melanoma cells	51
Fisetin	Thin-film hydration	Skin cancer	Binary ethosomal gel	Reduction in TNF-α, IL-1α, and tumor incidences compared to UV-exposed mice	52
Sulforaphane	Ethanol injection	Skin cancer	Ethosomes	Greater anticancer efficacy against SK-MEL 28 after 24 hours vs. free drug	53
(-)-Epigallocatechin-3-gallate	Thin film hydration	Skin cancer	Ethosomes & transethosomes	Reduced tumor size, oxidative stress markers, and lipid peroxidation; effective against epidermoid carcinoma cell line A431	54
Celecoxib	Thin layer evaporation	Antitumor activity	Transfersomes & ethosomes	Enhanced drug penetration compared to suspensions and liposomes	55
Paclitaxel	Ethanol injection	Squamous cell carcinoma	Ethosomes	Increased anti-proliferative and anti-apoptotic activity vs. free drug	37
Transcutaneous Tumor Vaccine/anti-programmed cell death protein 1 (aPD-1) Monoclonal Antibody	Thin layer evaporation	Transcutaneous immunization (Melanoma suppression)	Ethosome-loaded nanofibrous patch	Targeted dendritic cells, boosted DC maturation, suppressed melanoma; synergy with aPD-1 led to increased CD4+ and CD8+ T-cell infiltration and IL-12 production	56
Silver nanoparticles (AgNPs) & Tasar Silk Sericin Proteins	Ethanol injection	Non-melanoma skin carcinoma	Bioengineered ethosomes	AgNPs induced ROS production, disrupted mitochondrial stability triggered apoptotic/ necrotic cell death, and caused DNA double-strand breaks	57

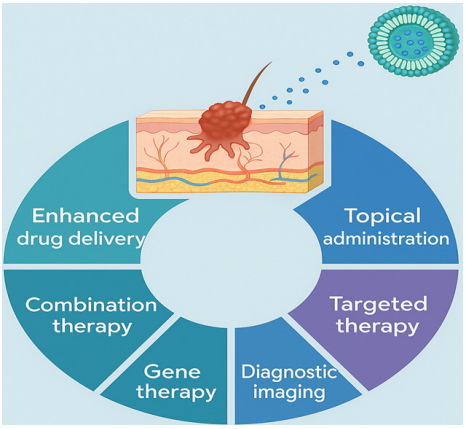

Fig. 5. Potential applications of ethosomes in skin cancer therapy.

Taken together these properties have placed ethosomes as functionally distinct entities compared to traditional topical carriers, providing a platform of delivery well-suited to sustained, localized therapy as opposed to a temporary exposure to the surface.

Ethosomal systems have been utilized to a wide variety of therapeutic and cosmeceutical applications, such as anti-aging agents, corticosteroids, antifungals and antimicrobials, in which increased skin penetration and augmented local efficacy has been reported consistently. Recipes of acyclovir or clindamycin, such as ones, have been shown to possess better dermal delivery and therapeutic efficacy than traditional topical dosage forms.[59,60]

More recent studies have applied ethosomal delivery to macromolecular cargos, including peptides, proteins, small interfering RNA, and plasmid DNA, fostering the use of more specific intervention approaches to inflammatory and oncological skin disease with the help of ethosomal delivery.[61] The concept of co-encapsulation has also been extended to realize synergistic outcomes in the complex disease context, such as skin cancer and drug-resistant infection.[58]

Formulation wise, ethosomes can be tailored to a variety of dosage conditions such as gels, creams, patches, and sprays with ease of administration in a non-invasive manner and acceptance by the patient. Their ability to combine with a large variety of excipients and the demonstrated potential to scale-up makes them relevant in localized and transdermal delivery, and future clinical studies should further specify how they can be used in precision topical treatment.[62]

Targeted delivery of chemotherapeutics

Based on these wider uses, ethosomal carriers have recently been of growing interest in the local delivery of anticancer agents to the skin. The optimal treatments to use in such cutaneous malignancies like melanoma, squamous cell carcinoma and basal cell carcinoma are the long-term exposure of the drug to tumor tissue at the lowest levels of systemic toxicity. Ethosomes can help in achieving this need by incorporating superior skin permeation with superior drug loading capacity, controlled release and localization in the application site. Their deformable, ethanol-containing structure facilitates penetration to deeper layers of the cutaneous tissues, and they pass the diffusional barrier posed by the SC.[58]

An example of a therapeutic application of this method, the small, polar antimetabolite 5-FU has been used to demonstrate the therapeutic relevance of this method in the treatment of actinic keratosis and basal cell carcinoma. Even though 5-FU as topical creams and solutions is clinically available, traditional formulations of the drug mainly limit the drug to the upper layers of skin, hindering its effectiveness in deeper or more aggressive diseases. The encapsulation of 5-FU in ethosomal has been demonstrated to increase skin penetration and intradermal retention of the compound, increasing its aptitude in topical chemotherapy. Moreover, circumstances also indicate that 5-FU may be used in synergy with immunomodulators like imiquimod in treating melanoma and this further suggests that ethosomal systems have the potential to aid combination therapy in cutaneous oncology.[63]

Nevertheless, with its clinical potential, standard topical preparations of 5-FU largely restrict the penetration and retention of the drug to the superficial tissue in the skin, and not deep tissue tumors. The limitation is mostly because of the SC, the most outer epidermal layer that composed of keratin corneocytes that are embedded within a highly ordered lamellar lipid matrix that immensely limits the penetration of drugs.[63] Ethosomal encapsulation has been shown to overcome this barrier by enhancing intradermal penetration and prolonging drug residence time, thereby improving therapeutic efficacy, particularly in aggressive or deeply infiltrating cutaneous malignancies.

Lipid lamellae and corneocytes together constitute the primary skin barrier to drug entry and permeation, restricting transdermal transport to molecules with a log octanol-water partition coefficient of 1–3, melting point below 200°C, and molecular weight below 1000 Da. To overcome this barrier, vesicular carriers including liposomes, transfersomes, niosomes, and ethosomes have been developed. Conventional rigid liposomes fail to traverse intact skin, depositing the majority of drug on the surface rather than within deeper layers. While niosomes and transfersomes improve transdermal penetration, their limited skin drug deposition renders them less suitable for local skin cancer treatment. Ethosomes, by contrast, demonstrate superiority in both transdermal penetration and skin deposition simultaneously. Ethosomes on the contrary exhibit the best of transdermal penetration and skin deposition at the same time. In a representative in vivo study, 5-FU-loaded ethosomes exhibited a Cmax of 12.9 ± 1.1 μg/mL at 1.5 ± 0.1 h (Tmax), with AUC_{0-24} and $AUC_{0-\infty}$ values of 106.2 ± 9.7 μg·h/mL and 188.9 ± 43.8 μg·h/mL, respectively. Compared with conventional formulations, ethosomes significantly enhanced drug exposure and promoted sustained skin retention, resulting in progressive drug accumulation for up to 24 h.[64]

In vitro release studies demonstrated marked differences between free 5-FU and ethosomal formulations. Free 5-FU exhibited rapid and nearly complete drug release

within 6 h, indicating a burst-release profile. In contrast, ethosomal formulations provided sustained drug release, with cumulative release values of 78.01%, 86.09%, and 100% after 24 h for formulations containing 5%, 10%, and 15% ethanol, respectively. The differences in release behavior were statistically significant (ANOVA, $P<0.05$), confirming the controlled-release capability of ethosomal carriers. Enhanced drug release was strongly correlated with entrapment efficiency and drug-loading capacity ($r=0.999$ and $r=0.998$, respectively), while vesicle size and ethanol concentration also influenced release kinetics. Drug release followed a diffusion-controlled mechanism best described by the Weibull model. Transdermal permeation was markedly enhanced by ethosomes over the neat drug. Previous work showed that over 20% permeation in 24 h could be obtained using ethosomes containing over 5% ethanol, and ethosomes containing 5% ethanol permeated less but retained more drug in the skin. The hydrophilic nature of neat 5-FU was confirmed by the poor skin penetration. Interestingly, the results of FTIR imaging demonstrated that 5% ethanol ethosomes deposit more drug on the epidermis level, while 10% and 15% penetrate deeper in dermis level. Higher concentrations of ethanol induce permeation of the drug from the vesicles to the donor compartment, yet less drug was retained in the skin tissue with higher-ethanol vesicles, hinting at a trade-off between retention and penetration achieved with varying ethanol concentration and vesicle attributes.[64]

Ismail et al established the superiority of the ethosomal releases compared to the conventional dosage forms using brucine (BRU), a natural alkaloid which has been reported to have anticancer effect against skin malignancy. As indicated by (Fig. 6), BRU suspension displayed an almost extensive burst release (~100 patients) after 2 hours, but BRU-loaded ethosomes and ethosomal gel displayed an extensively prolonged, biphasic release curve, releasing about 51 and 34 per cent of drug after 6 hours, respectively ($P<0.05$). The BRU-charged gel exhibited an intermediate release profile (~69 at 6 h), which confirms that it is not the gel matrix, but ethosomal encapsulation that is the key determinant of long-term drug release. This slow release kinetics supports the nanodepots hypothesis, according to which ethosomes do not rapidly dump their contents via transmembrane diffusion through intact bilayers, an ability that is especially beneficial to maintain therapeutic drug levels in the tumor site without excessive exposure to the body system.[65]

Ethosomal delivery of Paclitaxel fundamentally changes the Paclitaxel kinetics of transdermal delivery, to a steady-state flux in squamous cell carcinoma models. This is a 23 fold difference in permeation when compared to hydro-alcoholic suspensions, which have a quick flux initiation in the first 60 minutes. In addition to superficial flux, an important dermal nanodepots, Paclitaxel is sequestered in the deeper layers of the skin ethosomes increase the Paclitaxel absorption of the skin by 24 folds, as compared to conventional formulations. This intense concentration is directly proportional to excellent anti-proliferative

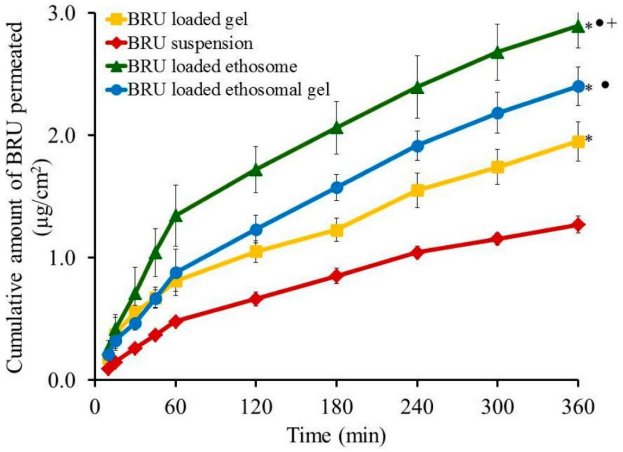

Fig. 6. Cumulative skin permeation of brucine (BRU) from ethosomal gel, plain gel, and drug suspension over time. Data expressed as mean ± SD (n = 3). *$P<0.05$ vs. BRU suspension; ● $P<0.05$ vs. BRU-loaded gel; + $P<0.05$ vs. BRU-loaded ethosomal gel. Adapted from Ismail et al,[65] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

effects in DJM-1 cells. Free paclitaxel requires a lot of concentration and a long duration of exposure (72 h) to induce 40% cell death whereas ethosomal Paclitaxel induces a lot of antagonistic proliferative activity at a mere 24 hours. These results highlight the ability of ethosomal scaffolds to reduce the therapeutic dose and hasten the process of apoptotic signaling in skin malignancies.[37]

Electrostatic hybridization process of polyethyleneimine (PEI)- modified ethosomes (Eth-PEI) and sodium cholate-modified ethosomes (Eth-SC) offers a useful controlled stoichiometric method to deliver doxorubicin (DOX) curcumin (CUR) in a concurrent manner, through transdermal delivery. As proven by Ma et al, this charge-modulated assembly neutralizes the detrimental effect of cationic toxicity of PEI which was indicated by the sustained viability of the L929 fibroblast lineages even when incubated with 120 μg/mL. The dual-loaded Eth-PEI/Eth-SC (7:3) complex inhibited tumor growth as it reduced tumor size to 279 mm^3 in B16 melanoma murine models compared to the PBS control (1065 mm^3) and had an inhibition rate of 46.38% ($P<0.01$). This was the improved therapeutic index with a 100 percent survival rate, as opposed to the high mortality rate in the monotherapy and control groups. These hybrid vesicular complexes enhance both the localized drug-lipid interactions required to penetrate the pathological dermal architecture of aggressive cutaneous malignancies by promoting the simultaneous intradermal flux of antineoplastics and chemosensitizers.[66]

Ethosomal systems have been shown to have significant potential as topical delivery of chemotherapeutic agents that have no inherent potential due to poor skin penetration or systemic toxicity. Investigations utilizing 5-FU, paclitaxel and DOX reveal that ethosomal encapsulation increases intradermal retention, regulates the release of drugs as well as the therapeutic efficacy compared to the traditional topical formulations. Here, ethosomes enhanced skin retention and controlled releases in the case of 5-FU, which favors local therapeutic approaches especially at the

optimal ethanol levels. The paclitaxel-ethosomes showed improved skin permeation and antiproliferative effects on carcinoma cells and reduced doses were used effectively. DOX ethosomes, particularly when used together with curcumin, were synergistically cytotoxic, exhibited strong tumor growth inhibitory effects as well as increased survival in melanoma mouse models, highlighting the ability of ethosomes to maximize efficacy whilst minimizing systemic exposure.[37,62,64,66]

These benefits are further demonstrated by preclinical studies. The ethosomal gel loaded with mitoxantrone (~78 nm; −55 mV) exhibited a significantly improved skin permeation, elevated cytotoxicity against B16 melanoma cells, and improved in vivo anti-melanoma activity with no apparent adverse effects which are backed up by an increase in cellular uptake and calreticulin translocation.[36] Ethosomes made of binary containing vismodegib had a high entrapment capacity (83.3%), high epidermal deposition (100.5 μg/cm^2), released over 24 h, and a high level of tumor regression in models of basal cell carcinoma in comparison to oral and traditional topical formulations.[50] Equally, the ethosomes loaded with cytarabine resulted in a 20-fold elevation in skin flux and a > 4 fold elevation in skin deposition as compared to liposomes, and sustained plasma exposure and increased cytotoxicity, negatively free of normal lymphocytes.[67] Ethosomes co-loaded with berberine chloride and evodiamine also exhibited high encapsulation efficiency (> 90%), epidermal accumulation and better anti-melanoma activity, which underscores the possibility of synergistic localized therapy.[51]

Moolakkadath et al formulated fisetin-loaded binary ethosomes (~100 nm, ~90% entrapment efficiency) optimized via Box–Behnken design for dermal chemoprevention in UV-exposed mice. The ethosomes achieved deeper skin penetration than conventional gels and reduced tumor incidence from 96% (control) to 49%, accompanied by marked reductions in inflammatory cytokines and oxidative stress markers, confirming strong anti-carcinogenic and antioxidant efficacy.[52]

Cristiano et al investigated sulforaphane-loaded ethosomes as ultra deformable vesicular carriers for skin cancer treatment. Ethosomes composed of 40% ethanol and 2% phospholipon 90G showed mean sizes below 400 nm and excellent stability. In vitro permeation studies on human skin membranes confirmed enhanced sulforaphane delivery, which translated into improved antiproliferative activity against melanoma cell lines compared to free drug.[53]

Across several studies, bioactive and drugs ((-)-epigallocatechin-3-gallate, celecoxib, paclitaxel) as well as immunotherapeutics and bioengineered AgNP-sericin systems were formulated into ethosomes/transethosomes using thin-film hydration, thin-layer evaporation or ethanol-injection methods. These reports consistently found superior outcomes versus free drug or conventional carriers enhanced skin permeation and cellular uptake, greater antiproliferative/antitumor efficacy (reduced tumor size; lowered oxidative stress and lipid peroxidation), ROS-mediated cytotoxicity for AgNPs, and improved immune activation (DC maturation, increased CD4+/CD8+ infiltration and IL-12 production).[37,54-57]

Role in photodynamic therapy and immunotherapy

Advancements in skin cancer therapies like photodynamic therapy (PDT) and immunotherapy have been made through the use of ethosomal systems. Skin tissues are damaged through Apoptosis and necrosis in PDT treatment using a photosensitizer and light of certain wavelength.[68] Reactive oxygen species (ROS) is produced and has the potential to become cytotoxic. Despite PDT having the potential to treat skin cancers, the bioavailability of the photosensitizer as well as skin penetration is often low, making the clinical efficacy of PAN lower than expected. Ethosomes are in the form of ultra-deformable vesicles and enable the fluid dynamic effect of ethanol on the SC to enhance trans dermal delivery of hydrophobic 5-ALA, porphyrins and chlorins.[68-70] Studies have proven that ethosomal encapsulation improves drug stability and controlled release. Fewer off-target effects and better phototoxic reactions are made possible by increased accumulation of photosensitizing drugs in the tumor tissues. Ethosomes' photosensitizer delivery efficacy and high permeation efficiency help surpass side effects of conventional topical PDT making it possible to deliver medications into deeper skin layers and overcome most hurdles of transdermal drug delivery systems.[68,71]

Aside from PDT, ethosomes are also being studied for the novel delivery of immunotherapeutic agents to skin tumors. It aims to employ the immune system's cells and molecules to locate and destroy the cancer, but its use in skin cancer is hampered by the inability of big compounds such as cytokines, peptides, and checkpoint inhibitors to penetrate the skin barrier.[37] Ethosomal vesicles stabilized with immunostimulatory agents are capable of inducing changes in tumor microenvironment relative to antigen presentation, T-cell activating, and cytokine releasing. A number of researchers have shown synergistic effects using ethosomal Immunotherapeutics and PDT. Tumor destruction through ROS generation prompts an immune mediated response against residual cancer cells. This approach aids in effective tumor clearance by enduring anti-tumor immunity, thereby, low chances of recurrence in aggressive forms of skin cancer like melanoma. Hence, ethosomes prove to be adaptable and pioneering techniques that address the drawbacks of existing strategies based on PDT and immunotherapy devising a new strategy for skin cancer treatment that is simpler and more precise.[71,72]

Challenges and future perspectives

A search of ClinicalTrials.gov (search terms: "ethosome", "ethosomal", "ethosomes" combined with "skin cancer", "melanoma", "basal cell carcinoma", "squamous cell carcinoma", "cutaneous malignancy", and "non-melanoma skin cancer"; accessed April 2026) returned no registered clinical trials evaluating ethosomal formulations for the treatment of any form of skin cancer. The few

registered ethosome trials in dermatology address non-oncological conditions, namely chronic plaque psoriasis (NCT03348462, anthralin ethosomal gel) and keloids (NCT05893108, losartan ethosomal gel). Registered topical nanocarrier trials in cutaneous oncology are limited to non-ethosomal systems, such as T4N5 liposomal lotion for non-melanoma skin cancer chemoprevention (NCT00089180) and topical paclitaxel nanoparticle therapy for cutaneous metastases (NCT03101358). This translational gap between strong preclinical anticancer data and absent registered ethosomal oncology trials remains the principal hurdle for clinical adoption.

Clinical translation of ethosomal drug delivery systems in skin cancer remains poorly attempted despite strong preclinical evidence. One of the main issues is the stability of formulation. Ethanol-containing bilayer on which the ethosomal penetration efficiency relies and concentrates on enhances the susceptibility to vesicle fusion, aggregation, and drug leakage especially when it is not stored and handled in ideal conditions. The information about long-term stability that demonstrates the real-world distribution and use are still uncommon and are a serious gap. Scalability of manufacturing is another obstacle. Ethosomal properties are very sensitive to processing variables and small scale-up variations can lead to a high degree of variability in the vesicle size, drug loading and release properties. It is thus necessary to have strong, repeatable manufacturing plans in order to develop commercially. Regarding safety, despite the generally good tolerance of ethosomes, the impact of reiterated or extended topical application particularly those dealing with ethanol-mediated barrier modification need to be evaluated systematically on a long-term basis. The risk of regulatory uncertainty also contributes to translation as there are no guidelines on ethosomes in particular, and the current system of nanomedicine does not take account of ethanol-based vesicular systems comprehensively. Moreover, lack of standardized methodologies to measure drug leakage, retention and long-term performance restricts cross-study comparison and regulatory confidence. Factors that affect patients, such as interindividual differences in skin physiology and formulation acceptability, are also not researched enough, although they influence clinical outcome. The more important focus of future activities should be put on stability improvement, scalable production, and clinically relevant safety testing. The new approaches, such as stimuli responsive and hybrid ethosomal system and data-driven optimization of the formulation could be beneficial to overcome the existing constraints and bring ethosomes to the precision treatment of skin cancer.

Ethosomal systems have generally demonstrated a favorable safety profile across in vitro, animal, and limited human studies. Histopathological evaluations have shown no significant alterations in epidermal or dermal structure following topical application, and minimal inflammatory response has been reported. In vitro studies also indicate that empty ethosomal carriers are non-cytotoxic, suggesting that the vesicular system itself does not contribute to off-target toxicity.[37]

Although clinical data in skin cancer are limited, evidence from dermatological applications supports their tolerability. Studies in human volunteers have reported good skin compatibility with no significant erythema or irritation following application of ethosomal formulations. Clinical evaluations, including topical delivery of acyclovir and anthralin-loaded ethosomal gels, have similarly demonstrated minimal adverse effects, with only mild and transient sensations such as slight burning at the site of application.[37,73]

However, certain considerations remain important. The relatively high ethanol content may cause temporary irritation, particularly in sensitive skin. In addition, modified ethosomal systems containing surfactants or edge activators may carry an increased risk of irritation. Furthermore, long-term safety data, especially under conditions of repeated application for cancer therapy, are still limited. These aspects highlight the need for further systematic evaluation to support clinical translation.

Conclusion

Ethosomal drug delivery systems are an attractive system to deliver drugs locally for skin cancer therapy as this allows greater penetration of drugs into the skin, prolonged local retention of drugs and increase in therapeutic efficacy. This is due to their ability to deliver various anticancer agents across SC with their deformability due to the presence of ethanol, which outperforms traditional lipid carriers in preclinical models. The use of ethosomes has also been proven to be useful in chemotherapeutic delivery as well as in a combination method of photodynamic and immunomodulatory. However, issues of stability, mass production, regulatory compatibility, and long-term safety still undermine clinical adoption. It will be vital to address these problems with the help of rational design of forms, standardized evaluation models, and translationally-oriented research. It is anticipated that further development of next-generation ethosomal systems and in particular with responsive or personalized capabilities will further reinforce their role in localized and precision-based skin cancer therapy.

In addition to these considerations, it is important to highlight that the practical application of ethosomal systems depends on achieving a balance between enhanced skin penetration and formulation stability. While preclinical studies have demonstrated promising outcomes, further work is required to translate these findings into clinically relevant products. Future research should focus on improving formulation robustness, ensuring reproducibility during scale-up, and establishing standardized evaluation methods. Moreover, the use of advanced skin models that better represent tumor-altered conditions may provide more reliable insights into therapeutic performance. Such efforts will be essential to support the successful clinical development of ethosomal systems for skin cancer therapy.

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Competing Interests

The authors declare that they have no known conflicting financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declaration of AI-assisted Tools in the Writing Procedure

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, grammar correction, and improvement of manuscript readability. The authors carefully reviewed and verified all AI-assisted content for scientific accuracy and completeness. No AI tool was used for data generation, data analysis, interpretation of results, preparation of figures, or formulation of scientific conclusions. The authors take full responsibility for the final content of the manuscript.

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