



Review Article

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Ethosomes as delivery system for treatment of melanoma: a mini-review

<https://doi.org/10.1515/oncologie-2023-0177>

Received May 9, 2023; accepted June 28, 2023;
published online July 13, 2023

Abstract: Many dermatological diseases still do not have an adequate treatment, such as melanoma. The treatments are usually lengthy, complex, with low cure rates and with severe side effects. This leads to low patient compliance, generating recurrence and/or worsening of the disease. Ethosomes, which are phospholipid-based vesicles containing ethanol, have shown great potential as drug delivery systems for the treatment of melanoma and other skin diseases. The unique structure of ethosomes allows for enhanced skin penetration and efficient delivery of therapeutic agents to the target site, improving the efficacy of treatment. The use of ethosomes in melanoma treatment holds promise for overcoming the limitations of conventional therapies, offering the potential for improved patient outcomes, reduced treatment duration, and minimized side effects. In this mini-review we present the advances, challenges, limitations and advantages, and future perspectives of the use of ethosomes in the treatment of the melanoma.

Keywords: ethosomes; lipid vesicular carriers; transdermal delivery; transdermal drug delivery

Introduction

Drug delivery systems are important components in the strategy to carry drugs for the treatment of several diseases,

including cancer and other dermatological conditions [1]. Among these, ethosomes are a type of nanocarrier used for topical and transdermal applications because they combine the properties of their organic components (phospholipids and ethanol 20–45 %) to overcome low skin permeability and achieve therapeutic efficacy while reducing adverse effects [2].

Ethosomes

Ethosomes (lipid vesicular carriers) were developed as novel lipid carriers, composed of phospholipids, ethanol, and water (Figure 1). These vesicular systems can be defined as the second generation of liposomes, characterized by greater malleability, stability and ability to trap hydrophilic and hydrophobic molecules. They possess attractive properties in terms of cost-effectiveness and ease of application [3].

Alcohol induces a mechanism in which it interacts with the polar region of the lipid molecules, resulting in a reduction of the melting point of the corneal layer lipids (Figure 2). Due to the fluidizing effect of alcohol on lipids, ethosomes can penetrate the stratum corneum and reach the deeper layers of the skin [4].

Among the techniques for preparing ethosomes, the literature highlights simple and low-cost methods: (I) thin film hydration [5]; (II) mechanical dispersion [6]; (III) a cold method based on dropwise addition of bidistilled water to an ethanol solution [7] and (IV) single-step injection [8].

The commercialization of ethosome technology began in 2000 and is an increasingly evolving field. Several commercial products based on ethosomal technology have been developed. These include Nanominox®, a product containing minoxidil, a hair growth promoter, from Sirene of Germany; Supravir® cream, for the treatment of the herpes virus, from Trima of Israel; Cellutight EE®, a topical cream for cellulite, from Hampden Health of the USA; Decorin® cream, an anti-aging cream, from Genome Cosmetics, Pennsylvania, U. S.; the Noicellex®, topical anti-cellulite cream, from Novel Therapeutic Technologies of Israel and

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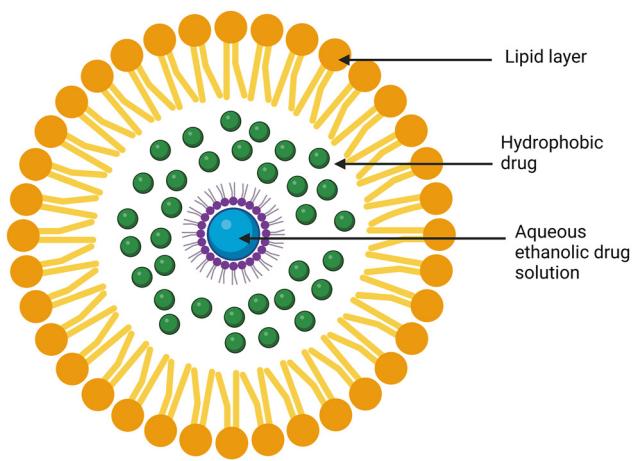


Figure 1: Ethosome structure.

Skin genuity[®], for the treatment of cellulite from Physonics, Nottingham, UK [9].

Table 1 shows some therapeutic applications of ethosomes. In a study conducted by Paolino and colleagues, ethosomes were formulated using commercially available Phospholipon 90, ethanol, active molecules, and water through dropwise addition of water [10]. They observed that the dimension varied depending on the composition used,

and ethanol typically reduced the mean diameter. Their formulation led to an increase of the percutaneous permeation of the active ingredient, both *in vitro* and *in vivo*, with good tolerability in human volunteers.

Mishra and collaborators prepared ethosomes using soya phosphatidylcholine, ethanol, antigen and/or probes through thin film hydration [15]. They observed that the antigen-loaded ethosomes exhibited higher skin permeability compared to conventional liposomes and soluble antigen. This finding may have implication to the development of vaccines.

The literature highlights the main advantages of ethosome systems, including the improvement of photo-protection and stability of encapsulated actives, as well as the reduction or elimination of topical adverse effects (irritation). They also enhance therapeutic efficiency by allowing gradual release and longer duration on the skin. Disadvantages are cited, such as the risk of non-enzymatic hydrolysis and microbiological contamination of the formulation. Another limitation includes temporal stability, as these vesicles may form aggregates or undergo particle fusion over time when in aqueous medium [1]. Complementary discussion about limitations can be found elsewhere [16].

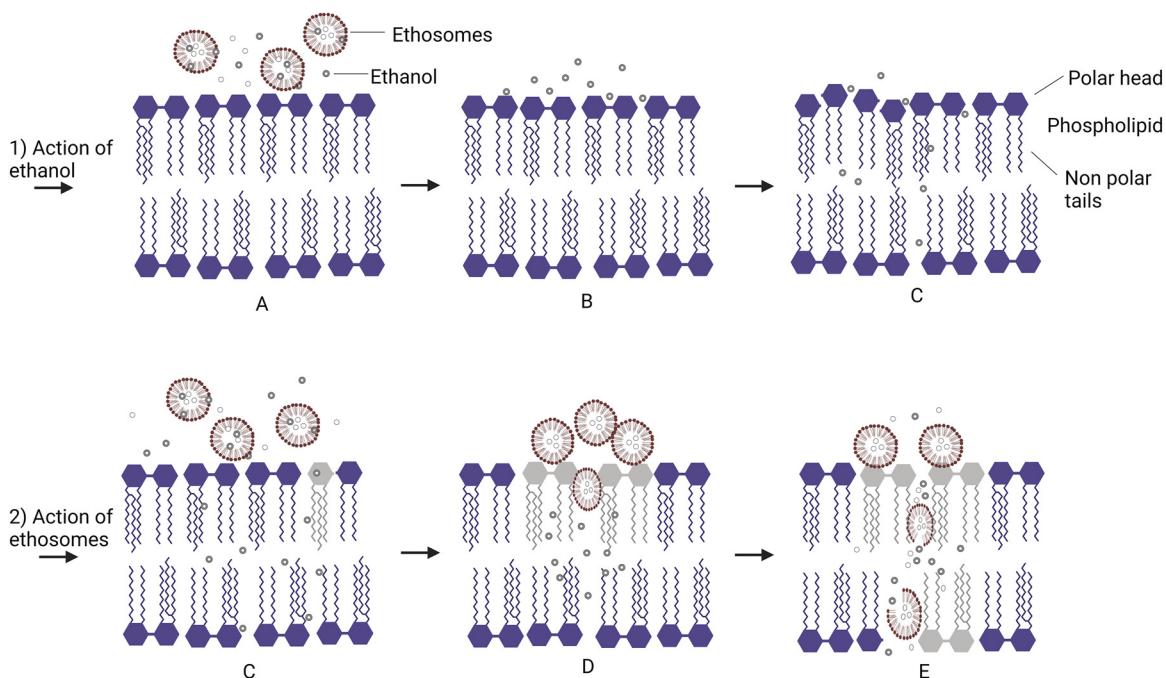


Figure 2: Proposed permeability mechanism of ethosomes: (A) *Stratum corneum* lipid layer; (B) ethanol interacts with lipid molecules; (C) ethanol increases lipid layer density; (D) interaction of ethanol and ethosomes on *stratum corneum*; (E) penetration and release of ethosomes into deep layers of skin.

Table 1: Therapeutic applications of ethosomes as drug delivery system.

Author	Study	Result
Touitou et al. [10]	Compared the skin permeation of testosterone-loaded ethosomes through rabbit pinna skin with the commercially available testosterone transdermal patch (Testoderm® patch). Further, a non-adhesive formulation of testosterone was designed to reduce the application area	<i>In vitro</i> and <i>in vivo</i> studies have demonstrated a better skin permeation and bioavailability of testosterone from the ethosome formulation. With the ethosome formulation, the application area required to produce the effective plasma concentration was 10 times smaller than that required by the commercial gel formulation.
Dayan e Touitou [11]	Prepared ethosome formulation of the psychoactive drug trihexyphenidyl hydrochloride and compared its delivery with the classical liposome formulation for the treatment of Parkinson's disease.	Results indicated the superior potential of cutaneous permeation of ethosome-THP formulation and its use for better management of Parkinson's disease.
Lodzki et al. [12]	Prepared a transdermal distribution of Cannabidiol-ethosomal formulation for the treatment of rheumatoid arthritis.	Encapsulation of Cannabidiol in ethosomes significantly increased its permeation and accumulation in the skin, and consequently, its biological activity.
Paolino et al. [13]	Investigated the potential application of ethosomes for cutaneous delivery of ammonium glycyrrhizinate. Ammonium glycyrrhizinate is useful for the treatment of various inflammatory skin diseases.	<i>In vitro</i> skin permeation experiments demonstrated that a significantly higher cumulative amount of drug was permeated in ethosomes (63.3 %) than in hydroalcoholic solution (22.3 %) and aqueous solution (8.9 %) of ammonium glycyrrhizinate.
Dubey et al. [14]	Developed optimized methotrexate-loaded ethosomes and the skin permeation profile of the developed formulation revealed enhanced permeation of rhodamine-loaded ethosomes to deeper skin layers.	Formulation maintained its penetration power after storage, and the vesicle-skin interaction study also highlighted the effect of increased penetration of ethosomes, with some penetration pathways being visually observed and corneocytes swelling.
Mishra et al. [15]	Ethosomes for transcutaneous immunization, and antigen-loaded ethosomes for transcutaneous immunization against hepatitis B.	HBSAg-loaded ethosomes were capable of generating a protective immune response, and their ability to cross and target the skin's immune milieu finds potential application in the development of a transcutaneous vaccine against hepatitis B virus.

Melanoma

Among the most prevalent dermatological conditions, melanoma stands out as the leading cause of death from skin cancer worldwide, causing significant psychosocial and emotional distress. Melanoma can arise from the factors: exposure to heat, solar UV rays, chemical agents and genetic predisposition. It presents the challenges regarding drug delivery across the skin barrier [17].

Melanoma is the most severe and aggressive form of skin cancer. It originates from the malignant transformation of melanocytes, which are cells responsible for producing melanin, a pigment that provides protection against ultraviolet radiation. The global incidence of melanoma has significantly increased over the years. It is considered multifactorial disease, involving the interaction of genetic, environmental, and behavioral factors. One of the most important socio-environmental risk factors associated with the development of melanoma is ultraviolet radiation exposure, known for its genotoxic effect [18].

Alteration of normal cellular physiological pathways is fundamental for carcinogenesis. In melanoma, these alterations are associated with the growth and amplification of atypical melanocytes, exhibiting characteristics such as growth factor auto-sufficiency, insensitivity to growth inhibitors, evasion of apoptosis, sustained angiogenesis, tissue invasion and metastasis. Melanoma is known to have one of the highest somatic mutation rates among the various types of cancers [19].

Melanoma presents significant clinical challenges and limitations in its treatment. The chances of cure using conventional treatments increase when the diagnosis occurs earlier. However, the thickness of the stratum corneum poses the first challenge, acting as a natural barrier that may require efficient skin permeation techniques and individualized dose adjustments to achieve optimal bioavailability in the affected tissue. Due to tumor heterogeneity, there is a lack of universal treatment response, limiting the options for certain melanoma subtypes. Patient compliance with the prescribed therapy is also crucial, particularly for topical applications that may involve daily multidose and a prolonged treatment duration [20–22]. Additionally, melanoma tumors may develop resistance to conventional treatments and side effects of conventional treatment also pose as limitations. Furthermore, the combinatory delivery of drug resistance inhibitor (MDR) siRNA and monoclonal antibodies via nanosystems is listed as promising approach, which extends the potential of ethosomes as delivery vehicle.

Application of ethosomes to melanoma

Ethosomes can encapsulate hydrophobic and hydrophilic drugs within their structure, enabling transdermal absorption. The absorption of drugs through the skin poses a challenge due to the protective barrier it provides. However, ethosomes, with their specific size and composition, possess permeabilizing properties that aid in overcoming this barrier, facilitating efficient delivery to melanoma cells in the skin. Recent studies highlighting the use of ethosomes in the treatment of melanoma are presented in Table 2.

As observed in Table 2, ethosomes are still underexplored as a delivery system for treating melanoma, indicating the potential application of this system in facilitating the treatment of this type of cancer. The results of these studies suggest that ethosomes may serve as an effective drug delivery system for melanoma treatment, owing to their ability to penetrate the skin and target melanoma cells, leading to enhanced therapeutic efficacy observed in the

studies. However, further clinical studies are needed to evaluate the safety and efficacy of ethosomes as a drug delivery system for melanoma treatment.

Nanotechnology has enabled the encapsulation of active molecules within supramolecular structures, such as nanospheres, nanocapsules, liposomes, solid lipid nanoparticles, polymeric micelles, ethosomes, and others. Each of these structures has advantages and disadvantages that can be explored based on the target and resource availability. Comparative studies of these nanostructures can be found in the literature, aiding in the selection of the most suitable system [28].

Future studies with ethosomes are expected to have an impact on the treatment and prevention of skin diseases. Ethosomes, containing alcohol, and their second-generation counterparts, transferosomes, containing surfactant, may carry chemoprotective actives such as natural antioxidants, acting as a step prior to cancer establishment in chemoprevention.

Immunotherapy has emerged as a promising approach for melanoma treatment, and mRNA vaccines, which introduce genetic material encoding specific tumor antigens expressed by melanoma cells, play a crucial role in this approach. The use of nanocarriers, including ethosomes, is essential for effective delivery in this application.

Additionally, gene and RNA interference (RNAi) therapy are other promising approaches for melanoma treatment, involving the introduction of genetic material into cells using nanocarriers, with ethosomes being a potential choice for this task.

In summary, ethosomes serve as a drug delivery system that can play important roles in targeting skin disorders. With further research, we can expect the development of novel therapeutics utilizing ethosomes to improve patient outcomes.

Table 2: Application of ethosomes to melanoma.

Author	Study	Results
Ma et al. [23]	Proposed the elaboration of ethosomes modified with polyethylenimine and sodium cholate with the simultaneous carriage of doxorubicin (cytotoxic agent) and curcumin (chemopreventive agent).	Inhibited murine B16 melanoma <i>in vitro</i> and <i>in vivo</i> .
Lin et al. [24]	Co-encapsulated berberine chloride (dyslipidemic agent) and evodiamine (thermogenic agent) in ethosome for the treatment of melanoma.	Obtained synergistical cytotoxicity <i>in vitro</i> against the B16 cell line.
Fang et al. [25]	Compared the permeability of 5-aminolevulinic acid encapsulated in liposomes and ethosomes for the treatment of melanoma.	Observed higher delivery capacity of ethosomes compared to liposomes.
Ismail et al. [26]	Encapsulated brucine (a natural anti-inflammatory and analgesic alkaloid) in ethosomes.	Obtained enhanced <i>in vitro</i> anticancer activity compared to the unencapsulated drug against human melanoma A375.
Kandil et al. [27]	Formulated the encapsulation of magnesium ascorbyl phosphate in ethosomes by factorial planning and subsequently incorporated into carbopol gel.	Gels showed controlled permeation and clinically and statistically significant reduction in melanin level after one month.

Acknowledgments: Authors would like to thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, as well as Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for fellowships.

Research funding: This work was partially supported by FAPEMIG (RED-00053-21) and CNPq (311195/2022-9).

Author contributions: All authors confirm contribution to the paper as follows: study conception and design: W.R.B., G.D.T., and F.P.; data collection: L.N.G., W.R.B., N.P.S., and M.G.S.P.; draft manuscript preparation: L.N.G., W.R.B., N.P.S., M.G.S.P.; manuscript revision: E.L.C.C.C., G.D.T., and F.P. Authors reviewed the results and approved the final version of the manuscript.

Competing interests: The authors declare that they have no conflict of interest.

Availability of data and materials: Not applicable.

Ethics approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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