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EDITED BY
Rovshan Khalilov,
Baku State University, Azerbaijan

REVIEWED BY
Dilpreet Singh,
Indo-Soviet Friendship College of Pharmacy, India
Van-An Duong,
Gachon University, South Korea

*CORRESPONDENCE
Sankha Bhattacharya,
sankhabhatt@gmail.com

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Ethosomes and their monotonous effects on Skin cancer disruption

Prafull Shinde[1], Amit Page[2] and Sankha Bhattacharya [2]*

[1]Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM'S NMIMS, Vile-Parle (W), Mumbai, Maharashtra, India, [2]Department of Pharmaceutics, School of Pharmacy and Technology Management, SVKM'S NMIMS, Shirpur, Maharashtra, India

Skin cancer is one of the most prominent diseases, affecting all continents worldwide, and has shown a significant rise in mortality and prevalence. Conventional therapy, including chemotherapy and surgery, has a few drawbacks. The ethosomal systems would be thoroughly reviewed in this compilation, and they would be classified based on constituents: classical ethosomes, binary ethosomes, and transethosomes. Ethosomes systems are model lipid vesicular carriers with a substantial portion of ethanol. The impacts of ethosomal system components, preparation techniques, and their major roles in selecting the final characteristics of these nanocarriers are comprehensively reviewed in this chapter. The special techniques for ethosomes, including the cold approach, hot approach, injection method, mechanical dispersion method, and conventional method, are explained in this chapter. Various evaluation parameters of ethosomes were also explained. Furthermore, ethosomal gels, patches, and creams can be emphasised as innovative pharmaceutical drug formulations. Some hybrid ethosomal vesicles possessing combinatorial cancer therapy using nanomedicine could overcome the current drug resistance of specific cancer cells. Through the use of repurpose therapy, phytoconstituents may be delivered more effectively. A wide range of *in vivo* models are employed to assess their effectiveness. Ethosomes have provided numerous potential skin cancer therapeutic approaches in the future.

KEYWORDS

skin cancer, ethosomes, drug delivery, ethanol, characterization, phospholipid

Introduction

Cancer is among the main causes of mortality globally, based on chronic tissue damage, host-environment interactions, etc. Repeated exposure to carcinogens like smoke, UV rays, and infections causes a variety of genetic (mutations), epigenetic (loss of heterozygosity), and global transcriptome alterations (through inflammatory pathways), all of which are associated with an increased risk of developing cancer. The previous 10 years have seen an upsurge in the incidence and prevalence of cancer, which has presented a significant challenge to medical experts. According to World Health Organization (WHO) data, there will be a worrying 45% increase in cancer deaths worldwide by 2030, with India contributing 70% of those fatalities. The most

Abbreviations: WHO, World Health Organization; BCC, Basal Cell Carcinoma; SCC, Squamous Cell Carcinoma; NMSC, Non-melanocytic Skin Cancer; UV, Ultraviolet; ROS, Reactive Oxygen Species; CSCC, Cutaneous Squamous Cell Carcinoma; MMS, Mohs Micrographic Surgery; PG, Propylene Glycol; TEM, Transmission Electron Microscopy; SEM, Scanning Electron Microscopy Micrograph; DSC, Differential Scanning Calorimetry; aPD1, Protein 1 Monoclonal Antibody; TCI, Transcutaneous Immunisation; EVO, Evodiamine; BBR, Berberine Chloride; AUC, Area under Curve; CARPA, Complement Activation-Related Pseudo Allergy; EMA, European Medicines Agency; US FDA, US Food and Drug Administration.

prevalent malignant condition, especially in Caucasians, is skin cancer (Garrett-mayer, 2019). Each year, more than a million new cases are recorded globally. The cells from whence they arise and their clinical characteristics are used to designate the various forms of skin cancer. The much more prevalent varieties are malignant melanoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC), collectively known as non-melanocytic skin cancer (NMSC) (World Health Organization, 2022).

Topical medication delivery using a liposomal formulation has drawn a great deal of attention in the last 10 years. When administered in non-occluded environments, deformable liposomes and transferosomes the first generation of elastic vesicles—were shown to penetrate intact skin while delivering a therapeutic concentration of medicines (Honeywell-Nguyen and Bouwstra, 2003). They were initially presented by Ceve and Blume in 1992. The medication is known to be carried into and through the skin *via* lipid vesicles made of phospholipids and non-ionic surfactants. The lipids in the skin help to maintain the skin's barrier functions and stop medications from being absorbed into the body. Lipid vesicles may act as non-toxic penetration enhancers due to their amphiphilic nature. Additionally, hydrophilic (Biju et al., 2006), lipophilic, high-and low-molecular-weight compounds can be enclosed in the vesicles (Essa et al., 2003). Furthermore, it is theorised that these lipid-rich vesicles transport a considerable amount of drug through the skin, improving the systemic absorption of medication. It has been discovered and shown that ethosomes, which are lipid vesicular networks that contain ethanol in some really high concentrations, are extremely effective at increasing the skin absorption of a wide range of medications (Touitou et al., 2000).

This review intends to assemble and explain major research employing these nanovesicular drug delivery for skin cancer. This review article discusses many forms of skin cancer and their incidence. Nanotechnology methods provide a chance to improve the therapies for different forms of cancer. Also, a discussion related to both traditional and non-conventional therapy. Then there's skin cancer's problems (Borgheti-Cardoso et al., 2020). The physiological hurdles that obstruct regional and national therapy will be highlighted. Multidrug resistance, as well as systemically distributed ethosomal vesicles with many types of anticancer formulation. Then, based on the route of administration, the designs and optimal properties of ethosomes for successful drug delivery in skin cancer therapy will be presented. The several ethosomal formulations that have been described for the management of basal cell carcinoma, squamous cell carcinoma, and melanoma are presented in the review that follows. These formulations have been utilized for systemic and transdermal drug administration in skin cancer. Characteristics of the skin barrier are also highlighted in respect on current clinical care for skin cancer, with major focus on non-surgical therapeutic treatments. This review also discusses the relationships of ethosomes with skin tissue, the many pathways for drug administration *via* the skin, and a comprehensive investigation of the ethosomes topical permeability. This paper describes the various methods of topical or dermal distribution and gives prospective researchers guidelines for topical formulation invention.

Skin as a barrier

The human body skin has more efficient delivery of drugs by barrier "our insides in and the outside out". Skin has some barrier issues to selected drugs passing through for topically applied various types of formulations containing active pharmaceutical agents. The skin surrounds the body's surface, integrates with the environment, and protects the inner organs against direct contact from the outside atmosphere (Williams and Barry, 2004) It has the property of altering the entry of chemical and biological agents because of the stratum corneum (subcutaneous) that functions to regulate heat through regulation such as blood flux, perspiration, and sweating (Harman et al., 1966). Lipid bilayers are composed of primary ceramides sequentially arranged on the flattened corneocyte layer of skin. The largest organ in the body is the skin, which covers almost 10 percent of the total body mass; it weighs an average of 4 kg and covers an area of 2 m^2 (Mishra et al., 2013). Schematic structure of skin layer represented in Figure 1.

The drug penetration through the skin is mainly by the stratum corneum layer. It follows different pathways through penetration, which helps in delivering drugs through novel drug delivery systems. It has the ability to enter through the skin and trigger the necessary action.

Some permeation enhancers such as Piperine and also some vascular carriers facilitate transport across the skin (Jantarat et al., 2018). Pathway through drug absorption *via* skin represented in Figure 2.

The following stages are associated with drug penetration through the skin.

- Product sorption by the stratum corneum
- Drug penetration even by intact skin
- Drug absorption by the capillary network throughout the dermal papillary layer

The permeation level throughout the skin (dQ/dt) is provided by

$$dQ/dt = Ps\,(C_d - C_r)$$

This Equation helps to determine the permeation rate of a drug through delivery system.

Based on their content, ethosomes as a carrier can transfer topically administered abilities to different skin layers. Dissolution from the inside and release from the preparation are typically two-step processes by which a medicinal substance is released from a topical application and transferred to the blood circulation (Barua and Mitragotri, 2014), primarily *via* the lipidic intercellular route through the SC by partitioning into the viable aqueous epidermis. The healthy epidermis diffuses into the upper dermis, the papillary dermal layer, and is eventually absorbed by the vascular system.

Skin cancer

Skin cancer is a form of cancer that develops on the skin surface as a result of the proliferation of aberrant cells. Cancer cells have the ability to spread across the body and invade other organs. In recent years, non-melanoma and melanoma, the two most common kinds of skin cancer, the most common cause of skin cancer is sensitivity to ultraviolet (UV) radiation. Specific genes are inactivated by UV radiation, whereas oncogenes are activated. Although skin cancer is not the most lethal type of cancer, it is the most frequent in the United States and many other nations (Misak et al., 2013). Melanoma accounts for the great majority of skin cancer mortality despite accounting for just a small percentage of skin cancer rates. Early

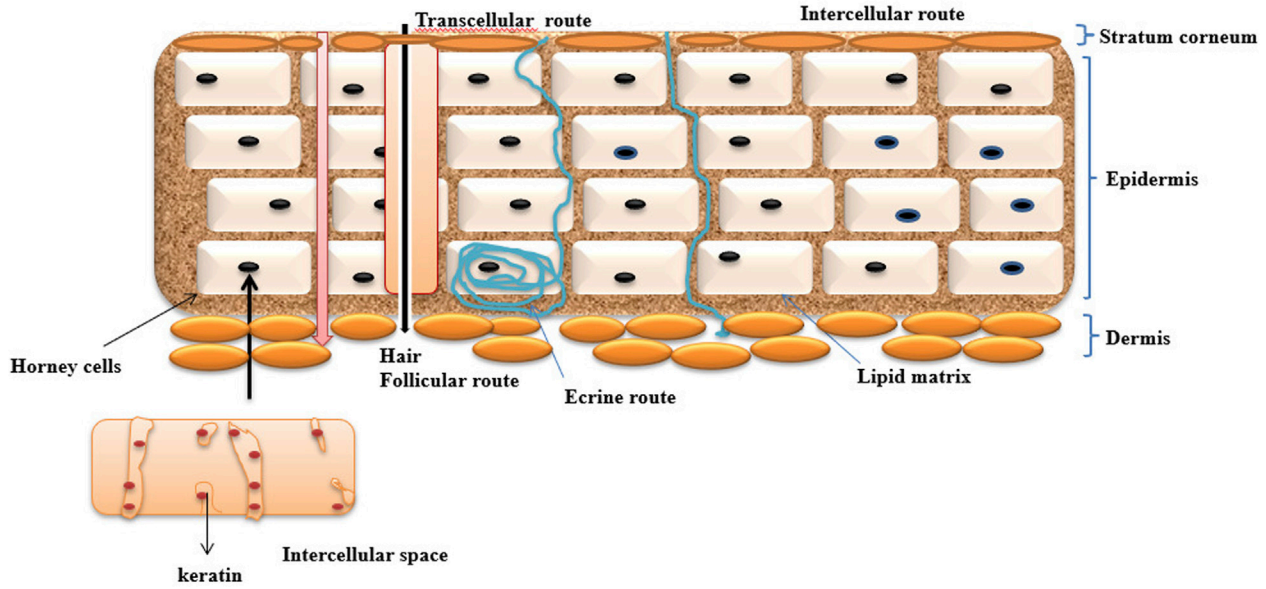

FIGURE 1
Structure of skin layer.

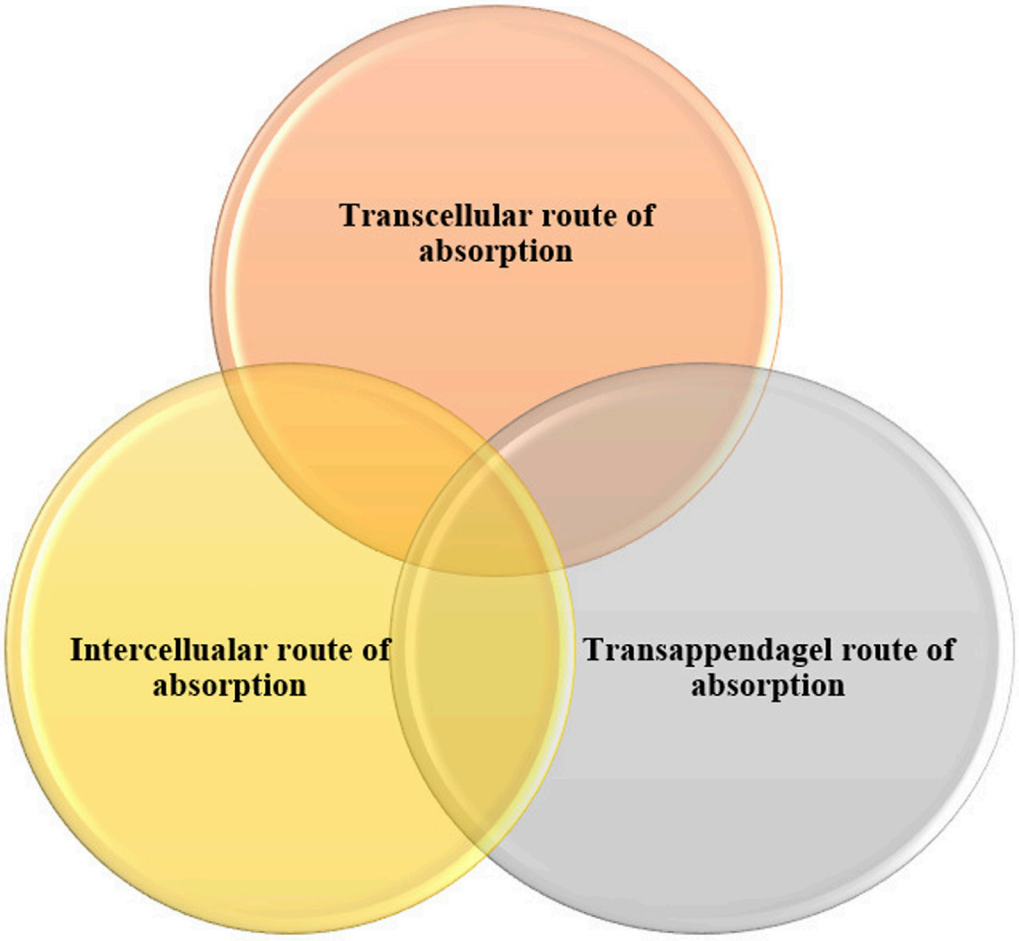

FIGURE 2
Pathways through absorption of drug by skin.

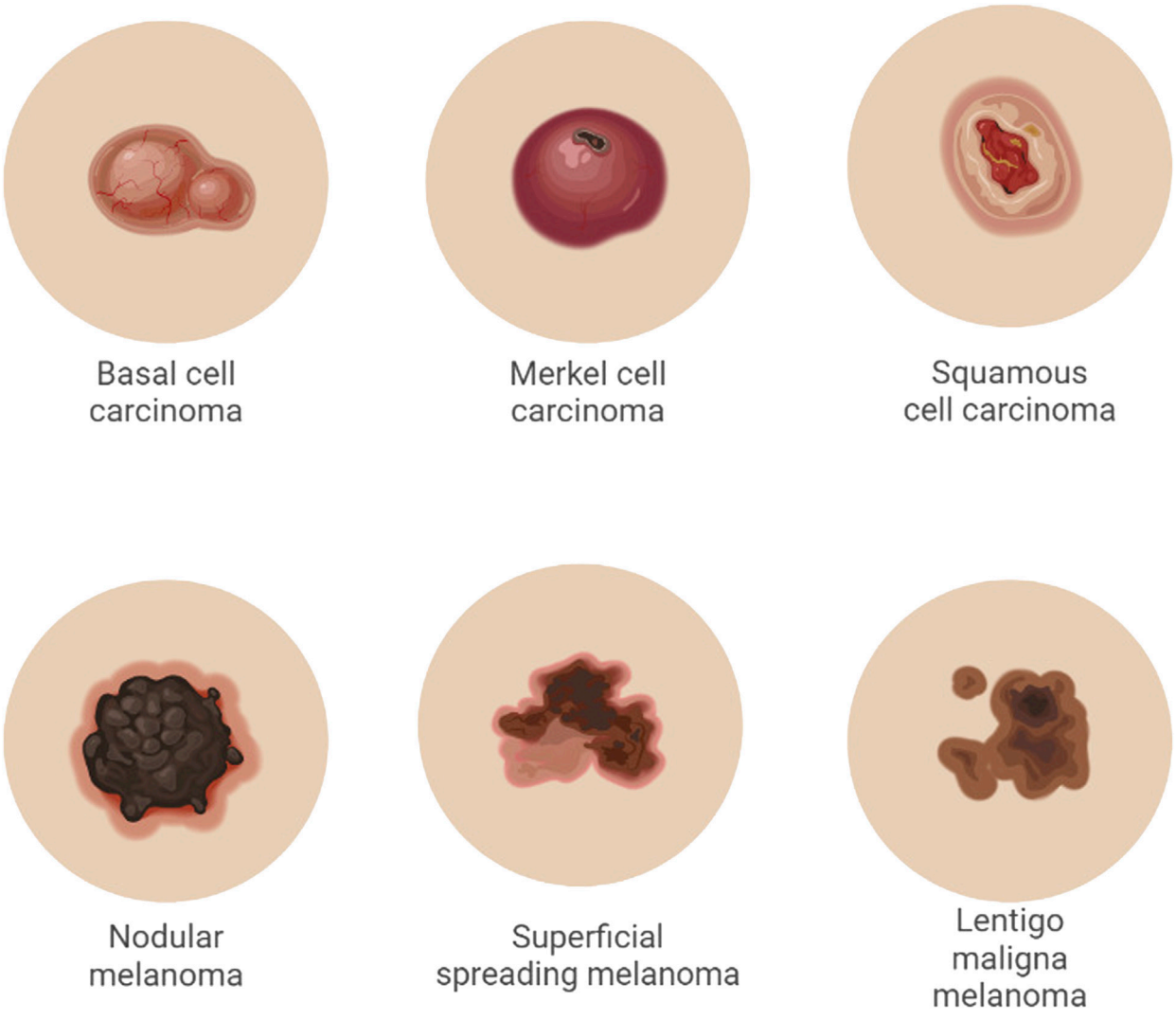

FIGURE 3
Types of carcinoma.

detection and treatment of skin cancer may be biopsied at a preliminary phase and has a 99 percent survival probability, but metastasized melanoma kills 80 percent of patients within 5 years of diagnosis (Jerant et al., 2000). Basal cell carcinoma and squamous cell carcinoma are the two most frequent kinds of skin cancer. For these restricted conditions, excision is the gold standard therapy. They can, however, spread to regional lymph nodes and distant sites in extremely rare circumstances. Melanoma is still a major cause of death and morbidity in the U.S. and across the globe. Greater UV radiation exposure and increased monitoring have both been blamed for the rise in melanoma cases. There has been no change in the behavior of young adults who use indoor tanning despite increased public knowledge of the hazards of extreme UV exposure in connection to melanoma formation Beds. The following are some of the current public health programs aimed at lowering melanoma rates: sunlight exposure should be reduced, sunscreen should be used more frequently, and indoor tanning should be avoided, particularly for kids. Lowering the rates of illness and death linked to melanoma will necessitate a combined public and healthcare professional effort (Carr et al., 2020).

Melanoma is a cancer that starts in the melanocytes and is classified as cutaneous or non-cutaneous depending on how it manifests (Garbe and Leiter, 2008) the most common type of melanoma mentioned below.

- Superficial spreading melanoma (70%) prevalent type of melanoma is Before vertical (invasive) growth, it passes through lateral (radial) growth.
- Nodular melanomas (15%–30%) are fast-expanding raised or polypoid lesions that are frequently blue or black in colour and have an early vertical development phase.
- Lentigo malignant melanoma (4%–10%) is more prevalent in older people who have had their skin exposed to the Sun for a long time. It usually starts as a tiny macule that looks like a freckle. It expands over time, becoming darker, more asymmetrical, and exhibiting a vertical development phase.
- Acral lentiginous (5%): tumours most usually appear on the palms, soles, subungual, and mucosal surfaces. Melanoma can develop in any non-cutaneous place with melanocytes, such as

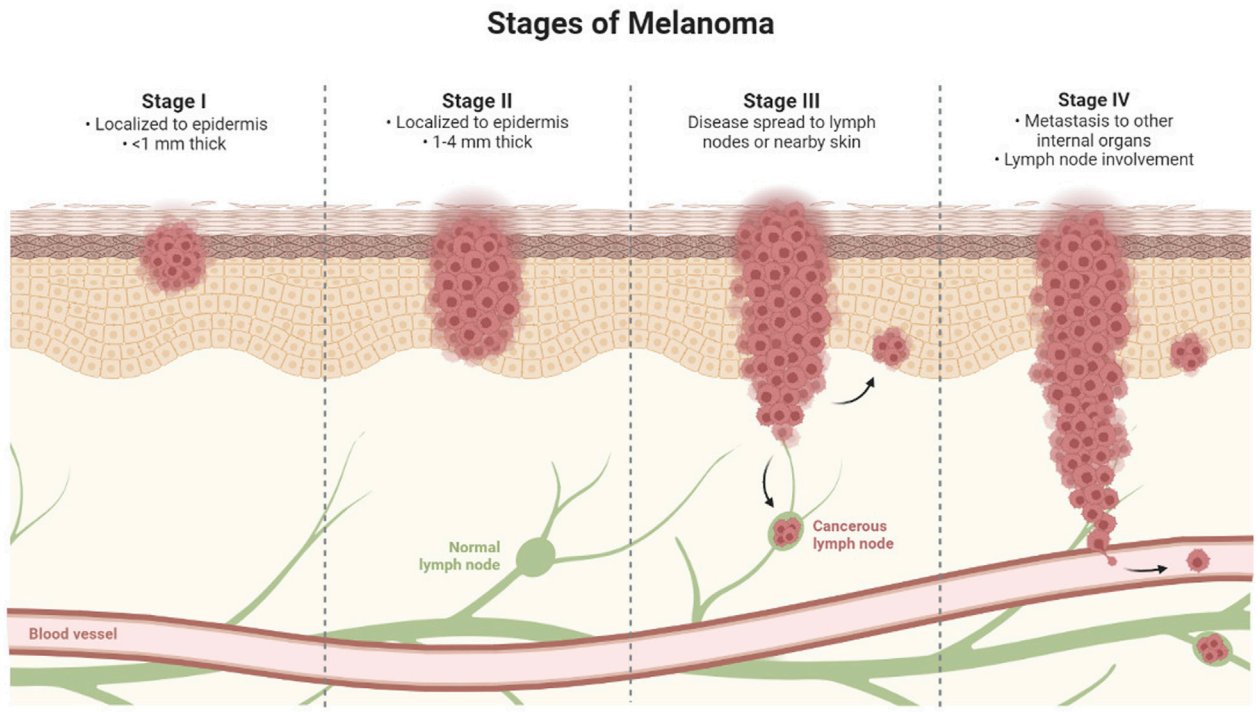

FIGURE 4
Stages of melanoma.

the ophthalmic, gastrointestinal, genitourinary, and nasopharyngeal areas. Type of carcinoma represented in Figure 3.

According to a study published in the journal Cancer, they are far less prevalent than cutaneous melanoma. The National Cancer Data Base has information for 84,836 people with cutaneous cancer. Melanoma of the skin and non-cutaneous melanoma (Carr et al., 2020). Intermittent Sun exposure and ultraviolet (UV) radiation have been linked to the development of melanoma, basal cell, and squamous cell carcinoma in several studies (Nagore et al., 2010; Bauer et al., 2011). UVA radiation stimulates the creation of reactive oxygen species (ROS), which leads to the degradation of deoxyribonucleic acid (DNA), lipids, and proteins during oxidative reactions (Viros et al., 2014). Indoor tanning also hastens the progression of melanoma, with non-exposed people having a 16–20 percent increased risk of developing the disease. Even in the absence of mutations, the major melanoma mutations (BRAF, MEK, NRAS, and MAPK) cause different melanogenic pathway functions. However, due to the restrictive strategy associated with several medications, a novel technique is particularly needed (Feoktistova and Panayotova-Dimitrova, 2017). Surgery, radiation treatment (Kalekhan et al., 2022), immunotherapy (Nanamori and Sawada, 2022), systematic therapy, and antibody therapy (Esnault et al., 2022) are among some of the therapeutic interventions for malignant melanoma. In addition, because of the specific delivery of therapeutic components, nanotechnology-based approaches have emerged as promising strategies in melanoma treatment (Portugal et al., 2021). Stages of Melanoma represented in Figure 4.

Non-melanoma skin cancer

Melanoma and non-melanoma skin cancer (NMSC) incidence have been rising in the U.S. for the past 2 decades, and this has been linked to increased ultraviolet radiation exposure (UVR). With these escalating rates, it has become much more crucial than ever to take preventative actions to lower the incidence and encourage timely diagnosis of these tumours (Trager et al., 2022). Patients with non-melanoma skin cancer are the most highly prevalent cancer form. Because the growth of non-melanoma skin cancer follows molecular pathways, it leads to effective therapy options for NMSC (Didona et al., 2018). NMSC is typically categorised into two categories: BCC and SCC.

Basal cell carcinoma

Basal cell carcinoma, also known as Jacob's ulcer, rodent ulcer, basal cell epithelioma, or basalioma, is a cancer that arises from the epidermis's basal membrane (Sari et al., 2020). Out of all the skin cancers, BCC is a really limited malignancy that requires lineage-specific immunohistochemistry investigation for accurate identification and is recommended to be totally excised at an early stage (Bisceglia et al., 2020). It occurs in 80% of patients, primarily in the head and neck areas. BCC seldom metastasizes, although it does regularly invade and destroy tissue locally, resulting in high morbidity (Cameron et al., 2019). It has been discovered that genetic disposition increases skin ageing and shares molecular pathogenic characteristics in the establishment of BCC (van der Poort et al., 2020).

The Hedgehog (HH) pathway, which is normally suppressed in adults, plays a vital role in organogenesis, patterning, proliferation, survivability, and differentiation (Rimkus et al., 2016). It had been *Drosophila* was the source of the discovery. The HH is an abbreviation for "highly Three factors might trigger a signalling cascade. Various ligands involved in this pathway include: The Indian hedgehog (SHH), the Sonic hedgehog (SHH), and the Hedgehog of the Desert SHH, on the other hand, is very common. Research expressed in human tissues. These are the ligands. Binds to the Patched-1 (PTCH) receptor (Skoda et al., 2018). On the surface of the membrane, there is a receptor for target cells, which usually works by obstructing Another membrane is smoothened (SMO). The HH signalling cascade is then initiated (Pak and Segal, 2016; Doheny et al., 2020).

The inhibition of SMO is lifted, resulting in a cascade of stimulation that is abnormal. SMO plays a crucial function in this process; it works as a signalling molecule *via* triggering the HH signalling cascade, which results in the transcription regulators' activation for homolog 1 of the glioma-associated oncogene (Gli1), which stimulates angiogenesis and the expression of target genes Bcl-2, Cyclin-D1, and Myc (Bcl-2, Cyclin-D1, and Myc) (Pak and Segal, 2016). When there is not any The SHH ligand is inert, the HH pathway is dormant, and PTCH is inactive. Gli1-targeting is prevented by inhibiting SMO activity. transcription of genes. The HH route is implicated in the process. In the control of cell growth and differentiation. It is active throughout embryogenesis but not in adulthood. tissues. The HH pathway's abnormal activation Increased cell proliferation and survival are associated with the pathophysiology of several solid tumours, especially BCC (Luca et al., 2044).

BCCs were advised to be divided into two categories: "simple to treat" and "difficult to treat," with distinct treatment choices for each. Treatment methods that have been licenced and are indicated for both local and systemic therapy, such as imiquimod and 5-fluorouracil. New medicinal medicines that have undergone clinical studies are explored. Targeted treatments, such as GLI antagonists or anti-VEGFR drugs, immunotherapies, such as checkpoint inhibitors, recombinant cytokines, or silencing RNA, and intralesional virotherapies using modified adeno-as well as herpes viruses (Cameron et al., 2019).

Squamous cell carcinoma

The second leading cutaneous cancer that requires rapid detection is squamous cell carcinoma. The definition of clinical characteristics, diagnostic procedures, grading, staging, surgery, and non-invasive therapy must all be updated. Topical therapy, cryosurgery, photodynamic therapy, and radiation are among the non-surgical current therapies (Dreyfuss et al., 2022).

Cemiplimab and pembrolizumab are two anti-PD1 medicines that have been authorised by the Drug Administration for the treatment of established CSCC and have shown therapeutic significance and long-lasting responses, as well as an acceptable safety profile. Immune system blockers are now the gold standard of therapy for both laCSCC and mCSCC. Anti-PD1 immunotherapy's safety and efficacy in both lcscc and mcscc must be validated in real-world research (de Jong et al., 2022).

Immunotherapeutic drugs such as PD-1 inhibitors are utilised to treat advanced cutaneous squamous cell carcinoma (cSCC) (Aboul-Fettouh et al., 2022). The anticancer activity, increased lifespan, antiviral, and tolerability decrease of a cisplatin-and imiquimod-loaded pro-vesicular dual-drug delivery system were all excellent, demonstrating the benefit of localised drug administration and adjusted combination treatment (Gupta et al., 2014). Topical formulations encased in polymeric and lipid nanoparticles, nanoemulsions, dendrimers, and liposomes have higher skin penetration and stability, resulting in improved effectiveness (Ravikumar and Tatke, 2019). The skin deposition ability of nobiletin vesicular systems was effectively created and compared. Small particle size, high entrapment for nobiletin, and high skin deposition ability were exhibited in composite penetration booster vesicles made of phospholipids, transcutol P as a penetration enhancer, and chitosan (Bayoumi et al., 2021).

Conventional treatments and limitations of the conventional dosage form for skin cancer

The rising incidence of skin cancer throughout the world necessitates a variety of treatment choices (Simões et al., 2015). Chemotherapy, immunotherapy, surgical excision, radiation, Mohs micrographic surgery (MMS), and cryotherapy are among the well-known therapies for skin cancer.

Chemotherapy is administering cytotoxic medications to patients in order to extend their lives and enhance their quality of life. After oral delivery, only a tiny percentage of medications reach the therapeutic location, decreasing therapeutic effectiveness. Frequent doses are necessary to compensate for the loss of therapeutic potential and sustain the optimal drug concentration at the therapeutic site, which may lead to drug resistance and produce harmful adverse effects due to recurrent medication use (Mou et al., 2016). In a conventional chemotherapy procedure, cytotoxic medicines are frequently given in conjunction with two or more additional treatments to help patients tolerate the side effects of other adjuvants and obtain greater therapeutic efficacy. With so many pharmaceuticals being used in patients, the possibility of a drug–drug interaction is unavoidable. According to (Buajordet et al., 2001), drug–drug interactions are expected to be the cause of mortality in 4% of cancer patients. There were five incidences of anticancer agents being used in cancer treatment that resulted in fatal side effects (Marks et al., 2001). The graph depicts the total number of Research papers related ethosomes for skin cancer has reviewed from 2008 to until December 2022 mentioned in Figure 5. Also included summary of research articles work for treatment of skin cancer represented in Table 1.

To study the current scenario of scientific investigations heading towards the development of a potential strategy for the diagnosis, management, and treatment of skin cancer, we have analysed all the relevant data or work through the Pub Med search engine.

We searched for the number of research papers done related to ethosomes drug delivery system. The number of articles searched with the set of keywords applied with available data is ethosomes for skin cancer.

Ethosomes, invented by Touitou in 1996 (Touitou, 1996), are non-invasive lipid vesicular agents that have been well for attaining effective skin administration and have nanometric dimensions (Touitou et al., 2000; Bendas and Tadros, 2007; Ibrahim et al., 2019). They include phospholipids in the range of 2%–5% and a high concentration of ethanol in the range of 20%–40% (Romero and Morilla, 2013). The combined potential of lower chain alcohol and soft

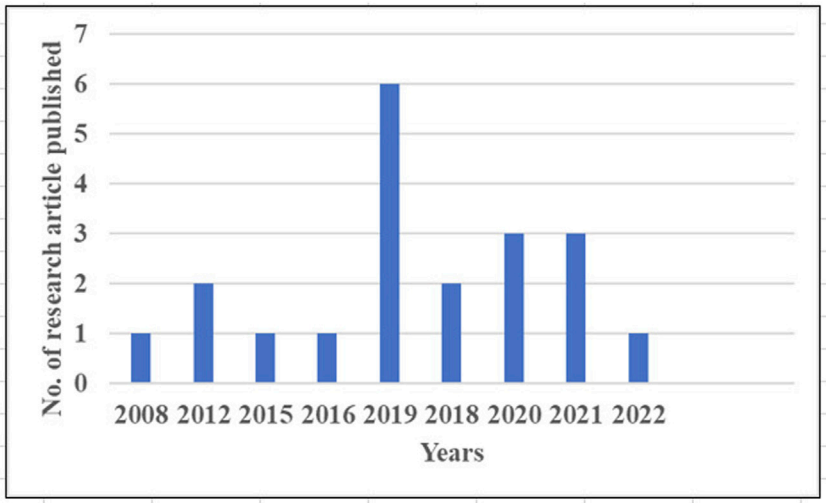

FIGURE 5
Number of research articles published on ethosomes used for treatment skin cancer from 2008 to 2022.

pliable vesicles, the former essential for imparting fluidic characteristics (Blume et al., 1993) and membrane permeability, and the latter penetrating the deep dermal layers (Elsayed et al., 2007), results in a bigger penetration response in the lipid environment. Because of their structural shape and amount of lipid bilayers, ethosomes act as entrapment carriers for hydrophilic (buspirone), lipophilic (simvastatin), and large molecular weight compounds (Bayerl et al., 2002; Godin and Touitou, 2003; Fu et al., 2019). Numerous studies have supported conclusions that ethosomes improve encapsulation efficiency, permeability, and drug deposition. Structure of ethosomes represented in Figure 6.

Benefits of Ethosomal Drug Delivery (Godin and Touitou, 2003; Abdulbaqi et al., 2016; Pandey et al., 2017; Garg and Jain, 2022).

Ethosomes improve the drug's transdermal and dermal absorption *via* the skin.

- Ethosomes serve as delivery systems for a wide range of medications.
- In terms of quantity and depth, ethosomal systems are significantly effective in delivering a fluorescent probe (quantum dots) to the skin.
- Low risk profile: Because the toxicity profiles of the ethosomes components have been thoroughly documented in the scientific literature, there is no risk associated with large-scale medication development using this technique.
- Excellent patient compliance—The semisolid form (gel or cream) in which the ethosomes medicines are administered results in high patient compliance. Iontophoresis and phonophoresis, on the other hand, are somewhat difficult to utilise, which will impact patient compliance (Ferrara et al., 2022).
- High market appeal for items utilising patented technologies ethosomes manufacturing is rather easy and does not require any expensive technical inputs.
- The ethosomes technology is passive and non-passive, and it is now commercialized.

Limitations

Ethosomal administration is intended to give a constant stream of drug over a period of time rather than a bolus of drug all at once (Laïb and Routh, 2008; Rakesh and Anoop, 2012; Patel et al., 2013).

- Adequate drug solubility allows drug penetration into the dermal microcirculation and circulatory system under both lipophilic and lipophobic conditions.
- The drug's molecular weight should be such that it can be absorbed *via* the skin.
- Adhesive may or may not be suitable for all skin types.
- It will not be economically viable.
- The yield of interest is negligible.
- Excipients and enhancers may cause dermatitis or skin irritation in the vicinity of the pharmaceutical distribution structure.
- When ethosomes are submerged in water and the shell locking fails, the ethosomes cluster and disintegrate.
- There is a loss when transitioning from the organic to aqueous phase.

Mechanism

Ethosomal possibilities are eco-friendly, and a rise in ethanol content produces negatively charged moieties that promote stability, bioavailability, and penetrability. Going to follow ethosomal delivery, it has been noted that the stratum corneum's lipidic component is disrupted, inter-cellular lipid is extracted and disintegrated, and the skin's horny layer's temperature dependence is changed (Bindu Madhavi et al., 2013). Extensive research has confirmed the findings about ethosomes' enhanced entrapment efficiency, penetrability, and drug-deposition. The interdigitated, flexible ethosome vesicles are capable of creating pathways through the unstructured SC

TABLE 1 Summary of reported research based on ethosomes for treatment of skin cancer.

Name of drug	Formulation components	Vesicle size (nm)	PDI	Entrapment efficiency	*In vitro*	*In vivo*	Ref
Metformin	Lecithin, cholesterol ethanol and isopropyl alcohol	124 ± 14.2 and 560 ± 127	-	97.8 ± .23% and 99.4 ± .24%	Permeation 1660 ± 32.4 μg/cm^2, while the steady-state flux was 3.61 μg/cm^2/h. The percent of cumulative permeation was 97.6%	Significant antitumor activity against the skin cancer compared to the application of free metformin in male Swiss albino mice. Histopathological study proved efficacy of the Metformin ethosomal gel in skin cancer	Mousa et al. (2022)
Brucine	Ethanol, soy lecithin, cholesterol and hydroxy propyl methyl cellulose	145.6	.259	72.9%	Drug loaded ethosomes and gel was 68.87 ± 3.9, 50.87 ± 4.5 and 33.67 ± 3.92% respectively over a period of 6 h	Ethosomal gel formulation was enhanced by 1.89 ± .12 folds value .4 ± .03 μg/cm^2 ·h	Ismail et al. (2021)
Sericin	Soya-lecithin, chitosan coated nanoparticles, milliQ water, ethanol	177.7 ± 3.14 and 192 ± 4.1	-	62.15 ± 1.02%	It showed that 56%–59% release sericin	Exhibited the stimulation of IgM secretion with T cell-mediated immune response	Nayak et al. (2021)
Tocotrienol	Phospholipid, Cholesterol, Polysorbate 80, ethanol	64.9 ± 2.2 to 79.6 ± 3.9	0.24	66.8 ± 1.9% and 68.5 ± 1.2%	After 48 h was 1.03 ± .24 mg cm^{-2} with a flux of .03 ± .01 mg cm^2 h^{-1}. HaCat cells showed significantly higher cell viability than the pure drug	Drug flux across the Strat-MVR and the epidermal membrane was found to be higher than that across full-thickness skin	Nair et al. (2021)
Curcumin (CUR) and sulphoraphane (Sulph)	Phospholipon 90G, ethanol and phosphate buffer saline pH 7.4	125.67 ± 10.43	0.296 ± 0.0051	72.67 ± 8.02%	Average flux of drug loaded ethosome 54.72 ± 5.45 and 59.83 ± 6.09 μg/cm^2/h	Co-delivered CUR and Sulph from NG retained in the epidermis was 256.67 110 μg/g of skin which was significantly higher compare to free CUR and Sulph drug solution 50 and 50.34 μg/g of skin	Soni et al. (2020)
Naringin	Cholesterol, soy phosphatidylcholine (SPC), ethanol	142.5 ± 5.6	.199 ± .007	33.79 ± 1.35%	Amount of naringin retained in skin from the creams batch-1 and batch-2 was 13.35 ± .33 and 17.68 ± .42 μg/cm^2, respectively	Amount of naringin retained in the skin at the end of 4 h was found to be 550.2 ± 5.7 ng/cm^2	Gollavilli et al. (2020)
Vismodegib	Phospholipon 90G, cholesterol, phosphate buffer (pH 5.5), ethanol, IPA and propylene glycol	419.44 ± 7.64	-	83.28 ± .54%	Drug release of 52.07 ± .64%	A steady-state flux of 3.55 ± .02 μg/cm^2/h and skin deposition of 100.5 ± 1.53 μg/cm^2. Optimum drug loaded gel higher concentration of the drug in the epidermis than in the dermis	Calienni et al. (2019)
Meso-tetrakis(N-methyl-4-pyridyl) porphyrin (TMPyP)	Soybean Phosphatidylcholine, ethanol, propylene glycol, isopropanol	115 ± .81	-	74.64% ± 0.9	Penetrating amount of TMPyP gradually increased with a steady-state flux of 3.92 μg cm^{-2} h^{-1}. Significant higher percentage of cumulative TMPyP release after 3 h from preparation	Histopathological findings which revealed more obvious tumor degeneration and necrosis by TMPyP ethosomal gel.The mean survival time of the group was significantly greater than that of the group tumor-bearing mice	Fadel et al. (2020)

(Continued on following page)

TABLE 1 (*Continued*) Summary of reported research based on ethosomes for treatment of skin cancer.

Name of drug	Formulation components	Vesicle size (nm)	PDI	Entrapment efficiency	*In vitro*	*In vivo*	Ref
5-Fluorouracil	Soya phosphotidylcholine (SPC), ethanol, USP phosphate buffer saline pH 7.4. Fluorescent based ethosomes prepared by FITC	128.9 ± 0.4	0.07 ± 0.01	91.6 ± 0.15%	By using Microwave approach to increase the drug or ethosomes penetration into the skin. Higher amount of drug retention at the end of 24 h of skin permeation higher (395.4 ± 7.9 lg) compared to ethosomes used alone (281.3 ± 13.9 lg)	*In vivo* studies analysed that model of SKMEL-28 human melanoma cells permeation of drug from ethosomes found to be reduced with male Sprague Dawley rats having their skin treated with microwave	Rahim Khan and Wong, (2018)
Orthosiphon stamineus (EXOS) extract	Sophorolipid, ethanol, water	189.9 nm	0.404	77.23 ± 5.9%	During *Ex-vivo* trans diffused solution after application of the hydroethanolic based gel of EXOS and the standard extract of O.S leaves (SLOS) was 0.11 ± .009 µg/mL and .23 ± 0.01 µg/mL. Cell line model B16Fl0 melanoma cells used Cytotoxic test on confirmed the safety of SLOS *in vitro*	It showed 97.07 ± 0.6% anti-tumor activity during 16 days in albino mice	Nazari Vishkaei et al. (2018)
Fisetin	Phospholipid 90G, ethanol, and propylene glycol	99.89 ± 3.24	-	89.23 ± 2.13%	Binary ethosomes gel in both epidermis and epidermis were found to be higher drug conc. than the conventional gel	Drug loaded ethosomes gel had shown marked decrease in the levels of TNF-α and IL-1α to 553 ± 12 pg/mL and 934 ± 15 pg/mL due to reduction in inflammatory response. UV exposure resulted in significant decrease in the amount of glutathione and catalase enzyme by 24% and 45%, respectively average tumour volume recorded for UV only treated mice group was 7.0 ± 2.0 mm^3 whilst that of binary ethosomes gel treated group was 2.0 ± 1.0 mm^3. significant reduction in epidermal cell invasion with reduction of epidermal thickness	Moolakkadath etal. (2019)
(−)epigallocatechin-3-gallate (EGCG)	PEG 400 (in PEVs) or Tw80, phospholipid, methanol, ethanol	205.9 ± 50.98	.504	74.7%	Skin deposition and preservation of the inherent antioxidant properties of EGCG with good physical stability	Inhibitory effect on epidermoid carcinoma cell line (A431) in addition to reduced tumor sizes in mice, Histopathological analysis and biochemical quantification of skin oxidative stress biomarkers; glutathione, superoxide dismutase and catalase, as well as lipid peroxidation	El-Kayal et al. (2019)
Curcumin	Phospholipon® 90G, ethanol, phosphate buffer saline PH 7.4	247 ± 5.25 and an entrapment efficiency of	0.19 ± 0.16	92.24 ± 0.20%	Drug permeation studies proved the superiority of ethosomes over the	-	Krishna Kollipara et al. (2019)

(Continued on following page)

TABLE 1 (*Continued*) Summary of reported research based on ethosomes for treatment of skin cancer.

Name of drug	Formulation components	Vesicle size (nm)	PDI	Entrapment efficiency	*In vitro*	*In vivo*	Ref
					traditional liposomes in terms of the amount of drug permeated and deposited in skin layers. *In vitro* cytotoxicity and cellular uptake studies revealed that curcumin ethosomes have significantly improved cytotoxicity and cellular uptake in A375 human melanoma cell lines		
Sulforaphane	PL90G, ethanol, and water	227 ± 3	.01 ± .01	87.5 ± 2.5%	Anticancer activity on SK-MEL 28 after 24 h and concentrations compared with the free drug	-	Cristiano et al. (2019)
Ferrous Chlorophyllin	% Soya bean phosphatidyl choline, Cremophor-A25, ethanol, distilled water	383 ± 8.1	.3 ± 0.1	78.2 ± 1.35%	Ethosomes had deeper localization owing to the presence of ethanol and edge activator in ethosomal composition, that increases the fluidity of the vesicles and give them the ability to penetrate deep into different skin layers	Skin retention studies in albino mice showed no significant difference in skin retention of Fe-CHL compared to PC/CHI	Nasr et al. (2019)
Mitoxantrone (MTO)	Soya phospholipids, ethanol, water, hydroxypropyl methylcellulose	78 +- 4.8	-	-	Higher *in vitro* permeability across the Wistar rat skin than MTO aqueous solutions	Significant cytotoxicity and higher *in vivo* anti-melanoma effect than MTO solutions in Melanoma-bearing mouse	Yu et al. (2015)
5-Fluorouracil	SPC, ethanol, phosphate buffer saline pH 7.4	110 and 130	less than 0.1	-	Increased skin drug penetration and retention of low ethanol ethosomes and provided lower drug permeation	-	Khan and Wong, (2016)
Paclitaxel (PTX)	PL-90G, absolute ethanol, water	102.0 ± 5.50	less than 0.1	82.0 ± 1.60%	Cell death findings were always in very good agreement with MTT test (cell proliferation) data (Figure 5), thus showing similar results by using two independent assays	Sustained release of PTX, suitable for *in vivo* administration	Paolino et al. (2012)
5-Fluorouracil (5-FU)	Phospholipid, ethanol, Carbopol 934P	<200	less than 1	-	Drug deposition was found to increase 5.9- and 9.4-fold on treatment with ethogel in comparison with the marketed cream and drug solution, respectively. Antitumor activity Cytoselect 96-well cell transformation assay revealed a large reduction in tumor	Increasing the local bioavailability of 5-FU in comparison to the marketed formulation	Puri and Jain, (2012)

(Continued on following page)

TABLE 1 (*Continued*) Summary of reported research based on ethosomes for treatment of skin cancer.

Name of drug	Formulation components	Vesicle size (nm)	PDI	Entrapment efficiency	*In vitro*	*In vivo*	Ref
					density with treatment with the 5-FU		
5-aminolevulinic acid	Phospholipids (60°C for PE), ethanol solution or aqueous solution	<200	less than 1	8%–66%	Penetration ability of ethosomes was greater than that of liposomes	Enhancement ratio of ethosome formulations did not significantly differ between the non-irradiated and irradiated groups	Fang et al., (2008)

and eventually delivering medication into the deep skin layers. The fusion of ethosomes might then lead to the transdermal absorption of medicines. Lipids from the skin. As a consequence, it is anticipated that drugs will be discharged at different sites along the penetration route (Godin et al., n.d.; Heeremans et al., 1995; Touitou et al., 2000; Touitou and Godin, 2007; Verma et al., n.d.). Permeation mechanism of drug from ethosomes represented in Figure 7.

According to research, ethanol and phospholipids work together to improve the skin absorption of drugs in ethosomal compositions. Ethanol changes the configuration and lowers the density of skin lipids by fluidizing the lipid bilayers of the stratum corneum and ethosomal vesicles at the same time. As a result, the altered stratum corneum structure will be penetrated by the very flexible and soft vesicles of an ethosomal system, providing a passage through the skin.

This would be followed by the "ethosomes effect," which is the releasing of the medication into the deep layers of the skin through lipid penetration and permeation by the formation of new routes as a result of the flexibility and merging of ethosomes with skin lipids (Verma and Fahr, 2004). These vesicles merge with cell membranes in the deeper layers of the skin to deliver the therapeutic agent. Certain research suggests that transethosomes may offer better skin-permeation abilities than conventional ethosomes. This is due to the presence of ethanol in transethosomes as well as the edge activator or penetration enhancer, both of which work together to promote vesicular elasticity and skin-lipid alteration.

Types of Ethosomes

When compared to traditional liposomes, those vesicle nanocarriers have already shown superior delivery of drugs due to their smaller particle size, negative zeta-potential, greater encapsulation efficiency, and enhanced stability. Nevertheless, a new generation of ethosomes—binary ethosomes and transethosomes—was created in search of a more effective skin permeation mechanism. Types of ethosomes represented in Figure 8.

Conventional or classical ethosomes are a version of traditional liposomes and are made up of phospholipids, water, and ethanol in high concentrations (up to 45% w/w). For topically applied drug administration, conventional ethosomes were reportedly better than conventional liposomes because they had a smaller size, negative potential, and higher entrapment efficiency. In contrast, traditional ethosomes are up to a high standard liposome in terms of skin penetration and stability characteristics (Zhang Z et al., 2012; Sarwa et al., 2013; Jain et al., 2015; Tiwari et al., 2016). Drugs bound in traditional ethosomes have had molecular weights ranging from 130.077 Da to 24 kDa (Mishra et al., 2010; Zhou et al., 2010; Li et al., 2012).

Binary ethosomes were first discovered in 2010 by Zhou et al. Binary ethosomes include two alcohols in addition to ethanol, usually propylene glycol (PG) and isopropyl alcohol (Dave et al., 2010; Akhtar and Pathak, 2012; Zhang J. P. et al., 2012; Shen et al., 2014). PG is a well-known penetration enhancer, and it has greater viscosity and hygroscopicity in comparison to ethanol, as well as non-toxicity, decreased skin inflammation, and stability.

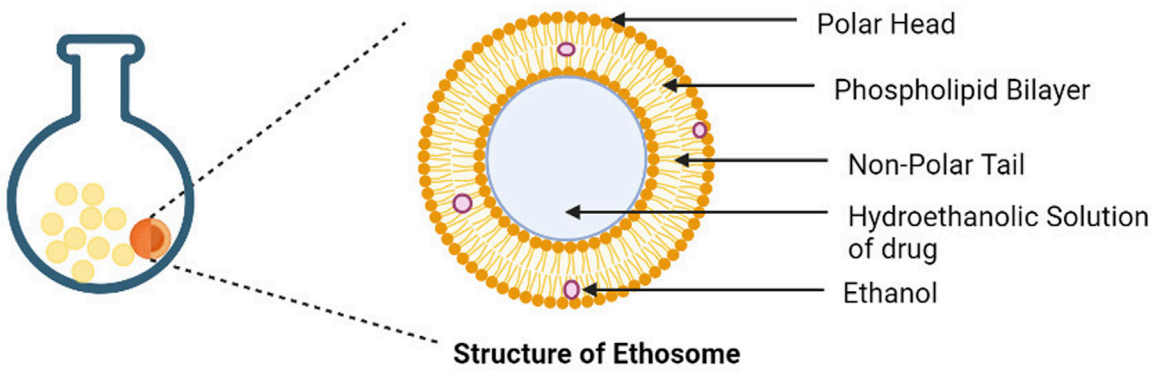

FIGURE 6
Structure of ethosomes.

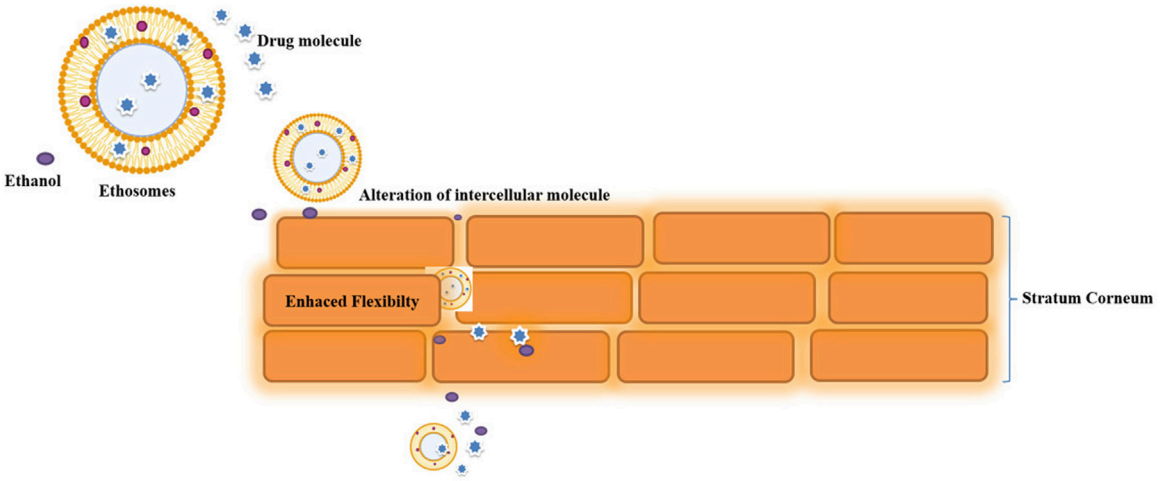

FIGURE 7
Permeation mechanism of drug from ethosomes.

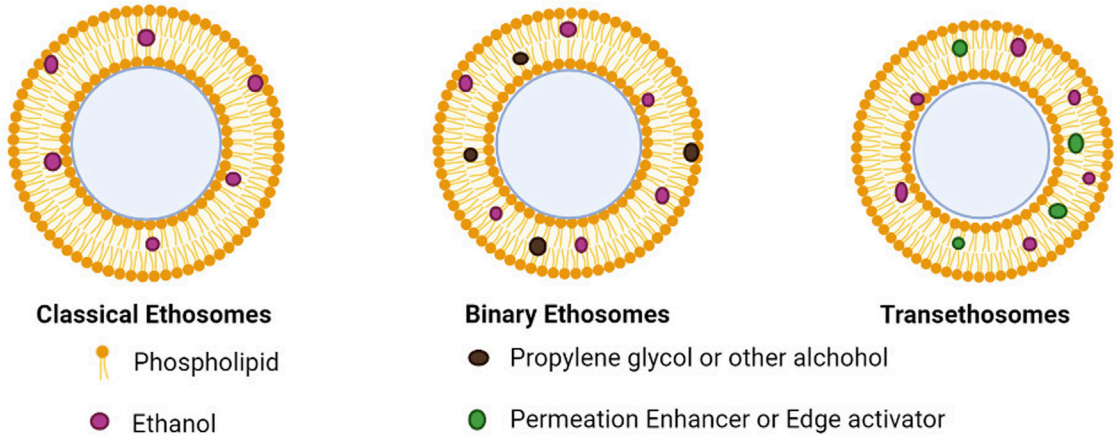

FIGURE 8
Types of ethosomes.

Transethosomes

Introduced a new generation of ethosomes with the goal of Because they included both ethanol and edge activators, the transethosomes offered the benefits of both ethosomes and deformable liposomes (Song et al., 2012). Transethosomes comprise an edge activator or a penetration enhancer in addition to the identical ethosome makeup by using surfactant (Song et al., 2012). In addition to phospholipid and water, edge activators make deformable liposomes more flexible by disrupting the lipid bilayers. Utilizing this technique, drugs in the low to high molecular weight range may be easily captured. The bioactive agent releases its payload slowly and gradually since it is preserved by encapsulation. They have a highly effective trapping ability since they are biodegradable and biocompatible. The formulation does not need any additional adjuvant and no complicated method of preparation for it (Honeywell-Nguyen and Bouwstra, 2003; Trotta et al., 2004; Song et al., 2012).Because ethanol has a natural tendency to fluidize the cellular membranes, its contact with the lipids of both skin and vesicles has rendered the latter more flexible. Because of this ethanol property, medicines bound in ethosomes were able to penetrate through stratum corneum barriers relatively rapidly.

Impacts of ethosomal components, preparation techniques, and their major roles in shaping the final properties

Composition of ethosomes

Phospholipid

The composition of the ethosomal system has made use of phospholipids from various sources. When creating an ethosomal system, selecting the appropriate phospholipid type and concentration is crucial since it affects the vesicle's size,

entrapment efficiency, Zeta-potential, stability, and penetrating abilities (Abdulbaqi et al., 2016).

In order to enhance the safety and efficiency of anthralin, liposomes and anthralin-loaded ethosomal formulations were examined. The drug encapsulation efficiency was substantially enhanced from 97.2 .4% to 99.2 .2% when the drug/lipid ratio was raised from 10% to 25%. As compared to the 10% ratio, the drug encapsulation efficiency significantly increased with further increases in the ratio to 50% and 75%. However, there was no statistically relevant difference in the drug encapsulation efficiency between 25%, 50%, and 75% (Paliwal et al., 2019).

Phospholipids can be derived from a variety of sources, including natural, semisynthetic, and synthetic materials, and can be included in concentrations ranging from 5% to 10% of the final ethosome content (Ascenso et al., 2013). Several phospholipids have been employed in prior ethosomal formulations, including PC, dipalmitoylphosphatidylcholine (DPPC), phosphatidylethanolamine (PE), Phospholipon® 90, and Lipoid S 100 (Abdulbaqi et al., 2016). Skin permeation also depends on the selected phospholipid, as it will combine with the skin lipid bilayer and enable the vesicle to produce tiny openings inside the SC (Arora and Nanda, 2019). In an ethosomal formulation, phospholipid concentrations generally range from .5% to 5%. Vesicular size will increase slightly or considerably when phospholipid concentration rises, while entrapment efficiency will rise significantly. (Chen et al., 2011; Prasanthi and Lakshmi, 2012).The relationship is valid up to a specific concentration, beyond which an increase in phospholipid concentration has no further impact on the effectiveness of entrapment (Naeff, 1996; Vanic, 2015).

Ethanol

Ethanol effectively increases penetration. Despite giving the vesicles distinctive qualities in regards to size, potential, stability, and other factors, they play a crucial role in ethosomal systems. Improved skin permeability and effective trapping (Prasanthi and Lakshmi, 2012). It has been observed that ethosomal systems contain ethanol concentrations of 10%–50% Several studies have found that increasing the ethanol content reduces the size of the ethosomes.

The concentration of ethanol in the vesicle membrane was presumably the cause of the greater entrapment effectiveness of lamivudine in ethosomes compared to liposomal formulation. Due to an increase in membrane fluidity, the entrapment efficiency as the ethanol concentration was raised from 15% to 45% (Paolino et al., 2012). However, subsequent ethanol ingestion would likely have rendered the vesicle membrane more permeable, decreasing the effectiveness of trapping. With a rise in ethanol concentration from 15% to 60%, the size of the vesicles shrank. The solution with 15% ethanol included the biggest vesicles, measuring 207 nm in size, whereas the preparation with 60% ethanol contained the smallest vesicles, measuring 72 nm in size. Since different quantities of ethanol are present, there is a substantial variability in the size of ethosomal compositions. Ethanol most likely modifies the system's net charge and gives it some stearic stabilisation, which may induce the average vesicle size to decrease (Dubey et al., 2007)

During the process of formulation, the concentration of ethanol should be optimised because even at small concentrations, the effectiveness of entrapment will be relatively low, and at very high concentrations, the ethosomal membrane will be more permeable even though phospholipids can quickly dissolve in ethanol, which results in a significant decrease in entrapment efficacy. Composition of ethosomes represented in Table 2.

Cholesterol

Since cholesterol is a solid steroid molecule, adding it to ethosomal systems improves the stability and effectiveness of drug entrapment. In addition to preventing loss, it decreases vesicular fusion as well as permeability. It is typically applied at a concentration of 3%. At a fixed drug/lipid ratio of 10%, the impact of the cholesterol/PL-90G ratio on the drug encapsulation efficiency showed that the drug encapsulation efficacy was considerably reduced as the cholesterol concentration of the vesicles increased. As a result, when the CH/PL-90G ratio rose from .3:0.7 to .4:0.6, drug encapsulation efficiency dropped substantially from 97.2% to 94.8% (Fathalla et al., 2020). Cholesterol (30 mol%) was added into the ethosomes to convey an ordering arrangement of the phospholipids' acyl chains and cause a condensing effect. Stearylamine (0–5 mol%) was also incorporated in order to assess the effect of cationic lipids in the formulation (Arora and Nanda, 2019).

Other alcohols

Propylene glycol (PG)

A common penetration enhancer is PG. It influences the ethosomal features of size, entrapment effectiveness, permeability, and stability when utilised in the formation of binary ethosomes at concentrations between 5% and 20%. When PG is added to ethosomal solutions, particle size is reduced much more, in contrast to ethosomal systems lacking PG.

Other additives in ethanol

To avoid vesicle aggregation and improve the stability of the formulation, diacetyl phosphate is frequently used. In the ethosomal, it is employed in quantities ranging from 8% to 20% of the overall phospholipid content. It was recently discovered that diacetyl phosphate-containing ethosomal compositions all resulted in vesicles with strongly negative potential.

In different experiments, the positive-charge agent stearyl amine was utilised in ethosomal preparations. The first research used an ethosomal system that contained mycophenolic acid and a 2:1:1 mass ratio of phosphatidylcholine, cholesterol, and stearyl amine. When Stearyl amine was added to the ethosomal formulation, it resulted in a significant rise in vesicular size, a decrease in entrapment effectiveness, a change from a negative to a positive-potential charge, and aggregation of both the vesicles within 1 week.

Methods of preparation

Different ethosomes kinds may be prepared using straightforward procedures that are simple to significantly increase without the use of expensive machinery. There are two common ways to create ethosomes (the cold technique and the hot method), although alternative approaches, such as the traditional mechanical-dispersion method and the transmembrane pH-gradient approach, have additionally been described. (Dubey et al., 2007).

Classic method

The medication and phospholipid are dissolved in ethanol and heated in a steam bath to 30°C + 1°C. In a sealed container, the lipid

TABLE 2 Ethosomes class of excipients and its role.

Type of class	Examples	Role
Phospholipids	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline, 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine, Distearyl phosphatidyl choline	Vesicles forming agent
Alcohol	Ethanol, Isopropyl alcohol	Providing the softness of vesicle membrane and act as penetration enhancer
Stabilizer	Cholesterol	Enhances the stability and entrapment efficiency
Other alcohol	Propylene glycol, IPA	Act as a skin penetration enhancer
Dye	Rhodamine-123, Rhodamine red, Fluorescene Isothiocynate (FITC), 6-Carboxy fluorescence	For Evaluation of formulation
Vehicle	Carbopol 934	Act as a gelling agent

mixture is poured with double-distilled water in a fine mist while being constantly stirred at a speed of 700 rpm. Over three repetitions of passing through a polycarbonate membrane using a hand extruder, the final vesicle solution is homogenised (Dubey et al., 2007)

Traditional mechanical dispersion technique

In a round-bottom flask, soy phosphatidylcholine is dissolved in a solution of chloroform: methanol (3:1). A thin lipid layer is formed on the flask wall when the organic solvents are evaporated using a rotating vacuum evaporator just above the lipid annealing temperature. The deposited lipid film is then cleaned of any remaining solvent combination by placing the container's components under a vacuum for the night. By revolving the flask at the appropriate temperature, hydration is accomplished with various concentrations of a hydroethanolic medium containing the medication (Dubey et al., 2007).

Hot method

This process requires heating the phospholipids in a water bath at 40°C until they generate a colloidal solution, and then dispersing them in water. In a different vessel, ethanol and propylene glycol are mixed and heated to 40°C. After both solutions reach 400°C, the organic component is added to the aqueous phase. Depending on how hydrophilic or hydrophobic the medication is, it is dissolved in ethanol or water. An ethosomal formulation's vesicle size can be decreased to the required size *via* exploratory sonication or even the extrusion method. (United States Patent, 2022).

Cold method

This technique for ethosomal preparation represents the most popular and commonly utilised one. The medication, phospholipids, and other lipid components are mixed in ethanol with continuous stirring at room temperature. In a water bath, the mixture is heated to 30°C. In another vessel, the water has been heated to 30°C before being added, combined, and swirled for 5 minutes. If desired, the ethosomal formulation's vesicle size can be reduced by sonication or extrusion in order to increase its extensibility. Furthermore, the mixture needs to be better refrigerated. (Verma and Pathak, 2010).

Transmembrane pH-Gradient method

The creation of empty binary ethosomes and the active loading of the medication are two distinct processes in this procedure. First, an alcoholic phase made up of PG and ethanol dissolves the phospholipid (for instance, PC). With continual 700 rpm stirring, a citrate buffer solution is progressively added to the initial solution. Throughout this procedure, the system is maintained at a temperature of around 30.1°C before being cooled to ambient temperature. The generation of blank binary ethosomes is now complete. In order to efficiently scatter and dissolve the drug, it is then actively injected into the ethosomes and the system is continually agitated at 700 rpm. This process involves two distinct steps: preparing an empty the ethosomal system's internal phase (acid) and exterior portion (alkaline) can be separated by a pH gradient that can be generated by adjusting by introducing a .5 M sodium hydroxide (NaOH) solution to the pH outside. The system is then incubated under an opportune moment and climate, enabling the union. Drugs actively pass through the lipid bilayer of ethosomes and get imprisoned in the vesicles (Abdulbaqi et al., 2016; Zhang et al., 2016).

Evaluation parameters of ethosomes

The development of ethosomes can make use of a wide range of compounds. Phospholipids, whether with or without cholesterol, are frequently the chief constituent. The composition of the vesicle is a crucial element that directly affects the physical and chemical characteristics of the ethosome, affecting a number of aspects, including the bilayer's flexibility, size, charges, and thermodynamic state. The following is an assessment of some of these characteristics.

Vesicle size and shape

A photomicrograph, transmission electron microscopy (TEM), or scanning electron microscopy (SEM) micrograph may all be used to see the vesicle form clearly. The transmission electron microscope photograph of the sample verifies the three-dimensional shape or structure of the phospholipid vesicles to be unilamellar and multilamellar. Utilizing Zetasizer may be used to determine

whether phospholipids and ethanol concentration influence the size and dispersion of ethosome vesicles. The formulation's ingredients can affect the ethosomes' size, which can range from hundreds of nanometers to microns. The ethosomes' size and zeta potential are influenced by a number of parameters. Although ethanol alters the system's net charge and gives it a little more degree of stearic stability, which may eventually induce a drop in the average vesicle size, the mean vesicle diameter is diminished (Pathak and Kumari, 2013; Hallan et al., 2021).

Transition temperature

Differential scanning calorimetry (DSC), a technique that also measures ethanol-skin phospholipid interaction, which is a characteristic related to the fluidizing impact of ethanol on the phospholipid bilayers, may be used to measure the glass transition of the vesicular lipid structures (Mbah et al., 2021).

Ethosomes-skin interaction study

Ethosomes skin interaction can be analyze using differential scanning calorimeter (DSC) and FTIR. A 12-h penetration test was carried out. The Untreated was another skin sample placed on Franz's cell. Afterward, skin samples were taken from both Franz cells. Taken and wet, dried, and chopped into small pieces and analysed using DSC and FTIR.

Entrapment efficiency

After extracting the drug that's not trapped inside the ethosomes, the drug that was may be estimated. The dispersion can be centrifuged in a cooled centrifuge to release the unentrapped medicine from the ethosomes (Raghuvanshi et al., 2022). The samples will be centrifuged in a centrifuge machine for 3 hours at a temperature of four degrees. The silt that had formed a layer of vesicles was ruptured, and the sediment was then filtered to determine the concentration of drug inside the ethosomes vesicles using HPLC or UV spectroscopy. After a reasonable dilution using the proper medium, the supernatant will also be collected, and the drug concentration will be calculated using the formula below.

$$Entrapment\ Efficiency\ (EC) = Amount\ of\ drug\ retained\ by\ the\ vesicle / Total\ amount\ of\ drug\ added \times 100$$

Vesicle elasticity measurement

The extrusion method was used to gauge the ethosome vesicles' flexibility (Mbah et al., 2021). A stainless-steel filter device with a 50 mm diameter was used to extrude the ethosomal formulation through a filter membrane with a pore size of 100 nm while applying a pressure of 2.5 bar. It was calculated how many vesicle suspensions were extruded in 5 min.

$$2E = J^{*}(r/r)vp$$

E is the elastic property of the vesicle, J is the volume of suspension extruded in 5 minutes, and r and r are the size of the vesicle following v are extrusion and the pore diameter of the filter membrane, respectively.

Stability study

While keeping the vesicles at 4°C and .5°C, the stability of the vesicles was evaluated. During 180 days, the vesicles' size, zeta potential, and entrapment effectiveness can be analysed.

Other parameters

Confocal laser scanning microscopy can be used to assess the ethosomal preparation's penetration ability into the epidermal layers (Niu et al., 2019). Investigations into the capacity of the ethosomal formulation to increase the penetration of both hydrophilic and hydrophobic compounds through the skin in both *in vitro* and *in vivo* settings have shown that it is superior to traditional liposomes. According to numerous researchers, ethosomes-formulated medications penetrate the skin 5–10 times better than traditional liposomal therapies.

Ethosomes incorporated in suitable dosage form

The great majority of research that has been published has examined ethosomal systems while they were initially suspended. Even though ethosomal suspension has a strong alcohol content, further incorporating the system into an appropriate vehicle for dermal or transdermal delivery has some benefits, including trying to prevent ethanol evaporation; extending the skin's time in interaction with the drug; increasing the drug's therapeutic effectiveness; improving the stability and shelf life of the final form of administration; and improving patient compliance. Innovative pharmaceutical compositions have been created using ethosomal systems in a variety of vehicles, including ethosomal gels, transdermal patches, and creams.

Ethosomes in gel form

The pH, viscosity, spreadability, and extrudability of ethosomal gels are their unique features. For integrating ethosomal systems, carbopol and hydroxypropyl methylcellulose in all of their associated categories are the two most frequently employed gel-forming agents. These polymers provide the necessary viscosity and bioadhesive characteristics, and it has been proven that they are acceptable with ethosomal systems. In comparison to conventional or commercially available gels or creams, researchers have looked into how effectively medications from ethosomal gels penetrate the skin and the way they are released effectively. One of the research targets reach that ethosomes nanocarrier system for the transdermal administration of complex molecular protein drugs has superior clinical potential application and gives a potentially useful design for ethosomal gels with a variety of transdermal delivery drugs (such as Chinese medicine, vaccines, and interleukins) for further investigation (Fu et al., 2019).

Brucine based ethosomal formulation was well designed in the proposed investigation utilising a response surface approach and included in an HPMC gel foundation. The produced BRU-loaded ethosomal gel showed sufficient nanoscale vesicular size, adequate physical properties, adequate encapsulation efficiency, and optimal flux. Whenever BRU was incorporated into ethosomal gel, the *in-vitro* release of BRU was significantly impacted (Ismail et al., 2021).

In vivo studies were based on mice exhibiting chemically induced skin cancer responded significantly to topical treatment of metformin ethosomal gel (Mousa et al., 2022).

One of the research works has proposed on ethosomal gel with itraconazole in it offered improved transdermal flow, a shorter lag

time, excellent entrapment efficiency, and a minimal chance for skin irritation. The drug's ethosomal formulation kills cytotoxic cells more rapidly and thoroughly. Drugs incorporated into ethosomal gels have a longer half-life and a more potent cytotoxic action than free drugs (Saraf and Kumar Gupta, 2018).

Ethosomes in patch form

Although moulds are required to prepare ethosomal patches, their manufacture and assessment are more difficult than those for ethosomal gels. *In vitro* and *in vivo*, examined the transdermal delivery capabilities of Testoderm's ethosomal patches with non-ethosomal patches of testosterone. Both patches had the same size and pharmaceutical composition. The drug deposition just on skin was obtained from the ethosomal patch and was seven times more powerful than the patch of testoderm. Since they allow the administration of ethosomes in occlusive circumstances, ethosomal patches have several benefits over ethosomal gels and creams in respect of expected penetration.

Ethosomes in cream form

Based on all of the previous research, adding ethosomal systems to appropriate delivery methods such as gels, patches, and creams enhances the medication or agent delivered *via* the ethosomal systems' skin penetration. The incorporation of ethosomal systems is best suited for the vehicles discussed—gels—while creams may be favoured for cosmetic preparations.

Developing and evaluating naringin-loaded ethosomes has proposed work that have been enable the antioxidant potential of naringin to absorb free radicals. These ethosomes was then be included in sunscreen lotions that comprise zinc oxide (ZnO) and titanium dioxide (TiO2) to ensure desired skin penetration as well as skin retention (Gollavilli et al., 2020).

Modification of ethosomes for transdermal delivery

Transcutaneous immunization patches have achieved impressive transdermal medication release. Also had an inhibitory impact on tumour development in mice having melanoma and significantly increased the antitumor effect of aPD-1 without inducing systemic toxicity. This method provides a simple and non-intrusive method for enhancing the effectiveness of immune checkpoint inhibitor treatment (Song et al., 2022). Co-administration of the protein 1 monoclonal antibody (aPD1) and transcutaneous immunisation (TCI) patch can successfully slow the growth of tumours by raising levels of interleukin-12, TNF-, and IFN-, as well as encouraging the infiltration of cytotoxic T lymphocytes into cancerous tissue. After changing the mannose molecule on the exterior of the ethosome *via* electrostatic adsorption and layer-by-layer self-assembly techniques, a unique mannosylated chitosan (MC)-modified ethosome was developed throughout this work that has the capability to target dendritic cells. The plasmid (pIL-12@Eth-MC) encoding the IL-12 gene was demonstrated to induce the maturation of DCs *in vitro*. The TCI patch was created by electro spraying nanoparticles loaded with pIL-12@Eth-MC onto the surface of SF-PVA nanofibers.

New investigation on transcutaneous immunisation delivery method was created with electro-spraying microspheres carrying ethanol-mannosylated polyethyleneimine onto the electro spun SF-PVA nanofibrous patch (Hong et al., 2021). The transcutaneous medication administration demonstrated by the composite patches was effective. The use of the transcutaneous tumour vaccination combined with the anti-programmed death-1 monoclonal antibody greatly inhibited the proliferation of melanoma. Additionally, the combination of therapy led to a synergistic antitumor effect, which may be explained by the therapies' enhanced infiltration of $CD4^+$ and $CD8^+$ T cells.

Research has investigated based on synergistic effect of 5-fluorouracil (5-FU) ethosomes with microwave (Khan and Wong, 2016). 5-FU retention in skin from ethosomes was enhanced *in vitro* and *in vivo* by which was before skin by microwave energy at 2450 MHz for 2.5 min. Nevertheless, compared to single ethosomal administration *in vivo*, the transdermal penetration flux and total drug absorbed into the systemic circulation were significantly decreased. The human melanoma cell line SKMEL-28 was pre-treated with microwave energy at 2450 MHz for 2.5 min alone, in addition to in conjunction with ethosomes, which significantly reduced the viability of the cells compared to those receiving plain 5-FU (Khan and Wong, 2016).

Repurpose therapy for the treatment of skin cancer

After just a few weeks of receiving traditional anti-cancer medications, patients build up a high tolerance, which facilitates metastatic spread and recurrence. Therefore, it is crucial to find new anti-skin cancer medications and combinations.

Mice with chemically induced skin cancer respond significantly to topical treatment of metformin ethosomal gel. This was demonstrated by presenting a high percentage of the EE%, lowest vesicle size, maximum ZP, and maximum DR% utilising the Box-Behnken design of "a three-level three-factor." When isopropyl alcohol was combined with ethanol to create ethosomes, ethanol's capacity to solubilize lecithin was enhanced, which improved the stability and efficiency of the ethosomes vesicles (Mousa et al., 2022).

One of research were met to the physicochemical and technical evaluation of the stable sulforaphane-loaded ethosomes® and transfersomes®, always one ethosomal formulation demonstrated a satisfactory ability to hold and release the medication as well as effectively penetrate the skin. Furthermore, the disparities in the ways that ethosomes® and transfersomes® work seem to indicate that they are less effective than one another as melanoma therapy agents (Cristiano et al., 2019). In contrast to transferosomes and drug solutions, ethosomes® increased the *in vitro* percutaneous penetration of sulforaphane. This result is most likely attributable to the inclusion of ethanol in the ethosomes® constitution, which may interact among carriers and stratum corneum lipids.

Therapeutic potential of herbal ethosomes

Most phytochemical constituents fail to attain bioavailability due to low absorption due to low absorption, even after showing potential therapeutic benefits. High molecular sizes with low lipid solubilities are the primary causes of phytoconstituents poor absorption and hence limited bioavailability. The bioavailability of such plant active ingredients or

extracts is significantly increased when they are incorporated into vesicular carriers like ethosomes (Natsheh et al., 2019). Ethosomes are specifically crafted and constructed vesicles that contain a significant amount of ethanol. This gives them extra flexibility and competence enough to respond to peripheral stress by fluidizing and upsetting the lipids inside the stratum corneum. Ultimately, it results in the effective deep skin penetration of medicinal substances. (Sultana et al., 2018).

New innovation that successful in producing ethosomes that were co-loaded with evodiamine (EVO) and berberine chloride (BBR). The targeted ethosomes were at least 90% entrapment efficient for both BBR and EVO, with a mean size of 171 nm. Incorporating various *in vitro* applications to human skin, it was established that these ethosomes could carry medication to the basal layers of human skin. Furthermore, when BBR and EVO were co-loaded in ethosomes, B16 cells in cultured cells showed better anti-melanoma activities. The enhanced drug bioavailability and synergistic interactions between BBR and EVO may be responsible for this elevation in anticancer activity. This finding showed the ethosomes' usefulness as possible dermal carriers for the management of melanoma (Lin et al., 2020).

A controlled release of the lipophilic chemical curcumin was accomplished by loading it into the vesicles and then introducing it into the ethosomal gel. The ethosomal gel had good skin deposition, which allowed curcumin to stay in the skin's deeper layers for a longer duration of time, aiding in the full destruction of melanoma cells without inflicting significant discomfort to the skin (Krishna Kollipara et al., 2019).

Also, with the use of Carbopol-934 and sodium CMC as the polymer, camptothecin extracted from Nothapodytes nimmoniana may be administered by transdermal route in the form of a herbal gel formulation. These herbal gel preparations can efficiently deliver camptothecin in the treatment of dermatological cancer as evidenced by anticancer activity mostly on epidermoid carcinoma cell line-A431, which has an IC50 value of 48.03 g/mL and a percent apoptosis of 54.67 4.58, respectively. This is due to the controlled release that can be achieved by utilising these herbal gel formulations for a longer duration of time (Galatage et al., 2020).

Stability of ethosomes

The compositions long-term stability was evaluated in terms of their particle size and entrapment capability. Fundamentally, choosing the right lipid composition seems to be crucial for producing stable ethosome dispersions with the best possible pharmacological and medicinal properties. When it comes to liposomes, a variety of alterations could take place after storage. When stored, liposomes tend to merge and expand into larger vesicles, which creates a significant problem with medication leakage from the vesicles. The predisposition of the neutral liposome to aggregate is likely due to the absence of electrostatic repulsion, but with ethosomes, ethanol modifies the system's net charge and confers some degree of steric stabilisation, increasing the dispersion's stability against agglomeration and possibly reducing the mean vesicle size.

Since the membranes are more flexible, increasing the ethanol content from 15% to 45% improves the entrapment efficiency. However, ethanol concentrations above 45% may cause the vesicle membrane to become more permeable, decreasing the effectiveness of entrapment. Thus, the ethosomes become unstable as a result. The natural and/or chemically synthesised phospholipid sources are used to make the ethosomes lipid component. Unsaturated fatty acid-containing phospholipids are understood to go through oxidative processes. Then Changes in permeability can be caused by reactive species. Bilayers in the ethosomes. Lipids can undergo oxidative breakdown that can be reduced by safeguarding the lipid formulation by including antioxidants like light tocopherol Additionally, lipid hydrolysis results in lyso-PC formation. Lyso-PC addition increases the permeability of ethosomes, making it imperative to maintain a minimum in a particular preparation (Patel, 1990; Touitou et al., 2000; Rao, 2019).

IN-VIVO studies

Several researchers have evaluated ethosomal systems including skin permeability, pharmacokinetics, pharmacodynamics, safety, and skin irritation studies utilising highly variable *in vivo* studies and models using humans, rabbits, rats, mice, and guinea pigs. Several *in vivo* investigations have revealed that ethosomal systems may penetrate intact skin and transport compounds with various physicochemical properties in therapeutic levels to the blood circulation. Some research based on In- vivo studies mentioned in table 1.

In this study, scientist has investigated the ethosomal system filled with Sonidegib (SNG) and its pharmacokinetic and pharmacodynamic impacts in mice. Sonidegib Loaded Ethosomes (SNE) gel had a substantially higher AUC (210.80 7.83 g/h/mL) than oral SNG (66.14 4.53 g/h/mL), which was a difference of 3.18 folds. The AUC for SLE gel was 2.34 times (significantly) higher than that of the AUC for SLL gel (90.04 vs 5.52 g/mL). In comparison to oral SNG (.132 .07 h), the elimination rate constant (K) with SLE gel (.0547 .05 h1) was much lower. The skin deposition concentration of the gel developed had a much greater half-life (t0.5) compared to that of the SLE gel (12.54 h) (Gamal et al., 2021).

The increased bioavailability and longer plasma profile of the revised nimodipine loaded (NIM-ETH) gel formulation indicated a decrease in the dose and frequency of administration, which would affect patient tolerance, price, and dose-dependent negative impacts. Although the NIM-ETH gel formulation had maintained plasma concentration for roughly 36 h as seen by a drop in Cmax and delayed tmax of 3.61 ng/mL and 12 h, respectively, oral preparation's pharmacokinetic parameters following oral administration culminated in Cmax of 8.1 ng/mL and Tmax of 5 h. When contrasted to the oral formulation (Nymalize oral preparation), the mean AUC0 to AUC0-1 of NiM-Eth gel rose from 4.1 to 5.9 folds, which also was determined to be the outcome of first pass effect (Moideen Muthu Mohamed et al., 2022).

The safety of conventional ethosomes has been established in both human and animal *in vivo* safety investigations, and they have great skin tolerability (Dubey et al., 2007; Ascenso et al., 2013; Zhang et al., 2014). In specific, for the other two classifications of ethosomal systems (binary ethosomes and transethosomes), which encompass penetration enhancers and/or edge activators in their preparation and may therefore increase the probability of developing harmful skin reactions, such as discomforts or erythema, more than that *in vivo* safety studies are needed to assess the short- and long-term impacts of repeated trials of these nanocarriers mostly on skin.

Ethosomes safety issues and their clinical applications in the treatment of skin cancer

According to *in vitro* and preliminary investigations, the majority of the vesicular drugs approved for the management of skin cancer appear to

be safe, with minimal systemic risk. It was discovered that the treatment of skin cancer greatly benefits from the use of ethosomes. Ethosomes can lessen the negative side effects of chemotherapy while enhancing the efficiency of anticancer activity (Yingchoncharoen et al., 2016). However, the use of lipid nanoparticles has resulted in instances of complement activation-related pseudo allergy (CARPA). The complementing system is activated upon initial exposure to lipid additives, resulting in the pseudo allergy taking place when there has been no previous sensitization. Despite a large number of *in vivo* and preclinical studies that support their therapeutic application in the management of skin cancer, there is presently less information available on the topical application of vesicular drug delivery systems in the clinical-trial phases. In dermatological illnesses, such as skin cancer, topical medication may reduce local drug concentration and adverse effects when compared to systemic administration. The stratum corneum of the skin, however, functions as an efficient barrier, and its effectiveness is influenced by a particular compound's importance in relation to a chemical property's molecular size (Jain et al., 2017). The European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) respectively, authorised Vismodegib, a top-notch Hedgehog Signalling Activation Inhibitor, for the treatment of BCC in 2012 and 2013 (Calienni et al., 2019). The clinical potential, safety, and efficacy of vesicular delivery of drugs against skin cancer should always be assessed by clinical research.

Future prospects

Since the discovery of ethosomes, vesicular research on transdermal medication administration has entered a new phase. Numerous studies indicate that ethosomes have a bright future. It is efficient in improving the transdermal administration of several substances. Additionally, this research will enable improved control over medication release in real time, enabling doctors to render the treatment more efficient. Ethosomes provide the opportunity to distribute modest, non-invasive packages. Since drug molecules have a medium to large size, it may be inferred logically that ethosomal compounds have a bright future in efficient dermal/transdermal transporting bioactive agents.

Conclusion

Transethosomes are a new generation of ethosomal systems that have been developed as a result of substantial and ongoing study. These transethosomes have been proven to have superior vesicular characteristics and skin-permeation capabilities compared to traditional ethosomes. Transethosomes give the formulator the most flexibility to alter the ethosomal characteristics in accordance with the necessary study requirements by altering the edge activators and/or penetration enhancers. To improve skin-permeation and therapeutic results, ethosomal systems must be included in appropriate vehicles such as gels, patches, and creams. Additional research is needed to improve the ethosomal system's stability. The potential of ethosomal systems in cutaneous and transdermal distribution is being demonstrated by the outcomes of *in vivo* experiments and clinical trials.

Author contributions

PS, AP, and SB were involved in the conception and design, or analysis and interpretation of the data; drafting of the paper, revising it critically for intellectual content; and SB did final approval of the version to be published. PS, AP, and SB agree to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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