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Review

Lipid vesicles for skin delivery of drugs: Reviewing three decades of research

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Abstract

Since liposomes were first shown to be of potential value for topical therapy by Mezei and Gulasekharam in 1980, studies continued towards further investigation and development of lipid vesicles as carriers for skin delivery of drugs. Despite this long history of intensive research, lipid vesicles are still considered as a controversial class of dermal and transdermal carriers. Accordingly, this article provides an overview of the development of lipid vesicles for skin delivery of drugs, with special emphasis on recent advances in this field, including the development of deformable liposomes and ethosomes.

Keywords: Lipid vesicles; Liposomes; Deformable liposomes; Ethosomes; Skin delivery; Topical; Transdermal; Mechanism of action

Contents

1. Introduction

Although the skin as a route for drug delivery can offer many advantages, including avoidance of first-pass metabolism, lower fluctuations in plasma drug levels, targeting of the active ingredient for a local effect and good patient compliance (Williams, 2003), the barrier nature of skin makes it difficult for most drugs to penetrate into and permeate through it (El Maghraby et al., 2001b). During the past decades there has been wide interest in exploring new techniques to increase drug absorption through skin (Barry, 2001; Williams, 2003; Honeywell-Nguyen and Bouwstra, 2005). Topical delivery of drugs by lipid vesicles has evoked a considerable interest.

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Since the first paper to report the effectiveness of liposomes for skin delivery was published by Mezei and Gulasekharam (1980), conflicting results continued to be published concerning their effectiveness, enhancing the controversy of liposomes as dermal and transdermal drug delivery vehicles (Honeywell-Nguyen and Bouwstra, 2005). The first therapeutic, using lipid vesicles on the skin, was commercialized shortly before the year 1990, and contained the anti-mycotic agent, econazole. A few other, relatively simple, liposome-based dermal products followed (Cevc, 2004; Song and Kim, 2006). Recently, it became evident that, in most cases, classic liposomes are of little or no value as carriers for transdermal drug delivery as they do not deeply penetrate skin, but rather remain confined to upper layers of the stratum corneum.

Intensive research led to the introduction and development (Cevc and Blume, 1992), over the past 15 years, of a new class of lipid vesicles, the highly deformable (elastic or ultraflexible) liposomes, that have been termed Transfersomes®. Several studies have reported that deformable liposomes were able to improve in vitro skin delivery of various drugs (El Maghraby et al., 1999, 2001a; Trotta et al., 2002, 2004; Boinpally et al., 2003) and to penetrate intact skin, in vivo, transferring therapeutic amounts of drugs (Cevc and Blume, 2001, 2003, 2004), with efficiency comparable with subcutaneous administration (Cevc et al., 1995, 1998; Paul et al., 1995; Cevc, 2003).

Ethosome is another novel lipid carrier, recently developed by Touitou et al., showing enhanced skin delivery (Touitou et al., 1997, 2000a; Dayan and Touitou, 2000; Ainbinder and Touitou, 2005; Paolino et al., 2005). The aim of this article is to provide an overview of lipid vesicles as carriers for skin delivery of drugs, with special emphasis on recent advances in this field, including the development of deformable liposomes and ethosomes.

2. Conventional (traditional) liposomes

Liposomes are lipid vesicles that fully enclose an aqueous volume (Williams, 2003). Lipid molecules are usually phospholipids with or without some additives. Cholesterol may be included to improve bilayers characteristics of liposomes (Vemuri and Rhodes, 1995); increasing microviscosity of the bilayers, reducing permeability of the membrane to water soluble molecules, stabilizing the membrane and increasing rigidity of the vesicles. Many methods for preparation of liposomes are described in the literature (Weiner et al., 1989; Barenholz and Crommelin, 1994; Vemuri and Rhodes, 1995). Most commonly, the film hydration method is used (Williams, 2003).

2.1. Conventional liposomes as carriers for dermal and transdermal drug delivery

The potential value of liposomes for topical therapy was first introduced by Mezei and Gulasekharam (1980). In this study, greater four- to five-fold triamcinolone acetonide concentrations in the epidermis and dermis, with lower systemic drug levels, were observed when the drug was delivered from liposomal lotion in comparison with conventional formulations of the same drug concentration. Similar findings were also observed for triamcinolone acetonide liposomal gel formulations (Mezei and Gulasekharam, 1982) and for progesterone and econazole (Mezei, 1985). After these first papers, a large number of studies were initiated and conflicting results continued to be published concerning liposomes effectiveness. Several in vivo and in vitro transport studies reported that conventional liposomes only enhanced skin deposition, with mostly reduction (or no effect) in percutaneous permeation or systemic absorption, of hydrocortisone (Wohlrab and Lasch, 1987, 1989; Kim et al., 1997), other corticosteroids (Fresta and Puglisi, 1997), lidocaine (Foldvari et al., 1990), tretinoin (Masini et al., 1993), ciclosporin (Egbaria et al., 1990b), among others (Bernard et al., 1995; Fresta and Puglisi, 1996; Katahira et al., 1999). These results suggested that conventional liposomes were useful for topical dermal delivery of these drugs. Several recent studies suggested other applications and confirmed improved skin deposition (Ferreira et al., 2004; Puglia et al., 2004; Ramon et al., 2005; Kitagawa and Kasamaki, 2006).

Lipid composition, method of preparation and thermodynamic state of the bilayers of liposomes, were all shown to greatly affect skin deposition behavior of liposomes (Bouwstra and Honeywell-Nguyen, 2002; El Maghraby et al., 2006). Application of a lipophilic fluorescent label in liquid-state liposomes resulted in a deeper skin penetration than when applied in gel-state liposomes (Van Kuijk-Meuwissen et al., 1998a,b). Dermal delivery with skin-lipid liposomes was shown to be more effective than delivery with phospholipid vesicles (Egbaria et al., 1990a,b, 1991; Fresta and Puglisi, 1996, 1997; Yu and Liao, 1996; Liu et al., 2004). A decrease in cholesterol content in vesicular bilayers, which increases fluidity of the bilayers, resulted also in an increase in drug transport across the stratum corneum. Other physico-chemical properties, such as size, charge and lamellarity may also influence the effectiveness of liposomes as delivery vehicles (Yu and Liao, 1996; Katahira et al., 1999; Ogiso et al., 2001; Liu et al., 2004; Manosroi et al., 2004; Choi and Maibach, 2005; Sinico et al., 2005), although probably to a lesser extent (Honeywell-Nguyen and Bouwstra, 2005) with wide controversy among different studies.

Liposomes have also been used to target therapeutic and cosmetic agents to skin appendages, especially to the pilocebaceous units (hair follicles with their associated sebaceous glands) (Lauer et al., 1996). Carboxyfluorescein (fluorescent hydrophilic dye) encapsulated liposomes resulted in dye deposition in hair follicles, whereas in case of aqueous solution, the dye appeared only in the stratum corneum (Lieb et al., 1992). Several other studies showed that liposomes can effectively target drug delivery to skin appendages (Bernard et al., 1995; Li and Hoffman, 1997; Hoffman, 1998; Agarwal et al., 2000; Ciotti and Weiner, 2002; Jung et al., 2006; Tabbakhian et al., 2006).

Although some authors suggested conventional liposomes as suitable carriers for transdermal delivery of some drugs (Yu and Liao, 1996; Deo et al., 1997; Liu et al., 2004), it became recently evident that, in most cases, classic liposomes are of little or no value as carriers for transdermal drug delivery as they do not deeply penetrate skin, but rather remain confined to upper layers of the stratum corneum. Confocal microscopy studies showed

that intact liposomes were not able to penetrate into granular layers of the epidermis (Kirjavainen et al., 1996).

2.2. Mode of action of conventional liposomes

A variety of possible mechanisms exist for enhanced skin delivery of drugs from liposomes. They include the intact vesicular skin penetration, the penetration enhancing effect, the adsorption effect and the penetration of liposomes through the transappendageal route.

First, the concept of intact vesicular skin penetration was suggested in the first reports on liposomes as skin drug delivery systems (Mezei and Gulasekharam, 1980, 1982). Penetration was reported to reach the vascular dermis. It was suggested that because the liposomes were large, they were not able to enter the capillary circulation and thus acted as reservoirs for the drug at the site of action. Conceptually, it was difficult to conceive that large lipid vesicles could penetrate the densely packed stratum corneum in great numbers (El Maghraby et al., 2006). Consequently, many workers have tested this hypothesis. Electron micrography, applied using liposomes loaded with colloidal iron (an electron dense marker) to guinea-pigs, showed the presence of intact liposomes in the dermis (Foldvari et al., 1990). Although large multilamellar vesicles were applied, most of liposomes detected in the dermis were unilamellar. The authors proposed that liposomes could penetrate the epidermis carrying the drug into skin and that large multilamellar vesicles could lose their external bilayers during penetration. They added that liposomes could be adsorbed intact on the skin surface before penetration, with a possibility that some vesicles might rupture. Small unilamellar lipid vesicles produced better input of aqueous radiolabelled inulin and radiolabelled lipid bilayer components (Fresta and Puglisi, 1996) and tocopherol acetate (Natsuki et al., 1996) into deeper skin strata compared with large multilamellar vesicles suggesting the dependence of skin deposition on vesicle size, which supported the concept of intact vesicular penetration as a possible mechanism for improved skin accumulation.

However, contrary to the previous findings, investigating the effect of vesicle size of liposomes on skin deposition of ciclosporin, showed that the intermediate size and not the small size entities resulted in higher skin deposition, indicating that intact liposomes did not penetrate the skin (Du Plessis et al., 1994a) (greater flux from smaller vesicles is expected in case of intact vesicular skin penetration). Improved delivery of a fluorescent lipid bilayer marker into deep stratum corneum after skin pretreatment with empty vesicles was similar to that obtained from carriers encapsulating the marker, suggesting that improved delivery was not due to intact vesicular penetration and supporting a second possible mode of action, the penetration enhancing effect (Kirjavainen et al., 1996). It was suggested that liposome lipids penetrate into the stratum corneum by adhering onto the surface of the skin and, subsequently destabilizing, and fusing or mixing with the lipid matrix (Kirjavainen et al., 1996). Thereafter, they may act as penetration enhancers, loosening the lipid structure of the stratum corneum and promoting impaired barrier function of these layers to the drug, with less well-packed intercellular lipid structure forms, and with subsequent increased skin partitioning of the drug (Kirjavainen et al., 1999a). The extent of interaction between lipid vesicles and skin was highly dependent on the lipid composition of the liposomes (Kirjavainen et al., 1996). This could explain the observation that dermal delivery with skin-lipid liposomes is more effective than delivery with phospholipid vesicles. Several studies suggested this possible penetration enhancing effects (Hofland et al., 1995; Korting et al., 1995; Zellmer et al., 1995). However, Du Plessis et al. (1994b) studied the influence of in vivo skin pretreatment with liposomes on the topical absorption of hydrophilic (inulin) and hydrophobic (hydrocortisone) drugs. On the contrary, they found that pretreatment did not give advantages of encapsulated drug, showing no enhancement for both hydrophilic and lipophilic drugs, concluding that liposomes-stratum corneum interaction hypothesis was invalid and suggesting that liposomes must at least be applied concomitantly with the drug or the drug must be encapsulated within them.

Adsorption and fusion of liposomes onto the skin surface was also demonstrated, resulting in the formation of lamellae and rough structures on the top of the outermost corneocytes (Abraham and Downing, 1990; Hofland et al., 1995). This could increase the driving force for permeation of liberated molecules. However, the collapse of vesicles on skin surface may form an additional barrier, reducing permeation of hydrophilic molecules encapsulated in the vesicular aqueous core. This mechanism cannot also account for the increased delivery of macromolecules (Williams, 2003).

An important role of the transappendageal route in improving skin delivery of drugs by liposomes was also suggested (Du Plessis et al., 1992). Recent studies suggested that the role of the transappendageal route is limited to improved vesicular penetration into (i.e. targeting) but not necessarily through hair follicles and that it plays no major role in improving liposomal transdermal delivery (El Maghraby et al., 2001b, 2006).

3. Highly deformable (ultraflexible or elastic) liposomes

Intensive research led to the introduction and development, over the past 15 years, of a new class of highly deformable (elastic or ultraflexible) liposomes that have been termed Transfersomes®. While conventional liposomes were reported to have mainly localizing or rarely transdermal effects, deformable liposomes were reported to penetrate intact skin, carrying therapeutic concentrations of drugs, but only when applied under non-occluded conditions (Cevc and Blume, 1992).

Deformable liposomes (Transfersomes®) are the first generation of elastic vesicles introduced by Cevc and Blume (1992). They consist of phospholipids and an edge activator. An edge activator is often a single chain surfactant, having a high radius of curvature, that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers (Cevc, 1996; Cevc et al., 1996; Honeywell-Nguyen and Bouwstra, 2005). Sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80 and dipotassium glycyrrhizinate were employed as edge activators (Cevc et al., 1998; El Maghraby et al., 1999, 2000a,b; Trotta et al., 2004; Garg et al., 2006; Oh et al., 2006). Preparation of deformable liposomes

involves methods similar to those used in preparation of traditional liposomes. Most commonly, the film hydration method is used. The effects of incorporation of different edge activators on physicochemical properties (including vesicle size, entrapment efficiency, zeta potential, among others) of deformable liposomes were extensively investigated in several studies (Jain et al., 2003; Lee et al., 2005; Elsayed et al., 2007; Oh et al., 2006). The interaction between edge activators and liposomes was also investigated (El Maghraby et al., 2000a, 2004). In a recent study, the provesicular approach, proposed to enhance the stability of vesicles, has been extended to deformable liposomes and proultraflexible lipid vesicles of levonorgestrel were developed and investigated (Jain et al., 2005).

3.1. Deformable liposomes as carriers for dermal and transdermal drug delivery

Several studies (Table 1) have reported that deformable liposomes were able to penetrate intact skin in vivo, transferring therapeutic amounts of drugs (Cevc and Blume, 2001, 2003, 2004), including macromolecules, with efficiency comparable with subcutaneous administration (Cevc et al., 1995, 1998; Paul et al., 1995; Cevc, 2003). Other in vitro skin permeation and/or deposition studies (Table 2) confirmed that deformable liposomes were able to improve skin delivery of various chemical entities. Although most of these results were indicative of improved transdermal delivery, in vitro transport rates (Table 2) were usually much lower than exceptional high transport rates reported in vivo (Table 1). Deformable liposomes resulted in 14–17-fold increase in maximum oestradiol flux through human cadaver epidermis with 9.2–11-fold increase in skin deposited drug (El Maghraby et al., 1999); however, total oestradiol permeated and skin deposited after 12 h did not exceed 2% of the applied dose, a value much lower than reported in vivo results for some drugs. Deformable liposomes were also reported to improve both in vitro skin permeation and deposition of cyclosporin A (Guo et al., 2000a), methotrexate (Trotta et al., 2004), melatonin (Dubey et al., 2006), among others (Table 2). They significantly improved ketotifen skin delivery, with greater improvement of ketotifen skin deposition than improvement of ketotifen skin permeation, hence were suggested to be more useful for dermal than for transdermal delivery of ketotifen (Elsayed et al., 2007). They were also reported to improve only skin deposition of 5-fluorouracil (El Maghraby et al., 2001a) and dipotassium glycyrrhizinate (Trotta et al., 2002), hence were considered only useful for dermal delivery of these drugs.

Reported success of deformable liposomes to deliver macromolecules and proteins such as insulin through intact human skin with efficiency comparable with subcutaneous administration (Paul et al., 1995, 1998; Cevc et al., 1998; Cevc, 2003) led to their introduction as possible carriers for non-invasive gene delivery and transcutaneous immunization. Kim et al. (2004) investigated deformable cationic liposomes, prepared using a cationic lipid, 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), and sodium cholate, as gene delivery system. In vitro transfection efficiency of plasmid DNA was assessed by the expression of green fluorescent protein (GFP). This formulation was capable of transfecting several cell lines. It was also tested for in vivo transfection efficiency and its retention time within the organs, by applying the complexes on hair-removed dorsal skin of mice, non-invasively. It was found that genes were transported into several organs for 6 days once applied on intact skin, suggesting promising properties for non-invasive gene delivery. In another study, deformable liposomes prepared using egg-phosphatidylcholine could also deliver genetic materials (GFP) into mice transdermally (Lee et al., 2005). Gupta et al. (2005a,b) investigated deformable liposomes as carriers for topical immunization. Deformable lipid vesicles were used for the non-invasive delivery of tetanus toxoid (TT). The immune stimulating activity of these vesicles was studied by measuring the serum anti-tetanus toxoid IgG titer following topical immunization. The immune response was compared with the same dose of alum adsorbed tetanus toxoid (AATT) given intramuscularly, topically administered plain tetanus toxoid solution, and a physical mixture of tetanus toxoid and deformable liposomes again given topically. An in vivo study revealed that topically administered tetanus toxoid-loaded deformable liposomes, after secondary immunization, elicited an immune response (anti-TT-IgG) comparable with that produced by intramuscular AATT. Fluorescence microscopy revealed the penetration of deformable liposomes through the skin to deliver the antigen to the immunocompetent Langerhans cells (Gupta et al., 2005b). Mishra et al. (2006) also investigated deformable liposomes as carriers for transcutaneous immunization by measuring immune response elicited by topically applied hepatitis B surface antigen (HBsAg) loaded deformable liposomes, compared to the intramuscularly administered alum-adsorbed HBsAg solution, topically applied plain HBsAg solution and physical mixture of HBsAg and deformable liposomes. Results indicated that transcutaneous immunization via deformable liposomes induced robust systemic and mucosal antibody response against HBsAg as compared to other formulations. The fluorescence microscopy results suggest prominent skin permeation and biodistribution, demonstrating efficient delivery of antigens to the immunocompetent Langerhans cells and lymphatics, justifying deformable liposomes potential for improved vaccine delivery.

A second generation of elastic vesicles, consisting mainly of non-ionic surfactants, was introduced by van den Bergh (1999). These surfactant-based elastic vesicles were shown to be also more effective than rigid vesicles in enhancing skin penetration of various chemical entities (Honeywell-Nguyen et al., 2002, 2003a,b; Honeywell-Nguyen and Bouwstra, 2003).

3.2. Mode of action of deformable liposomes

Several studies investigated possible mechanisms by which deformable vesicles could improve skin delivery of drugs. Two mechanisms were proposed (Honeywell-Nguyen et al., 2003a; Honeywell-Nguyen and Bouwstra, 2003). First, vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin (mechanism 1). Second, vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify the intercellular lipid lamellae. This

Table 1
Summary of some reported in vivo studies investigating efficiency and applications of deformable liposomes as carriers for skin delivery of drugs

Drug (or marker)	Subjects/species	Criteria under investigation	Results
Radiolabelled lipid (Cevc and Blume, 1992)	Mice	Fate of lipids	30 ± 10% of applied lipids were found in subdermis and up to 6–8% in blood.
Lidocaine (Planas et al., 1992)	Rats	Anesthetic action using heat and pressure stimuli	130% increase in heat stimulus reaction (withdrawal time) relative to controls.
Lidocaine and tetracaine (Planas et al., 1992)	Humans	Pinprick method	Effectiveness was similar to subcutaneous injections of similar drug quantities.
Human serum albumin or gap junction protein (Paul et al., 1995, 1998)	Mice	Immune response	Deformable liposomes encapsulated proteins elicited an immune response (similar or slightly higher antibody titer than s.c. injections of the same immunogen formulation) while standard liposomes were ineffective for delivering the proteins.
Cyclosporin A (Guo et al., 2000a)	Mice	Drug serum concentration	Deformable liposomes resulted in serum drug concentration of 53.43 ± 9.24 ng/mL after 2 h and 154.37 ± 27.15 ng/mL after 8 h. Conventional vesicles failed to deliver measurable amount of drug into blood.
Insulin (Cevc et al., 1998; Cevc, 2003)	Mice and humans	Fate of radiolabelled iodinated insulin and hypoglycemic action	Insulin was transferred across intact skin evolving significant systemic hypoglycemia with efficacy comparable (at least 50%) to s.c. injection, but with approximately 45–145 min delay. Mixed micelles and conventional liposomes were ineffective.
Insulin (Guo et al., 2000b)	Mice	Hypoglycemic action	Drop in blood glucose was 21.4 ± 10.1% at 1 h, reached 61.4 ± 8.9% at 5 h and >50% for 18 h. Conventional vesicles, insulin solution, and saline had no hypoglycemic effect.
Diclofenac (Cevc and Blume, 2001)	Mice, rats and pigs	Biodistribution of radiolabelled drug	Deformable liposomes were superior to commercial gel, producing longer effect, 10 times higher concentrations in subcutaneous tissues, deeper penetration into soft tissue, delivering therapeutic quantity of diclofenac into treated knee with reasonable reproducibility and therapeutic drug levels in systemic blood circulation could also be reached. This was not observed when the drug was used in a gel.
Triamcinolone acetonide (Cevc and Blume, 2003; Fesq et al., 2003), hydrocortisone and dexamethasone (Cevc and Blume, 2004)	Mice and humans	Biodistribution of radiolabelled corticosteroid and measuring suppression in chemically induced murine ear oedema	Deformable liposomes were superior to commercial formulations, producing better targeting, prolonged anti-inflammatory action at greatly reduced dose (by at least 10-fold for triamcinolone and dexamethasone and 3–5-fold for hydrocortisone), diminishing drug's abrasion sensitivity (increase general robustness of drug effect), and hence improving therapeutic risk–benefit ratio.
Levonorgestrel proultraflexible liposomes (Jain et al., 2005)	Female Sprague-Dawley rats	Endometrial weight and mucosal thickness	After 9 days, proultraflexible liposomes significantly increased endometrial weight (2.8- and 2.2-fold) and mucosal thickness (4.3- and 2.5-fold). Data are compared with plain drug solution and proliposomes, respectively.
		Inhibition of luteinization	After 9 days, the percent inhibitions, compared to control, were 85.7 ± 5.2, 45.2 ± 2.8 and 14.3 ± 1.6% for proultraflexible liposomes, proliposomes and plain drug solution treated rats, respectively.
		Drug serum concentration (Pharmacokinetics)	Proultraflexible liposomes produced 8-fold higher plasma concentration, relative to plain drug, within 4 h that was maintained over 48 h. AUC (0–24 h) after application of proultraflexible liposomes was nearly 4 times higher than that after application of proliposomal formulation and nearly 18 times higher than after plain drug application.
Ethinylestradiol (Garg et al., 2006)	Female albino rats	Anti-ovulatory activity	Significant anti-ovulatory activity of ultraflexible liposomes was observed as compared to traditional liposomes and plain drug solution given orally and topically. In case of ultraflexible liposomes, the disintegration of the graffian follicle starts right from the first day. After 9 days, no new follicles could be found developing and the ovary was filled with remnants of theca externa, theca interna and stromal tissue. In case of traditional liposomes, process of ova disintegration and follicles development inhibition was comparatively delayed. Plain drug solution applied topically did not show any prominent effect of steroid.

Table 1 (*Continued*)

Drug (or marker)	Subjects/species	Criteria under investigation	Results
		Endometrial weight and mucosal thickness	After 9 days, ultraflexible liposomes significantly increased endometrial weight (8.1- and 2.9-fold) and mucosal thickness (17.0- and 5.5-fold). Data are compared with plain drug solution and traditional liposomes, applied to skin, respectively.
		Drug serum concentration (Pharmacokinetics)	AUC (0–72 h) obtained after transdermal application of ultraflexible liposome (134.91 ± 2.11 pg h/mL) was significantly high when compared with plain drug solution given orally (15.94 ± 0.32 pg h/mL), non-flexible liposomal formulation (19.61 ± 0.86 pg h/mL) and plain drug solution applied to the skin (0.16 ± 0.02 pg h/mL). Drug levels were maintained in blood for much longer time, indicating a longer plasma half-life for the ultraflexible liposomal formulation.
Low-molecular-weight heparin (LMWH) (Song and Kim, 2006)	Hairless mice	Concentration in stratum corneum using tape stripping and in vivo localization	Concentration in stratum corneum was in the following order: LMWH solution ≈ anionic flexosomes (flexible liposomes) > neutral flexosomes ≫ cationic flexosomes. Concentration in viable epidermis was in the following order: cationic flexosomes ≫ LMWH solution > neutral flexosomes > anionic flexosome.
Zidovudine (Jain et al., 2006)	Rats	Pharmacokinetics	AUC (0–24 h) for elastic liposomal formulation (12.63 ± 1.2 μg h/mL) was found to be nearly 12-fold higher than the control (0.83 ± 0.2 μg h/mL). Elastic liposomes resulted in substantially higher accumulation of the drug in target RES organs that play a key role in AIDS pathogenesis by providing long-term reservoir for the virus.

will facilitate penetration of free drug molecules into and across the stratum corneum (mechanism 2).

The first mechanism was put forward by Cevc et al. for deformable liposomes (Cevc and Blume, 1992, 2001; Cevc et al., 2002). They proposed that the driving force for the vesicles entering the skin is xerophobia (the tendency to avoid dry surroundings) (Cevc and Blume, 1992). The important difference between deformable liposomes and traditional liposomes is the high and stress-dependent adaptability of such deformable vesicles, which enables them alone to squeeze between the cells in the stratum corneum, despite the large average vesicle size (Cevc et al., 2002). Thus, they can trespass the intact skin spontaneously, under the influence of the naturally occurring, in vivo tanscutaneous hydration gradient (Cevc and Blume, 2001), intact without permanent disintegration (Cevc et al., 2002). Using confocal laser scanning microscopy, Schatzlein and Cevc (1998) have suggested that there were two different penetration pathways, the intercluster pathway and the intercorneocyte pathway (honeycomb-like system, Fig. 1). The authors have suggested that these regions contained structural irregularities within the intercellular lipid lamellae, and that these irregularities can function as 'virtual channels' through which elastic vesicles can penetrate. It was also claimed that intact deformable liposomes penetrated through the stratum corneum and through

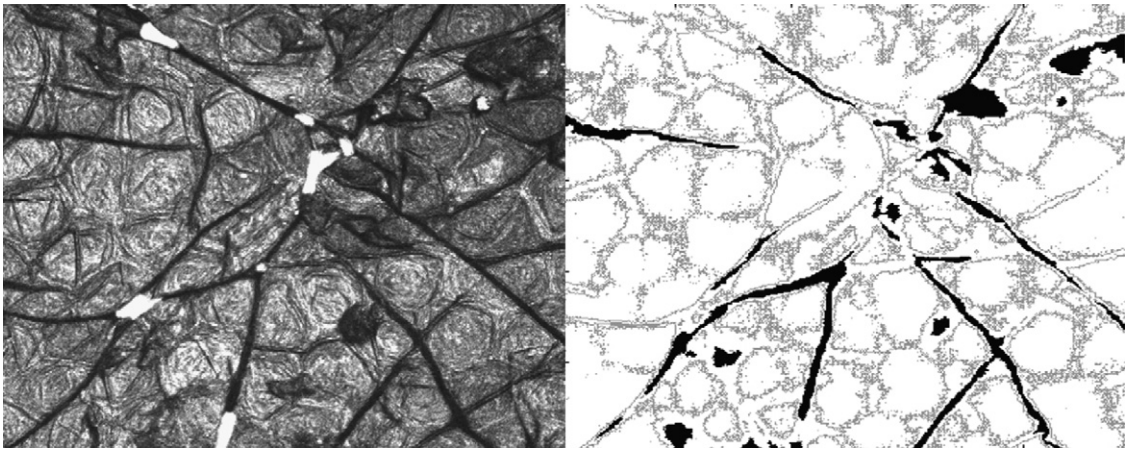

Fig. 1. (Left) surface of a living nude mouse skin with ~200 μm × 200 μm area, as measured with the confocal laser scanning microscopy in reflectometry mode. (Right) Characteristic fluorescence distribution in inter-cluster (black) and inter-corneocyte (grey) regions of the skin, with a relatively low and high respective penetration resistance (from Cevc, 2004).

Table 2
Summary of deformable liposomes in vitro skin permeation/deposition studies

Drug (or marker)	Vesicular composition[a] (w/w)	Tissue used	Enhancement ratio[b]		Remarks
			Permeation	Deposition	
Oestradiol (El Maghraby et al., 1999)	SPC:SCh (86:14%) SPC:S 80 (86.7:13.3%) SPC:T 80 (84.5:15.5%)	Heat separated human epidermis	17 (2.1[c]) 17 (2.1[c]) 14 (1.7[c])	9.4 (1.1[c]) 9.2 (1.1[c]) 11 (1.3[c])	Containing 7% ethanol
Cyclosporin A (Guo et al., 2000a)	SPC: SCh (1:0.28)	Kunming mouse abdominal skin	Flexible liposomes delivered $1.16 \pm 0.26\ \mu g/cm^2$ after 8 h and amounted to $1.88 \pm 0.06\ \mu g$ 24 h later. Conventional liposomes failed to transfer cyclosporin A after 24 h	16.1 (2.5[c])	The receptor compartment contained 20% ethanol adjusted to isotonic by 0.9% NaCl
5-Fluorouracil (El Maghraby et al., 2001a)	SPC:SCh (84:16%)	Heat separated human epidermis	No improvement	10.2 (8.5[c]) (12 h) 8.21 (6.2[c]) (36 h)	Containing 7% ethanol
Dipotassium glycyrrhizinate (Trotta et al., 2002)	PC:KG (2:1) PC:KG (4:1) PC:KG (8:1) HPC:KG (2:1) HPC:KG (4:1) HPC:KG (8:1)	Full-thickness pig ear skin	Negligible flux, below detection limit	5.0 5.9 5.2 5.3 5.5 5.4	
Diclofenac (Boinpally et al., 2003)	SPC:SCh (44:15%)	Heat separated human epidermis	(2.0[c])	Not determined	Containing 10% ethanol
Methotrexate (Trotta et al., 2004)	PC:KG (2:1) PC:KG (4:1) HPC:KG (2:1) HPC:KG (4:1)	Full-thickness pig ear skin	5.2 (8.5[c]) 2.9 (4.8[c]) 5.9 (5.9[c]) 4.1 (4.1[c])	3.3 (3.5[c]) 2.7 (2.9[c]) 3.4 (3.2[c]) 2.9 (2.7[c])	
Levonorgestrel proultraflexible liposomes (Jain et al., 2005)	SPC:SCh (85:15%) SPC:SDCh (85:15%)	Abdominal female Sprague-Dawley rat skin	353.7 (1.9[c]) 494.4 (2.7[c])	Not determined Not determined	PC + Surfactant:alcohol:aqueous phase (5:4:5, w/w/w)
Low-molecular-weight heparin (Song and Kim, 2006)	DOTAP:T 20 (86.9:13.1%) EPC:T 20 (86.9:13.1%) EPC:DCP:T 20 (82.9:4:13.1%)	Full thickness hairless mouse skin	400 43 30	Not determined Not determined Not determined	
Retinol (Oh et al., 2006)	EPC:T 20 (6:1)	Dermatomed human cadaver skin Epidermis skin model	(≈5.5[c]) (≈3.8[c])	Not determined Not determined	Drug suspension showed no skin permeation
Ethinylestradiol (Garg et al., 2006)	SPC:SDCh (85:15%) SPC:T 60 (85:15%) SPC:S 60 (85:15%) SPC:S 65 (85:15%)	Female albino Sprague-Dawley rat skin	15.19 (1.79[c]) 14.95 (1.76[c]) 17.24 (2.03[c]) 17.90 (2.11[c])	Not determined Not determined Not determined Not determined	
(+)-Catechin (Fang et al., 2006)	EPC:CH:Deoxycholic acid (4:1:0.25)	Female nude mouse skin	4.75 (1.24[c])	0.22	Containing 15% ethanol
Melatonin (Dubey et al., 2006)	SPC:SDCh (86:14%)	Dermatomed abdominal human cadaver skin	12.19 (4.70[c])	4.17 (2.22[c])	
Ketotifen (Elsayed et al., 2007)	SPC:T 80 (84.5:15.5%)	Albino rabbit pinna skin	1.6 (1.7[c])	2.2 (1.2[c])	Containing 7% ethanol

[a] DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane (cationic lipid); EPC, egg-phosphatidylcholine; SPC, soya-phosphatidylcholine; HPC, hydrogenated phosphatidylcholine; DCP, dicetyl phosphate; CH, cholesterol; SCh, sodium cholate; SDCh, sodium deoxycholate; KG, dipotassium glycyrrhizinate; T, Tween; S, Span.
[b] Unless otherwise indicated, relative to drug solution or suspension.
[c] Relative to traditional liposomes.

the underlying viable skin into blood circulation (Cevc et al., 2002).

Several studies supported that deformable liposomes and surfactant-based elastic vesicles may act as carrier systems. Pre-treatment of skin membranes with empty deformable liposomes did not enhance oestradiol flux, while application of oestradiol entrapped in vesicles resulted in 14–17-fold increase in flux relative to control (El Maghraby et al., 1999). Interestingly, fluxes of oestradiol from large and small vesicles were similar in this study, providing no evidence of intact vesicle penetration. However, in another study, using two fluorescently labeled substances (a hydrophilic fluorescent compound and a lipophilic one), reduction of vesicle size improved deposition into deeper strata and penetration through skin, with large structures improving deposition only (Verma et al., 2003b). Additionally, the vesicular components as lipid solution in 90% propylene glycol in water improved oestradiol epidermal permeation but were inferior to vesicles, suggesting that lipid components should be in the form of vesicles for optimum effects (El Maghraby et al., 2000b). Similarly, pre-treatment of surfactant-based elastic vesicles did not improve the transport of pergolide and rotigotine and higher entrapment efficiency resulted in higher drug transport supporting that surfactant-based elastic vesicles may act also as carrier systems (Honeywell-Nguyen et al., 2003a; Honeywell-Nguyen and Bouwstra, 2003). Song and Kim (2006) examined skin surface charge changes with low-molecular-weight heparin cationic flexible liposomes (flexosomes, prepared using a cationic lipid, 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), and Tween 20) in Franz diffusion cells. The surface charge of skin with low-molecular-weight heparin cationic flexosomes decreased from 7.5 to −13.7 mV in 12 h, while that of the intact skin was constant at −10 mV, suggesting that cationic flexosomes could pass the stratum corneum as intact structures.

Recently, an in vivo electron microscopic study demonstrated a fast (within 1 h of application) partitioning of intact surfactant-based elastic vesicles into human stratum corneum, but almost no vesicles could be found in the deepest layers of the stratum corneum (Honeywell-Nguyen et al., 2000). Transmission electron microscopy and freeze-fracture electron microscopy demonstrated also major morphological changes in the intercellular lipid lamellar structure with no changes in the viable epidermis ultrastructure, after application of surfactant-based elastic vesicles (van den Bergh et al., 1999b). Using two-photon fluorescence microscopy, thread-like channels (Fig. 2), much finer than channels reported for deformable liposomes (Schatzlein and Cevc, 1998), were visualized. They might serve as penetration pathways; however, no dye (fluorescence) could be detected in the viable epidermis (van den Bergh et al., 1999b).

Recent evidence showed that the water gradient across the skin may not be linear and there may be a relatively ‘dry’ region within the stratum corneum (Williams, 2003). It was also noticed that even in fully hydrated state, the water content in the lowest stratum corneum layers close to the viable epidermis is much lower than in central regions of the stratum corneum. Therefore, it was expected that, as a result of the osmotic force, vesicles will not penetrate beyond the level of the lowest layers in the stratum corneum (Bouwstra and Honeywell-Nguyen, 2002). Results of studies showing that deformable liposomes only improved skin deposition of some drugs (El Maghraby et al., 2001a; Trotta et al., 2002) could support this proposal. Drugs will penetrate further solitary. Thus, drugs have to be released from vesicles in order to reach the systemic circulation (Honeywell-Nguyen and Bouwstra, 2003).

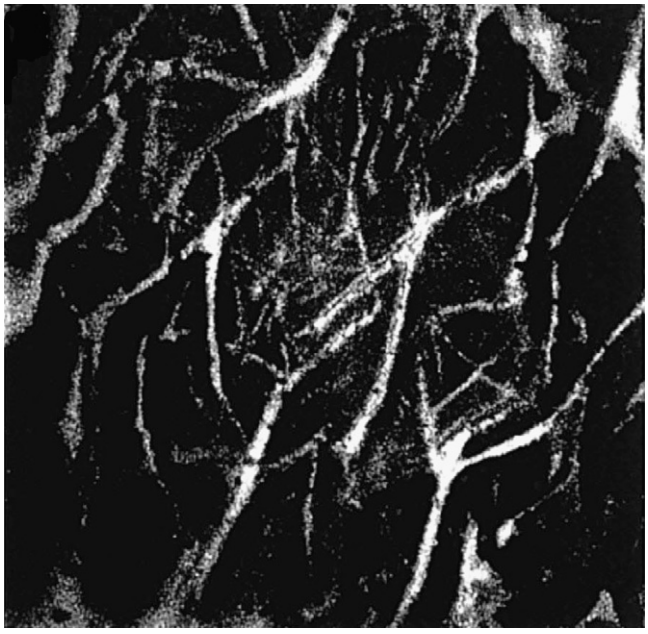

Fig. 2. Two-photon fluorescence microscopy image (95 μm × 95 μm) recorded at a depth of 10 μm in skin treated with surfactant-based liquid-state elastic vesicles after 1 h of application (from van den Bergh et al., 1999b).

Several studies suggested also a possible penetration enhancing mechanism (mechanism 2) for deformable liposomes and for surfactant-based elastic vesicles. van den Bergh et al. (1999a) showed that pre-treatment of hairless mouse skin with surfactant-based elastic vesicles increased the diffusion of ^3H_2O compared to pre-treatment with a buffer control, indicating a possible penetration enhancing mechanism. Deformable liposomes were able to carry both the entrapped and the non-entrapped hydrophilic fluorescent compound, carboxyfluorescein, into the stratum corneum and possibly to deeper layers of the skin (Verma et al., 2003a), suggesting also a possible penetration enhancing effect. However, deformable liposomes with carboxyfluorescein only inside the vesicles exhibited a higher penetration into deeper skin layers than that exhibited by vesicles with carboxyfluorescein only outside the vesicles which might support the hypothesis of intact vesicle permeation, although the total amount of carboxyfluorescein that penetrated into deeper skin layers and into the receiver compartment did not exceed 0.2% of the dose applied (Verma et al., 2003a). In another study, deformable liposomes with ketotifen only outside the vesicles significantly improved ketotifen skin permeation and deposition over deformable liposomes with ketotifen only entrapped inside the vesicles, suggesting that the penetration enhancing effect may be of greater importance in the enhanced skin delivery of ketotifen by deformable liposomes (Elsayed et al., 2006). Deformable liposomes improved only skin deposition of

dipotassium glycyrrhizinate as skin fluxes were below detection limit, whereas skin deposition increased 4.5-fold in comparison to an aqueous control (Trotta et al., 2002). Deformable liposomes also improved only skin deposition of 5-fluorouracil with little or no effect on the drug permeation (El Maghraby et al., 2001a), hence are only useful for dermal delivery of these drugs. The limited partitioning into the acceptor phase indicates that deformable vesicles are not carrying the associated drug into the acceptor phase. In addition, the percentage of 5-fluorouracil that penetrated the skin was higher than the drug entrapment efficiency which strongly suggested that liposome components may have altered skin structure, thus enhancing 5-fluorouracil transmission through human epidermis (El Maghraby et al., 2001a) (i.e. possible penetration enhancing mechanism). It should be noted that, to the best of our knowledge, all studies that suggested a possible penetration enhancing mechanism involved hydrophilic drugs or hydrophilic fluorescent compounds.

The possible role of shunt route penetration in skin delivery of oestradiol from deformable liposomes was also studied using a novel technique (El Maghraby et al., 2001b). The study involved monitoring the liposomal delivery of oestradiol through epidermal membranes and comparing this with penetration through a sandwich of stratum corneum (SC) and epidermis, where the additional SC formed a top layer. As the orifices of the shunts occupy only about 0.1% of the total skin surface area, there is a negligible chance that shunts in the two membranes will superimpose. It was therefore assumed that the top layer of SC would block most of the shunts available in the bottom membrane. Results revealed a reduction in the drug flux after using SC/epidermis sandwiches compared with epidermis. The reduction was close to the theoretical expectations calculated assuming that the SC/epidermis sandwich has a thickness double that of the epidermis (i.e. neglecting the presence of the nucleated epidermis). This indicates absence of any significant role for the shunt route in skin delivery of oestradiol from liposomes.

El Maghraby et al. (2006) suggested that the reasons for variable effects and explanations may arise from different vesicle compositions, alternative methods of preparation which result in vesicles having diverse characteristics with respect to size, lamellarity, charge, membrane fluidity and elasticity and drug entrapment efficiency, and the selection of skin membranes (man or animal, in vivo or in vitro). Other aspects of the experimental design (such as receptor solution composition) and the technique used in evaluation may have profound effects on the recorded action.

Based on results of reviewed studies, we could support that both the intact vesicular permeation into the stratum corneum and the penetration enhancing effect play a role in the enhanced skin delivery of drugs by deformable liposomes under non-occlusive conditions and suggest that one of the two mechanisms might predominate according to the physico-chemical properties of the drug. The transport of the drug, carried by deformable liposomes, into the stratum corneum bypassing the main barrier for drug permeation will considerably improve skin delivery. This role may be of great effect in improving skin deposition. However, several factors might contribute to or contribute against this role in improving transdermal flux. Drug release from the vesicles in the stratum corneum is an important step (Honeywell-Nguyen and Bouwstra, 2003) that will affect transdermal flux. The rate and amount of released drug is a balance between two factors: (1) drug affinity to vesicles, and (2) drug solubility in lipids of the stratum corneum (Honeywell-Nguyen and Bouwstra, 2003). As reported in a recent study, poor drug release resulted in retention of the drug within vesicles in the stratum corneum and elastic vesicles served as a slow release depot system (Honeywell-Nguyen and Bouwstra, 2003).

For hydrophilic drugs, the penetration enhancing effect seems to play a more important role in the enhanced skin delivery than in case of lipophilic drugs (as for many penetration enhancers), since permeation of hydrophilic molecules tends to be relatively slower and hence more enhanceable (Williams and Barry, 1991; Williams, 2003). The intact vesicle penetration mechanism will have also an important role specially in improving skin deposition. However, as previously mentioned, drug release from vesicles in the stratum corneum is an important step that affects transdermal flux (Honeywell-Nguyen and Bouwstra, 2003). We therefore suggest that, regarding transdermal permeation, hydrophilic drugs might not be necessarily entrapped in vesicles for optimum effects. On the contrary, entrapment of hydrophilic drugs might result in a slow release, where phospholipids might form an extra lipid barrier, decreasing the flux of these hydrophilic drugs. This could explain results of a recent study (Elsayed et al., 2006), where entrapment of ketotifen failed to improve its transdermal permeation. This also could explain the variation in reported results regarding improvement of skin permeation of hydrophilic drugs by deformable liposomes (Table 2), as some studies showed improved transdermal permeation, while others showed little or no effect (El Maghraby et al., 2001a; Trotta et al., 2002).

However, for a lipophilic drug, the penetration of the drug into the stratum corneum associated with (solubilized in) vesicular lipid bilayers, bypassing the primary barrier for drug permeation, is expected to have a more important role in improving both skin deposition and transdermal permeation (possibly due to higher solubility in lipids of the stratum corneum and hence relatively faster release). Penetration enhancing mechanism might also play a role in the enhanced skin delivery of lipophilic drugs; however, it is not the main factor operating (El Maghraby et al., 2000b). Therefore, lipophilic drugs should be entrapped in vesicles for optimum skin delivery. Based on this suggestion, highly deformable carriers need to be designed and tested on a case by case basis. Further studies, using different hydrophilic and lipophilic agents, are to be carried out to further prove this suggestion.

4. Ethosomes

Ethosome is another novel lipid carrier, recently developed by Touitou et al. (2000a,b), showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water (Touitou et al., 2000a). Although, liposomal formulations containing up to 10% ethanol and up to 15% propylene glycol were previously described (Foldvari et al., 1993), the use of high ethanol content was first described by Touitou et

al. (1997) for ethosomes. Due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulations. However, ethosomes which are novel permeation-enhancing lipid vesicles embodying high concentration (20–45%) of ethanol were developed and investigated. Ethosomes are most commonly prepared as described by Touitou et al. (2000a). Briefly, the lipids and the drug are dissolved in ethanol. The aqueous component is added slowly in a fine stream at constant rate in a well-sealed container with constant mixing. Mixing is then continued for additional few minutes.

Several studies investigated the effect of ethanol on physicochemical characteristics of the ethosomal vesicles (Dayan and Touitou, 2000; Touitou et al., 2000a; Lopez-Pinto et al., 2005; Elsayed et al., 2007). One reported characteristic of ethosomes is their small size relative to liposomes, when both are obtained by preparation methods not involving any size reduction steps (Dayan and Touitou, 2000). This reduction in vesicle size could be explained as a result of incorporation of high ethanol concentration. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease (Touitou et al., 2000a; Lopez-Pinto et al., 2005). The size of ethosomal vesicles was reported to increase with decreasing ethanol concentration in the ethanol concentration range of 20–45% (Touitou et al., 2000a). The effect of phospholipid concentration on the size of ethosomal vesicles was also investigated (Touitou et al., 2000a; Elsayed et al., 2007). Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles (Touitou et al., 2000a) as well as by the presence of ethanol in ethosomes, which allows for better solubility of many drugs.

4.1. Ethosomes as carriers for dermal and transdermal drug delivery

Ethosomes were reported to be effective at delivering molecules to and through the skin to the systemic circulation. Enhanced delivery of chemicals from the ethosomal carrier was observed in permeation experiments with fluorescent probes. The amphiphilic fluorescent probe D-289 was used to study skin penetration from trihexyphenidyl HCl ethosomes into nude mouse skin, after non-occlusive application (Dayan and Touitou, 2000). Confocal laser scanning micrographs (Fig. 3) showed that classic liposomes did not facilitate probe penetration into the skin, rather, resulted in only a small reservoir in upper layers of skin. Using hydroethanolic solution, a relatively deep penetration (up to about 132 μm) was observed, but of a relatively very low fluorescence intensity. The use of ethosomal system resulted in an increase in both depth (up to about 170 μm) of penetration and fluorescence intensity. Assuming fluorescence intensity can be taken as a relative measure of probe in the skin, the skin depot of probe from ethosomes was 18 times that of liposomes, and 10 times that of hydroethanolic solution. Similar results were obtained with the lipophilic fluorescent probe, rhodamine red (Touitou et al., 2000a).

Ethosomes were reported to improve in vivo (Table 3) and in vitro (Table 4) skin delivery of various drugs. Contrary to deformable liposomes, ethosomes were able to improve skin delivery of drugs both under occlusive (Dayan and Touitou, 2000; Ainbinder and Touitou, 2005; Lopez-Pinto et al., 2005; Paolino et al., 2005) and non-occlusive conditions (Dayan and Touitou, 2000; Elsayed et al., 2007).

4.2. Mode of action of ethosomes

Although the exact process of drug delivery by ethosomes remains a matter of speculation (Dayan and Touitou, 2000), most likely, a combination of processes contribute to the enhancing effect (Touitou et al., 2000a). Ethanol is a well-known permeation enhancer (Williams, 2003). However, previous studies (Dayan and Touitou, 2000; Touitou et al., 2000a) that compared permeation enhancement of drugs from ethosomal systems versus hydroethanolic solutions showed that permeation enhancement from ethosomes was much greater than would be expected from ethanol alone. A synergistic mechanism was suggested between ethanol, vesicles and skin lipids (Touitou et al., 2000a). However, the literature is lacking a study comparing skin delivery from ethosomes to skin delivery from traditional liposomes on skin pretreated with hydroethanolic solution. This

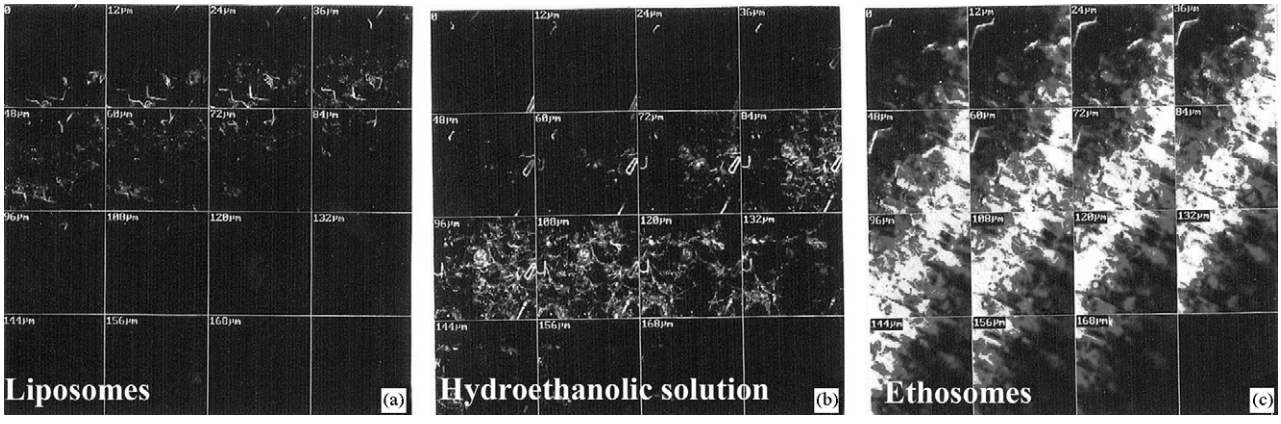

Fig. 3. Confocal laser scanning microscopy micrographs of mouse skin, after application of the fluorescent probe D-289 in traditional liposomes, hydroethanolic solution and ethosomes (from Dayan and Touitou, 2000).

Table 3
Summary of some reported in vivo studies investigating efficiency and applications of ethosomes as carriers for skin delivery of drugs

Drug	Criteria under investigation	Subjects/species	Results
Acyclovir (Horwitz et al., 1999)	Clinical efficacy in treatment of recurrent herpes labialis	Humans	Time to crusting of lesions and time to loss of crust were shorter with the ethosomal acyclovir than with the commercial cream (Zovirax cream).
Testosterone (Touitou et al., 2000a)	Pharmacokinetics	Rabbits	After application for 5 days (new patch applied daily), AUC was 125% greater with ethosomal patch than with commercially available patch.
Testosterone (Ainbinder and Touitou, 2005)	Pharmacokinetics	Male Sprague-Dawley rats	AUC was about 64% greater with ethosomes than with commercial gel.
Cannabidiol (Lodzki et al., 2003)	Suppression of carrageenan-induced aseptic paw edema (anti-inflammatory action)	Male mice	Development of edema was prevented entirely only in pretreated (ethosomal patch) group of mice. Delta in paw thickness of pretreated mice was statistically different from that of the non-pretreated mice starting from 1 h post-carrageenan injection and lasting until the end of the inflammation course.
Erythromycin (Godin and Touitou, 2005)	In vivo antibacterial efficiency	*S. aurues* inoculated mice skin	Ethosomal erythromycin resulted in complete inhibition of infection while hydroethanolic erythromycin solution caused deep dermal and subcutaneous abscesses within 5 days after challenge.
Ammonium glycyrrhizinate (Paolino et al., 2005)	Suppression of chemically induced erythema (anti-inflammatory action)	Human volunteers	Ethosomes reduced the erythema more rapidly with respect to drug solutions. Ethosomes also showed sustained effect.

Table 4
Summary of ethosomes in vitro skin permeation/deposition studies

Drug	Tissue used	Enhancement ratio		Remarks
		Permeation[a]	Deposition	
Sotalol (Kirjavainen et al., 1999b)	Heat separated human epidermis	7.1[c]	Not determined	Franz diffusion cells
Sodium salicylate (Kirjavainen et al., 1999b)	Heat separated human epidermis	3.8[c]	Not determined	Franz diffusion cells
Propranolol (Kirjavainen et al., 1999b)	Heat separated human epidermis	1.4[c] (not statistically significant)	Not determined	Franz diffusion cells
Trihexyphenidyl HCl (Dayan and Touitou, 2000)	Male nude mouse dorsal skin	51[b], 4.5[c], 87[e]	4.6[b], 1.4[c], 1.4[e]	Side-by-side diffusion cells
Minoxidil (Touitou et al., 2000a)	Male nude mouse abdominal skin	45[c], 35[d], 10[f]	7[c], 5[d], 2[f]	Side-by-side diffusion cells
Minoxidil (Lopez-Pinto et al., 2005)	Rat abdominal skin	1.2[e] (Addition of cholesterol significantly improved skin delivery from ethosomes)	Not determined	Franz diffusion cells
Testosterone (Touitou et al., 2000a)	Rabbit pinna skin	30 (relative to commercial patch)	7 (relative to commercial patch)	Franz diffusion cells
Testosterone (Ainbinder and Touitou, 2005)	Dermatomed cadaver human skin	6.4 (relative to commercial gel)	Not determined	Franz diffusion cells
Azelaic acid (Esposito et al., 2004)	Synthetic membranes	Release rate was higher from ethosomes than from liposomes. Ethosomes having the highest ethanol concentration released the drug more rapidly	–	Diffusion through synthetic membranes
Zidovudine (Jain et al., 2004)	Rat skin	15.1[c], 10.9[d], 12.9[e], 7.7[f]	Not determined	Keshary-Chien diffusion cell
Ammonium glycyrrhizinate (Paolino et al., 2005)	Human epidermis	Ethosomes improved cumulative drug permeated after 24 h and reduced lag time relative to aqueous solution, hydroethanolic solution, and mixture of empty ethosomes/hydroethanolic drug solution	Not determined	Franz diffusion cells
Ketotifen (Elsayed et al., 2007)	Rabbit pinna skin	1.2[b], 1.4[c], 1.2[e]	3.3[b], 6.2[c], 1.7[e]	Franz diffusion cells

[a] Estimated based on cumulative amounts permeated at the end of the experiment or on flux data.
[b] Relative to aqueous solution.
[c] Relative to hydroethanolic solution.
[d] Relative to absolute ethanol.
[e] Relative to traditional liposomes.
[f] Relative to lipid ethanolic solution.

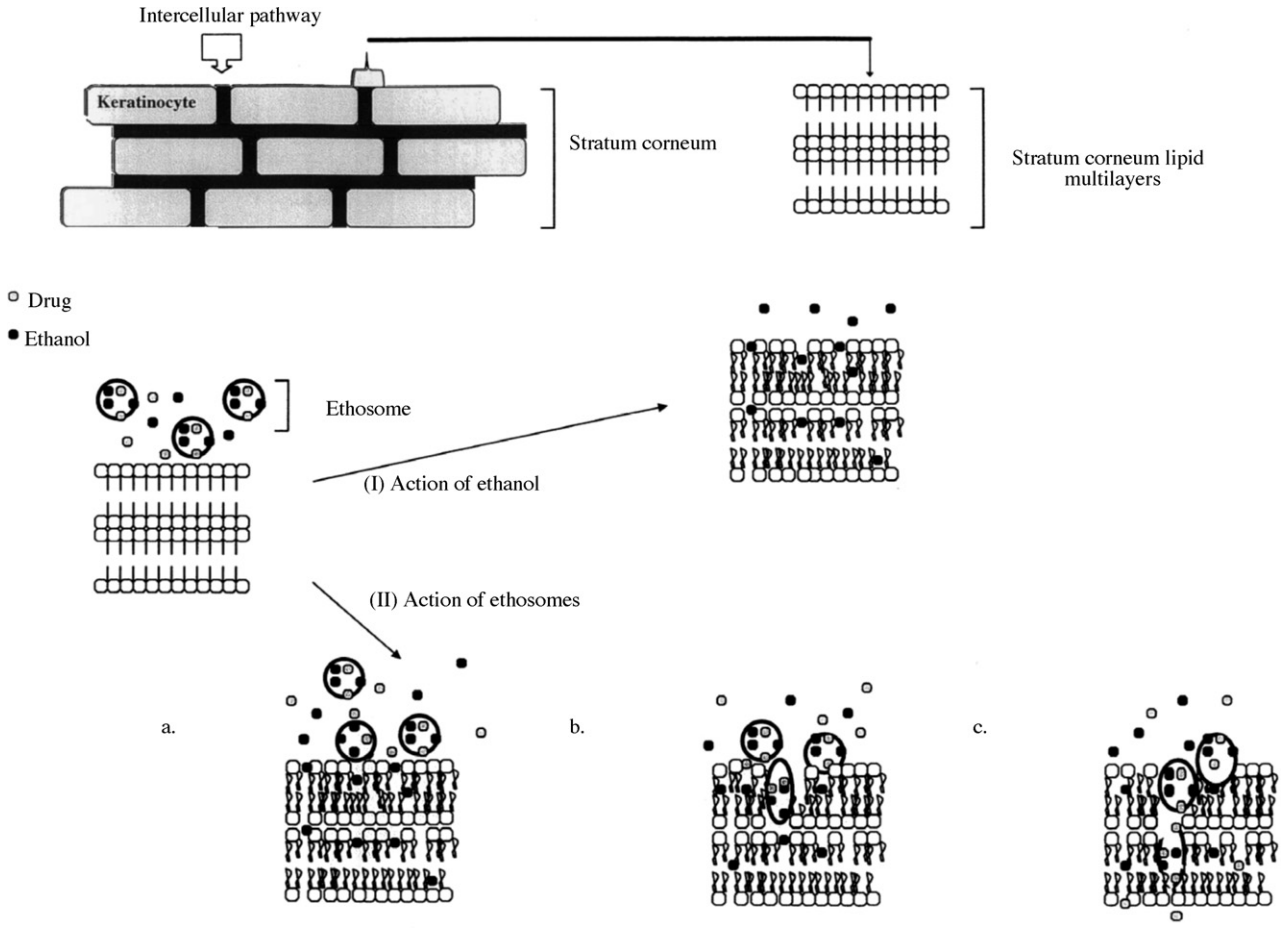

Fig. 4. Proposed model for skin delivery from ethosomal systems (from Touitou et al., 2000a).

study can further demonstrate whether improved skin delivery from ethosomes, relative to traditional liposomes, is a result of an additive ethanol penetration enhancing effect or a synergistic mechanism exists.

Fig. 4 illustrates a hypothetical model, suggested by Touitou et al. (2000a), of how ethosomes may enhance penetration of drugs through stratum corneum lipids. The stratum corneum lipid multilayers, at physiological temperature, are densely packed and highly conformationally ordered. Ethanol interacts with lipid molecules in the polar headgroup region, resulting in a reduction in the T_m of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar headgroup environment can result in an increase in the membrane permeability. In addition to the effects of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier. Ethanol may also provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into deeper layers of the skin. The interdigitated, malleable ethosome vesicles can forge paths in the disordered stratum corneum. The release of drug in the deep layers of the skin and its transdermal absorption could then be the result of fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway. In a recent study (Elsayed et al., 2006), ethosomes with ketotifen only entrapped inside the vesicles improved significantly ketotifen skin permeation over ethosomes having the drug both inside and outside the vesicles and over ethosomes with ketotifen only outside the vesicles. Ethosomes, with ketotifen only outside the vesicles, were not able to improve skin delivery of non-entrapped ketotifen relative to hydroethanolic solution. This suggested that ketotifen should be incorporated in the ethosomal vesicles for optimum skin delivery.

In summary, the effect of ethanol on stratum corneum lipids and on vesicle fluidity as well as a dynamic interaction between ethosomes and the stratum corneum may contribute to the superior delivery properties described.

5. Future perspectives

Studies will continue to further improve skin delivery of drugs using lipid vesicles. Special emphasis seems to be given for skin delivery of proteins and other macromolecules and for transcutaneous immunization.

The potential of lipid vesicles for transdermal iontophoretic drug delivery was first reported by Vutla et al. (1996). Few limited studies followed, investigating such approach, using either traditional or deformable liposomes (Fang et al., 1999; Essa et al., 2002, 2004; Boinpally et al., 2004; Han et al., 2004). The exact benefits remain unclear, as a result of paucity of reported investigations; however, it appears that combining iontophore-

sis with lipid vesicles facilitates drug delivery to deeper layers of skin including enhanced transfollicular delivery. Studies will continue to further investigate such a promising approach.

In our laboratory (Elsayed, 2006), a new liposomal system, the propylene glycol-embodying liposomes (PG-liposomes), composed of phospholipid, propylene glycol and water, was recently introduced, developed and investigated, as a carrier for skin delivery of drugs. PG-liposomes of the local anesthetic, cinchocaine, showed high entrapment efficiency and were relatively more stable than other liposomal formulations, including deformable liposomes and ethosomes. In vivo skin deposition studies, carried out using rabbit dorsal skin, indicated that cinchocaine PG-liposomes were superior, as carriers for skin delivery, relative to other liposomal formulations. PG-liposomes showed improved skin deposition, even relative to deformable liposomes and ethosomes, suggesting that PG-liposomes, recently developed, may have promising future as carriers for skin delivery of drugs (data to be published soon).

The near future may also hold the emergence of new commercial liposome-based topical products. IDEA AG, the biopharmaceutical company, developing targeted therapeutics based on the novel Transfersome® carriers, has an attractive portfolio of proprietary Transfersome® applications with its leading compound, IDEA-033, currently in phase III trials in Europe for the treatment of peripheral pain. IDEA-033 (∼100 mg of ketoprofen in Transfersome® gel) is expected to become the first, truly effective, topical analgesic on the market for treating peripheral chronic pain, such as that caused by osteoarthritis. Studies comparing safety and efficacy of IDEA-033 (applied epicutaneously, twice daily) with that of celecoxib 100 mg (oral, twice daily) in treating signs and symptoms of osteoarthritis of the knee indicated similar efficacy but with much lower systemic drug exposure (data obtained from IDEA AG; http://www.idea-ag.de/web/en/press_releases/PressRelease_2005_11_eng_text.html, November 2005, accessed 2 December 2006). IDEA-033 is expected to give the medical community an effective and safe alternative to oral non-steroidal anti-inflammatory drugs for suppressing pain associated with muscle conditions.

NTT, Novel Therapeutic Technology Inc., is another biopharmaceutical company, having a portfolio of pharmaceutical formulations based on ethosomes technology, including formulations for the treatment of alopecia, deep skin infection, herpes, hormone deficiencies, inflammation, post-operative nausea, atopic dermatitis and erectile dysfunction.

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