Expert Opinion

Vesicular carriers for dermal drug delivery

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The skin can offer several advantages as a route of drug administration although its barrier nature makes it difficult for most drugs to penetrate into and permeate through it. During the past decades there has been a lot of interest in lipid vesicles as a tool to improve drug topical delivery. Vesicular systems such as liposomes, niosomes, ethosomes and elastic, deformable vesicles provide an alternative for improved skin drug delivery. The function of vesicles as topical delivery systems is controversial with variable effects being reported in relation to the type of vesicles and their composition. In fact, vesicles can act as drug carriers controlling active release; they can provide a localized depot in the skin for dermally active compounds and enhance transdermal drug delivery. A wide variety of lipids and surfactants can be used to prepare vesicles, which are commonly composed of phospholipids (liposomes) or non-ionic surfactants (niosomes). Vesicle composition and preparation method influence their physicochemical properties (size, charge, lamellarity, thermodynamic state, deformability) and therefore their efficacy as drug delivery systems. A review of vesicle value in localizing drugs within the skin at the site of action will be provided with emphasis on their potential mechanism of action.

Keywords: dermal drug delivery, elastic and deformable vesicles, ethosomes, liposomes, niosomes

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1. Introduction

The skin forms an effective barrier between the organism and the environment, preventing invasion of pathogens and fending off chemical and physical assaults as well as the unregulated loss of water and solutes. It is made up of several anatomically distinct layers, namely the stratum corneum (SC), the viable epidermis (divided into the granulosum, spinosum and basale layers) and the dermis. Although the skin represents one of the major sites for non-invasive delivery of therapeutic agents, most drugs cannot penetrate into or through this organ owing to its impermeability [1].

The physical barrier is mainly localized in the SC and consists of protein-enriched dead cells (corneocytes with a cornified envelope and cytoskeletal elements as well as corneodesmosomes) and lipid-enriched intercellular domains (lamellar sheets composed of approximately equimolar concentrations of free fatty acids, cholesterol and long chain ceramides). The SC barrier function is dependent not on one single component but on its total architecture, which has been described by P. Elias as the 'bricks and mortar' model where the bricks are the cornecytes and the mortar refers to the lipid rich matrix [2]. The nucleated epidermis also contributes to the barrier through tight, gap and adherent junctions as well as through desmosomes and cytoskeletal elements. During epidermal differentiation, lipids are synthesized in the keratinocytes and extruded into the extracellular domains, where they form extracellular lipid-enriched layers. The cornified cell envelope, a tough protein/lipid

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polymer structure, resides below the cytoplasmic membrane on the exterior of the corneocytes. Ceramides A and B are covalently bound to cornified envelope proteins and form the backbone for the subsequent addition of free ceramides, free fatty acids and cholesterol in the lipid matrix of the SC. The lipids are organized as multiple lipid bilayers that form regions of semi-crystalline gel and liquid crystals domains [3].

Dermal and transdermal delivery requires efficient penetration of active compounds through the skin barrier by a passive diffusion process. A molecule applied on the skin surface may use two diffusional routes to penetrate: the transappendageal and the transepidermal routes.

The transappendageal route includes transport through the sweat glands and hair follicles with their associated sebaceous glands. Although these routes were traditionally considered of minor importance because of their relatively small area, recent research has indicated that the pilosebaceous units may contribute significantly to topical drug delivery by acting as a low resistance pathway for ions and large polar molecules that hardly permeate through the SC.

Moreover, the hair follicles and sebaceous glands are associated with various dermatological disorders such as acne, alopecia and several skin tumors. Therefore, there is a great interest in the pilosebaceous units as targets for localized drug delivery as well as shunts for transdermal delivery, even if the specific role of the follicular pathway in dermal drug absorption is difficult to explain owing to the lack of an adequate animal model to distinguish follicular and non-follicular transport [4].

Through the epidermis there exist two pathways: transcellular (across the corneocytes and the lipid matrix) and intercellular (across the lipid domains between the corneocytes). The structure of the skin and its barrier function have been extensively described in the literature, and it is generally accepted that the intercellular route provides the principal pathway for the permeation of most drugs [5,6]

Liposomal and niosomal vesicles are promising drug delivery systems for topical administration. The use of vesicles to deliver drugs to the skin can offer some advantages over classical topical dosage forms. The function of vesicles as transdermal or dermal delivery systems is controversial. Transdermal delivery occurs when a molecule diffuses through the skin layers and reaches the systemic circulation, while dermal delivery should only be used to define targeting to skin sites with minimal systemic absorption. Many authors report that specially designed vesicular carriers can be used to increase penetration and permeation through the SC for systemic delivery, but they can also be used to localize the drug in the skin layers for local treatment in the context of various skin diseases [7].

A review of vesicle value in localizing drugs within the skin at the site of action will be provided with emphasis on their potential mechanism of action. The use of vesicles as suitable carriers for transdermal delivery of drugs will be briefly reported as several exhaustive reviews on this topic have been written recently [7-10].

2. Vesicular carriers for dermal delivery of drugs

Vesicular carriers can be divided into two main classes: liposomes (vesicles mostly made up of phospholipids, discovered by Bangham in 1960 [11]) and non-ionic surfactant vesicles (discovered by L'Oreal in the 1970s and called niosomes [12]). In the last 15 years, new classes of lipid vesicles were introduced by different researchers. In 1992, Cevc and Blume [13] introduced the first generation of the highly deformable, elastic liposomes, referred to as Transfersomes® (IDEA AG, Munich, Germany). A second generation of elastic vesicles, mainly consisting of non-ionic surfactants, was introduced in 1999 by van den Bergh [14]. In 1997 Touitou *et al.* developed ethosomes [15], new soft vesicular carriers mainly consisting of phospholipids and ethanol, and in 2004 Fahr and colleagues developed invasomes [16,17], composed of phosphatidylcholine, ethanol and a mixture of terpenes as penetration enhancers.

2.1 Conventional vesicles

The term 'conventional' liposomes is generally used to distinguish classical phospholipid-based vesicles from the new vesicle formulations. Therefore, in this review, the term 'conventional' liposomes is used as an alternative to the term 'classic' liposomes to refer to the first class of vesicles prepared with any type of phospholipids. However, phospholipids used in liposomal formulations can vary greatly for type and purity. Indeed, besides liposomes prepared with pure phospholipids, several lecithin mixtures with different amounts of lysophospolipids and impurities are used. Lysophospholipids as well as fatty acids can modify vesicular bilayer properties in such a way that in several cases the features of 'conventional' liposomes may be very similar to those of elastic and deformable vesicles. In other words, components of the lecithin products, especially lysophospholipids, can act as 'edge activators' and, therefore, in these cases a clear distinction between 'conventional' and 'deformable' vesicles may be difficult.

The first investigation on the applicability of liposomes as carriers for both enhancing the skin concentration and lowering the systemic absorption of drugs was described by Mezei and Gulasekharam in 1980 [18]. They reported that the liposomal triamcinolone acetonide, when compared with a control lotion, provided higher drug deposition in rabbit skin (epidermis and dermis), lower concentration in the thalamic region (a possible site of adverse effect) and reduced urinary excretion. Later, in 1982, the same authors [19] demonstrated that treatment with the above liposomal formulation, incorporated in a hydrocolloid gel vehicle, provided higher dermal and epidermal triamcinolone acetonide concentrations when compared with the free drug incorporated in the hydrocolloid gel.

In 1988, in a US Patent [20], Mezei demonstrated that a number of liposomal formulations containing lipid-soluble drugs (such as econazole, progesterone and minoxidil) in a multiple-dose topical treatment provided a higher drug concentration in the skin of rabbits and guinea pigs and decreased biodisposition

in plasma and remote sites when compared with several conventional dosage forms (creams, lotions, ointments and pastes). The higher drug concentration in the skin was a result of a lower drug clearance and a decreased barrier property of the SC due to both the miscibility of the liposomal phospholipids with the skin lipids and the hydration of the keratin layer by liposomal occlusive action. Moreover, Mezei suggested that the epidermal lipid bilayers could serve as pathways for penetration of intact liposomes. These large multilamellar vesicles are able to protect the encapsulated drug from the metabolizing enzymes but cannot penetrate the blood vessels, therefore, reducing skin clearance (metabolism and uptake into the blood circulation) [21].

In 1985, Patel [22] found that topically applied liposomes may act as sustained release vehicles for drugs into the epidermis. After application of free and liposome methotrexate on the skin of nude mice, he observed that the entrapped drug was retained in the skin two- to threefold more than the free methotrexate, while the pattern for the blood concentration was the opposite, the liposomal form provided lower drug concentration than the free form.

From the 1980s onwards, the feature of conventional liposomes to act, when topically applied, as a skin drug 'localizer' rather than as a drug 'transporter' has been confirmed by several authors [7,8,10,23]. Among the great variety of candidates for liposome encapsulation (antifungal, antibiotics, disinfectant, immunosuppressive agents), there are three classes of drugs that are studied more often for dermal vesicular delivery: anesthetics, corticosteroids and retinoids [24].

The potential of liposomal local anesthetics to provide anesthesia when applied on the intact skin has been investigated extensively. In 1988 Gesztes and Mezei [25] obtained a prolonged local anesthetic effect by applying tetracaine in liposomes, whereas a commercial cream was ineffective.

In 1990, Foldvary *et al.* [26] studied the fate of liposome-encapsulated lidocaine after topical application to explain the mechanism by which vesicular carriers can provide higher drug delivery in the site of action (i.e., the skin) and lower blood concentration. Lidocaine applied on the forearm of human volunteers produced greater local anesthetic effect in the liposomal form than in a cream form (Dermabase®; Paddock, Laboratories Inc., Minnesota, USA). Moreover autoradiography demonstrated higher concentration of ^{14}C-lidocaine in the epidermis and dermis of guinea pigs treated with liposomal lidocaine as opposed to lidocaine in Dermabase cream. By means of electron microscopy, these authors observed the presence of intact liposomes in the skin, but only small unilamellar vesicles even when they applied multilamellar vesicles on the skin surface. From these results, they concluded that in the liposome–skin interaction process more than one event takes place. Multi- and unilamellar vesicles can be adsorbed intact to the skin surface before their penetration, but some liposomes can rupture on the skin surface. The penetration of smaller vesicles is more probable, but it is possible that the intradermally localized unilammelar or oligolamellar vesicles are derived from multilamellar liposomes that lost their outer bilayers during penetration.

After these first papers, the superiority of liposomal local anesthetic formulations over the conventional dosage forms has been demonstrated by several investigators [27-33]. In several research papers, Wohlrab and Lasch [34-36] studied the applicability of liposomal hydrocortisone as a selective drug delivery system for cutaneous administration of glucocorticoids. With the liposomal form, they obtained a considerably better concentration–time skin profile than with the corresponding hydrocortisone ointment as well as a decreased serum concentration and urinary excretion of hydrocortisone. To explain their results, these authors also investigated the mechanism by which the penetration of liposomal drugs is promoted and, in particular, they attempted to visualize how deep intact liposomes can penetrate into human skin by a fluoromicro graphy technique. They concluded that intact liposomes are confined only in the horny layer and do not penetrate deeper. As for the mechanism responsible for the liposomal drug penetration, they suggested that the liposomes produce a fluidization of the intercellular domains in the SC [37].

Interesting results for corticosteroid dermal delivery were found by Fresta and Puglisi [38], who prepared unilamellar skin-lipid liposomes as carriers for three different corticosteroids: hydrocortisone, bethametasone and triamcinolone. In this study the authors pointed out that several factors, such as lipid composition, size and thermodynamic state of the lipid bilayer, can strongly affect skin deposition behavior of conventional liposomes. Vesicles were made up of phospholipids or skin lipids (SC lipid-based liposomes) and radiolabeled in the bilayer structures and in the aqueous compartment. The results obtained showed that SC lipid-based liposomes could permeate the SC to a greater extent than phospholipid liposomes, deliver a greater amount of drug to the deeper strata (epidermis and dermis) and avoid systemic absorption and, hence, organ distribution and renal elimination. Vesicle size is important in determining the extent of absorption: the greater the liposome mean size the poorer the permeation. Finally, when fluid liposomes made up of unsaturated lecithins were used, a percutaneous absorption was obtained instead of the dermal delivery [39].

The assumption that a decrease in the particle size of the liposomes would result in an increase of the amount of drug found in the deeper skin strata supports the concept of intact vesicular penetration as one of the possible mechanisms for improved skin accumulation. On the contrary, Esposito *et al.* [40] reported that the permeability coefficient of methyl nicotinate is inversely related to the liposome size, and Du Plessis *et al.* [41] showed that the intermediate particle size of 300 nm resulted in the highest reservoir in the deeper skin layers, a fact that confirms that topical drug delivery is influenced by the size of liposomes [42] but also suggests that intact liposomes do not penetrate the skin.

Several conventional liposome formulations have been developed to improve physicochemical and biological properties

of retinoids. Different investigators reported an increased skin accumulation of *trans*-retinoic acid *in vitro* and a reduced irritancy *in vivo* after treatment with these carriers. Foong *et al.* [43] reported greater tretinoin concentration in the epidermis and dermis after application of liposomal formulations compared with conventional creams. In one report, Masini *et al.* [44] found that the percentages of tretinoin in the epidermis and dermis (*in vitro* studies) were significantly higher with liposomes than with gel formulations. Furthermore, Schäfer-Korting *et al.* [45] demonstrated that liposomal formulations with lower tretinoin concentrations than commercial topical preparations had the same efficacy and lower skin irritancy in humans. Recently Sinico *et al.* [46] showed that tretinoin skin penetration and permeation is influenced by vesicle composition and structure. In this study the authors compared *trans*-retinoic acid dermal delivery from liposomes prepared with low and high transition temperature phospholipids using soy phosphatidylcholine and hydrogenated soy phosphatidylcholine. Therefore, at the temperature of the skin (i.e., 32°C) liposomal bilayers should be in a different thermodynamic state.

Experiments of dermal delivery were performed *in vitro* in occlusive conditions and in comparison with three different controls: a hydroalcoholic solution, an oil solution and a commercial formulation of *trans*-retinoic acid (Retin-A®; Janssen-Cilag, Milan, Italy). Furthermore, transmission electron microscopy (TEM) in combination with osmium tetroxide (OsO_4) was used to visualize the pig skin structure after the liposomal administration. Results of this study showed that vesicle size and lamellarity did not affect tretinoin delivery through the pig skin. In fact, for each composition the permeation profile was very similar for both multi- and unilamellar vesicle dispersions. These results seem to confirm the findings of Du Plessis *et al.* [41], which suggest that intact penetration of liposomes does not occur. Moreover, the authors pointed out some interesting data related to vesicle composition and charge. Surprisingly, they obtained higher drug permeation when *trans*-retinoic acid was delivered in vesicles made from phospholipids with the higher Tc (transition temperature), while drug retention into the skin was favored when the main component of liposomes had a Tc lower than skin temperature. It is well recognized that the thermodynamic state of the liposomal bilayers plays a fundamental role in the drug transport rate through the skin *in vitro*. Incorporation of drugs in gel-state liposomes is known to result in a slower skin permeation rate than in fluid state vesicles [47]. However, it is also well known that cholesterol content may play a crucial role for the effective delivery of liposomal substances into the skin [48]. Results obtained during this work were explained as the consequence of the presence of both cholesterol and the amphipathic drug tretinoin in the liposomal bilayers. TEM analyzes in this work did not show any evidence of intact vesicular structures in the pig skin, but only vesicle adsorption or mixing and fusion with the SC surface. Adsorption and fusion of vesicles on the skin surface, which resulted in formation of lamellae and rough structures on the top of the outermost corneocytes, had already been demonstrated by other researchers [49,50]. This behavior could increase the driving force for permeation of released molecules. However, vesicles could collapse on the skin surface, forming an additional barrier that can reduce permeation of the hydrophilic drug encapsulated in the aqueous liposomal core.

Although, in the last decades, research concerning lipid-based vesicles as suitable carriers to overcome the skin barrier has been centered on phospholipid systems, the advantages offered by non-ionic surfactant systems (i.e., lower costs, higher stability and greater availability of surfactant classes) have led researchers to the investigation of niosomes as an alternative.

Niosomes seem to be an interesting drug delivery system in the treatment of dermatological disorders because their topical application can increase the residence time of drugs in both the SC and the epidermis, while reducing the systemic absorption of the drug. They are thought to improve the horny layer properties, both by reducing transepidermal water loss and by increasing smoothness through replenishment of lost skin lipids [51]. Since the first reports from L'Oreal laboratories in the 1970s [12,52], much research has been carried out into the vesicle forming ability of an ever increasing number of amphiphilic lipids, with different chemical structures and different composition of the hydrophilic and hydrophobic moiety [53-61].

In 1994, an interesting investigation on the permeation of estradiol from vesicular formulations composed of non-ionic *n*-alkyl polyoxyethylene ether surfactants was carried out by Hofland *et al.* [62]. When the skin was pre-treated with empty vesicles, the authors observed significantly higher estradiol fluxes compared with untreated SC. However, estradiol fluxes obtained in pre-treatment experiments appeared to be significantly lower than those obtained by the direct application of the estradiol-saturated vesicles. From these results, two mechanisms are proposed to play an important role in vesicle–skin interactions, namely a penetration enhancing effect of surfactant molecules and a direct effect of the vesicular structures that are most probably caused by adsorption of the vesicles at the SC–suspension interface.

The mechanism of interaction of niosomes with the skin has been extensively studied by several authors [47-51] and it has become clear that the same penetration mechanisms assumed for liposomes may apply to niosomes.

In the literature we can find several works comparing liposomes and niosomes as carriers through the skin [63-67]. In 2001 Agarwal *et al.* [65] developed dithranol-entrapped liposomal and niosomal systems for topical application. The results of an *in vitro* permeation study clearly showed the enhancing effect on drug penetration and permeation of both liposomal and niosomal encapsulation in comparison with a conventional cream. Furthermore, the permeation of dithranol is observed to be more pronounced in the case of liposomes with respect to niosomes. The authors explained the improved

skin penetration of the drug and the consequently enhanced drug transport using liposomes and niosomes on the basis of the presence of drug molecules in a solubilized state. Moreover, they supposed that the higher permeation of dithranol in the case of liposomes reflects the influence of vesicle composition on drug transport through the skin. They claimed that liposomal phospholipids can be regarded as natural constituents of skin lipids (a fact that is at least controversial) and, possibly for this debatable reason, proved to be better in generating and retaining the required physicochemical state of the skin for enhanced permeation. This may be attributed to the ability of phospholipids to form vesicles alone, owing to the presence of two non-polar lipid chains and a polar head group in their molecular structure, which help the spontaneous self-assembly into closed bilayer systems [48,68]. Moreover, this improves their capacity to produce a long-lasting effect. Niosomes may serve well in the initial stages but are not better than liposomes as their constituents (i.e., non-ionic surfactants) are not structured to spontaneously self-aggregate to form vesicles. They require other components such as cholesterol to modulate the critical packing parameter in order to acquire the shape, and once deformed during the penetration process may not regain the same required structures. This explains why niosomes are comparatively less efficient in sustaining the suitable skin status for drug transfer. In contrast, some different results were found by Manconi *et al.* in a paper [66] where they compared cutaneous delivery of tretinoin incorporated in both niosomes and liposomes with a commercial formulation of the drug. In this work, niosomes were prepared using two different alkylpolyglucosides (octyldecylpolyglucoside Oramix®CG110 and decylpolyglucoside Oramix®NS10), which vary in terms of different grades of polymerization and the nature of the lipophilic moiety, while liposomes were made of soy phosphatidylcholine. Data obtained for *in vitro* penetration and permeation showed that composition of niosomes is very important for improving cutaneous or transdermal delivery of a lipophilic drug such as *trans*-retinoic acid. In fact, while Oramix®NS10 (hydrophilic–lipophilic balance (HLB) = 11) vesicles gave the highest accumulation values and a low permeation rate, Oramix®CG110 (HLB = 16) niosomes showed higher fluxes and very low accumulation values. This study gave evidence that interactions between skin and vesicles mainly depend on physicochemical properties of the major component of the vesicular bilayer. As discussed by other authors [51], the polar moiety of the amphiphile plays the main role with respect to interaction with skin lipids. Oramix®CG110, owing to a very strong hydrophilic head group, is not able to penetrate significantly into the SC. However, when penetration occurs its highly polar head can strongly perturb the SC intercellular lipid bilayer giving rise to a facilitated pathway for the drug, which can reach the dermis and receiver compartment more easily and in a greater quantity. In 2002, Carafa *et al.* [67] carried out a study focused on a novel formulation of niosomes entrapping lidocaine in the form of a free base (LID) and a hydrochloride (LIDHCl). Vesicles were prepared from polyoxyethylene sorbitan monolaurate (Tween 20) and cholesterol. Permeation of LIDHCl-loaded vesicles through mouse abdominal skin showed a higher flux and a shorter lag time with respect to classical liposome formulations, while LID permeation rate was quite similar for niosomes and liposome formulations.

Although most topical liposome research, as extensively reported above, have focused on drug deposition into the skin layers, several studies have also demonstrated the possibility of obtaining a drug depot localized in the skin appendages via liposomes. In particular, different authors reported that liposomes were capable of enhancing the topical delivery of small hydrophilic compounds, which selectively accumulated in the pilosebaceous units. Carboxyfluorescein in liposomes was targeted into the hair follicles of hamster ears providing better deposition than aqueous solutions of the dye, even when these solutions contained penetration enhancers (propylene glycol, sodium lauryl sulfate, ethanol) or a mixture of the same lipids used to form the liposomes [69]. Similar results were obtained by incubating mouse skin histoculture, complete with hair follicles, with an aqueous calcein solution and liposome-entrapped calcein [70]. Also the deposition of γ-IFN into the skin of humans, hairless mice and hamsters was greater from liposomes compared with aqueous solutions. Moreover, the greatest appendage deposition was observed in skin with the highest follicular density, namely the hamster skin, suggesting the follicular pathway as the preferential route for liposomal delivery of hydrophilic drugs [71].

All these studies also show that skin deposition behavior of conventional vesicles is affected by several factors such as lipid composition, vesicle structure and the thermodynamic state of the lipid bilayer. However, the experimental conditions (i.e., working in occlusive or non-occlusive conditions, selection of skin membrane, receptor fluid composition and evaluation technique) can also modify the fate of the topically applied vesicular drug. In particular, working in occlusive or non-occlusive conditions can influence the penetration depth of the vesicular drug. In fact, it has generally been reported that occlusive conditions are likely to enhance drug accumulation into the skin layers while they are detrimental to transdermal drug delivery [47,78].

2.2 Elastic and deformable vesicles

Over the last two decades, the lack of ability of conventional liposomes to deliver drugs across the skin has led to intensive research in the field with the introduction and development of new classes of lipid vesicles. In the early 1990s, the so-called elastic, highly deformable or ultraflexible vesicles were introduced with the aim of improving (*trans*)dermal drug delivery.

In particular, Transfersomes, introduced by Cevc *et al.* [13,68,72,73], were the first generation of this new class of lipid vesicles. As with conventional liposomes, they mainly consist of phospholipids but also contain a surfactant (sodium cholate, sodium deoxycholate, different Spans and Tweens,

dipotassium glycyrrhinizate) that acts as an 'edge activator' capable of destabilizing the lipid bilayer thus increasing its deformability. Transfersomes are prepared by the same techniques as traditional liposomes, with the lipid film hydration method being the most used.

Deformable vesicles have been reported to penetrate intact skin *in vivo*, carrying therapeutic concentrations of drugs (including macromolecules), with an efficiency similar to subcutaneous administration, which only occurs when the elastic vesicles are topically applied in non-occlusive conditions [73-75]. Cevc *et al*. have reported that Transfersomes can act as carrier systems because of their high deformability and adaptability, which allow them to squeeze through small pre-existing channels in the SC despite their larger size [13,73,76]. The authors also suggested that the driving force for skin penetration of deformable vesicles is the osmotic gradient across the skin. Topically applied Transfersomes dehydrate on the skin surface by evaporation and thus an osmotic pressure difference is originated between the highly hydrated regions inside the skin and the dehydrated skin surface, which makes Transfersomes enter the SC moving into the deeper skin strata to avoid dehydration [74,75]. Therefore, occlusive conditions are detrimental to the Transfersome's capability to deliver drugs through the skin, as in this situation the osmotic effect would be removed. Another possible explanation was recently hypothesized by El Maghraby *et al*. : over-hydration of the skin can swell the corneocytes and thus close or at least minimize the size of shunts that may play a role in liposomal skin delivery [77].

Using confocal laser scanning microscopy, Schatzlein and Cevc [76] suggested two different penetration pathways: the intercluster and intercorneocyte pathways (honeycomb-like system). Within the intercellular lipid lamellae, these regions contain structural irregularities that can act as 'virtual channels' through which the deformable vesicles can penetrate because of their high flexibility owing to the edge activator molecules [76]. The authors also argued that intact deformable liposomes penetrated through the SC and viable epidermis into the blood circulation [13]. However, Bouwstra and Honeywell-Nguyen [78] noticed that even in a fully hydrated state, the water content in the lowest SC layers close to the viable epidermis is much lower than in the central regions of the SC. Thus, they suggested that, as a result of the osmotic force, vesicles will not penetrate beyond the level of the lowest SC layers (see below).

Deformable vesicles have been studied for transdermal delivery of several important drugs that also do not have the physicochemical properties required to be delivered across the skin (e.g., insulin) [73,79]. However, transdermal drug delivery is beyond the scope of this review and, as written above, these studies have been exhaustively reviewed by different authors recently [7,10,77,80,81].

Although most studies have reported that highly deformable liposomes are especially able to enhance transdermal drug delivery, this class of lipid vesicles has also been reported to improve *in vitro* drug skin deposition with a higher effectiveness than conventional vesicles for dermal drug delivery [82-90].

Several studies on skin delivery from elastic liposomes were carried out by Barry's group [82-85]. In particular, in 2001 the authors compared deformable vesicles and conventional liposomes as skin delivery systems for 5-fluorouracil (5-FU). Results showed that the Transfersomes were better than liposomes for *in vitro* skin delivery of 5-FU and also that they were able to only improve skin deposition without any appreciable effect on drug permeation. The limited drug partitioning into the receptor phase led the authors to conclude that deformable vesicles are not acting as carrier systems. Moreover, the percentage of 5-FU that penetrated the skin was higher than the drug entrapment efficiency, which suggests that vesicle components may have altered the skin structure, thus enhancing 5-FU diffusion through the human epidermis. In conclusion, the authors suggested a possible penetration enhancing mechanism and concluded that these vesicles are useful only for dermal delivery of this drug [85].

Trotta *et al*. prepared and tested deformable liposomes containing soy phosphatidylcholine or hydrogenated lecithin mixed with dipotassium glycyrrhizinate (KG), a compound with emulsifying properties extracted from the liquorice root [86]. The presence of KG increased liposome elasticity as shown by extrusion experiments through pores of poly carbonate membrane with a diameter three times smaller than vesicle size. *In vitro* permeation and skin deposition studies carried out using full-thickness pig ear skin showed that KG skin fluxes were negligible while skin deposition increased 4.5-fold when compared with aqueous solution. Therefore, they also concluded that deformable vesicles are useful only for dermal drug delivery.

In accordance with what had been reported for lecithin: cholate vesicles by Kirjavainen *et al*. [91], the authors suggested two possible reasons to explain the obtained results. First, the liposomes are able to penetrate through the interstices of the SC under the influence of the transcutaneous hydration force. Second, the enhancement effect could also be due to fusion of vesicles with the skin, thanks to the increased fluidity of the lipid bilayers containing KG.

These authors also studied the same deformable vesicles as carriers for skin delivery of methotrexate. At the end of the skin permeation assays using the deformable liposomes, up to 50% of the applied dose of methotrexate was found in the skin, suggesting that liposomes containing KG could be of value for topical administration of methotrexate in the treatment of psoriasis. This capability was suggested to be dependent on the self-regulating carrier deformability [87]. More recently, Trotta *et al*. studied other elastic vesicle formulations by using different molecules as edge activators such as sucrose monopalmitate, dodecylcarbonate γ-cyclodextrin as well as sodium cholate, KG and polysorbate 80. Formulations were tested for topical delivery of different model drugs (i.e., acyclovir, progesterone, α-tocopherol) [88-90]. In all these studies, an enhanced skin deposition of the tested drugs was found

in comparison with conventional vesicles. Moreover, the entrapment of α-tocopherol in elastic or conventional liposomes increased the drug photostability thus confirming the capability of lipid vesicles to protect the entrapped drugs [92].

Elsayed *et al.* investigated traditional liposomes, deformable liposomes and ethosomes as carriers for skin delivery of a hydrophilic model drug, ketotifen fumarate (KT) [9,93]. Deformable vesicles were prepared with soy phosphatidylcholine and Tween 80 as the edge activator. Results showed that conventional liposomes only improved skin deposition of KT while both ethosomes and deformable liposomes improved skin delivery of KT with greater improvement of drug skin deposition than drug skin permeation [93]. To shed some light on the possible mechanism by which deformable liposomes and ethosomes could improve skin delivery of KT under non-occlusive conditions, these authors investigated *in vitro* permeation and skin deposition behavior of the deformable liposomes and ethosomes using rabbit pinna skin [9]. For this purpose, Elsayed *et al.* tested formulations having the drug both inside and outside the vesicles (DL-In/Out), having KT only inside (DL-In) and having KT only outside (DL-Out). Results showed that only DL-Out significantly improved both KT skin permeation and accumulation in comparison with DL-In. Moreover, DL-Out significantly improved drug skin deposition over DL-In/Out with only a slight improvement in KT permeation. In addition, DL-In did not improve significantly ($p > 0.05$) skin deposition and especially skin permeation over aqueous control. All these results led the authors to conclude that in the enhanced skin delivery of KT by deformable liposomes the main mechanism would be the penetration enhancing effect. However, the authors did not exclude intact vesicle penetration into the SC but they suggested that one of the two mechanisms might predominate according to the physicochemical properties of the drug. For hydrophilic drugs, such as KT, the penetration enhancing effect seems to play a more important role in enhanced skin delivery than in the case of lipophilic drugs, as permeation of hydrophilic molecules tends to be relatively slower and hence more enhanceable [9].

Recently, El Maghraby *et al.* studied new deformable vesicles in which penetration enhancer molecules (i.e., oleic acid and limonene) were used as edge activators [94] and a new kind of elastic vesicle, called invasomes, were introduced by the Verma and Fahr groups [16,17,95]. Invasomes are vesicles composed of phosphatidylcholine, ethanol and a mixture of terpenes as penetration enhancers. Invasomes containing 3.3% ethanol and 1% of a terpene mixture (cineole:citral:D-limonene = 45:45:10) were able to significantly enhance skin penetration and SC deposition of the highly hydrophobic photosensitiser temoporfin (mTHPC) when compared with liposomes without terpenes and conventional liposomes. Invasomes were also shown to be efficient in delivering mTHPC to deeper skin layers [16,17]. Successively, the same authors developed new mTHPC-loaded invasomes to further enhance the drug penetration. The ratio between D-limonene, citral and cineole was varied in the standard terpene mixture and single terpenes were also used. Invasomes containing 1% cineole provided the highest drug deposition in the SC and deeper skin layers, indicating that incorporation of a single terpene into invasomes could also give efficient nanocarriers for mTHPC [95]. These results have been explained as the consequence of a synergistic effect of liposomes, ethanol and terpenes. The authors proposed the following mechanism: one part of the vesicles is fragmented during their penetration into the upper skin layers and the released terpenes and phospholipids, which may act as penetration enhancers, fluidize the intercellular lipids also with the contribution of ethanol. Ethanol and terpenes in invasomes are proposed to increase the invasome flexibility (i.e., deformability), which was shown by the presence of deformed vesicles using cryoelectron microscopy. The perturbed organization of SC lipids, the high deformability of the invasomes and the presence of the transepidermal osmotic gradient might facilitate the penetration into the SC of some small intact invasomes, which were not fragmented during their penetration. According to previous studies by the same group [16,96], some of the small invasomes may have either reached deeper SC layers intact or passed through the SC following small hydrophilic channels present in the intercellular space of the SC. Or the invasomes may have followed the follicular transport pathway, as suggested by other authors [75,76,97].

In 1999, van den Bergh *et al.* introduced a new generation of elastic vesicles: the elastic, non-ionic surfactant vesicles composed of the bilayer-forming surfactant L-595 (sucrose laurate ester) and micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester) [14]. Incorporation of a micelle forming surfactant would result in a destabilization of the lipid bilayer, thereby increasing the vesicle elasticity [98]. These deformable niosomes were shown to be more effective than rigid vesicles in enhancing the penetration of $3H_2O$ across hairless mouse skin *in vitro* [98] and pergolide, lidocaine and rotigotine across human skin *in vitro* [99-102]. Results obtained with pergolide and rotigotine suggested that elastic vesicles are better than rigid vesicles in enhancing the transport of drug molecules. Moreover, results showed that for optimal delivery drug molecules must be entrapped within the deformable non-ionic surfactant vesicles. Consequently, this indicated that elastic vesicles act as drug carrier systems and not solely as penetration enhancers.

Using human skin, van de Bergh *et al.* also investigated the elastic vesicle transport. They examined the *in vitro* penetration pathways of a lipophilic fluorescent label linked to the deformable non-ionic surfactant vesicle formulations and rigid vesicles [103]. This study demonstrated that the elastic vesicles could alter the penetration pathway of the fluorescent label. Moreover, the results gave the evidence of vesicle material transport through thread-like channel structures but van den Bergh *et al.* visualized a much finer network of channels than the honeycomb-like system of intercluster and intercorneocyte pathways described by Schatzlein and Cevc [76].

Interestingly, the microscopic images obtained using the non-ionic surfactant vesicles illustrated that the lipophilic fluorescent label was always confined to the SC. This suggests that the components of deformable, non-ionic surfactant vesicles do not travel beyond the SC thus contradicting remarks by Cevc *et al.* that Transfersomes could even reach the systemic circulation [13]. Therefore, once the elastic vesicles partition into the SC, the drug is released and only the free molecules can diffuse and reach the inner skin strata [103,104].

This suggestion was also confirmed by an *in vivo* study carried out by Honeywell-Nguyen *et al.* to assess the distribution profiles of elastic and rigid vesicle material in human skin [105]. For this purpose, ketorolac-loaded elastic and rigid vesicle formulations were applied in non-occlusive conditions. A deuterium-labeled phospholipid was incorporated into these vesicles as a marker for the vesicle material. Data obtained by tape-stripping showed that elastic vesicle material can rapidly enter the deeper layers of the SC and can reach almost the SC-viable epidermal junction, in contrast to rigid vesicle material that did not penetrate deep into the SC. Furthermore, the elastic vesicles were better than the rigid vesicles in the enhancement of ketorolac transport into human SC. The distribution profile of ketorolac in the deeper SC layers was, however, different from that of the vesicle material. This suggested that once the elastic vesicles partition into the SC, ketorolac is released from the vesicles. The elastic vesicles are superior to the rigid vesicles in terms of both vesicular transport into the SC and therapeutic potential as a skin delivery vehicle [105].

In summary, the studies described above clearly show that elastic vesicles have higher properties than conventional vesicles in terms of both interactions with animal and human skin and improved skin delivery of different molecules. Although the exact mechanism by which elastic vesicles are able to transport molecules into and through the skin is still unclear, two main mechanisms have been proposed. First, intact vesicles enter the SC carrying their content into and through the SC. Second, vesicles act as penetration enhancers: vesicle bilayers enter the SC modifying intercellular lipid lamellae and hence facilitate penetration of free drug molecules into and across the SC. As mentioned above, the first mechanism was suggested by Cevc and several other studies support that deformable vesicles as well as surfactant-based elastic vesicles may act as carrier systems. Results obtained by several authors allow us to conclude that elastic vesicles could penetrate intact into the skin to a certain extent. However, the question that still needs a definite answer is how deep the vesicles penetrate into the skin. Although many studies have been supporting the penetration enhancing effect, others do not exclude both mechanisms.

2.3 Ethosomes

A new kind of flexible vesicle was introduced by Touitou *et al.* [15,106,107]. These vesicles are very different from other lipid vesicles in their composition, structure, mechanism of action and delivery properties. Ethosomes – soft lipid vesicles mainly composed of phospholipids, ethanol and water – may be uni- or multilamellar vesicles, with mean size ranging from 30 nm to microns. Their name was chosen to emphasize the presence of high concentrations of ethanol (20 – 45%). Owing to the interdigitation effect of ethanol on lipid bilayers, it was thought that high concentrations of ethanol are destructive to liposomal structures. However, the existence of vesicles as well as the ethosome structure was demonstrated by several techniques including ^{31}PNMR, TEM and scanning electron microscopy. Because of the presence of alcohol, ethosomes are able to entrap molecules with different physicochemical properties with high entrapment efficiency [106]. It has been shown that the physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the SC into the deeper layers of the skin than conventional liposomes *in vitro*, in animals and clinical studies [106-109]. Furthermore, the ethosomal carrier is also able to provide an effective intracellular delivery of both hydrophilic and lipophilic molecules [110] and facilitate the penetration of an antibiotic peptide (i.e., bacitracin) within fibroblast cells [111]. Enhanced delivery of hydrophilic and lipophilic fluorescent probes was observed when compared with different controls (hydroalcoholic solutions, liposomes and ethanolic phospholipid solutions). Indeed, ethosomal calcein and rhodamine were able to penetrate deeper into nude mouse skin than the controls [106,110,111]. Moreover, ethosomes were able to deliver highly lipophilic molecules such as cannabinoids, testosterone and minoxidil through the skin [106-108] as well as polypeptides and proteins such as insulin [108,112].

Using differential scanning calorimetry and fluorescence anisotropy Touitou *et al.* gave the evidence of the soft malleable structure of these vesicles, suggesting that it could be related to the fluidizing effect of ethanol on the phospholipid bilayers [106,111-113].

Moreover, the alcohol interferes with lipid organization of the SC and a mechanism for enhanced skin delivery by ethosomes was proposed. In particular, the authors suggest that the fluidizing effect of ethanol on the lipid bilayers of the SC together with the softness of the ethosomal carrier gives them the capability to penetrate the perturbed SC lamellae more easily thus promoting delivery of the actives into the deep layers of the skin and through the skin. Therefore, the soft vesicles penetrate the fluidized bilayers of the SC creating a pathway through the skin [106]. These claims are supported by a work of Elsayed *et al.* where, working in non-occlusive conditions, it was shown that the capability of ethosomes to improve skin delivery of ketotifen was confined to the case where the drug is incorporated within the ethosomal carriers [9]. For these reasons, ethanol's fluidizing properties on the ethosomal bilayers, together with their induced increased flexibility, seem to substantiate the fact that ethosomes tend to penetrate into deeper skin layers more easily.

To investigate the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate, useful for the

treatment of various inflammatory based skin diseases, Paolino *et al.* studied ethosomes made with phospholipids and ethanol at various concentrations [114]. Percutaneous permeation of ethosomal ammonium glycyrrhizinate was evaluated *in vitro* under occlusive conditions and through human SC and viable epidermis membranes in comparison with drug solutions in water or in a water–ethanol mixture and a physical mixture of free ethosomes incorporated in a water–ethanol drug solution. Experiments to evaluate both the *in vivo* efficacy of the ethosomal ammonium glycyrrhi zinate and the prolonged release properties of this carrier were also carried out. Results showed that ethosomes increased the percutaneous permeation of ammonium glycyrrhizinate both *in vitro* and *in vivo*; thus improving the anti-inflammatory activity of this drug in an *in vivo* model of skin erythema. In addition, *in vivo* experiments showed that ethosomes can ensure a sustained release of the drug and hence a prolongation of its therapeutic activity, which can be related to an accumulation of ammonium glycyrrhizinate in the skin [114].

Recently, interesting *in vitro* and *in vivo* studies have shown ethosomes to be efficient carriers for erythromycin delivery to bacteria localized within the deep skin strata with eradication of staphylococcal infections [113,115]. Moreover, therapy with ethosomal erythromycin applied to the skin of *Staphylococcus aureus*-infected mice was as effective as systemically administered erythromycin, suggesting a new possibility to treat deep dermal infections by local application of antibiotic in the ethosomal carrier [115].

Recently, Dubey *et al.* investigated ethosomes as carriers for topical delivery of the highly hydrosoluble, anti-psoriac drug methotrexate. *In vitro* skin permeation experiments through dermatomed human cadaver skin showed that ethosomes were able to enhance both methotrexate flux and skin deposition in comparison with the controls (hydroethanolic solution, conventional liposomes and plain drug solution). The vesicle skin interaction study also highlighted the penetration enhancing effect of ethosomes with some visual penetration pathways and mild swelling of corneocyte [116].

Finally, an interesting difference with elastic, deformable vesicles has to be underlined: ethosomes have been shown to enhance dermal and transdermal delivery of drugs both under non-occlusive [9,107] and occlusive conditions [114,116].

As a result of the above mentioned literature, all studies carried out with ethosomes have shown that these vesicles enable efficient transport of different active compounds into the deep strata and through the skin.

3. Expert opinion

Whether, and to what extent, vesicular carriers can provide an efficient dermal delivery system is a rapidly growing research topic, which, at present, animates an interesting scientific debate because several conflicting results on this argument have recently been published. By way of example, even the definite mechanism whereby vesicle dermal drug delivery is achieved represents one of the most controversial issues in skin research. Having said that, it is a fact that vesicular systems provide variable effects according to their composition (i.e., liposomes, niosomes, ethosomes, elastic and deformable vesicles), method of preparation, entrapped drug and skin type. This variability makes vesicles a highly efficient carrier for delivering drugs to the skin for different target sites, ranging from the skin surface to the systemic blood circulation. A variety of possible mechanisms have been proposed for the enhanced skin delivery of drugs from vesicles. They include the intact vesicular penetration, the penetration enhancing effect the adsorption and fusion of liposomes on the skin surface and the vesicle penetration through the transappendageal route.

As for conventional liposomes, the possibility that intact vesicles penetrate deeper than the SC seems to be acceptable only in presence of a diseased skin. We believe that liposomes may strongly affect the structure of the intra- and intercellular regions of the healthy skin by adsorption on surface and both fusion and mixing with the SC lipid matrix. Thus, in our opinion, these carriers should be used especially in the local treatment of skin disorders. To support our point of view, we observe that most topical liposomal formulations, which have been developed from the research to the industrialization phase, carry drugs mainly targeted to the dermal region of the skin. In the late 1980s, the first liposome-based topical formulation available in the market (Pevaryl Creme, Janssen-Cilag, Buckinghamshire, UK) contained the antimycotic agent econazole. Shortly afterwards, several other liposome formulations for dermal delivery of local anesthetic, antifungal, anti-inflammatory and anticancer drugs were developed and produced, confirming the appeal of these products for pharmaceutical companies.

As for ultradeformable vesicles, several authors reported the ability of these structures to penetrate the healthy skin thus enhancing both drug deposition and permeation, although it remains a difficult task to evaluate the real extent of the penetration. Because of their elasticity and deformability, they seem to be a useful carrier for transdermal delivery of drugs. More recently, IDEA AG, a pharmaceutical company that owns Transfersomes technology, has been testing in the clinic the deformable vesicles for the targeted transdermal delivery of anti-inflammatory drugs. Ethosomes too can penetrate the skin and improve dermal, transdermal and follicular drug delivery. Pharmaceutical formulations based on ethosomes technology have been developed by Novel Therapeutic Technology, Inc., for the treatment of several diseases (i.e., alopecia, erectile dysfunction, dermatitis) by topical application of drugs.

In conclusion, we believe that the topical use of lipid vesicles can support a large variety of relevant developments and medical applications because their delivery enhancing properties can be easily modulated by changes in composition

and structure. Moreover, vesicles as dermal drug delivery systems provide plenty of opportunities for innovative research aimed at both increasing efficiency and reducing toxicity of drugs through simple topical application.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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