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Transfersomes for transdermal drug delivery

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Transfersomes® (Idea AG) are a form of elastic or deformable vesicle, which were first introduced in the early 1990s. Elasticity is generated by incorporation of an edge activator in the lipid bilayer structure. The original composition of these vesicles was soya phosphatidyl choline incorporating sodium cholate and a small concentration of ethanol. Transfersomes are applied in a non-occluded method to the skin and have been shown to permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. They have been used as drug carriers for a range of small molecules, peptides, proteins and vaccines, both *in vitro* and *in vivo*. It has been claimed by Idea AG that intact Transfersomes penetrate through the stratum corneum and the underlying viable skin into the blood circulation. However, this has not been substantiated by other research groups who have extensively probed the mechanism of penetration and interaction of elastic vesicles in the skin. Structural changes in the stratum corneum have been identified, and intact elastic vesicles visualised within the stratum corneum lipid lamellar regions, but no intact vesicles have been ascertained in the viable tissues. Using the principle of incorporating an edge-activator agent into a bilayer structure, a number of other elastic vesicle compositions have been evaluated. This review describes the research into the development and evaluation of Transfersomes and elastic vesicles as topical and transdermal delivery systems.

Keywords: colloids, elastic vesicles, liposomes, skin penetration enhancement, ultradeformable vesicles

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1. Introduction

The skin represents an ideal route of drug administration in terms of accessibility and ease of application. Topical application of creams and lotions for cosmetic and therapeutic effects in the skin and local tissues has been used for thousands of years. More recently, transdermal delivery of drugs for systemic effect has been developed with a variety of transdermal therapeutic systems (mainly patches) now available. However, the range of molecules that can achieve therapeutic amounts at their target site following application to the skin is severely limited. This is due to the effective barrier properties of intact skin, which is primarily associated with the outermost layers of the epidermis, namely the stratum corneum. Research since the 1960s has generated an understanding of the processes that are involved in percutaneous penetration and have provided insights into approaches to enhance penetration. This has allowed the optimisation of therapy for sites that are associated with the skin and underlying tissues and to expand the use of transdermal delivery for systemic outcomes.

2. Review

2.1 Drug delivery through the skin

A penetrant that is applied to the skin surface has three potential pathways across the epidermis: i) through the hair follicles and associated sebaceous glands; ii) through

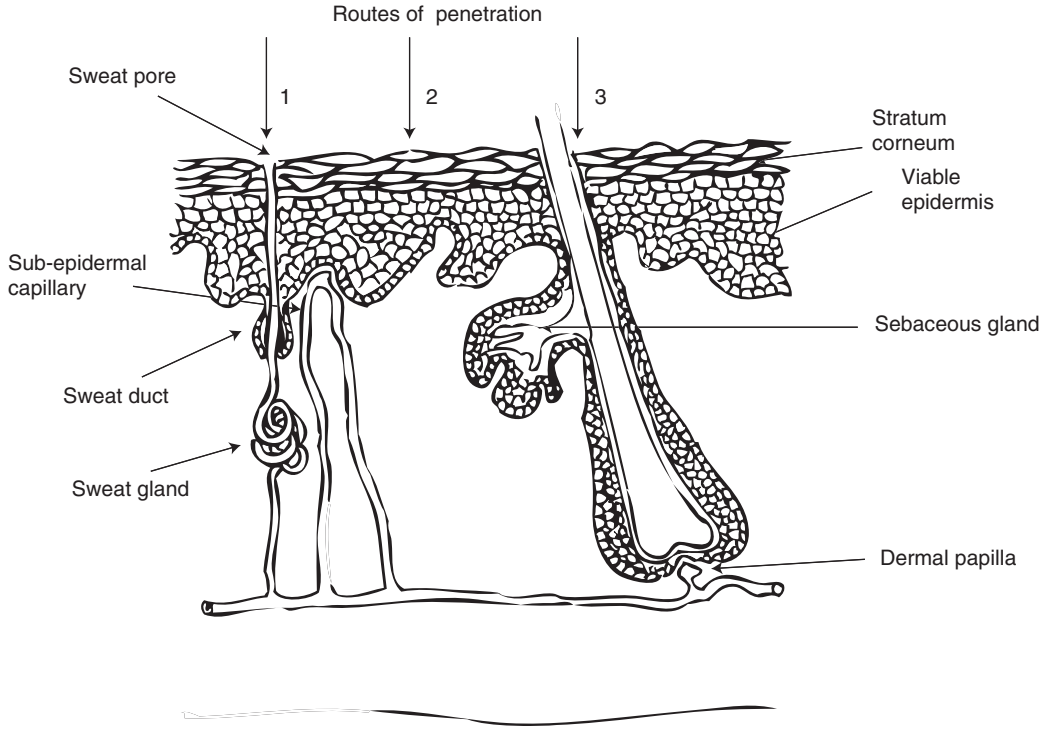

Figure 1. Simplified representation of the skin showing routes of penetration: 1) through the sweat ducts; 2) directly across the stratum corneum; and 3) through the hair follicles.
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sweat ducts; or iii) across the continuous stratum corneum (**Figure 1**). As the fractional surface area of the appendages is only ~ 0.1% [1], this route makes a negligible contribution to steady-state drug flux into and across the skin. However, entry via the appendages may contribute in the early time period between drug application and the establishment of steady-state flux. In addition, it may be an important route for ions, large polar molecules, polymers and colloidal particles.

The stratum corneum, although only 10 – 15 layers of keratinocytes thick, constitutes an effective barrier to both the ingress and egress of molecules. It comprises a 'brick and mortar'-like structure of keratin-filled keratinocytes (bricks) in an intercellular matrix (mortar), which is composed primarily of long-chain ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulfate and sterol/wax esters [2,3]. The keratinocytes are polygonal, elongated and flat, and are ~ 0.2 – 1.5 μm in thickness and 34 – 46 μm in diameter. The intercellular lipid matrix is generated by keratinocytes in the mid to upper part of the stratum granulosum, discharging their lamellar contents into the intercellular space. Within the stratum corneum, this extruded material associates into lipid bilayers with the hydrocarbon chains aligned and the polar head groups dissolved in an aqueous layer (**Figure 2**). A recent review by Menon *et al.* concerning the structure and barrier function of the stratum corneum provides considerable detail of the existing knowledge [4]. The stratum corneum structure is different to other biological membranes by virtue of its lipid composition and the lipid-packing structure, which contribute to the relative impermeability in comparison to other biological membranes. A domain mosaic model, with segregated areas of crystalline/gel domains and borders of more fluid liquid crystalline regions, has been proposed to take account of the heterogeneity of lipid packing in the stratum corneum bilayers [5]. It has been proposed that the more fluid liquid crystalline domains may provide permeation pathways within the stratum corneum lipid bilayers. In addition, proteins and desmosomes exist within the lipid bilayers and may also provide pathways of reduced diffusional resistance. The presence of water is essential for maintaining stratum corneum barrier integrity and is involved in the generation of natural moisturising factor, which acts as a plasticiser to prevent cracking.

The route by which penetrant molecules diffuse through the stratum corneum has been extensively researched, and there has been considerable debate over the relative contributions of the intercellular and transcellular routes (**Figure 2**). However, it is now generally accepted that the intercellular lipid route is the primary permeation pathway for the majority of small, uncharged molecules [6]. The transcellular

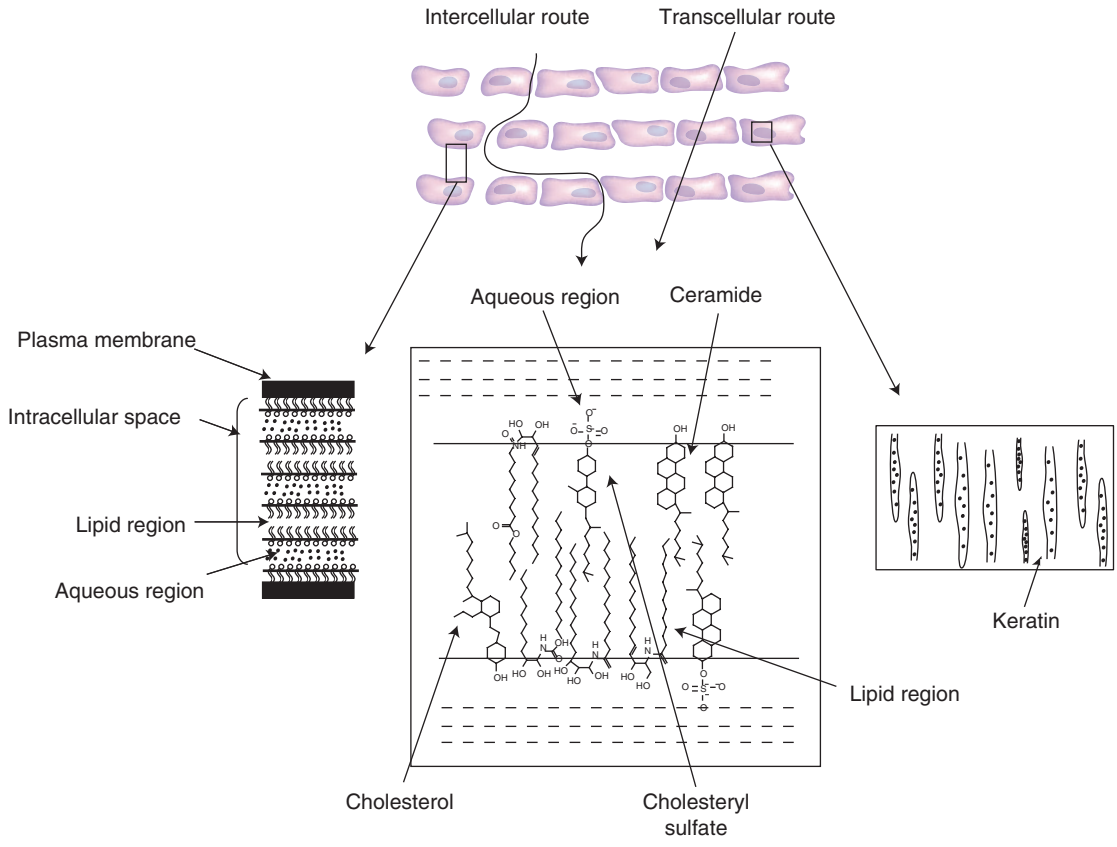

Figure 2. Diagrammatic representation of the stratum corneum and the intercellular and transcellular routes of penetration.
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route may predominate for highly hydrophilic molecules, but the bilayered lipid regions that the molecule must traverse between the keratinocytes remains as the rate-limiting barrier for permeation. Indeed, the use of solvents to remove lipids from the stratum corneum increases drug flux, even for highly hydrophilic molecules [7].

Significant research effort has been focused on developing strategies to enhance skin permeation in order to expand the application of transdermal delivery to a wider range of drugs. These strategies include optimisation of drug and vehicle properties, and modification of the stratum corneum by chemicals or electrical/external force methods [8,9]. One such formulation-based method is the encapsulation of penetrant molecules into vesicles.

2.2 Vesicles as delivery systems for the skin

Encapsulation of active ingredients into vesicles is used in many cosmetic products and has been used as both a formulation and a marketing tool. Cosmetic applications include humectants (such as glycerol and urea), sunscreen and tanning agents, enzymes, antiageing and acne agents (such as retinol), antimicrobials, steroids, hyaluronic acid and natural products. What these preparations have in common is that their target sites are within the skin layers or appendages. Examples of available vesicle-based drug products are less common, although it has been a very active research area with many publications in the literature and patent filings in recent years. A variety of colloid systems have been used for encapsulating penetrant molecules, including liposomes, deformable liposomes (such as Transfersomes® [Idea AG]), niosomes, ethosomes, stratum corneum lipid liposomes, cerasomes and solid lipid nanoparticles.

Liposomes are colloidal particles that are formed as concentric biomolecular layers that are capable of encapsulating both polar and non-polar drugs. Mezei and Gulasekharam first reported their potential for skin delivery, demonstrating four- to fivefold greater permeation of the steroid triamcinolone acetonide from a liposomal lotion as compared with an ointment containing equal drug concentration [10]. The most common liposome composition is phosphatidylcholine from soybean or egg yolk, although many other ingredients have been evaluated [11]. Addition of cholesterol acts to stabilise the structure; thereby, generating more rigid liposomes. Following application to the skin surface, liposomes are generally reported to accumulate in the stratum corneum, upper skin layers and in the appendages, with minimal penetration to deeper tissues

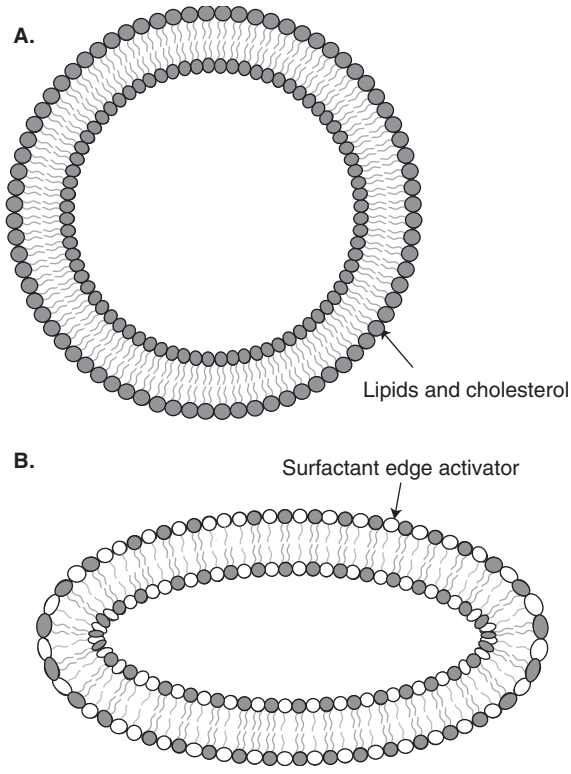

Figure 3. Rigid liposomes consist of double chain lipids in the presence or absence of cholesterol (A) and Transfersomes consist of double chain lipids and an edge activator such as sodium cholate (B).

or the systemic circulation [12-16]. Therefore, classical liposomes are not useful as transdermal delivery systems.

Other vesicle compositions have been investigated to develop systems that are capable of carrying drugs and macromolecules to deeper tissues and/or the systemic circulation. Ethanol is known to act as a skin-penetration enhancer and was first included in liposomes to form ethosomes by Touitou [101]. There are many reports in the literature that demonstrate enhanced penetration of a range of drugs encapsulated in ethosomes to deep tissues and to the systemic circulation [17-20]. It has been proposed that inclusion of ethanol into the phosphatidylcholine structure fluidises the ethosomal and stratum corneum bilayer lipids; thus allowing the soft, malleable ethosomes to penetrate the skin [21]. Niosomes are vesicles composed of non-ionic surfactants that have been evaluated as carriers for a number of cosmetic and drug applications [22-24]. The delivery mechanism may also be a combination of the penetration-enhancement effect of the surfactant in the skin and the increased flexibility that it contributes to the vesicle structure. This is an active research area with frequent reports of new vesicle compositions (e.g., [25-27]) and vesicles being applied in combination with various other penetration-enhancement modalities, including iontophoresis and electroporation [28-31].

2.3 Transfersomes: highly deformable vesicle skin delivery system

A new class of liposomes termed Transfersomes was first described by Cevc [32] and has been the subject of numerous patents and literature reports since the 1990s. These belong to the category that is variously termed as deformable, highly deformable, elastic or ultra-flexible liposomes or vesicles. Although it is generally accepted that the permeation of conventional liposomes is limited to the outer layers of the stratum corneum, thus providing a drug- or cosmetic-localising effect within the skin, Transfersomes are claimed to permeate as intact vesicles through the skin layers to the systemic circulation. Transfersomes or deformable vesicles are reported to improve *in vitro* skin delivery of a range of drugs [33-35] and *in vivo* penetration to achieve therapeutic amounts that are comparable with subcutaneous injection [36]. As with liposomes, Transfersomes are composed of phospholipids such as phosphatidylcholine, but also contain surfactants, such as sodium cholate, deoxycholate, Span 80, Tween 80 and dipotassium glycyrrhizinate (**Figure 3**) [34,35,37,38,102]. The surfactant acts as an edge activator that destabilises the lipid bilayers and increases the deformability of the vesicle [14,39]. The formulation may also contain some ethanol (typically ≤ 10%) and a total lipid concentration of ≤ 10% in the final aqueous lipid suspension [37,40].

Traditional liposomes are typically 100 – 400 nm in diameter and have a rigid structure. These are too large to fit within the intercellular lipid domains of the stratum corneum and to permeate to the deeper layers of the epidermis. Due to the flexibility conferred on the vesicles by the surfactant molecules, Transfersomes are claimed to be able to squeeze through channels one-tenth the diameter of the Transfersome [37], allowing them to spontaneously penetrate the stratum corneum. Cevc *et al.* have suggested that the driving force for penetration into the skin is the osmotic gradient that is caused by the difference in water content between the relatively dehydrated skin surface (~ 20% water) and the aqueous viable epidermis [32,41]. A lipid suspension applied to the skin is subject to evaporation and, to avoid dehydration, Transfersomes must penetrate to deeper tissues. Conventional liposomes remain near the skin surface, dehydrate and fuse with the skin lipids, whereas deformable Transfersomes squeeze through stratum corneum lipid lamellar regions penetrating deeper to follow the osmotic gradient. Consequently, Transfersomes should not be applied under occlusion as this would decrease the osmotic effect [42].

2.4 Transfersomes: delivery of small molecules

Transfersomes have been used successfully as carriers for a range of drugs, including steroids, NSAIDs and local anaesthetics. Most of the early work by Cevc involved *in vivo* application to mice, rats and humans. Transfenac® (Idea AG), a topical diclofenac formulation based on the Transfersome approach, has been shown to provide therapeutically meaningful drug concentrations in target tissues following administration [33]. In

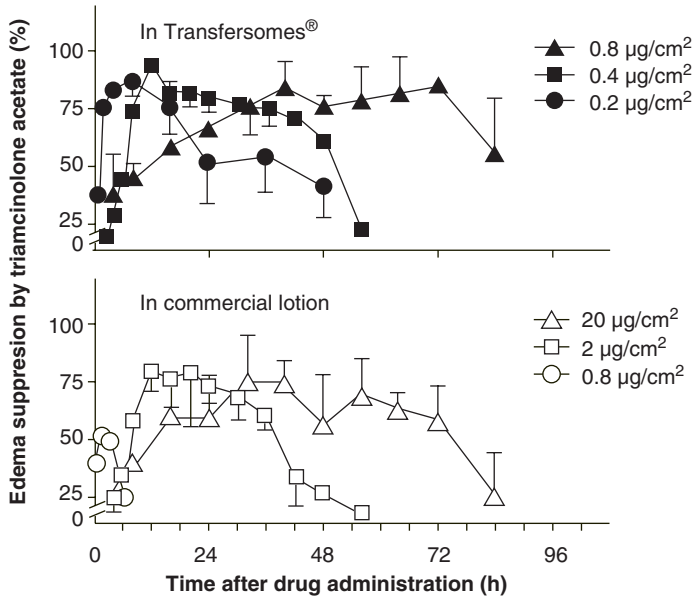

Figure 4. Time dependency of the suppression of arachidonic acid-induced ear oedema in mice, caused by an epicutaneous administration of triamcinolone acetonide in ultradeformable carriers. Mean values and their standard error are given.
Figure reprinted from [44], with permission from Elsevier.

comparison to a hydrogel formulation containing a higher diclofenac dose, the average intramuscular concentration was threefold higher than the Transfersome formulation (a Transfersome dose of 0.25 – 2.00 mg/kg of rat body weight achieved tissue concentrations between 0.5 and 2.0 µg/g; whereas a hydrogel formulation dose of 1.25 – 10.00 mg/kg body weight achieved a tissue level of < 0.5 µg/g muscle).

The distribution and biological activity of the corticosteroid triamcinolone acetonide was determined following topical administration in a Transfersome formulation and as commercially available lotion and cream products [43,44]. ^{3}H-labelled corticosteroid was applied in all of the formulations to permit measurement of the drug in the skin, blood and other organs of mice. The biological action was determined by measuring the suppression of chemically induced mouse ear oedema by the topically applied corticosteroid. The required dose of Transfersome-based steroid to suppress ~ 75% of murine ear oedema for at least 48 h was 0.8 µg/cm^2. A dose of 20 µg/cm^2 in a conventional topical product was required to provide similar biological activity (**Figure 4**). This could provide advantages with respect to increased therapeutic efficacy, while reducing side effects.

The efficacy of Transfersomes and deformable vesicles has also been evaluated by other research groups. For example, Jain *et al.* have reported that the *in vitro* permeation of dexamethasone applied as Transfersomes, across rat skin was nearly of zero order with no lag phase, which was in contrast to conventional liposome and ointment formulations, which both exhibited a lag phase [45]. This study group also evaluated the *in vivo* efficacy of the Transfersome formulation using a carrageenan-induced rat paw oedema model, reporting that a decrease in oedema was significantly greater for the Transfersome application than for traditional liposomes or for ointment application.

Recently, the same group reported increased transdermal flux, prolonged release and improved site specificity of zidovudine from elastic liposomes [46]. The elastic liposomal formulation showed a transdermal flux of 98.8 ± 5.8 µg/cm^2/h across rat skin as compared with 5.72 ± 0.30 µg/cm^2/h for the free drug and an $AUC_{0\text{-}12}$ that was nearly 12-fold higher. In addition, the administration of elastic liposome-encapsulated zidovudine resulted in a substantially higher accumulation of zidovudine in target reticuloendothelial system organs that are known to play a key role in the pathogenesis of AIDS.

El Maghraby *et al.* systematically examined the effect of vesicle composition and characteristics on skin permeation of a model compound [34,47-49]. These authors reported that the *in vitro* skin penetration of estradiol was further enhanced by ultradeformable vesicles (17-fold) as compared with conventional rigid liposomes (9-fold). Pretreating the skin with empty vesicles had a minimal effect on drug flux. The size of the highly deformable vesicles did not influence the enhancement effect, a finding that is in contrast to conventional rigid liposomes. This study also confirmed that the osmotic gradient was the main driving force for the transport of highly deformable vesicles, as the 17-fold increase in estradiol flux was reduced to a 6- to 9-fold increase under occlusion. This group also reported that ultradeformable vesicles were superior to conventional liposomes in the enhancement of 5-fluorouracil transport across skin *in vitro* [50]. Their experimental design

used an aqueous ethanolic receptor phase, which is believed to diffuse into the skin, disrupting deposited liposomes (if any) and, thus, releasing both bound and free drug to the receptor solution that serves as a model for the circulation. Based on their experiments, El Maghraby *et al.* suggested that ultradeformable vesicles improved skin disposition rather than penetration and, hence, are most useful for topical drug delivery.

Trotta *et al.* formulated the same conclusion based on their investigation of the drug flux of an anti-inflammatory agent that is used in dermatitis, dipotassium glycyrrhizinate, from deformable vesicles across pig skin [51]. The drug flux was below the assay detection limit, whereas skin deposition increased by 4.5-fold compared with an aqueous control. Using a similar approach and experimental protocol, they also reported that ≤ 50% of the administered dose of methotrexate in deformable vesicles was found in the skin. This group suggested that deformable liposomes may be useful for the topical administration of methotrexate in the treatment of psoriasis [35]. The formulation that was used by Trotta *et al.* differed from the Transfersome compositions of Cevc and El Maghraby, with lecithins as the phospholipids and dipotassium glycyrrhizinate as the surfactant.

The extent to which these vesicles increase permeation into or through the skin therefore remains controversial.

2.5 Transfersomes: skin delivery of peptides and proteins

Cevc *et al.* reported that Transfersomes could deliver insulin to the systemic circulation in therapeutic amounts equivalent to subcutaneous injection [36,52]. Insulin delivery from Transfersomes composed of phosphatidylcholine incorporating sodium cholate was compared with conventional liposomes and mixed micelles applied to the skin of both mice and humans. Given the size (a molecular weight of ~ 6000 Da) and polarity of insulin, passive permeation across intact human or animal skin is negligible. However, Cevc *et al.* reported that radiolabelled insulin delivered in Transfersomes permeated the skin to reduce blood glucose levels in mice. There was a 30-min lag time relative to a subcutaneous injection of the same formulation, but overall efficacy of delivery was comparable. Conventional liposomes and mixed micelles did not deliver insulin, demonstrating that the penetration achieved by Transfersomes was not due to the components of the formulation.

Similar success was also reported by Guo *et al.* using flexible vesicles that were composed of lecithin [53]. Conventional and flexible liposomes of similar size (74 and 87 nm, respectively) were applied unoccluded to mice abdominal skin *in vivo*. The percentage decrease in blood glucose levels by flexible vesicles was 21.4 ± 10.2% at 1 h, and reached 61.5 ± 9.0% at 5 h. Conventional vesicles, insulin solution and saline showed no hypoglycaemic effect. This group used a similar formulation to deliver ciclosporin A [54]. In this case they assessed the *in vitro* and *in vivo* mouse skin permeation of lecithin vesicles with and without the incorporation of sodium cholate and sodium cholate micelles, each containing ciclosporin A as a model peptide drug. Only the flexible vesicles transferred ciclosporin A to the receptor solution and blood circulation in measurable amounts. The authors also reported that hydration of the skin decreased the efficacy of the flexible vesicles in delivering the peptide across the skin.

The potential of Transfersomes for non-invasive vaccine delivery has been investigated [22]. In this study, comparison of the serum IgG antibody titre generated in response to administration of tetanus toxoid in Transfersomes, niosomes and conventional liposomes to the shaved skin of rats, and also an alum-absorbed tetanus toxoid given intramuscularly, was made. It was reported that two applications of tetanus toxoid in Transfersomes 28 days apart could elicit an immune response that was equivalent to that produced by intramuscular injection of alum-absorbed tetanus toxoid. In comparison, niosome and conventional liposome formulations elicited weaker immune responses. In addition, *in vitro* skin permeation of tetanoid toxin topical formulations was measured across nude rat skin over a 48-h period. The cumulative permeation of tetanus toxoid was ~ 16.4, 12.5 and 10.7% of the applied dose for the Transfersome, niosome and liposome formulations, respectively.

Successful delivery of proteinaceous antigens in elastic vesicles for transcutaneous vaccination has also been reported [55]. The immune response elicited by topically applied hepatitis B surface antigen (HBsAg)-loaded elastic liposomes was compared with the intramuscularly administered alum-adsorbed HBsAg solution, the topically applied plain HBsAg solution and the physical mixture of HBsAg and elastic liposomes. Elastic liposomes were shown to induce a robust systemic and mucosal antibody response against HBsAg as compared with other formulations. *Ex vivo* cellular uptake and fluorescence microscopy studies demonstrated skin permeation, biodistribution and efficient delivery of antigens to the immunocompetent Langerhan's cells and lymphatics.

The potential for Transfersomes to offer a means of delivering peptides, proteins and antigens through the skin is very exciting, but requires further investigation. Given the range of therapeutic applications that could be offered by elastic vesicle delivery systems, scientists have been encouraged to explore their mechanism of penetration enhancement and to attempt to develop an understanding of how these vesicles interact with the skin.

2.6 Other elastic vesicles

Cevc's original Transfersomes are composed of phosphatidyl choline in combination with the surfactant sodium cholate as an edge activator. Other compositions of elastic vesicles have also been developed and evaluated. For example, van den Bergh *et al.* introduced a series of elastic and rigid liquid-state vesicles, consisting of the bilayer-forming surfactant L-595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester) [56,57]. As discussed in Section 2.4, Trotta *et al.* formed flexible vesicles with

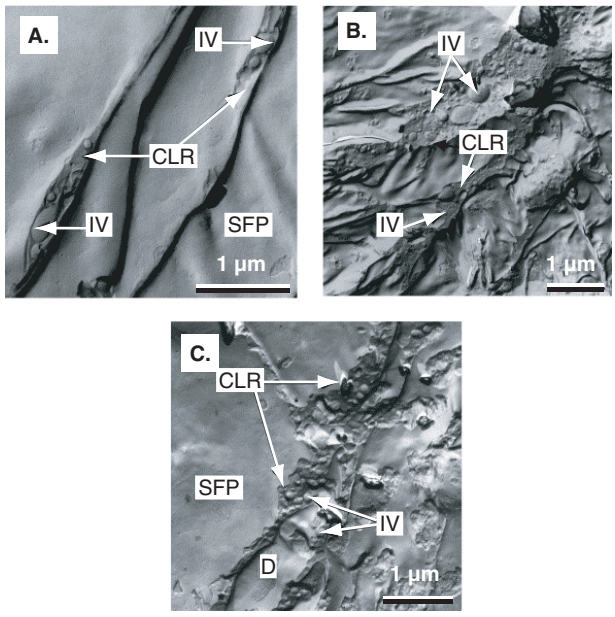

Figure 5. *In vivo* interactions between elastic vesicles and the human skin in the deeper layers of the stratum corneum. A. Micrograph of the ninth tape strip of skin treated with L-595/PEG-8-L/sulfosuccinate (50:50:5) elastic vesicles. **B** and **C.** Micrographs of the ninth tape strip of skin treated with L-595/Tween 20/sulfosuccinate (60:40:5) elastic vesicles. For both elastic vesicle compositions, channel-like regions can be seen containing vesicular structures. Although fused vesicle material is present, intact vesicles were also clearly seen. This strongly suggests that elastic vesicles can enter the deeper layers of the stratum corneum within 1 h of vesicle application.
Figure reprinted from [64] with permission from Elsevier.
CLR: Channel-like regions; D: Desmosome; IV: Intact vesicles; SFP: Smooth fracture planes.

lecithins as the phospholipids and dipotassium glycyrrhizinate as the surfactant [35,51]. El Maghraby *et al.* suggested that the surfactant could be replaced with chemical penetration enhancers such as oleic acid and limonene as the edge activators [48,58].

Recently, the provesicular approach that is proposed to enhance the stability of vesicles has been applied to deformable vesicles. Pro-transfersome gel formulations of levonorgestrel were prepared and characterised for vesicle shape, size, entrapment efficiency, turbidity, stability, *in vitro* drug permeation across rat skin and *in vivo* drug permeation using a biological assay of progestational activity. The effects of different formulation variables (type of alcohol, type and concentration of surfactant) on transdermal permeability profile were assessed. The optimised pro-transfersome formulation showed enhanced *in vitro* transdermal flux compared with a levonorgestrel solution (15.8 ± 0.4 and 0.03 ± 0.00 µg/cm^2/h, respectively) and enhanced the biological activity. The pro-transfersome formulation also showed good stability with no change in the liquid crystalline nature or the drug content after 2 months of storage [59].

The basic principle of the original Transfersome is to incorporate an agent into a bilayer structure to form a vesicle that is still capable of entrapping drug, but also being flexible to allow it to elongate in the stratum corneum. A number of compositions have been reported, but research continues into a range of materials that can be tailored to optimise vesicle properties for specific drugs and applications.

2.7 Transfersomes: interaction with the skin

First, Cevc's assertion that Transfersomes require a hydration gradient and, therefore, must be applied non-occluded to the skin has been widely substantiated. For example, a 17-fold increase in estradiol flux across human epidermal membranes *in vitro* was reduced to a six- to ninefold increase under occlusion [34]. Honeywell-Nguyen *et al.* demonstrated that intact elastic vesicles penetrate into the stratum corneum after non-occlusive application, but that very few intact vesicles were present in the deeper layers of the stratum corneum after occlusive administration [60]. However, Honeywell-Nguyen and Bouwstra have shown in their work that drug skin permeation from an occluded saturated buffer solution was higher than when compared with elastic vesicles formulations applied in an occluded or non-occluded method [60].

Despite the substantial interest in the mechanism of vesicular delivery over the past decade, there is still considerable debate as to whether Transfersomes and deformable vesicles act as carrier systems by penetrating intact through the skin [20,61,62]. The recent research into the mechanism of transdermal delivery of Transfersomes and elastic vesicles and evidence for how they interact with the skin is summarised in this section.

Using confocal laser scanning microscopy, Cevc and Schatzlein reported high fluorescence pathways located within the intercellular lipid lamellae of murine stratum corneum [42,63]. These authors suggested that these represent intercluster (clusters of 3 – 10 corneocytes) and intercorneocyte routes, and that these pathways act as virtual channels through which intact elastic vesicles could penetrate. Van den Bergh *et al.* reported no evidence of intact elastic vesicles (composed of l-595/PEG-8-L) in the deeper layers of the stratum corneum [56,57]. They found lamellar stacks in the intercellular lipid regions and also reported that the transport of a fluorescent label incorporated in elastic vesicles was through a fine meshwork of thread-like channels. The viable epidermis and dermis were unaffected, indicating that vesicle components remained in the stratum corneum. When the label was incorporated into conventional rigid liposomes and micelles, it was only detected in the superficial stratum corneum layers. The authors concluded that there is no evidence that vesicles or vesicle ingredients themselves rapidly penetrate into the viable epidermis.

The disadvantage of using fluorescent labels is that only the label rather than the vesicle can be visualised. Honeywell-Nguyen *et al.* used freeze-fracture electron

microscopy of tape-stripped human skin to visualise the applied vesicles and any changes in skin structure due to vesicle treatment [64]. Elastic (L-595/PEG-8-L/sulfosuccinate or L-595/Tween 20/sulfosuccinate) and rigid vesicles were applied non-occlusively to human skin for 1 h after which the stratum corneum was stripped sequentially and examined by freeze-fracture electron microscopy. Vesicles were found up to the ninth strip in the stratum corneum in channel-like regions (**Figure 5**). These regions were similar to the thread-like channels that were observed by van den Bergh [56,57]. No ultrastructural changes were seen in skin that was treated with rigid vesicles. In a subsequent study by Honeywell-Nguyen *et al.*, elastic vesicles were applied in a non-occluded method for 4 h, and vesicles were found to reach the fifteenth tape-strip, although extensive vesicle fusion was observed both at the skin surface and deeper layers of the stratum corneum [60]. Based on their studies probing the possible interaction between vesicles and the skin in an attempt to elucidate the mechanism of enhanced drug transport, the group of Bouwstra have demonstrated no evidence of intact elastic vesicles beyond the stratum corneum. Consequently, there remains scepticism regarding the claim by Cevc's group that intact Transfersomes penetrated through the stratum corneum and the underlying viable epidermis into the blood circulation.

3. Conclusion

It is clear that Transfersomes or elastic vesicles can deliver enhanced amounts of both small and large therapeutic agents into and through the skin. The exact mechanism by which transport occurs remains to be elucidated and evidence for transport of intact vesicles beyond the stratum corneum is lacking. However, there are increasing applications of enhanced delivery by elastic vesicle formulations that are being reported, with some products, such as Transfenac, nearing the market.

4. Expert opinion

Despite the attractiveness of the skin as a route of drug administration, the existing range of transdermal applications is limited due to the relative impermeability of the skin. Research over the past 40 years has provided a better understanding of the structure of the stratum corneum barrier and the mode of permeation of molecules across this region. Many approaches have been developed and evaluated for enhancing drug permeation across the skin, but these are often limited by cost, ease of application or toxicity. Liposomes are a logical carrier vehicle as they are capable of encapsulating a drug, negating its disadvantageous physicochemical characteristics, and presenting to the stratum corneum a lipid structure with some similarities to the lipid bilayers that they need to traverse. However, conventional liposomes are widely acknowledged to be unable to permeate intact beyond the first few layers of the stratum corneum.

The Transfersome structure offers the advantages of the liposome lipid bilayer while incorporating a surfactant that permits elasticity and deformation of the bilayer structure. This deformation allows the Transfersome or elastic vesicle to squeeze through small spaces; thereby facilitating permeation. Substantial research has been focused on identifying the transport pathway of Transfersomes and elastic vesicles through the stratum corneum. Ultrastructural changes in the form of thread-like channels associated with the presence of elastic vesicles have been identified within the stratum corneum. These channels were not seen in untreated skin or in skin treated with conventional rigid liposomes. It is likely that this ability to alter the structure of the stratum corneum is the mechanism by which elastic vesicles permeate the stratum corneum and, therefore, their mechanism of permeation enhancement. The presence of intact elastic vesicles well within the stratum corneum has been clearly demonstrated, but claims that Transfersomes can permeate through the viable epidermis to the blood circulation have not been substantiated.

Regardless of the debate over the precise mechanism of permeation of Transfersomes and elastic vesicles, the evidence for enhanced permeation of a wide variety of therapeutic molecules, ranging from small drugs to proteins, is impressive. These range from small molecules for applications such as cosmetic, dermatological or musculoskeletal pain, to proteins and peptides, including applications such as transcutaneous vaccination. So far, the main focus in trying to achieve transdermal protein and peptide delivery has been by application of physical enhancement technologies such as iontophoresis, electroporation or microneedles. A formulation-based method for enhancing transdermal protein delivery would be very attractive.

A number of Transfersome products are now in advanced clinical trials, such as IDEA-033 (Idea AG) for enhanced delivery of ketoprofen in the management of osteoarthritis (in Phase III trials). The deformable vesicle concept can be applied to a variety of compositions with the potential to optimise the permeability of a range of therapeutic molecules. There is considerable scope for exploring innovative compositions that incorporate suitable chemical penetration enhancers. It is likely that a number of Transfersome or elastic vesicle-based products for dermal and transdermal applications will be developed in the future.

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Patents

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101. TOUITOU ELKA: US5540934 (1996).

102. IDEA AG: US6165500 (2000).

•• **Key patent of Transfersomes® by inventor.**

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