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Drug delivery/formulation and nanotechnology

Vesicles as a tool for transdermal and dermal delivery

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Transdermal and dermal drug delivery is problematic because the skin, as a natural barrier, has a very low permeation rate. Therefore several methods have been assessed to increase this rate locally and temporarily. One approach is the use of vesicle formulations. In this paper the effectiveness of conventional and deformable vesicles as drug delivery systems as well as their possible mode of action as permeation enhancers or transdermal drug carriers will be discussed.

Introduction

The skin covers a total surface area of approximately 1.8 m² and provides the contact between the human body and its external environment [1]. Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic drug levels and therefore also reducing the systemic side effects. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs. This drug delivery route can have several advantages compared with oral drug administration. First of all, it circumvents the variables that could influence gastro-intestinal absorption such as pH, food intake and gastro-intestinal motility. Secondly, it circumvents the hepatic metabolism and is therefore suitable for drugs with a low bioavailability. Thirdly, transdermal drug delivery can give a constant, controlled drug input decreasing the variations in drug plasma

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levels, thus reducing the side effects particularly of drugs with a narrow therapeutic window.

Despite the many advantages of the skin as a site of drug delivery, only eight drugs are currently in the market in transdermal delivery system [2–9], namely clonidine, estradiol, nitroglycerine, fentanyl, testosterone, scopolamine, nicotine and oxybutinin. By far the most important reason for this is the low permeability of drugs in the stratum corneum, the outermost layer of the skin acting as the main barrier in the skin [10,11]. The structure of the stratum corneum is often compared with a brick wall, with the corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae [12] (Fig. 1). It has been generally accepted that the highly organized crystalline lipid lamellae play an essential role in the barrier properties of the stratum corneum [13–18]. Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin [19–21] or to increase the driving force for permeation of drugs across this skin barrier. One of the most controversial methods is the use of vesicle formulations as skin delivery systems.

Conventional vesicles as skin delivery systems

General

The basic structure of vesicles is illustrated in Fig. 2. Vesicles are water-filled colloidal particles. The walls of these capsules

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Glossary

Deformable non-ionic surfactant vesicles: vesicles prepared from mainly non-ionic surfactants using a surfactant composition that results in deformable bilayers.

Gel-state vesicles: the amphiphiles in the bilayers of these vesicles form a gel phase.

Liposomes: vesicles prepared from mainly phospholipids

Liquid-state vesicles: the amphiphiles in the bilayers of these vesicles form a liquid phase.

Non-ionic surfactant vesicles or niosomes: vesicles prepared from mainly non-ionic surfactants.

Transfersomes[®]: the walls of these vesicles consist of phospholipids and an edge activator, which results in deformable bilayers.

Vesicles: water-filled colloidal particles. The walls of these particles consist of amphiphilic molecules in a bilayer conformation.

consist of amphiphilic molecules in a bilayer conformation. In an excess of water these amphiphilic molecules can form one (unilamellar vesicles) or more (multilamellar vesicles) concentric bilayers [22]. Hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be asso-

ciated with the vesicle bilayer by hydrophobic and/or electrostatic interactions [23].

A wide variety of lipids and surfactants can be used to prepare vesicles. Most commonly, the vesicles are composed of phospholipids or non-ionic surfactants [24,25]. These are referred to as liposomes and niosomes or non-ionic surfactant vesicles, respectively. The composition of the vesicles influences their physico-chemical characteristics such as, size, charge, thermodynamic phase, lamellarity and bilayer elasticity. These physico-chemical characteristics have a significant effect on the behaviour of the vesicles and hence on their effectiveness as a drug delivery system.

The rationale for using vesicles in dermal and transdermal drug delivery is many folds [20,23]. Vesicles might

- (a) act as drug carriers to deliver entrapped drug molecules into or across the skin;
- (b) act as penetration enhancers owing the penetration of the individual lipid components into the stratum corneum and subsequently the alteration of the intercellular lipid lamellae within this skin layer;

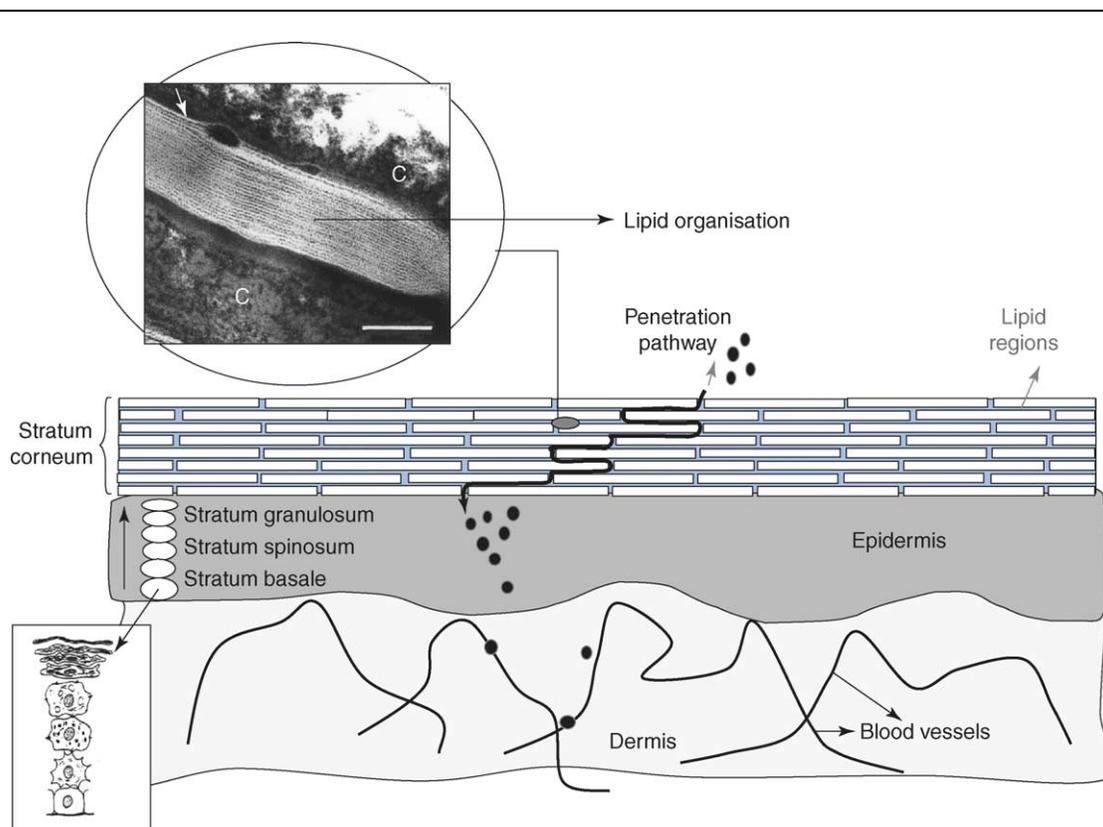
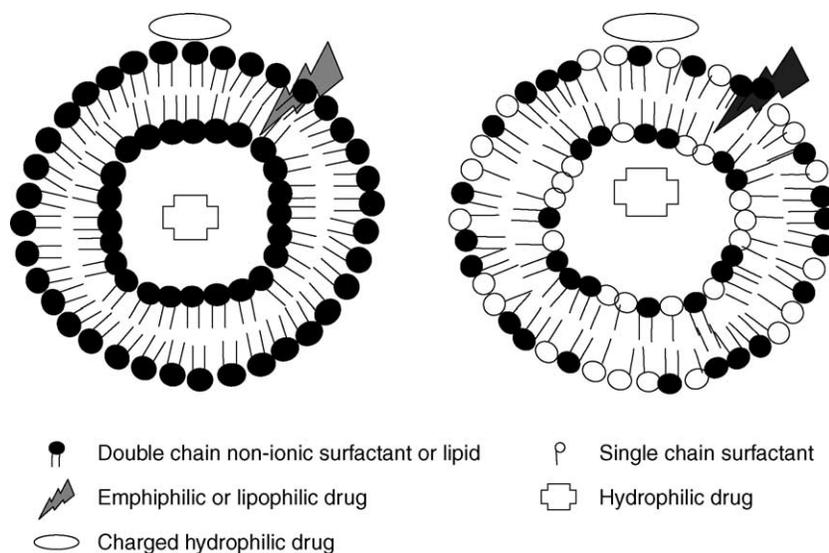


Figure 1. A schematic drawing of a skin cross-section [15]. The skin is composed of a dermis and an epidermis. In the basal layer of the epidermis cells proliferate. Upon leaving the basal layer cells start to differentiate and migrate in the direction of the skin surface. At the interface between stratum granulosum–stratum corneum final differentiation occurs, during which the viable cells are transformed into dead keratin filled cells (corneocytes). The corneocytes are embedded in lipid lamellar regions. Substances permeate mainly along the tortuous pathway in the intercellular lamellar regions. The thickness of the stratum corneum is approximately 15 μm . C = corneocyte filled with keratin. Bar = 100 nm.



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Figure 2. Charged hydrophilic, amphiphilic and lipophilic drug molecules can be associated with the bilayers of the vesicles, whereas hydrophilic substances can also be entrapped in the vesicles. Rigid vesicles consist of double chain non-ionic surfactants or lipids in the presence or absence of cholesterol (left image). Elastic vesicles consist of double chain surfactants or lipids and an edge activator. The edge activator is often a single chain surfactant (right image).

- (c) serve as a depot for sustained release of dermally active compounds;
- (d) serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system.

Despite extensive research and a great interest from the pharmaceutical and cosmetic industry, the use of vesicles is yet controversial. Two major questions are subjects for discussion:

- (1) What is the effectiveness of vesicles?
 - (a) Do they enhance drug deposition in the skin (dermal delivery)?
 - (b) Do they enhance drug transport across the skin (transdermal delivery)?
 - (c) Or do they enhance both dermal and transdermal delivery?
- (2) What is the interaction between vesicles and the skin?
 - (a) Do they act as penetration enhancers?
 - (b) Or do they act as drug carrier systems?

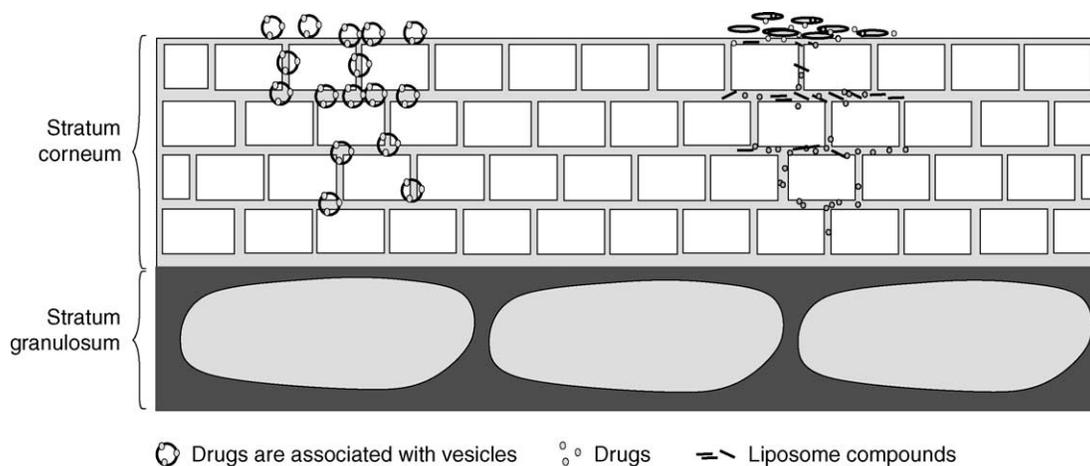
The answers to the two questions above are required to obtain a sound understanding of the mechanism of the action of vesicles and to select the most appropriate drug compounds to be delivered by vesicles. If vesicles act as carrier systems, they might be able to transport large molecular weight drugs, such as proteins into the skin or even into the systemic circulation. If they act as penetration enhancers, however, the main mode of action is a perturbation of the lipid organization in the stratum

corneum, thereby increasing the transport rate across the skin. The latter is only efficient for low molecular weight drugs. One of the most important characteristics of drug carrier systems is that drug and carrier should permeate along the same route across the skin (Fig. 3). In addition the vesicle material profile and the active compound profile in stratum corneum should be very similar.

The effectiveness of conventional vesicles as skin drug delivery vehicles **Early studies in the 1980s**

The first papers to report on the effectiveness of vesicles for skin delivery were published in the early 1980s. The reported data, however, were conflicting. Mezei and Gulasekharan reported that liposomal encapsulation of triamcinolone acetonide increased drug disposition in the epidermis and dermis [26,27]. Several other groups suggested a slower skin transport of highly polar compounds in vesicle formulations than in buffer solutions. The transport rate of lipophilic compounds was reported to be very similar to that of free drug solutions. Most groups concluded that liposomes did not act as transport system [28–33].

Conflicting results continued to be published concerning the effectiveness of vesicles, enhancing the controversy of vesicles as (trans)dermal delivery vehicles. Several transport studies have reported that vesicles only enhanced the drug disposal in the skin, suggesting that vesicles are only useful for topical dermal delivery [34–41]. Others, however, have suggested that application of drugs in vesicles could lead to therapeutic drug concentrations in the systemic circulation and are suitable candidates for transdermal delivery [42–48].



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Figure 3. If vesicles act as drug carriers the active ingredient and the vesicles should follow the same penetration route and should show a similar distribution profile in the stratum corneum (situation: left side). If vesicles act as penetration enhancers, the distribution profile and penetration pathway are not necessarily similar (situation: right side).

These inconsistent results can, at least in part, be explained by the fact that vesicles with different compositions and physico-chemical characteristics were used in different studies. So let us explain the physico-chemical characteristics of the vesicles in relation to their enhancing properties.

The effect of conventional vesicles on drug transport

Several studies have demonstrated that the vesicle composition and hence its physico-chemical properties can have a significant effect on drug permeation [32,49–51]. Several studies have demonstrated consistently that the phases of the amphiphiles formed in the membranes of the vesicles (a liquid or a gel phase) is an important feature, which plays a vital role in its effectiveness as a (trans)dermal delivery vehicle. Permeation studies *in vitro* have revealed that LIQUID-STATE VESICLES are more effective than GEL-STATE VESICLES in enhancing drug transport [51–54]. These results have recently been confirmed *in vivo* [55].

Other physico-chemical properties, such as size and lamellarity, might also influence the effectiveness of vesicles as a delivery vehicle, although probably to a lesser extent than the thermodynamic state [56,57].

The mode of action of conventional vesicles

Several mechanisms mediating the vesicle–skin interactions have been described in the literature. It has been suggested that vesicle–skin interactions can occur either at the skin surface or in the deeper layers of the stratum corneum. Hofland *et al.* and Abraham *et al.* have demonstrated adsorption and fusion of vesicles onto the skin surface, resulting in the formation of lamellae and rough structures on top of the outermost corneocytes [58–60]. Changes in the deeper layers

of the stratum corneum were observed only after treatment of the skin with liquid-state liposomes and non-ionic surfactant vesicles. No ultrastructural changes in the skin were found when gel-state non-ionic surfactant vesicles were applied [59,60]. The authors explained their results by a molecularly dispersed penetration of lipid or surfactant into the intercellular matrix. Studies with thermal analysis that enable to detect lipid phase transitions confirmed this mechanism [61]. This suggests that components of liquid state vesicles can enter the deeper layers of the stratum corneum where they can modify the intercellular lipid lamellae, whereas the components of gel-state vesicles remain on the skin surface. The superior mode of action of liquid-state vesicles for skin interactions is the most probable explanation for the fact that they are more effective in enhancing drug transport into and across the skin. This is in accord with a study that found a correlation between the skin penetration and the fluidity of the vesicle bilayers determined by electron spin resonance [62].

From the studies above there is no doubt that vesicular components can penetrate into the stratum corneum. However, it is still debated whether vesicles can enter the stratum corneum as intact entities. In fact only one group claimed that liposomes enter the stratum corneum intactly [26,27,63]. Remarkably, it was noticed [63] that these liposomes are ‘very flexible lipid vesicles’. As explained below, an increased deformability of the bilayers will have consequences for their interaction with skin.

In general, one can conclude that rigid liquid- and gel-state vesicles do not enter the stratum corneum as intact entities. However, the question remains whether highly deformable vesicles are able to enter the stratum corneum when applied in an optimal manner.

Elastic vesicles as skin delivery systems

General

These deformable vesicles are a novel type of liquid-state vesicles. Cevc *et al.* introduced the first generation of deformable vesicles, also referred to as TRANSFERSOMES[®] (Idea, Munich), consisting of phospholipids and an edge activator [49,64,65] (Fig. 2). An edge activator is often a single chain surfactant that destabilizes the lipid bilayer of the vesicles and increases the deformability of the bilayer by lowering its interfacial tension. Later, a second generation of elastic vesicles was introduced consisting of mainly non-ionic surfactants (B.A. Van den Bergh, PhD thesis, University of Leiden, 1999). Not only the physico-chemical characteristics of the vesicles, but also the mode of application plays a crucial role in the vesicles-skin interactions. Vesicles can be applied occlusively (covered by a patch to avoid water evaporation) or non-occlusively (exposed to the air, which results in evaporation of water). The difference in skin interaction between occlusive and non-occlusive application is of importance for deformable vesicles. Namely, Cevc *et al.* have suggested that the transport of Transfersomes[®] is driven by the osmotic gradient across the skin. Occlusion would eliminate this osmotic gradient and is therefore detrimental for the actions of the deformable vesicles [49,50].

The effectiveness of elastic vesicles as skin delivery vehicles

Several studies have shown that deformable vesicles were more effective than the conventional rigid vesicles in the enhancement of drug transport across animal and human skin. The first efficacy studies were performed using Transfersomes[®]. The hypothesis that Transfersomes[®] – applied under non-occlusive conditions – could penetrate the skin barrier and could subsequently be distributed throughout the entire body was tested [44]. The effectiveness of Transfersomes[®] was successfully demonstrated using model drugs, such as lidocaine, corticosteroids [46], diclofenac [66] and high molecular weight compounds, such as insulin [45]. Furthermore, it was suggested that Transfersomes[®] could be used for non-invasive transdermal immunisation [67,68]. When Transfersomes[®] labelled with a radioactive marker were applied onto the skin, radioactivity was observed in the liver. This indicates the presence of radioactive particles in the systemic blood circulation, which might suggest that Transfersomes[®] permeate across the skin. Most of these studies have been carried out *in vivo* in mice.

Other investigators have confirmed that deformable vesicles were more effective compared with rigid vesicles for (trans)dermal delivery. However, the exceptional high transport rates that were demonstrated by Cevc *et al.* were never achieved. Using human cadaver skin, El Maghraby *et al.* showed *in vitro* that Transfersomes[®] were superior to conventional rigid liposomes in the enhancement of oestradiol and 5-fluorouracil transport across skin [69–72]. However, an

aqueous ethanolic receptor phase was needed to liberate the drug from the skin into the ethanolic receptor phase that serves as a model for the blood circulation. El Maghraby *et al.* suggested that Transfersomes[®] only improved skin disposition, hence are only useful for dermal drug delivery. The latter was also concluded by Trotta *et al.* These studies showed that skin fluxes of dipotassium glycyrrhizinate were below the detection limit, whereas skin deposition increased 4.5-fold in comparison to an aqueous control [73]. However, their vesicle composition and sizes are different from those of Cevc and El Maghraby. In fact, the limited partitioning into the acceptor phase indicates that the ultra-deformable vesicles are not carrying the associated drug into the acceptor phase.

In 1998 deformable vesicles consisting mainly of non-ionic surfactants were introduced. The surfactant-based deformable vesicles have shown to be more effective than rigid vesicles in enhancing the penetration of ³H₂O across hairless mouse skin *in vitro* [74] and pergolide, lidocaine and rotigotine [75–78] across human skin *in vitro*. All the results with pergolide and rotigotine as model drugs have suggested that for optimal drug delivery it is essential to associate drug molecules with the DEFORMABLE NON-IONIC SURFACTANT VESICLES. This indicates that a penetration enhancing effect is not the main or the only mechanism of action of the deformable non-ionic surfactant vesicles but that these vesicles act as drug carrier systems.

The interactions of deformable vesicles with animal and human skin

The interactions of deformable vesicles with skin is one of the most debated, yet one of the most interesting issues in vesicular skin research. This debate was initiated by a paper published in 1992, reporting that Transfersomes[®] could penetrate the intact mouse skin and could travel as far as the systemic circulation [49]. The question that remains to be answered is: do deformable vesicles indeed act as carrier systems?

In order to answer this question penetration pathways in the stratum corneum were studied using fluorescent labels linked to Transfersomes[®] or to deformable non-ionic surfactant vesicle formulations [79,80]. Although in both studies evidence was obtained for the concept of vesicle material transport via channel-like structures, Van den Bergh *et al.* [80] have visualized a much finer network of channels (Fig. 4) than the honeycomb-like system of intercluster and intercorneocyte pathways published by Schatzlein and Cevc [79]. Interestingly, the microscopic images obtained using the non-ionic surfactant vesicles illustrated that the lipophilic fluorescent label was always confined to the stratum corneum. This suggests that the components of deformable non-ionic surfactant vesicles do not travel beyond the stratum corneum, which contradicts the observations by Schatzlein that Transfersomes[®] could even reach the systemic circulation.

Very recently three *in vivo* studies were performed with human volunteers. In two studies, tape stripping (a method to remove sequentially stratum corneum cell layers) was

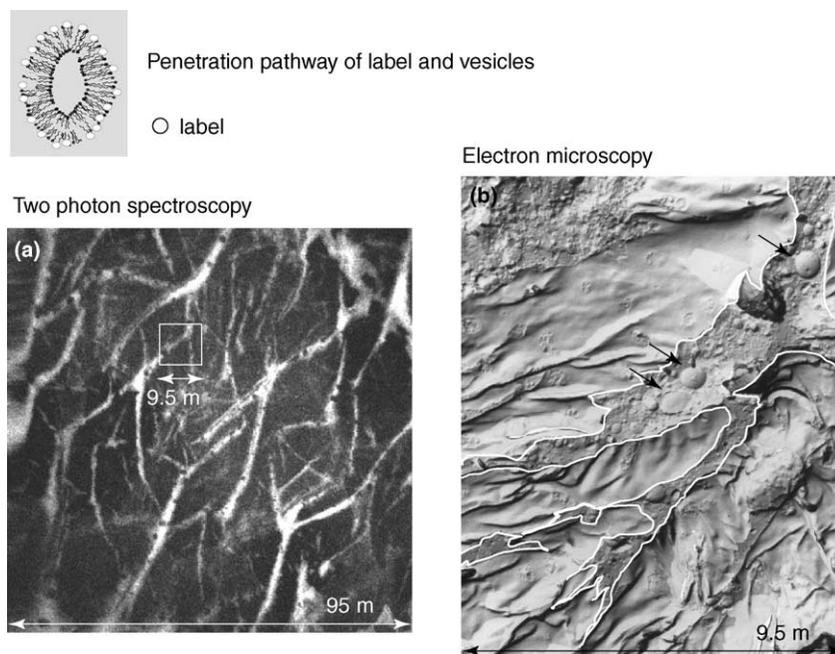


Figure 4. Left-hand image: a visual cross section ($95 \mu\text{m} \times 95 \mu\text{m}$) recorded at a depth of $10 \mu\text{m}$ in stratum corneum treated with elastic vesicles. After 1 h of application, already a fine meshwork of 'channels' was observed without any distinguishable polygonal cell contours, indicating that the fluorescent label applied associated with vesicles permeates in channel-like regions (fluorescence is indicated by the white arrows). The distance between these channels is approximately $5 \mu\text{m}$. The channels appear immediately after drying of the liquid-state vesicle suspension [80]. Right-hand image: Vesicles are visualized after 1 h non-occlusive application using freeze-fracture electron microscopy [81]. Note the differences in magnification compared with the left image. After 1 h of application, vesicles were visualized in human stratum corneum in channel-like regions. The size and shape of the channel-like regions in the right-hand image is very similar to that in the left-hand image, suggesting that elastic vesicles carry the fluorescent label into the stratum corneum. Vesicles are indicated by black arrows.

combined with electron microscopy to provide information on the permeation of intact deformable vesicles into the stratum corneum. The results were very remarkable and clearly indicated that the deformable non-ionic surfactant vesicles partition extremely fast into the deeper layers in the stratum corneum within 1 h (Fig. 4). Vesicles were visualized in channel-like structures in the intercellular regions [81,82], the size and appearance of which were similar to the channel network visualized after a model lipophilic fluorescent label linked to deformable non-ionic surfactant vesicles were applied [79]. The fact that (a) deformable non-ionic surfactant vesicles show a fast partitioning into the stratum corneum, (b) both model compounds and deformable non-ionic surfactant vesicles follow the same route through human stratum corneum, and (c) no other abnormalities were found in the intercellular lipid lamellae; all point to the fact that these deformable vesicles do not act as penetration enhancers but more probably act as drug carrier systems. When the application was changed from non-occlusive to occlusive, the presence of lipid plaques was frequently observed suggesting that occlusion impairs the transport of intact deformable vesicles into the skin. This is in agreement with the osmotic theory of Cevc and Blume [49]. In the third study tape stripping was used in combination with fourier transformed

infrared spectroscopy, which allows quantitative evaluation of the distribution profile of lipid material and the drug compound [83]. This study confirmed that deformable non-ionic surfactant vesicle material rapidly enters the deeper layers of the stratum corneum and could reach layers almost as deep as the stratum corneum-viable epidermal junction within 1 h. The distribution profiles of the vesicle material and the drug suggest that the model drug was associated with vesicle material in the upper and central layers of the stratum corneum but was not associated with vesicle material in the lowest layers of the stratum corneum and hence must have been partly released from these vesicles. This indicates that deformable non-ionic surfactant vesicles mainly remain in the stratum corneum and that drug molecules are released from these vesicles with subsequent transport of free drug molecules into the viable skin layers.

Conclusions

In summary, it is evident from the aforementioned studies that deformable vesicles have superior characteristics to rigid vesicles for the interaction with animal and human skin and for enhancing the drug transport across the skin. However, the exact mode and nature of deformable vesicle transport into and possibly across the skin is not yet fully understood.

Especially the degree of intact vesicle permeation across the stratum corneum and the partitioning of intact vesicles into the viable epidermis remains to be elucidated in more detail. In this respect, there are still inconsistencies in the results. All the studies performed with the surfactant-based deformable vesicles indicate an intact partitioning into the stratum corneum but very limited partitioning into the viable epidermis, if any. The *in vivo* studies with Transfersomes[®] suggest an intact partitioning of the vesicles into the viable epidermis and dermis. The *in vitro* transport with Transfersomes[®], however, does not indicate an intact partitioning into the receptor phase of the diffusion cell. The inconsistencies in the results observed might partly be owing to the differences in vesicle composition, vesicle preparation or to differences in skin structure from different species. For example, one has to realize that mouse skin is much thinner than human skin and deformable vesicles prepared from non-ionic surfactants do not necessarily act in the same way as deformable vesicles prepared from phospholipids and edge activators. However, a detailed knowledge of the mode of action is necessary in order to assess the full potential of elastic vesicles as skin delivery vehicles, such as the delivery of large molecules or the targeting to certain sites and cells within the skin. This is only possible when vesicles act as carrier systems and could give rise to the development of very interesting and novel transdermal drug delivery systems. Transport of vesicles into the viable epidermis or even to the general circulation, however, might also have some drawbacks. The presence of large amounts of lipid material in the viable epidermis will give rise to questions about skin irritation and toxicity. A vesicle formulation that rapidly enters the stratum corneum and remains in the deepest layers of the stratum corneum releasing their drugs or proteins might therefore also have some important advantages.

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