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Particle size of liposomes influences dermal delivery of substances into skin

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Abstract

In the present study, the influence of vesicle size on the penetration of two fluorescently labeled substances into the human skin was investigated. For the measurements either a hydrophilic fluorescent compound [carboxyfluorescein (CF)] or a lipophilic one [1,1′-dioctadecyl-3,3,3′,3′-tertramethylindocarbo-cyanine perchlorate (DiI)] were encapsulated into vesicles. Liposomal formulations were prepared by extruding the vesicles through polycarbonate membrane filters with pores of different sizes. In vitro penetration studies into human abdominal skin were performed by using the Franz diffusion cell and a standardized skin stripping technique in attempt to find an optimum size for topical drug delivery by liposomes. Confocal laser scanning microscopy (CLSM) was used to visualize the effect of penetration ability of liposomal DiI. The maximum DiI fluorescence in the skin was observed with smaller liposomes of 71 nm diameter. The liposomes with a size of 120 nm diameter showed statistically enhanced penetration of CF into the skin as compared to larger ones. The results indicated that the CF penetration was inversely related to the size of the liposomes, which was confirmed by the data of the confocal laser scanning microscopy studies.

Keywords: Topical delivery; Liposomes; Particle size; Carboxyfluorescein; Skin penetration

1. Introduction

The skin is the largest organ of our body (up to 16% of body weight) and consists of a complex layered structure, which forms a barrier between the body and the outside environment. However, this barrier remains slightly open and permeable to the environment to allow an exchange of heat, air, as well as fluids containing matter of very low molecular weight.

The skin is structured in three layers, namely the epidermis, the dermis and the subcutaneous layer. The outer layer of the epidermis, the stratum corneum (SC), covers the entire outside of the body. The horny cells of the SC are constantly being shed as thin scales and replaced with new layers from below. The SC is the rate-limiting barrier to percutaneous absorption

Abbreviations: CF, carboxyfluorescein; SC, stratum corneum; PI, polydispersity index; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; PBS, phosphate buffer saline; DOPE, dioleylphosphatidyl ethanolamine; lyso-PC, lyso-phosphatidylcholine; MLV, multilamellar liposomes; LUV, large unilamellar liposomes; CLSM, confocal laser scanning microscopy

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and protects the body against influences from the outer world. It impedes the evaporation of water from the tissue beneath and acts as a barrier to large amounts of water and foreign substances with which the skin comes in contact. The SC is composed of dense layers of dead, flattened cells filled with the fibrous protein, keratin, which is produced by the epidermis beneath. In its absence, for example, when the skin is denuded by a disease or even after repeated tape stripping, the absorption of drugs into the skin is increased.

Many factors govern the delivery of drugs and cosmetics into the skin from topically applied formulations. These factors include the size of the molecule, the lipophilicity of the component, type of formulation, presence of penetration enhancers and physical state of the SC. Liposomes, small vesicles composed of phospholipids, have been used for years to bring active ingredients into the skin. Several factors such as lamellarity, lipid composition, charge on the liposomal surface, mode of application and the total lipid concentrations have been proven to influence drug deposition into the deeper skin layers (Cevc and Blume, 1992; Weiner et al., 1989).

Mezei and Gulasekharam (1980, 1982), Singh and Mezei (1983) and Schaeffer and Krohn (1982) were the first to report the potential use of liposomes in topical applications for the skin and eyes. Many studies performed in the last decade showed significantly higher absorption rates (Michel et al., 1992; Cevc, 1996), as well as greater pharmacological effects for drugs applied to the skin entrapped in liposomes, as compared to conventional topical formulations (Skalko et al., 1992; Sharma et al., 1994). Two in vivo rabbit studies document comparisons between liposomal and conventional formulations of triamcinolone acetonide (Mezei and Gulasekharam, 1980, 1982). In both studies, the application of the liposomal preparations was associated with greater steroid concentrations in the epidermis and dermis and a lower systemic absorption than the regular formulations. Further, biodeposition studies in animals have demonstrated that liposomal encapsulation can improve the penetration of various molecules.

It is reported by several authors that the high elasticity of vesicles could result in enhanced drug transport across the skin as compared to vesicles with rigid membranes (Planas et al., 1992; Sentjurc and Gabrijelcic, 1995; Cevc et al., 1998; Paul et al., 1998; van den Bergh et al., 1999; Guo et al., 2000a,b). It seems that liposomes with a heterogeneous lipid composition, or in other words, with several coexisting domains exhibiting different fluidity characteristics in the bilayer (Vrhovnik et al., 1998) can be used to enhance the penetration of entrapped drugs into the skin. It is supposed that once in contact with skin, some budding of liposomal membrane might occur (Cevc et al., 1995; Vrhovnik et al., 1998). This could cause a mixing of the liposome bilayer with intracellular lipids in the SC (Egbaria et al., 1991), which may change the hydration conditions and thereby the structure of lipid lamellae. This may enhance the permeation of the lipophilic drug into the SC and ease the diffusion of hydrophilic drugs into the interlamellar spaces. On the other hand, it may be possible that some vesicles, which are deformable enough, will pass the SC as intact structure (Cevc and Blume, 1992; Cevc et al., 2002) or may accumulate in the channel-like regions in the SC (Honeywell-Nguyen et al., 2000) depending upon their compositions. Mode of application of the vesicles has been another issue of discussion initially addressed by Cevc and co-workers in 1992. They reported that the flexible vesicles work more efficiently under non-occlusive application. Non-occlusive application is the key to create a transepidermal osmotic gradient, which is believed to be the driving force for the transport of vesicles into the skin (Cevc and Blume, 1992).

There have been several methods reported in literature for percutaneous penetration enhancement and its quantification. These include diffusion experiments (Du Plessis et al., 1994), visualization by electron microscopy (Hofland et al., 1995; Korting et al., 1995) and micro dialysis (Schnetz and Fartasch, 2001). Micro dialysis and diffusion experiments provide information about the amount and the rate of drug penetration of the model compound, but do not give any information about the physiological effects of the model drug on cells and lipid organization. The visualization by electron microscopy provides detailed information about the structure of the cells and lipid organization in the skin, but does not provide information about the penetration pathways. Other techniques used are fluoromicrography (Yarosh et al., 1994; Kriwet et al., 1995) and CLSM. Fluoromicrographs of skin treated with fluorescently labeled liposomes demonstrated that the fluorescent marker remained in the SC or penetrated deeper in the epi-

dermis mainly along the hair shaft. A disadvantage of the fluoromicrograph technique is that the tissue needs to be (cryo)fixed, which may change skin lipid organization or may result in redistribution of the marker (Shotton and White, 1989). CLSM provides information about the localization and the permeation pathway of a fluorescent model compound in the tissue. The major advantage of CLSM is that the distribution of the fluorescent model compound in the sample can be visualized without cryofixing or embedding the tissue. However, in the case of penetration studies with liposomes, CLSM does not provide information about the permeation of the entire liposome, but only about the penetration of the fluorescent label (van Kuijk-Meuwissen et al., 1998).

It was assumed 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. The aim of this study was, therefore, to investigate the influence of liposome size on the transport of a hydrophilic, as well as a lipophilic fluorescent compound into the human skin by using the Franz diffusion cell and CLSM. For this purpose liposomes with a well-defined lipid composition and size were used in an attempt to find the best formulation for topical drug delivery.

2. Materials and methods

2.1. Materials

2.1.1. Lipids

Phospholipon 90 was purchased from Nattermann GmbH (Cologne, Germany), and α-tocopherol from Merck (Darmstadt, Germany).

2.1.2. Other chemicals and instruments

CF was purchased from Fluka Biochemika (Steinheim, Germany). Sodium cholate, chloroform, methanol were purchased from Merck. Tris ultra pure was purchased from ICN Biomedicals (Aurora, Ohio). DiI was purchased from Aldrich Chem. Co., USA. All other chemicals were of analytical grade and the water used was demineralised by means of a Milli-Q plant (Millipore, Darmstadt, Germany). The fluorescence spectrometer used was Perkin-Elmer—LS50B luminescence, Langen, Germany. Mini extruder Lipofast was from Avestin (Ottawa, Canada). The cryotome was from Vogel Cryotome AS 620 (Anglia-Scientific, UK).

2.2. Liposome preparation and characterisation

2.2.1. Liposome preparation

Liposomes were prepared by a conventional rotary evaporation method. Phospholipon 90 (10 wt.%), α-tocopherol (1 wt.%) and sodium cholate (weight ratio of surfactant to lipid = 0.28) (Cevc and Blume, 1992; Guo et al., 2000a) were dissolved in methanol and chloroform (1:1). This mixture was dried to a thin film in the presence of nitrogen under vacuum. This film was kept under high vacuum for 2 h to remove the traces of organic solvents. The film was hydrated with Tris buffer pH 7.0 containing CF (30 mmol) to make a lipid coarse suspension. Liposomes with DiI were prepared by the ethanol dissolution method (Shmuel and Edward, 1973). A mixture of lipid soluble components including DiI (0.5 mM) were dissolved in ethanol (25 wt.%) and phosphate buffer saline was slowly added to this mixture with agitation. The ratio of lipid and ethanol was kept constant (75:25 w/w) for all liposomes containing DiI. These large vesicles were pressed through 400, 200, 100, and 50 nm pore size polycarbonate membrane to produce liposomes of the desired size with the help of Avestin Mini hand extrusion device (MacDonald et al., 1991).

2.2.2. Liposomal size

The diameter of vesicles was measured by a Zetasizer instrument (Malvern Instruments, Malvern, UK). The polydispersity index (PI) was used as a measure of an unimodal size distribution. Small value of PI (<0.1) indicates a homogenous population, while a PI (>0.3) indicates a higher heterogeneity.

2.3. The skin

2.3.1. Skin dissection

Excised human skin from female patients, who had undergone abdominal plastic surgery, was used. Immediately after excision the subcutaneous fatty tissue was removed using a scalpel. The skin was wrapped in aluminum foil and stored in polyethylene bags at $-25\,^{\circ}C$ until use. Under these conditions the skin is stable with regard to the penetration of drugs, as well

the thickness of the SC, over a time period of 3 and 6 months, respectively (Harrison et al., 1984; Bronaugh et al., 1986; Schaefer and Loth, 1996). For penetration experiments, skin disks of 35 mm in diameter were punched out, thawed, cleaned with cotton, which was soaked with Ringer solution, and transferred onto the Franz diffusion cell.

2.3.2. Determination of the thickness of the skin and the SC

For determination of the thickness of the SC, the tapes were accurately weighed before and after stripping. The resulting layer thickness was calculated according to the following equation (Michel et al., 1992):

$$T = \frac{d}{a \times p}$$

T represents the thickness of SC removed (in μm), d is the difference in strip weight after and before stripping (in μg), a denotes the area of the strip (μm^2) and p is the density of the SC (1×10^3 μg/1×10^9 μm^3).

2.4. Penetration studies

2.4.1. Dosage regime and incubation times

Ten microliters each of liposomal preparation containing CF were applied non-occlusively per cm^2 onto the skin surface. The duration of the penetration studies lasted 14 h in a Franz diffusion cell. In the case of DiI liposomes the dose applied was 31.85 μl/cm^2 for 3 h.

2.4.2. Franz diffusion cell

On the Franz diffusion cell (Gauer Glas, Püttlingen, Germany), the skin sections were mounted with nominal surface areas of 3.14 cm^2 and a receiver compartment of a 12 ml capacity. The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed by phosphate buffer saline pH 7.4. The receptor fluid was mixed with a magnetic stirring bar at 500 rpm. Buffer was kept at 37 ± 1 °C by a water jacket. All bubbles were carefully removed between the underside of the skin and solution in the receiver compartment. To achieve higher reproducibility the skin was pre-hydrated with the basolateral receptor medium for 60 min before applying the formulation. The liposomal formulations were applied onto the skin surface area of 3.14 cm^2 with the help of micropipette. Minimum of three diffusion cells were used for each formulation. All experiments were carried out with non-occluded donor compartments. After 3 or 14 h the experiments were stopped and the diffusion set up was dismantled.

2.4.3. Skin stripping and skin cryosectioning

After incubation for a predetermined time period, the liposomal formulation was removed by wiping the skin with cotton. The skin was transferred into a special apparatus where it was mounted on cork discs using small pins. The stretching of the skin was important as it helped to overcome problems of furrow in the subsequent tape-stripping procedure. For this procedure the surface of the skin was covered with a Teflon mask containing a central hole of 15 mm in diameter. Proceeding from this hole the skin was stripped with 20 pieces of adhesive tape (size = 15 mm × 20 mm) (Tesa®, Beiersdorf, Hamburg, Germany). The tape was of sufficient size to cover the full area of the skin, which was in contact with the formulation. In a standardised procedure each tape was charged with a weight of 2 kg for 10 s and thereafter removed rapidly. The adhesive tape was weighed before and after stripping to determine the weight of SC removed by each strip.

After the tape stripping, the skin was rapidly frozen by liquid nitrogen, a specimen with a diameter of 13 mm was taken out of the stripped area and frozen on a metal block. The metal block was transferred into a cryomicrotome. The skin was sliced into surface parallel sections and pooled according to the following scheme: # 1 = first slice; # 2–5 = 6 × 20 μm sections; # 6–9 = 12 × 20 μm sections; # 10 = rest of the residual tissue. The thickness of the first and last samples was calculated from the total weight of the full thickness slices. Fig. 1 represents the schematic diagrams explaining skin stripping and skin cryosectioning.

2.4.4. Skin cryosectioning for CLSM

After an incubation period of 3 h on Franz diffusion cell, the skin pieces were sliced in sections of 7 μm thickness, perpendicular to the skin, with the help of cryomicrotome. These cross-sections were investigated for the amount of CF in the different skin layers by using a laser scanning confocal imaging system(“True confocal Scanner”, Leica TCS 4D, Microscope- Leitz DM R XE upright, Laser: Argon

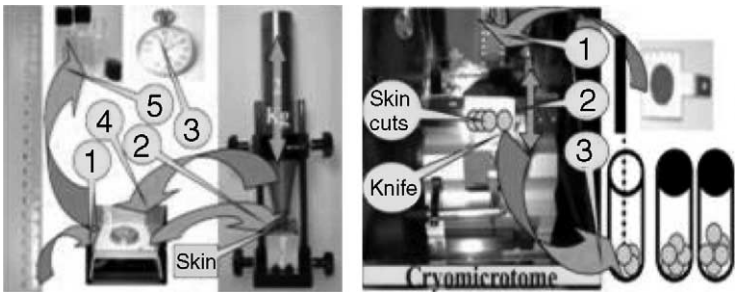

Fig. 1. Schematic diagrams explaining skin stripping (left) and skin cryosectioning (right).

Krypton emission Wavelengths of 488, 578 and 647, Filters: OG 590 for DiI and BP-FITC for CF and Programme Scanware 5.10).

2.4.5. Scoring

The fluorescence intensities of the CLSM images were semi-quantitatively blind-scored by five educated individuals and classified as follows: (−) no fluorescence, (±) very weak fluorescence, (+) weak fluorescence, (++) medium fluorescence, (+++) bright fluorescence.

2.5. Extraction of CF from the strips and skin samples

The CF was extracted from the adhesive tape and skin cuts with a mixture of ethanol and water pH 7.0 in 1:1 ratio. For extraction of CF from the adhesive tape, 2 ml of the extraction solvent was added to the each sample and was shaken for 1 h. For extraction of CF from the skin cuts, 2 ml of the extraction solvent was added to the each sample and this mixture was vortexed for 2 min, followed by probe sonication for 2 min. The samples were then shaken on a vibrator (MLW Labor technik, Ilmenau, Germany) for 2 h. After shaking, the skin samples were centrifuged at 5000 rpm for 30 min. All samples were quantitatively analysed by spectrofluorometry at an excitation wavelength 470 nm and emission wavelength at 520 nm.

2.6. Statistical data analysis

Data analysis was carried out with the software package Microsoft Excel, Version 97. Results are expressed as mean ± standard error, $n = 3$ independent samples. Statistically significant differences were determined using the Student's *t*-test and ANOVA with $P < 0.05$ as a minimal level of significance.

3. Results and discussion

The SC is built like a wall with protein bricks and lipid mortar (Elias, 1983). The intercellular lipids are important in controlling the percutaneous absorption (Elias et al., 1981; Williams and Elias, 1987). In the case of liposomes, the phospholipids may mix with the intercellular lipids and thereby cause the swelling of intercellular lipids without altering the multiple bilayer structure of the SC. These swollen lipids cause accumulation of the drug and thereby form an intracutaneous depot. Although the mechanism of enhancement using topically applied liposomes is not fully understood, drug disposition is primarily dependent on lipid composition, liposome lamellarity and surface charge (Couarraze and Wepierre, 1995). The mechanism by which the elastic vesicles penetrate the SC is explained by different authors. A considerable work on this aspect has been published by Bouwstra and co-workers. They demonstrated by visualisation studies on an electron microscopic level that the elastic vesicles are taken up very rapidly into human stratum corneum in vivo, most probably along a fine network of pre-existing channels. After 1 h of application, the vesicles have been visualised deep in the human stratum corneum (Honeywell-Nguyen et al., 2002a,b). Permeation studies demonstrated that elastic vesicles act as drug carrier systems and not as penetration enhancers (Honeywell-Nguyen et al., 2002). The vesicles carry thereby the associated drugs into the skin. All these studies strongly suggested that vesicles rapidly enter the stratum corneum in channel-like

Table 1
The amount of CF (expressed as percent dose applied ± S.E.) delivered from liposomes with different particle size into the different strata of human abdominal skin using Franz diffusion cell in vitro after 14 h non-occlusive application, $n = 3$

Average size of liposome (nm)	Stratum corneum	Deeper skin	Receiver compartment
120	72.879 ± 1.692	0.765 ± 0.079	0.268 ± 0.034
191	65.279 ± 1.736	0.164 ± 0.034	0.177 ± 0.026
377	61.194 ± 2.624	0.105 ± 0.009	0.113 ± 0.005
810	39.952 ± 2.638	0.023 ± 0.004	0.038 ± 0.003

regions and carry thereby drug molecules into the skin.

The influence of liposome size seems to be important, too, but no dedicated study was performed up to now to clarify this subject. Some authors have observed better penetration of hydrophilic drugs from reverse-phase evaporation vesicles than from MLV made of egg lecithin liposomes (Gabrijelcic et al., 1994). Esposito et al. (1998) reported that the permeability coefficient of methyl nicotinate is inversely related to the liposome size. Du Plessis et al. (1994) showed that the intermediate particle size of 300 nm resulted in both the highest reservoir in the deeper skin layers, as well as the highest drug concentration in the reservoir, confirming that topical drug delivery is influenced by the size of liposomes.

All the formulations used in this study showed a polydispersity index below 0.3 except for the 810-nm liposomes, indicating a good homogeneity for most of the liposomes. The smaller liposomes of 120 nm diameter showed statistically enhanced penetration of CF in the skin as compared to larger ones. Table 1 shows the amount of CF delivered from liposomes of different sizes into different layers of the skin. Figs. 2 and 3 represent the SC strip profile and skin depth profile, respectively. Fig. 4 represents the amount of CF delivered into the SC at an average thickness of 9.347 μm.

Liposomes with a size of 120 nm showed a maximum accumulation of CF in the SC, deeper skin layers, and also in the receptor compartment of the Franz diffusion cell, as compared to larger ones. The amount of CF in the SC was 1.12-, 1.19-, and 1.83-fold higher for small liposomes with a size of 120 nm as compared to 191, 377, and 810 nm, respectively. The amount of CF detected in the deeper skin layers was 4.68-, 7.29-,

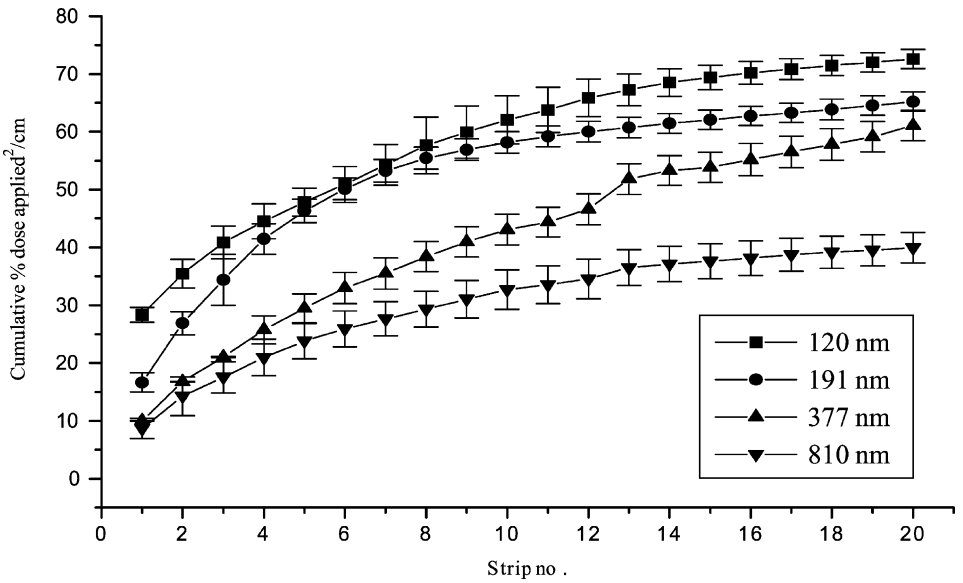

Fig. 2. SC strip profile of CF liposomes after 14 h of non-occlusive application (expressed as percent dose applied ± S.E.) $n = 3$.

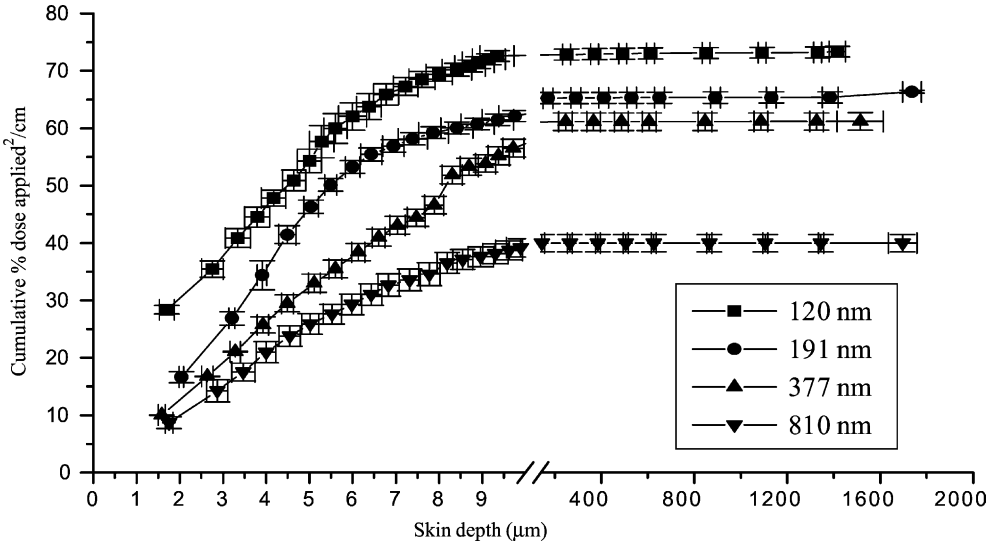

Fig. 3. Depth profile of CF liposomes across the human abdominal skin after 14 h of non-occlusive application (expressed as percent dose applied ± S.E.) $n = 3$.

and 33.57-fold higher than with larger liposomes and the amount recovered in the receptor compartment was 1.52-, 2.38-, and 7.11-fold higher. It is interesting to note that the smallest particle size 120 nm resulted in the highest amounts, i.e. 0.765% of CF in the deeper skin as compared to 0.02% with 810 nm liposomes. It thus appears as if the liposomes regulate the location of the depot effect of hydrophilic drugs into the skin. These results are in agreement with the previous reports (Esposito et al., 1998). These results let us conclude that larger vesicles may not penetrate well into the deeper layers of the skin and stay in/on the SC

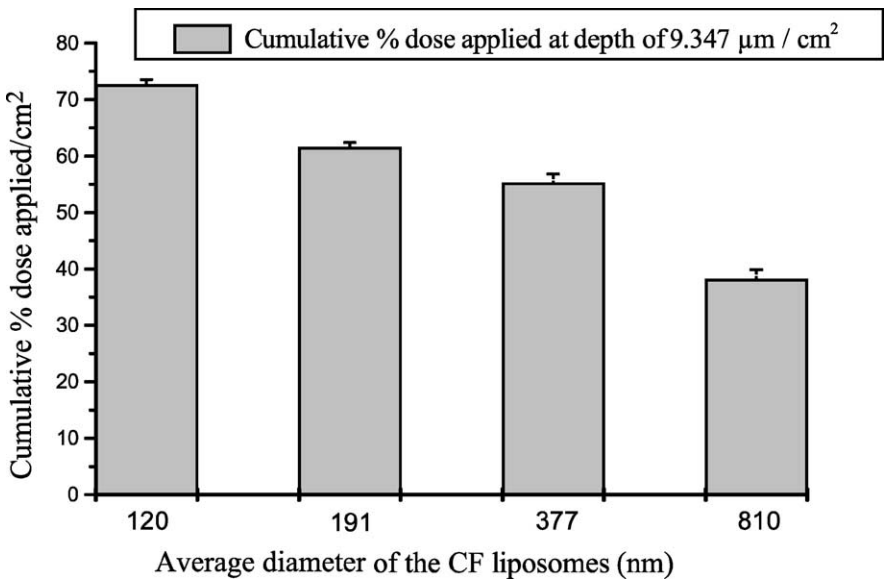

Fig. 4. The amount of CF delivered to the SC with a thickness of 9.347 μm (expressed as percent dose applied ± S.E.) $n = 3$.

forming a lipid layer after drying. It appears as if there might be an optimum particle size for optimal drug delivery. However, a more extensive study should be undertaken to find out the exact optimum particle size for each formulation.

In the last two decades, CLSM has been extensively used as a tool to visualize the fluorescent model compound in the skin. van Kuijk-Meuwissen et al. showed in 1998 that the dye applied non-occlusively in flexible liposomes penetrated deeper into the skin than after occlusive application (van Kuijk-Meuwissen et al., 1998). Kirjavainen et al. reported in 1996 that the fluorescence from liposomal compositions containing DOPE (dioleylphosphatidyl ethanolamine) were able to penetrate deeper into the SC than that from liposomes without DOPE. A pretreatment of skin with unlabeled liposomes containing DOPE or lyso-phosphatidylcholine (lyso-PC) enhanced the subsequent penetration of the fluorescent markers, N-Rh-PE and sulforhodamine B into the skin, suggesting possible enhancer activity (Kirjavainen et al., 1996). In 1997, Boderke et al. used CLSM to show that the amino peptidase activity was evenly distributed throughout the viable part of the epidermis, with enhanced fluorescence in the upper layers of the stratum granulosum, while dermis and SC showed

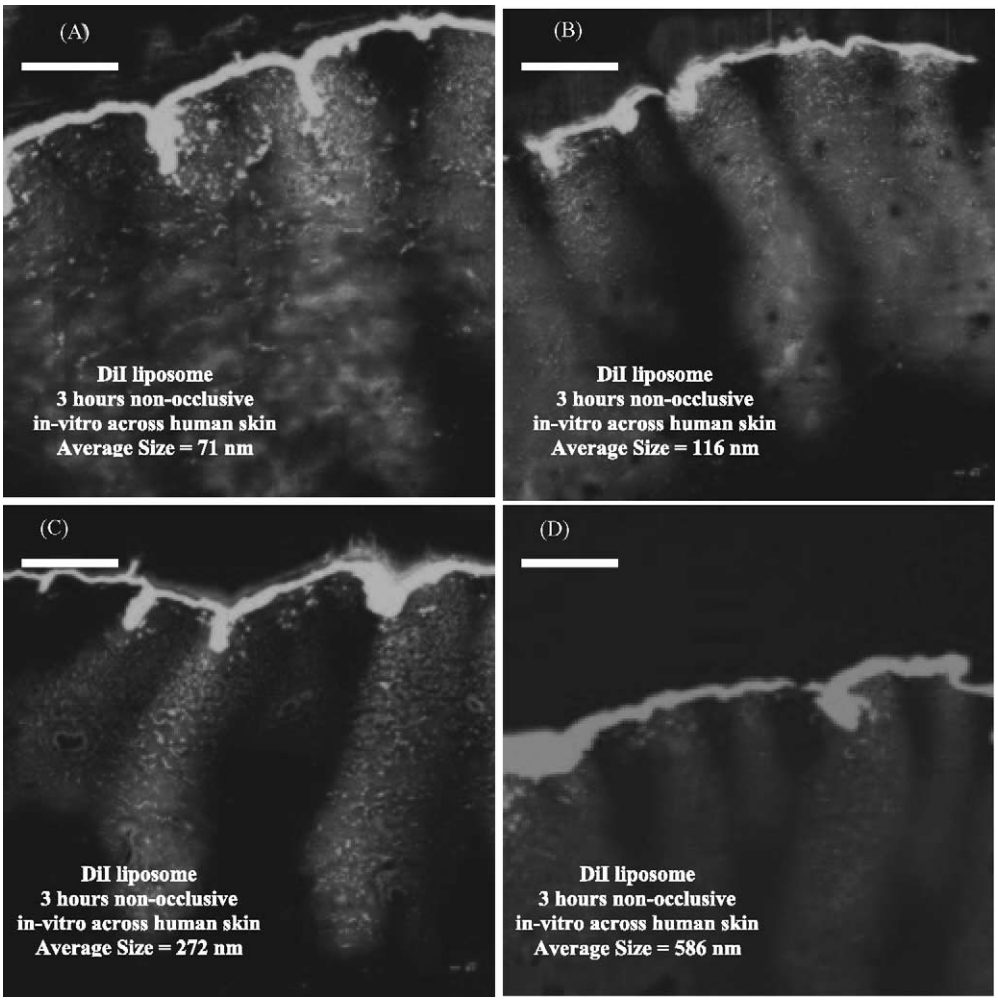

Fig. 5. CLSM images of a cross-section of human abdominal skin incubated in a Franz diffusion cell with liposomes containing the lipophilic label, DiI. The liposomes were applied non-occlusively for 3 h. Scale bar represents 100 nm.

considerably less amino peptidase activity (Boderke et al., 1997). In 1998, Zellmer et al. used CLSM to demonstrate that vesicles made of native human SC lipids rapidly interact with phosphatidylserine liposomes, weakly with human stratum corneum lipid liposomes and have no effect on PC liposomes (Zellmer et al., 1998). Vardaxis et al. (1997) employed CLSM to examine the structure of porcine skin and concluded that it provides valuable additional morphological information of material examined by conventional microscopy for wound healing studies (Vrhovnik et al., 1998). Zellmer et al. (1995) reported that neither the vesicles nor the fluorophore *N*-(lissamine rhodamine B sulfonyl) diacylphophatidylethanolamine (Rho-PE) penetrate into the human skin in a detectable amount. Turner and Guy showed in 1998 that iontophoresis significantly enhanced the delivery of calcein into hairless mouse skin, particularly via follicular structures (Turner and Guy, 1998). Simonetti et al. (1995) visualized diffusion pathways across the SC of native and in vitro reconstructed epidermis by using CLSM.

Fig. 5 represents CLSM images of the skin cross-sections perpendicular to the skin surface. Table 2 shows the blind scoring data from CLSM pictures. The images are taken after 3 h of the non-occlusive application of the liposomes containing DiI as lipophilic fluorescent label.

In all the images a very high fluorescence was observed in the SC, which is obvious as the fluorescent label DiI is highly lipophilic. In the case of larger vesicles, i.e. 586 nm liposomes, the fluorescence in the viable epidermis and dermis was very weak. However, in the case of liposomes with an average diameter of 272 nm, there was weak fluorescence observed in viable epidermis and dermis. The small sized liposomes of the average diameter 116 nm had shown weak to medium fluorescence in the viable epidermis and weak fluorescence in the dermis. The smallest liposomes with an average diameter of 71 nm had shown medium fluorescence in the viable epidermis and medium to weak fluorescence in the dermis. There has been great progress in the dermal liposomal delivery. Up to now there is no clear evidence, whether liposomes can pass intact into deeper layers of skin or not.

Table 2
Summarised blind scoring data from CLSM images

Average size of liposome (nm)	Stratum corneum	Viable epidermis	Dermis
71	+++	++	+
116	+++	++	+
272	+++	+	+
586	+++	±	±

CLSM images were taken from cross-sections of human skin that was non-occlusively treated with DiI labeled liposomes for 3 h. The images were scored on the depth and fluorescence intensities of DiI penetration in SC, viable epidermis and dermis. The fluorescence intensities of the CLSM images were semi-quantitatively blind-scored by five individuals and classified as: (−) no fluorescence; (±) very weak fluorescence; (+) weak fluorescence; (++) medium fluorescence; (+++) bright fluorescence.

This CLSM study indicates that the large vesicles with a size ≥600 nm are not able to deliver their contents into deeper layers of the skin. These liposomes stay in/on the SC and after drying they may form a layer of lipid, which may further strengthen the barrier properties of SC. The liposomes with size ≤300 nm are able to deliver their contents to some extent into the deeper layers of the skin. However, the liposomes with size ≤70 nm seems to be promising for dermal delivery as they have shown maximum fluorescence both in viable epidermis, as well as in dermis. In a growing number of topical studies, liposomes have been shown to target drugs to the pilosebaceous unit. It is now accepted that hair follicles and sebaceous glands may participate in skin penetration for a wide range of compounds (Lauer et al., 1995).

This study has shown that the fluorescent model compound can be visualized in deeper layers of the skin by making cross-sections perpendicular to the skin surface or by taking CLSM images. The method used in this study does not require fixation and embedding of the samples, and thereby reducing the redistribution of the label or tissue damage.

Until a suitable method of analysis is available, by which both the skin and the liposomes can be visualized, as well as distinguished, speculation about the mechanism of topical liposomal drug deposition will continue.

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