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Research paper

Modification of the conformational skin structure by treatment with liposomal formulations and its correlation to the penetration depth of aciclovir

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ABSTRACT

The stratum corneum (SC), top layer of the epidermis, is comprised mostly of lipids, which are responsible for the permeability properties of the SC and which protect the body from external agents. Changes in these skin micro constituents can be understood by instrumental methods such as attenuated total reflectance Fourier-transform infrared (ATR–FTIR) spectroscopy. The present work shows that different types of analyzed skin, dermatomed abdominal porcine skin, pig ear skin and human heat separated skin, influenced both the shape and the intensity of recorded spectra. The typical FTIR spectral bands of the conformation of the lipid aliphatic chains in the skin samples were altered after treatment with pure DPPC liposomes and chitosan (CS) coated DPPC liposomes, but not with aqueous CS-solution. The conformational change could be the reason for the variable permeability of the skin. This was confirmed by tape stripping on pig ear skin (imitating in vivo studies): the amount of aciclovir penetrating from polymer coated and polymer free liposomes was significantly higher under the skin surface in comparison with the aqueous CS-solution. Moreover, the addition of the polymer to liposomes induced a higher skin penetration than pure liposomes. One explanation might be the CS's stronger adhesion to the skin.

1. Introduction

The stratum corneum (SC) is a distinctive two compartment system, consisting of corneocytes embedded in a lipid matrix. The complex structure of the SC lipid bilayers is important in maintaining the barrier properties of the skin [1–3]. To understand the modification of the lipid organisation within the SC many techniques such as X-ray diffraction, IR spectroscopy, DSC, ^{2}H NMR spectroscopy and electron microscopy are applied [4–6]. Most of the experimental evidence is consistent with the domain mosaic model suggested by Forslind et al., in which the skin lipids are organised in ordered domains (orthorhombic (OR) and hexagonal (HEX)) connected by lipids in a disordered phase (liquid–crystalline (LIQ)) [7–9]. It has been proposed that these domains would have crucial impact on SC permeability: the permeability of a disordered phase is in general greater than that of the ordered one [5]. According to Wertz et al. the greatest flux would be at the boundaries of these two phases [7].

In the present study Fourier-transform infrared spectroscopy (FTIR) with attenuated total reflection (ATR) technique was performed. This is a highly suitable technique for determination of molecular vibrations of the components of the SC at the functional group level [8]. The FTIR method has already been used to characterize the phase transitions of the SC lipids. These transitions were detected by shifting of methylene (CH_2) bands of the lipid aliphatic chains [1,2,8,10]. Therefore, in a first step the characteristic CH_2 bands of dermatomed abdominal porcine skin, pig ear skin and human heat separated skin were compared and analyzed. In our last work the ATR–FTIR method was used to better understand interactions of the model drugs and polymers with DPPC liposomes [11]. Based on these results, our intention was to further understand interactions between skin and different formulations. For this purpose the following formulations were applied: DPPC liposomes, chitosan (CS) coated DPPC liposomes as well as aqueous CS-solution. The liposomal formulations were additionally visualised by cryo-TEM to assess whether the differences between polymer free and polymer containing liposomes could be seen. Because the previous studies showed higher aciclovir skin diffusion in vitro (Franz-cell model) from polymer coated versus polymer free liposomes additional penetration studies using the tape stripping method on pig ear skin were performed [11]. With this method it is possible to investigate the drug distribution and penetration layer by layer of topically applied drugs in the SC [12–14].

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2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphatydilcholine (DPPC) was purchased from Lipoid (Steinhausen, Switzerland). The product was synthetic Lipoid PC 16:0/16:0. The content of phosphatidylcholine was at least 99 % related to the dry weight. Chitosan (CS < 500 kDa) in the powder form was a gift from Syntapharm (Mülheim, Germany). The degree of deacetylation was determined by NMR with 95% [15]. Aciclovir was purchased from Fagron GmbH (Barsbüttel, Germany) All other chemicals used in this study were of analytical reagent grade and were used as received without any further purification.

2.2. Formulations

Skin samples for FTIR experiments were treated with DPPC liposomes and chitosan (CS) coated DPPC liposomes, prepared as previously described [11]. Particle size was measured by a Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom). The aqueous CS-solution was produced by dissolving the polymer in 0.01 M acetate buffer. The amount of CS used in formulations was 0.125%.

For the tape stripping procedure DPPC and CS coated liposomes were loaded with 1% aciclovir as reported [11]. Aciclovir, in aqueous CS-solution, was suspended in the same concentration.

2.2.1. Encapsulation of aciclovir in liposomes

Encapsulation of aciclovir in the liposomes was established by differential centrifugation method [16]. Briefly, liposomes and coated liposomes with aciclovir were centrifuged at 1200*g* for 2 h at 25 °C (Hermle Z323K, Wehingen, Germany). The supernatants were removed and dissolved in 96% ethanol. Aciclovir content in the supernatants and in the precipitates was quantified by HPLC method specified previously ($n = 3$) [11].

The encapsulation efficiency of aciclovir was calculated as described below:

$$\text{Encapsulation efficiency (EE\%)} = \frac{\text{Amount of bound drug}}{\text{Total amount of drug}} \times 100 = 24.38 \pm 0.24. \quad (1)$$

2.3. Skin preparation for FTIR

2.3.1. Abdominal porcine skin

The full thickness abdominal skin without hair was dermatomed to a thickness of 1.2 mm and then stored frozen at −20 °C. Skin samples with a dimension of about 7.5 cm^2 (ZnSe crystal dimension) were adopted for the experiment, which was performed ten times.

The following treatments were applied:

(a) Application of 40 μl distilled water.
(b) Application of 40 μl pure DPPC-liposomes.
(c) Application of 40 μl coated CS-DPPC liposomes.
(d) Application of 40 μl aqueous CS-solution.
(e) Untreated skin (control).

After the treatment, the skin samples were placed in PBS buffer and kept at 37 °C or 45 °C in a closed petri dish for 2 h in each case [5,10].

2.3.2. Pig ear skin

The same experimental procedure was repeated on pig ear skin prepared as described later (see Section 2.6.1).

2.3.3. Human skin

The human skin was obtained when excessive skin was removed from one healthy patient undergoing plastic surgery. The appropriately treated skin was heat separated and wrapped in filter paper. It was stored at −20 °C for not longer than 6 months before use [17].

2.4. Attenuated total reflectance Fourier-transform infrared (ATR–FTIR)

The penetration depth of IR is about 1 μm or less, which includes approximately 1–1.5 sheets of cell layers in the SC [10,18]. Infrared spectra of the prepared skin samples were obtained by using FTIR spectrophotometer (model: Tensor 27, Bruker Optics, Ettlingen, Germany) with a photovoltaic MCT detector at a temperature of 37 °C (physiological temperature) and 45 °C (transition temperature of SC lipids). To collect the spectra, the skin samples were placed stratum corneum down onto the ZnSe ATR crystal (tool: Bio-ATR I). To obtain the same intensity of spectra we put the cover glass on the sample. For data treatment we used the software OPUS 5.5.

2.5. Cryo-transmission electron microscopy (cryo-TEM)

Liposomes for cryo-TEM were frozen with a Leica EM GP immersion freezer (Leica Microsystems, Vienna, Austria) with its environmental chamber a 50 °C and 90% relative humidity. Four microliters of the specimen diluted to 2.5 mg/ml and pre-warmed to 50 °C was applied onto glow discharged EM grid coated with perforated Quantifoil R3.5/1carbon films (Quantifoil, Jena, Germany). After 30 s settling, the suspension was automatically blotted for 0.5–1.0 s with Whatman No. 1 filter paper and immediately plunged from the environmental chamber into liquid ethane. Subsequently, the specimens were handled only in the dehumidified working area and stored in liquid nitrogen prior to microscopy.

The vitrified specimens were visualised on a Tecnai F30 'Helium' (Polara) cryo-TEM (FEI Company, Eindhoven, Netherlands) operated at 300 kV and cooled with liquid nitrogen. Micrographs were acquired at nominal magnification of 31,000× at a defocus of −8.0 μm and captured with a Gatan US4000 CCD camera.

2.6. Tape stripping

2.6.1. Pig ear skin

The pig ears were obtained from local butcher and stored in the refrigerator at −20 °C. The night before the tape stripping procedure, the pig ears were stored in the fridge at 4 °C. On the day of procedure the skin surface was cleaned with distilled water and dried. The hair was carefully removed by scissors as closely as possible to the surface.

2.6.2. Tape stripping

The pig ears were fixed on a polystyrene support covered with aluminium foil, an area of 5 × 4 cm^2 was marked with a permanent marker and 5 μl/cm^2 of the tested formulation was applied. After 1 h tape stripping was performed as described previously using Corneofix® tape strips [12,19,20]. Briefly, each tape strip was pressed on the skin with the roller over the paper for 5 s and then removed in a single quick movement. Every tape strip was placed onto the special slide frame to determine the mass of the removed SC. The pseudo-absorption of the skin corneocytes, which

correlates with the protein amount on the removed tape strip, was established at 850 nm via infrared (IR) densitometer Squame-Scan™850A (Heiland electronic, Wetzlar, Germany) as described previously [21,22]. After the removal of 40 tape strips (corresponding to most of the SC) for all formulations, the model drug (aciclovir) was detectable up to the 10th strip. Therefore, only 10 strips were taken in all further experiments exhibited on five additional pig ears. To analyse aciclovir content in different layers of the skin, the tape strips (2×2 cm^2) were extracted with 4 ml distilled water (1 ml/cm^2), respectively. The tubes were sonicated on ultra sonic bath for 12 min and afterwards centrifuged for 10 min. Aciclovir content was detected by HPLC using the method described previously [11].

2.6.3. Transepidermal water loss (TEWL) measurements

The measurement of TEWL was used in a number of in vivo and in vitro experiments to provide information on a barrier property of the skin [23–25]. In our case, TEWL was recorded with closed chamber device (AquaFlux™, Biox, London, UK) before and after the tape stripping procedure on pig ear skin. Measurements were performed in triplicate, and AquaFlux® V6.2 software was used for data analysis.

2.7. Statistics

Results of all studies are presented as the means of at least three experiments ± SD. Data were exported to the GraphPad Prism statistics software package (GraphPad Prism Software, USA). Analysis groups consisted of independent mean values and the Gaussian distribution of the data was verified using the Kolmogorov–Smirnov test. A statistical analysis was performed using one-way ANOVA with post hoc Dunnet's or Tukey's test. *P*-values of <0.05 were considered significant.

3. Results and discussion

ATR–FTIR spectroscopy is a non-invasive technique for characterisation of the SC at a molecular level. Different attempts have been made to present IR spectra of skin [2,4,10]. In the present study IR spectra of dermatomed abdominal porcine skin, pig ear skin and human heat separated skin are compared (Fig. 1). The following skin bands obtained from skin intercellular lipids and keratin (corneocytes) were of interest: CH_2 asymmetric (~2920 cm^{-1}) and symmetric stretching vibration (~2850 cm^{-1}), CH_2 scissoring mode between 1470 and 1460 cm^{-1}, amid I (C═O) vibration at about 1640 cm^{-1} and amid II (C–N) vibration at about 1540 cm^{-1} as well as C═O stretching band from the fatty acids at about 1740 cm^{-1} [8,10] . In Fig. 1, it can be clearly seen that the human heat separated skin shows similar spectra to the dermatomed abdominal porcine skin as well as to the pig ear skin. These different skins have been employed for studying the influence of different formulation treatments on the characteristic vibration of the IR-spectra. For this purpose preliminary experiments were necessary. The amount of formulation applied, number of treatments and time of impregnation were optimised. The amount of 40 µl formulation to be used was established according to the length of time taken to be absorbed by the skin. This was in agreement with already published data [5,10]. Procedures using repeated applications and longer impregnation periods showed no significant differences in the obtained spectra (data not shown). According to the investigations undertaken, an application of 40 µl formulation impregnated with skin for 2 h was found to be optimal for spectra interpretation. In Tables 1 and 2, the band wavenumbers of different skin spectra (37 °C and 45 °C) impregnated with pure DPPC, CS coated liposomes as well as with the aqueous CS-solution and distilled water, respectively, are presented. The control modes (untreated skin) in all spectral regions, except the C═O region of pig ear skin and human skin, remained unaltered by an increase of temperature from 37 °C to 45 °C. This contrasts with published data showing a change across this temperature range [5,10,23].

Of particular interest for studying the interaction of the formulations with SC lipids are CH_2 stretching bands. Analyses of these bands provide information about conformational order (trans-gauche isomerisation) of the lipid alkyl chains [5,26,27]. After the treatment of dermatomed porcine skin and human skin with liposomal formulations, symmetric and asymmetric methylene stretching bands were shifted to significantly lower values compared with the control samples, indicating a lipid order–disorder transition from the gel to the liquid–crystalline state (Tables 1 and 2). This transition also involves an increase in the fluidity of the SC lipids that could increase the skin penetration [5]. However, the same treatments of pig ear skin showed the shift of symmetric stretching band to lower values only at 45 °C.

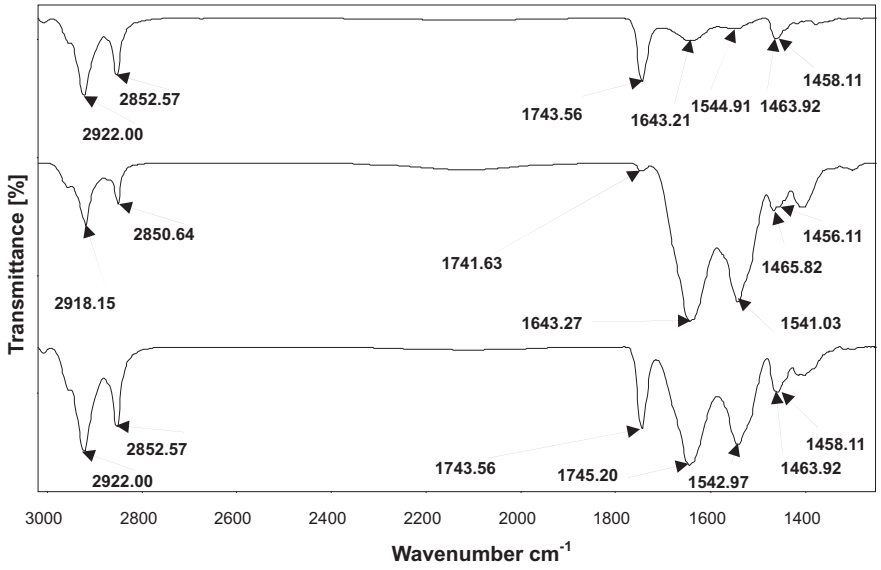

Fig. 1. Comparison of FTIR transmission spectra at 37 °C of untreated pig abdominal dermatomed, pig ear and human skin (from top to bottom).

Table 1
ATR–FTIR values of characteristic bands of different skin modes at 37 °C.

Impregnation	Dermatomed porcine skin modes (cm^{-1})				Pig ear skin modes (cm^{-1})				Human skin modes (cm^{-1})			
	CH_2 sym. stretching	CH_2 asym. stretching	C=O	CH_2 scissoring	CH_2 sym. stretching	CH_2 asym. stretching	C=O	CH_2 scissoring	CH_2 sym. stretching	CH_2 asym. stretching	C=O	CH_2 scissoring
Control	2922.0	2852.5	1743.5	1458.1 1463.9	2918.1	2850.6	1741.6	1456.1 1465.8	2922.0	2852.5	1743.5	1458.1 1463.9
Water	2922.0	2852.5	1743.5	1458.1 1463.9	2918.1	2850.6	1741.4	1456.1 1465.8	2922.0	2852.5	1743.5	1458.1 1463.9
DPPC	2918.1[a]	2850.6[a]	1742.2[a]	1456.1 1467.1	2918.1	2850.6	1738.4[a]	1456.1 1467.7	2920.0[a]	2850.6[a]	1743.5	1458.1 1465.8
CS-DPPC	2918.7[a]	2850.6[a]	1741.6[a]	1456.1 1465.8	2918.1	2850.6	1738.4[a]	1456.1 1467.7	2920.0[a]	2850.6[a]	1743.5	1458.1 1465.8
CS-solution	2922.0	2852.5	1743.5	1458.1 1463.9	2918.1	2850.6	1741.2	1456.1 1465.8	2921.3	2852.5	1743.5	1458.1 1463.9

Values are means of $n = 10$.
[a] Significantly decreased values compared with the control (Dunnet's test).

Table 2
ATR–FTIR values of characteristic bands of different skin modes at 45 °C.

Impregnation	Dermatomed porcine skin modes (cm^{-1})				Pig ear skin modes (cm^{-1})				Human skin modes (cm^{-1})			
	CH_2 sym. stretching	CH_2 asym. stretching	C=O	CH_2 scissoring	CH_2 sym. stretching	CH_2 asym. stretching	C=O	CH_2 scissoring	CH_2 sym. stretching	CH_2 asym. stretching	C=O	CH_2 scissoring
Control	2922.0	2852.5	1743.5	1458.1 1463.9	2918.1	2850.6	1743.5	1456.1 1465.8	2922.0	2852.5	1745.1	1458.1 1463.9
Water	2922.0	2852.5	1743.5	1458.1 1463.9	2918.1	2850.6	1743.5	1456.1 1465.8	2922.0	2852.5	1745.1	1458.1 1463.9
DPPC	2919.4[a]	2850.6[a]	1742.2[a]	1458.1 1465.8	2916.1[a]	2850.6	1738.4[a]	1456.1 1467.7	2918.7[a]	2850.6[a]	1742.9[a]	1456.1 1465.8
CS-DPPC	2918.7[a]	2850.6[a]	1741.6[a]	1458.1 1465.8	2916.8[a]	2849.3	1737.1[a]	1456.1 1467.7	2918.1[a]	2850.6[a]	1742.9[a]	1456.1 1465.8
CS-solution	2922.0	2852.5	1743.5	1456.1 1463.9	2918.1	2850.6	1741.2[a]	1456.1 1465.8	2921.3[a]	2852.5	1745.4[a]	1458.1 1463.9

Values are means of $n = 10$.
[a] Significantly decreased values compared with the control (Dunnet's test).

The region corresponding to CH_2 scissoring vibration provides information about the lateral packing of lipid alkyl chains in the SC. In all skin samples at both temperatures (37 °C and 45 °C) CH_2 scissoring band displayed a doublet, characteristic of the orthorhombic (OR) lattice, which is very important for the barrier function of the lipids (Tables 1 and 2) [10,28]. This doublet is caused by short-range interaction between the CH_2 in the lipid tails [23]. The spilt of the band is indicative of the degree of inter-chain interaction and the size of the domain with OR organisation. In the spectra of the pig ear skin the scissoring width was ~10 cm^{-1}

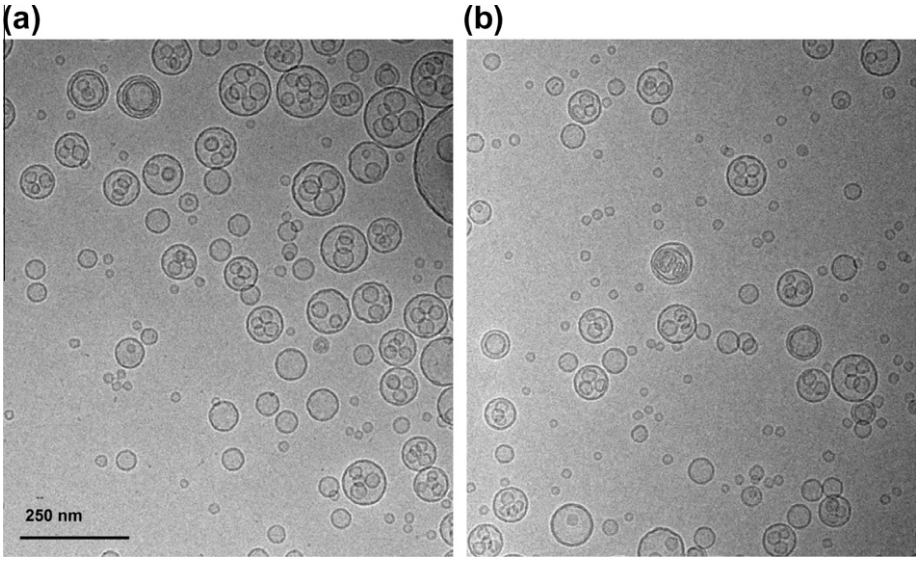

Fig. 2. Cryo-TEM micrographs of (a) pure DPPC liposomes and (b) CS coated DPPC liposomes.

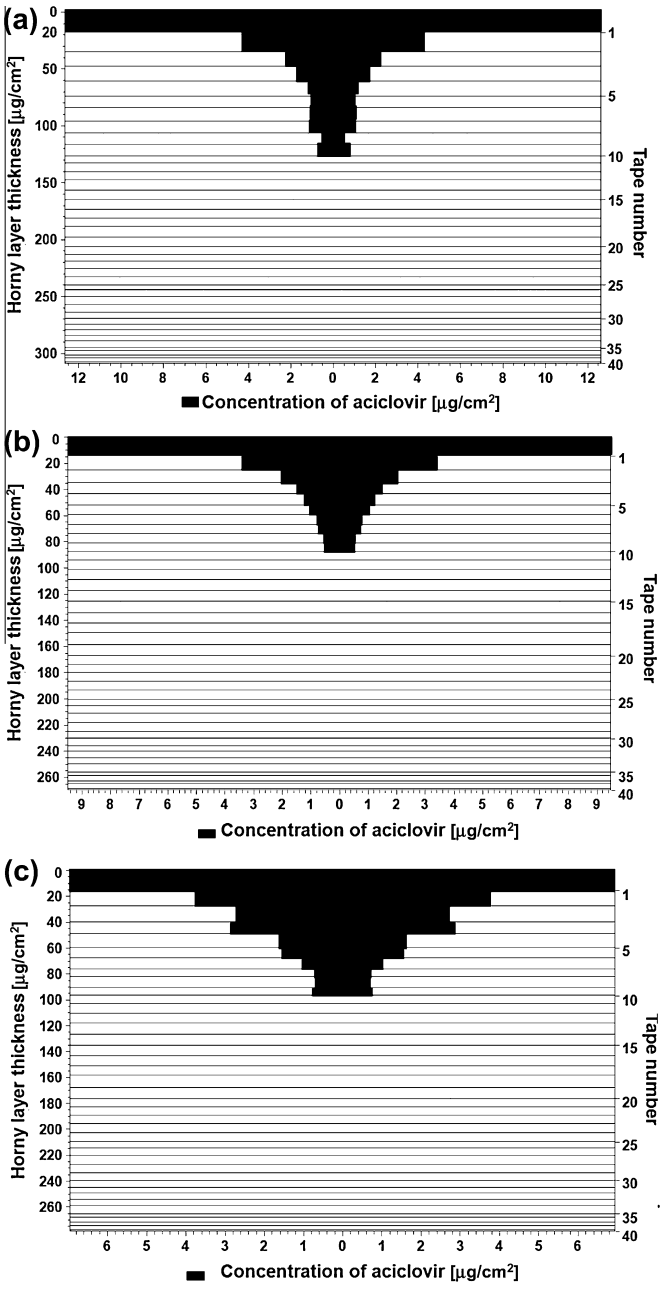

Fig. 3. Penetration profile of the aciclovir containing formulations: (a) aqueous CS-solution, (b) DPPC liposomes and (c) CS coated liposomes.

indicating a high content of OR phase. The corresponding value observed in dermatomed porcine skin and human skin was ~6 cm^{-1} displaying lower content of OR phase and higher conformational disorder of the lipid chains than in pig ear skin [2]. The treatment with liposomal formulations showed slight increase of width of the doublet at both temperatures (Tables 1 and 2). In conclusion, the FTIR data suggest that the SC barrier function is partially overcome by liposomal application.

In order to obtain more insight into the structure of the liposomes cryo-TEM was applied. As seen in Fig. 2 mainly multivesicular liposomes were present in the formulation [29]. The difference in liposomal structure induced by polymers could not be visualised by this technique. However, comparing Fig. 2a and b, CS containing liposomes seem to be more distant from each other. This could be explained by the positive charge of CS, which decreased the tendency of liposome-aggregation, resulting in enhanced stability.

Table 3
Aciclovir concentration in the first tape strip of the removed stratum corneum.

	Untreated skin	Skin treated with CS-DPPC	Skin treated with DPPC	Skin treated with CS-solution
Corneocytes (%)	19.80 ± 4.66[a]	21.02 ± 0.96[a]	18.77 ± 1.45[a]	24.32 ± 1.55[a]
Aciclovir (µg/cm²)	–	13.89 ± 2.79[b]	18.99 ± 2.51[b]	25.26 ± 2.85[b]

Values are means ± SD of six experiments.
[a] $P > 0.05$ (Tukey's test).
[b] $P < 0.05$ (Tukey's test).

Furthermore, tape stripping was performed on pig ears to establish the penetration depth of the model drug (aciclovir) from the formulations mentioned above (pure DPPC, CS coated liposomes and aqueous CS-solution). According to Herkenne et al. and Lindemann et al. data obtained in ex vivo on pig ear are comparable to those observed in vivo in humans [30,31]. Most of the SC was removed after approximately 40 strips, which was confirmed by TEWL values of 80 $gm^{-2}\,h^{-1}$ [32]. The maximal penetration depth of aciclovir was detectable until the 10th strip from all three tested formulations (Fig. 3a–c). The TEWL values measured before and after the tape stripping were increased from about 14 to 30 $gm^{-2}\,h^{-1}$. This is in accordance with findings of electron microscopy studies on humans, where liposomes did not penetrate deeper than the horny layer [33]. The findings of the present work are also in agreement with other studies, where particles greater than 10 nm (we produced liposomes of ~90 nm) did not penetrate the SC [34,35].

The concentration of aciclovir in SC was related to the amount of corneocytes on each tape strip analysed by IR densitometer [21]. As seen in Tables 3, the applied formulations did not significantly influence the amount of removed corneocytes in the first strip in comparison with untreated skin. Also a difference between the formulations considering the amount of removed corneocytes was not evident (Fig. 3a–c). Aciclovir from the aqueous CS-solution was detected in significantly higher amounts on the skin surface, which was presented by the first tape strip, than from liposomal formulations (Table 3) indicating the better aciclovir penetration from liposomes. This was expected since liposomes have been claimed to improve drug deposition within the SC [36]. As also seen in Table 3, CS coated liposomes induced significantly improved aciclovir penetration into the SC compared to pure liposomes. This was in agreement with our previously published data: aciclovir penetrated greater from CS coated liposomes than from CS free liposomes [11]. One of the reasons might be the positive charge of CS, leading to tight SC adhesion.

4. Conclusion

The FTIR results of this work appear to confirm the physiological similarities between pig skin and human skin: pig skin makes a good skin model for FTIR studies. Dermatomed abdominal porcine skin was especially suitable for this experiment due to its simple preparation. The skin treatments with liposomal formulations resulted in a partially decreased orthorhombic organisation or reduced barrier function. These data were confirmed by penetration studies. Using the tape stripping method the higher aciclovir penetration from CS coated liposomes versus polymer free liposomes was observed, verifying our previously published studies.

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References

[1] M. Lafleur, Phase behaviour of model stratum corneum lipid mixtures: an infrared spectroscopy investigation, Can. J. Chem. 76 (1998) 1501–1511.
[2] M. Boncheva, F. Damien, V. Normand, Molecular organisation of the lipid matrix in intact stratum corneum using ATR–FTIR spectroscopy, Biochim. Biophys. Acta 1778 (2008) 1344–1355.
[3] D. Kessner, A. Ruettinger, M.A. Kiselev, S. Wartewig, R.H. Neubert, Properties of ceramides and their impact on the stratum corneum structure: a review – part 2: stratum corneum lipid model systems, Skin Pharmacol. Physiol. 21 (2008) 58–74.
[4] D. Moore, M.E. Rerek, Insight into the molecular organisation of lipids in the skin barrier from IR spectroscopy studies of stratum corneum lipids, Acta Derm. Venereol. Suppl. 208 (2000) 16–22.
[5] G. Rodríguez, L. Barbosa-Barros, L. Rubio, M. Cocera, A. Diez, J. Estelrich, R. Pons, A. De la Maza, O. López, Conformational changes in SC lipids by effect of bicellar systems, Langmuir 18 (2009) 10595–10603.
[6] J.A. Bouwstra, G.S. Gooris, J.A. van der Spek, W. Bras, Structural investigations of human stratum corneum by small-angle X-ray scattering, J. Invest. Dermatol. 97 (1991) 1005–1012.
[7] P.W. Wertz, Lipid and barrier function of the skin, Acta Derm. Venereol. Suppl. 208 (2000) 7–11.
[8] Y. Obata, S. Utsumi, H. Watanabe, M. Suda, Y. Tokudome, M. Otsuka, K. Takayama, Infrared spectroscopic study of lipid interaction in stratum corneum treated with transdermal absorption enhancers, Int. J. Pharm. 389 (2010) 18–23.
[9] B. Forslind, S. Engström, J. Engblom, L. Norlén, A novel approach to the understanding of human skin barrier function, J. Dermatol. Sci. 14 (1997) 115–125.
[10] G. Rodríguez, L. Rubio, M. Cocera, J. Estelrich, R. Pons, A. De la Maza, O. López, Application of bicellar systems on skin: diffusion and molecular organization effects, Langmuir 26 (2010) 10578–10584.
[11] A. Hasanovic, C. Hollick, K. Fischinger, C. Valenta, Improvement in physicochemical parameters of DPPC liposomes and increase in skin permeation of aciclovir and minoxidil by the addition of cationic polymers, Eur. J. Pharm. Biopharm. 72 (2010) 148–153.
[12] H.J. Weigmann, S. Schanzer, A. Patzelt, V. Bahaban, F. Durat, W. Sterry, J. Lademann, Comparison of human and porcine skin for characterization of sunscreens, J. Biomed. Opt. 14 (2009) 1–6.
[13] J.J. Escobar -Chavez, V. Merino-Sanjuán, M. López-Cervantes, Z. Urban Morlan, E. Piñón-Segundo, D. Quintanar-Guerrero, A. Ganem-Quintanar, The tape-stripping technique as a method for drug quantification in skin, J. Pharm. Pharm. Sci. 11 (2008) 104–130.
[14] U. Jacobi, H.J. Weigmann, J. Ulrich, W. Sterry, J. Lademann, Estimation of the relative stratum corneum amount removed by tape stripping, Skin Res. Technol. 11 (2005) 91–96.
[15] H. Kählig, A. Hasanovic, B. Biruss, S. Höller, J. Grim, C. Valenta, Chitosan-glycolic acid: a possible matrix for progesterone delivery into skin, Drug Dev. Ind. Pharm. 1 (2009) 1–6.
[16] L. Montenegro, A.M. Panico, F. Bonina, Quantitative determination of hydrophobic compound entrapment in dipalmitoylphosphatidylcholine liposomes by differential scanning calorimetry, Int. J. Pharm. 138 (1996) 191–197.
[17] C. Valenta, J. Cladera, P. ÓShea, J. Hadgraft, Effect of phloretin on the percutaneous absorption of lignocaine across human skin, J. Pharm. Sci. 90 (2001) 485–492.
[18] S. Sakuyama, C. Hirabayashi, J. Hasegawa, S. Yoshida, Analysis of human face skin surface molecules in situ by Fourier-transform infrared spectroscopy, Skin Res. Technol. 16 (2010) 151–160.
[19] A. Teichmann, S. Heuschkel, U. Jacobi, G. Presse, R.H. Neubert, W. Sterry, J. Lademann, Comparison of stratum corneum penetration and localization of a lipophilic model drug applied in an o/w microemulsion and an amphiphilic cream, Eur. J. Pharm. Biopharm. 67 (2007) 699–706.
[20] H. Löffler, F. Dreher, H.I. Maibach, Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal, Br. J. Dermatol. 151 (2004) 746–752.
[21] R. Voegeli, J. Heiland, S. Doppler, A.V. Rawlings, T. Schreier, Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry, Skin Res. Technol. 13 (2007) 242–251.
[22] T. Hahn, S. Hansen, D. Neumann, K.H. Kostka, C.M. Lehr, L. Muys, U.F. Schaefer, Infrared densitometry: a fast and non-destructive method for exact stratum corneum depth calculation for in vitro tape-stripping, Skin Pharmacol. Physiol. 23 (2010) 183–192.
[23] J. Caussin, G.S. Gooris, M. Janssens, J.A. Bouwstra, Lipid organisation in human and porcine stratum corneum differs widely, while lipid mixture with porcine ceramides model human stratum corneum lipid organisation very closely, Biochim. Biophys. Acta 1778 (2008) 1472–1482.
[24] L.M. Russell, S. Wiedersberg, M.B. Delgado-Charro, The determination of stratum corneum thickness: an alternative approach, Eur. J. Pharm. Biopharm. 69 (2008) 861–870.
[25] R. Darlenski, S. Sassning, N. Tsankov, J.W. Fluhr, Non-invasive in vivo methods for investigation of the skin barrier physical properties, Eur. J. Pharm. Biopharm. 72 (2009) 295–303.
[26] R.D. Pensack, B.B. Michniak, D.J. Moore, R. Mendelsohn, Infrared kinetic/structural studies of barrier reformation in intact stratum corneum following thermal perturbation, Appl. Spectrosc. 60 (2006) 1399–1404.
[27] T.H. Prasch, G. Knübel, K. Schmidt-Fonk, S. Ortanderl, S. Nieveler, T. Förster, Infrared spectroscopy of the skin: influencing the stratum corneum with cosmetic products, Int. J. Cosmet. Sci. 22 (2000) 371–373.
[28] J. Caussin, E. Rozema, G.S. Gooris, J.W. Wiechers, S. Pavel, J.A. Bouwstra, Hydrophilic and lipophilic moisturizers have similar penetration profiles but different effects on SC water distribution in vivo, Experiment. Dermatol. 18 (2009) 954–961.
[29] R.H. Müller, G.E. Hildebrand, Pharmazeutische Technologie: Moderne Arzneistoffe, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany, 1998, pp. 219–222.
[30] C. Herkenne, A. Naik, Y.N. Kalia, J. Hadgraft, R.H. Guy, Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man, Pharm. Res. 23 (2006) 1850–1856.
[31] U. Lindemann, K. Wilken, H.J. Weigmann, H. Schaefer, W. Sterry, J. Lademann, Quantification of the horny layer using tape stripping and microscopic techniques, J. Biomed. Opt. 8 (2003) 601–607.
[32] X. Wu, B. Biatry, C. Cazeneuve, R.H. Guy, Drug delivery to the skin from sub-micron particles formulations: influence of particle size and polymer hydrophobicity, Pharm. Res. 26 (2009) 1995–2001.
[33] H.C. Korting, M. Schaller, Interaction of liposomes with human skin: the role of the stratum corneum, Adv. Drug Deliv. Rev. 18 (1996) 303–309.
[34] B. Baroli, M.G. Ennas, F. Loffredo, M. Isola, R. Pinna, M.A. López-Quintela, Penetration of metallic nanoparticles in human full-thickness skin, J. Invest. Dermatol. 127 (2007) 1701–1712.
[35] G. Sonavane, K. Tomoda, A. Sano, H. Ohshima, H. Terada, K. Makino, In vitro permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size, Coll. Surf. B: Biointerface 65 (2008) 1–10.
[36] G.M. El Maghraby, B.W. Barry, A.C. Williams, Liposomes and skin: from drug delivery to model membranes, Eur. J. Pharm. Sci. 34 (2008) 203–222.