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Effects of iontophoresis and electroporation on the stratum corneum

Review of the biophysical studies

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Abstract

This review focuses on the effects induced by iontophoresis and electroporation on the stratum corneum of the skin. Hence, the aims were: (1) to contribute to the understanding of the mechanisms of drug transport by these methods; (2) to evaluate the safety issues associated with current application. Complementary biophysical methods were used to provide a complete picture of the stratum corneum. Even though the mechanism of drug transport is believed to be different, i.e., electrophoresis for iontophoresis and creation of new aqueous pathways for electroporation, the effects on the stratum corneum detected minutes after current application are very similar. For both methods, the major findings were: (1) a disorganisation of the lipid bilayers of the stratum corneum; (2) an increase in skin hydration; (3) a larger decrease in skin resistance induced by electroporation as compared to iontophoresis. These changes were partly reversible and depended on the amount of electrical charges transferred. The mechanisms of stratum corneum perturbations are discussed. These perturbations could explain partly the increase in drug transport. If iontophoresis is considered as a safe method of drug delivery, the data augurs for the safety of electroporation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electroporation; Iontophoresis; Transdermal drug delivery; Stratum corneum; Skin; Biophysical methods

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1. Introduction

The barrier properties of the skin are due essentially to the outer layer of the skin, the stratum corneum (s.c.). The structure of the s.c. has been well characterized by microscopic, biophysical and bioengineering methods ([1], see Table 1). The s.c. consists of dead corneocytes filled with keratin and embedded in an extracellular matrix formed of multilamellar lipid bilayers.

Evidence that the barrier properties of the s.c. are

located in the extracellular regions has been concluded from visualization studies that revealed that penetration through the s.c. occurs mainly intercellularly [2] and from the reduced barrier function of the s.c. after extraction of its lipids [3] or stripping of the skin.

Several strategies have been developed to overcome the barriers of the s.c. and to enhance transdermal drug delivery by increasing the s.c. permeability and/or using a driving force. Chemical methods (e.g. chemical enhancers, liposomes) and physical meth-

Table 1
Biophysical techniques used to analyse the s.c. and corresponding information provided

Methods	Information provided
Electrical properties	Impedance → information on the permeation of conductive ions
FTIR	Infrared spectrum of s.c. → detection of conformational changes of the s.c. lipids or proteins and changes in water content
DTA	Measurement of T and ΔH of phase transitions → detection of modifications inside macromolecular organisations
X-ray	Measurements of the regular spacings → information on the lamellar (SAXS) and intralamellar (WAXS) lipid packing of the intercellular spaces and on the keratin
FFEM	Visualisation of the s.c. at an ultrastructural level
Fluorescence quenching	Analysis of the fluorescence intensity and lifetime of a fluorophore inserted within the lamellae structure of the intercellular lipids → information on the accessibility of the lipid lamellae to the quencher as a function of depth
TEWL	Measurement of the transepidermal water loss → estimation of the skin barrier function

ods (e.g. iontophoresis, electroporation or ultrasound) have been shown to increase transport of molecules across the skin.

Iontophoresis consists in the application of a low intensity ($< 0.5 \text{ mA/cm}^2$) electric field for minutes or hours. Iontophoresis drives molecules across the skin by electrostatic repulsion and/or electroosmosis during current application and induces as a secondary phenomenon structural and permeability changes in the skin (for review see Ref. [4]). Iontophoresis has attracted considerable interest for the systemic delivery of drugs including hydrophilic, charged drugs, peptides or oligonucleotides. It is generally considered as a safe procedure. It is currently undergoing phase I to III clinical trials and industrial scaling up for the production of miniaturized disposable systems.

The application of short ($< 1 \text{ s}$) high (50–500 V) voltage pulses to the skin has also been shown to enhance transdermal drug delivery by several orders of magnitude. It is believed that electroporation, i.e., the application of high voltage pulsing, involves the creation of aqueous pathways or “pores” for transport through lipid bilayers of the s.c. [5–8]. Besides, electrophoresis during the pulse and diffusion after pulsing also contribute to the transport of the drug across the electroporabilized skin [9,10]. Two different types of electrical pulsing protocols have been used: intermittent application of many “short” ($< 10 \text{ ms}$) high voltage pulses and low number (≤ 15) of “long” (100–500 ms) medium voltage pulses (50–250 V) [5,6]. In contrast to iontophoresis, the safety issues associated with the clinical use of electroporation are still under investigation [11].

The scope of this paper is to analyse the effects of electroporation on the s.c. and to compare these effects to those of iontophoresis, which is considered as quite safe. Understanding the effects of current on the s.c. is critical (i) to confirm or establish the therapeutic feasibility and clinical acceptability of iontophoresis and electroporation by evaluating the unwanted side effects; (ii) to determine the extent and if possible the mechanisms by which the barrier properties of the s.c. are impaired during and after current application.

This review will focus on the biophysical and ultrastructural studies of the s.c. after iontophoresis or electroporation. The various techniques and the

information provided by these techniques are summarised in Table 1. All the methods, except for the impedance measurements, have been used after voltage supply, that means that in general no measurements have been carried out “on line”. Since most of the techniques used, except for freeze–fracture electron microscopy (FFEM), are bulk methods, localised defects are unlikely to be detected. Potential changes detected by these techniques must therefore be largely spread over the fraction of s.c. investigated. If no changes are detected, it could be argued that: (i) no changes are induced; (ii) localised defects are induced or (iii) changes are induced but are highly reversible. Moreover, it has to be noted that in this review only the s.c. structure is considered. Besides the s.c. disorganisation, possible perturbations of the appendageal structures or viable skin might occur. The appendageal route has been involved as a significant pathway for iontophoretic transport. The importance of the follicular versus nonfollicular transport depends on the physical properties (e.g. molecular weight) of the penetrant [12]. Although involvement of appendages in transdermal transport by skin electroporation with medium voltage has been reported, transport occurs mainly through localized transport regions in the s.c. [13–15].

2. S.c. integrity after iontophoresis

2.1. Electrical skin barrier

The skin electrical properties have been appraised both *in vitro* and *in vivo* by numerous authors [16–21]. Iontophoresis has been shown to significantly decrease the skin electrical impedance (for review, see Ref. [22]). Changes in skin resistance occur over two time-scales: (a) rapid changes (beyond the microsecond [23]) and (b) slow resistance changes (up to hours). Furthermore, the rate of decrease in resistance has been shown to depend upon the applied voltage whereas the rate of recovery depends upon the duration and magnitude of the electrical field and upon the ionic strength of the solution [24]. Part of the drop in resistance is not reversible *in vitro* whereas the skin seems to recover fully *in vivo*.

2.2. Chemical skin barrier

Water and mannitol, have been used as model molecules to appraise the skin barrier alteration induced by iontophoresis *in vitro* [25–30]. Compared to passive diffusion through untreated skin, the transport of mannitol and water across a skin previously submitted to iontophoresis, is usually increased, suggesting an impairment of the barrier function of the skin following iontophoresis.

Various authors have suggested an influence of the current application and the donor solution composition on the permeabilization of s.c. and on the reversibility of the electrically-induced modifications [18–21,23,31,32].

2.3. IR spectrometry: hydration and conformation of lipids and proteins

A method to provide information on the conformational changes of lipids and proteins *in vitro* as well as *in vivo* is attenuated total reflectance-Fourier transform IR spectroscopy (ATR-FTIR). The main modification observed by ATR-FTIR after *in vivo* or *in vitro* application of various iontophoresis protocols is an increase in s.c. hydration [33–36]. The increase in skin hydration was demonstrated by the increase in ratio of the Amide I/Amide II absorbance and by the increase in normalised area under the 2106 cm^{-1} peak [37] (Fig. 1A).

Analysis of the CH_2 stretching peaks provides information on the potential lipid alkyl chain disordering [38–40]. No significant changes were observed *in vivo* [33,34,36] nor *in vitro* [35,41], even at high (e.g. 0.5 mA/cm^2) current densities. *In situ* measurements during current supply allowed rejection of a fast recovery of the lipid conformation [41] (Fig. 1B).

2.4. Thermal transitions in s.c.

Human s.c., when subjected to thermal analysis by differential scanning calorimetry (DSC) over the temperature range of $30\text{--}120^\circ\text{C}$ shows four major phase transitions occurring near 37°C (T_1), $60\text{--}65^\circ\text{C}$

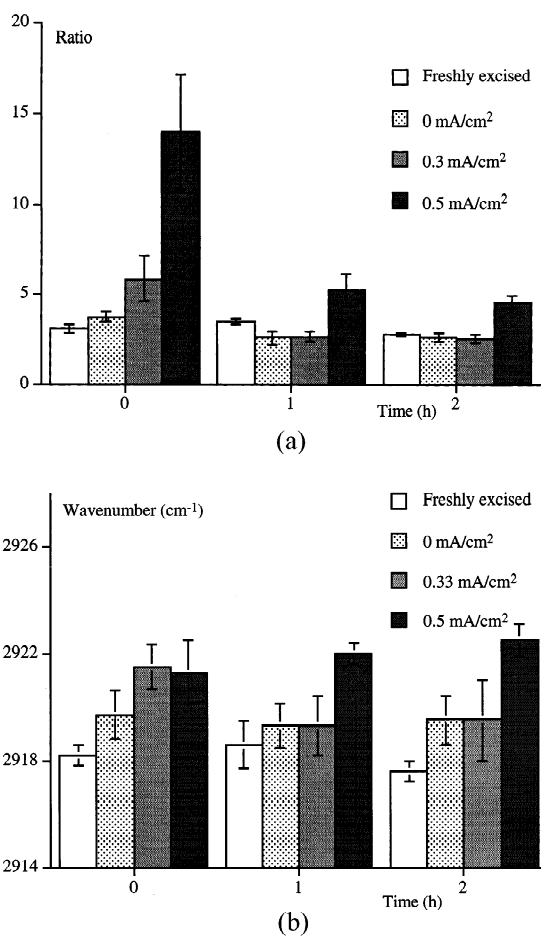


Fig. 1. (A) Intensity ratio of amide I/amide II absorbance as a function of time after current switching off. (B) Position of asymmetric stretching absorbance peak as a function of time after current switching off. Iontophoresis was performed during 6 h at a current density of 0 ($n = 6$), 0.33 ($n = 6$) or 0.5 mA/cm^2 ($n = 4$) and compared to freshly excised skin ($n = 5$) (adapted from Refs. [11,35]).

(T_2 attributed to lipids), $80\text{--}85^\circ\text{C}$ (T_3) and $95\text{--}110^\circ\text{C}$ (T_4 attributed to proteins) [42,43].

The results of the first DSC study after iontophoresis, reported no significant perturbation of either the proteins or the lipids [44]. In 1994, Clancy et al. studied the protein thermal behaviour. No significant changes were found [41]. More recently, Craane-van Hinsberg et al. [45] have investigated the

electrical properties of s.c. as a function of temperature. The main fall of s.c. resistance was observed at about 60°C (i.e. in the region of the second phase transition), and application of electrical current of 0.13 mA/cm² decreased this temperature [23,46]. Differential thermal analysis (DTA) was recently performed on human s.c. samples submitted to 6 h iontophoresis at 0.33 or 0.5 mA/cm² [11]. Two transitions were observed, a transition at approximately 60°C (T_2) and a combined transition (T_{3+4}) attributed to lipids and proteins. No significant difference in the temperatures of phase transition of T_2 and $T_{(3+4)}$ was observed after iontophoresis. Increase in the enthalpy of phase transitions was induced by 0.33 mA/cm² iontophoresis whereas at a current density of 0.5 mA/cm² the enthalpy of transition was similar to that of the control s.c. An increase in s.c. water content has also been shown to modify the thermal behaviour both in proteins and lipids [42,47].

2.5. Lipid organisation in s.c.

X-ray scattering can be used to characterize the s.c. structure. The X-ray scattering pattern of s.c. was studied after *in vitro* and *in situ* iontophoresis at small angle (SAXS, small angle X-ray scattering) which provides information on the repeated distance of the lamellae and at wide angle (WAXS, wide angle X-ray scattering) which gives information on the on lateral packing of the lipids, keratin and water content.

The results of the SAXS studies revealed that the peaks localised at 4.5 and 6.5 nm decreased in intensity after application of current densities of 0.33 and 0.5 mA/cm² [35]. According to Bouwstra and coworkers [48,49], the “4.5 nm” spacing corresponds to the third order of a “13.5 nm” periodicity. A decrease in intensity of the diffraction peaks was also observed after 1 h iontophoresis at 1.3 mA/cm² despite a 34 h time interval between electrical current exposure and X-ray analysis [50]. Decrease in intensity of the SAXS peaks is an indication of a lamellar disordering of the intercellular lipids. At a current density of 0.5 mA/cm², the decrease in peak intensities was reversible [35] (Fig. 2A).

No consistent changes of the s.c. scattering pattern

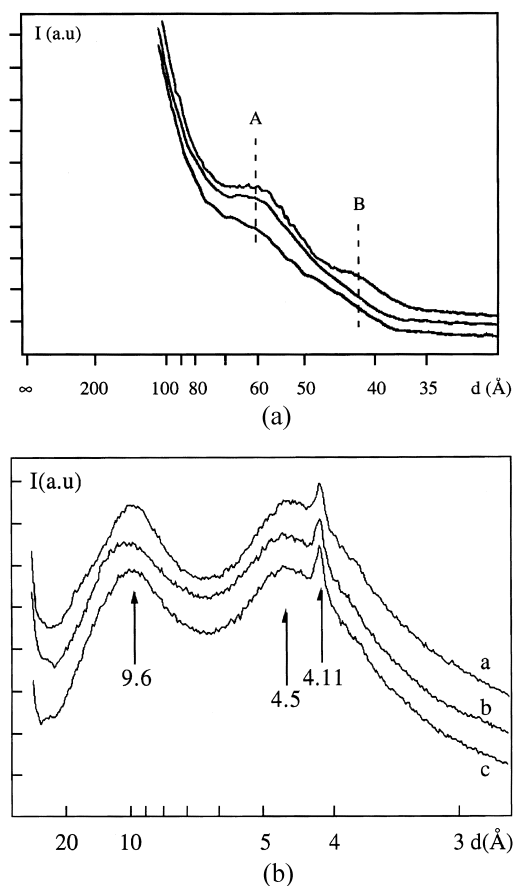


Fig. 2. (A) SAXS scattering intensity profiles along the equator of the three samples about 24 h after current switching off. (B) WAXS scattering intensity profiles about 24 h after current switch off. From top to bottom, the curves correspond to 0 (control; a), 0.33 (b) and 0.5 mA/cm² (c) current density applied during 6 h (adapted from Refs. [11,35]).

at wide angle were observed below 1 mA/cm² iontophoresis [23,50] (Fig. 2B).

2.6. Visualization of the s.c. ultrastructure

FFEM is used to visualize the s.c. ultrastructure. Using fractures parallel to the s.c. surface the intercellular lipid lamellae can be recognised by smooth planes. Sharp edges interrupting these smooth planes reflect cross fractures through the lamellae.

The ultrastructure of the s.c. after iontophoresis was studied by Craane-van Hinsberg et al. [50]. Low current densities did not affect the ultra structure of s.c. However, when increasing the current density, an increasing number of spherical deformations and larger areas of rough structures was observed, suggesting that the electric field can perturb the intercellular lamellar ordering in the s.c. Water pools were frequently detected. Furthermore, due to the coexistence of rough structures with lamellar structures at low current densities, it was proposed that electrical perturbations may start at discrete sites (Fig. 3).

2.7. *Transepidermal water loss (TEWL)*

The barrier function of the skin can be estimated by the measure of TEWL *in vivo*. Several investigators have demonstrated that iontophoresis does not enhance TEWL as compared to a control site. However, higher TEWL were sometimes reported straight after iontophoresis and were attributed to the skin hydration induced by iontophoresis [34,79].

2.8. *Transport pathways*

The transport pathway of ions in the s.c. has been investigated. Even though transappendageal transport has been shown to be important, nonfollicular transport through the s.c. has also been reported. HgCl₂ was mainly transported through the intercellular regions in the s.c. [51]. Turner et al. also demonstrated a significant nonfollicular transport of fluorescent polylysines permeant [12].

Fluorescence spectroscopy has been used to detect the diffusion of iodide ions. The fluorophores (i.e. anthroxyloxy fatty acid probes) incorporated in the s.c. lamellae are known to be quenched by iodide ions. Fluorescence quenching was increased with increasing iontophoretic current density, suggesting increased ion accessibility in the s.c. lamellae. At constant current, quenching decreased when the fluorophore was located deeper within the lamellae [52].

The results clearly demonstrate that the transport of ions does not occur only through the hair appendages, but also through the lipid regions in the s.c. In this respect it seems very relevant to examine the s.c. lipid organisation during and after iontophoresis.

3. **S.c. integrity after electroporation**

3.1. *Electrical skin barrier*

High voltage application induces a significant decrease in skin resistance [53] up to three orders of magnitude within microseconds during electroporation. The fast drop in skin resistance has been attributed to the extensive creation or enlargement of pathways for ionic current across the skin [10,53,54]. A slower further decrease could arise from thermal effects [10]. Skin resistance was shown to recover *in vitro* either partially or fully, according to the electrical protocol, to prepulse value in microseconds up to several hours [53].

3.2. *Chemical skin barrier*

Significant enhancements in water and mannitol permeation have been observed after electroporation suggesting a long-lasting permeabilization of the s.c. Indeed, postpulse diffusion has been shown to account for a significant part of the mechanism of drug transport by electroporation at least *in vitro* [9,55,56].

3.3. *IR spectrometry: hydration and conformation of lipids and proteins*

Electroporation increases the s.c. water content as demonstrated by the increase in intensity ratio of amide I/amide II observed after electroporation in the outer layers of s.c. using ATR-FTIR. However, the increase in s.c. hydration was not confirmed by analysis of the secondary OH stretching [11,33,34]. An increase in s.c. hydration was also observed after *in vitro* electroporation by thermogravimetry. The water content of the samples ranged from about 65% (for the control) up to 75% w/w (after electroporation), depending on the electrical protocol applied [57].

Since electroporation is believed to create permeabilized structures across the lipid bilayers of the s.c., particular attention was given to the lipid peaks analysis. No significant increase in the lipid fluidity of the alkyl chain could be detected after electroporation by analysis of the CH₂ asymmetric and symmetric stretching [11]. Nevertheless, these results do not

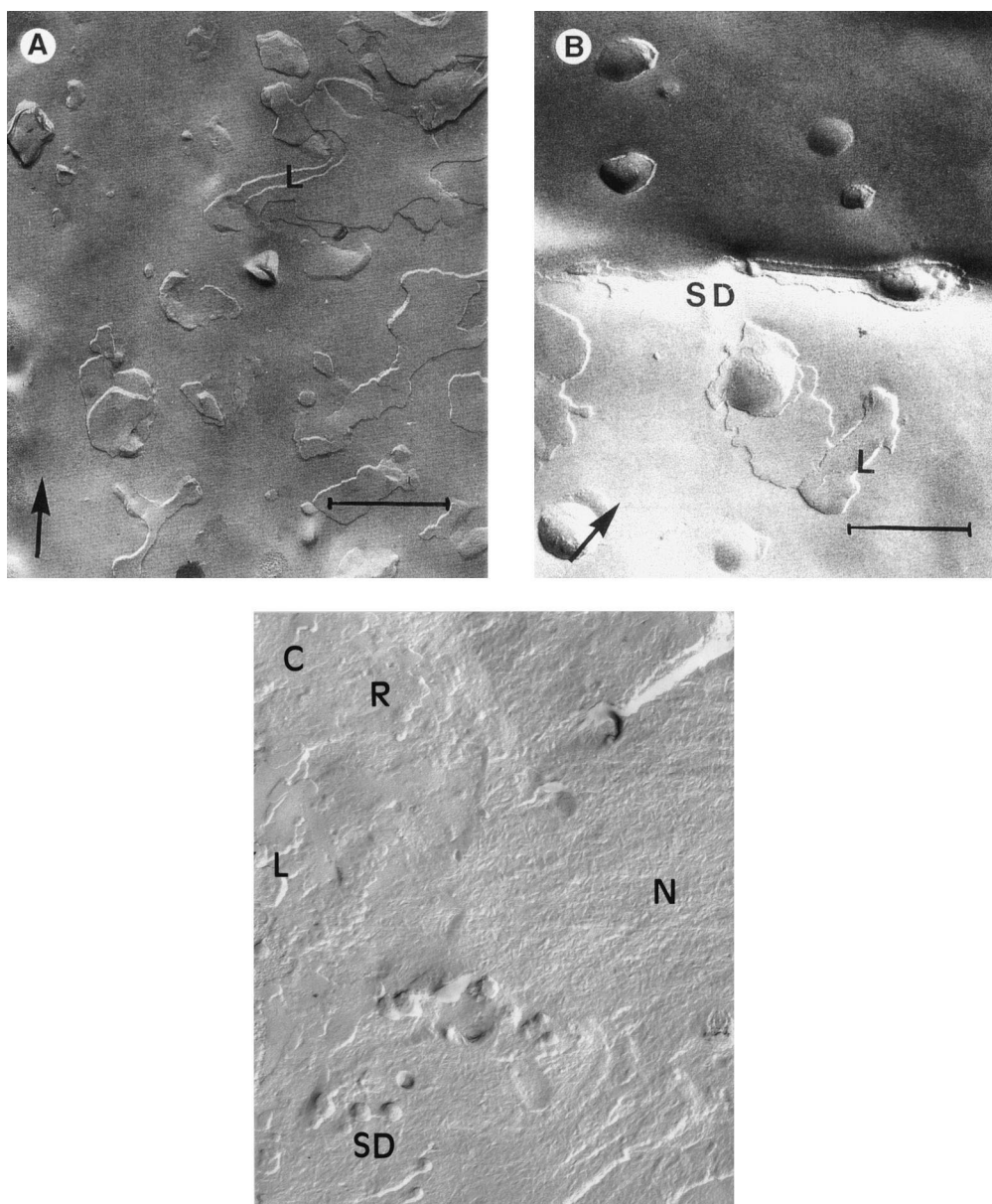


Fig. 3. (A) FFEM of control human s.c. (B) FFEM from human s.c. submitted to iontophoresis (0.13 mA/cm^2 1 h) [23]. (C) FFEM of human s.c. submitted to $20 \times (300 \text{ V} - 120 \text{ ms})$ high voltage pulses [57]; bar = $1 \mu\text{m}$; L, intercellular lipid lamellae; SD, spherical deformation; R, rough surface; N, network.

rule out the possibility of lipid fluidization due to electroporation since rapidly reversible and/or localised changes could be induced by electroporation (Fig. 4).

3.4. Thermal transitions in s.c.

No changes in transition temperature were detected after electroporation treatments.

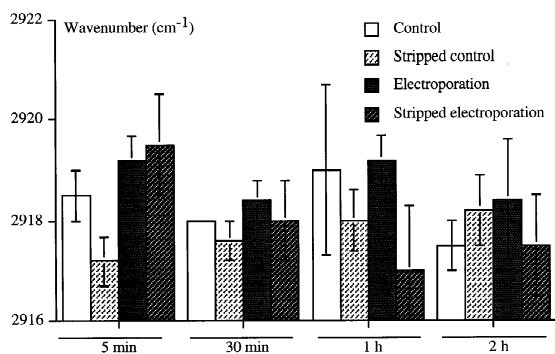


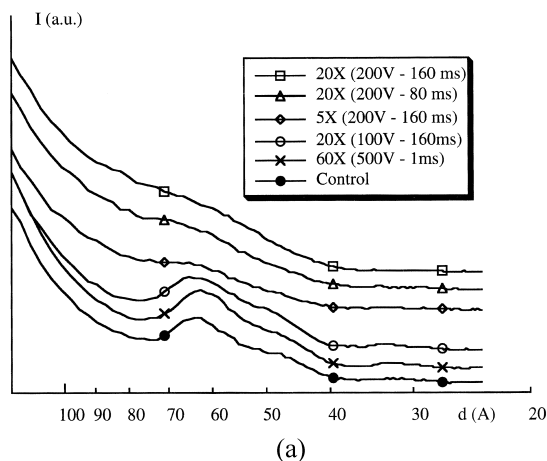
Fig. 4. Position of the asymmetric stretching peak as a function of electroperoration protocol and stratum corneum depth. Intact and stripped electroperated skin ($5 \times (250 \text{ V} - 300 \text{ ms})$) were compared to control samples or control stripped skin. The donor compartment was filled with citrate buffer 0.01 M, pH 3.5. N = number of pulses, V = voltage applied across the electrodes and τ = time constant (adapted from Ref. [11]).

A decrease in enthalpies of T_2 and $T_{(3+4)}$ phase transitions was measured after electroperoration with long pulses, suggesting a generalised rather than a localised effect. Since no change in the peak temperatures was observed, it was assumed that the transition entropy is also reduced, suggesting that electroperoration caused lipid disordering [43,58]. Since the $T_{(3+4)}$ phase transition is caused by proteins and lipids, a perturbation of the protein fine structure could not be excluded.

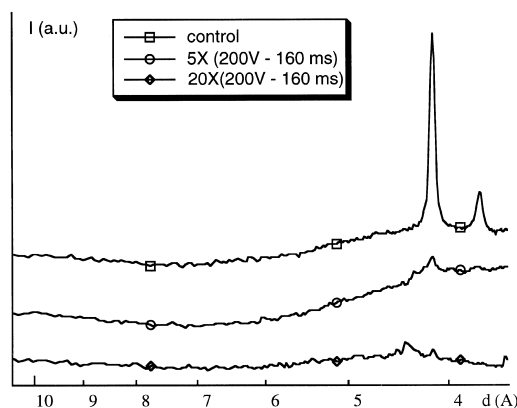
3.5. Lipid organisation in s.c.

Assuming that pulses were affecting the s.c. lipids, s.c. submitted to different electroperoration protocols were analyzed in vitro by X-ray diffraction [59]. A disordering of the lipid lamellar stacking and of the lipid lateral packing was observed after application of long pulses. The intensities of the 4.5 and 6.5 nm diffraction peaks strongly decreased after application of long pulses. Increasing the pulse number, the pulse duration or voltage enhanced the lamellar disordering. In addition the WAXS study revealed a decrease in intensity of the 0.375 nm and 0.411 nm reflections after high voltage pulses suggesting a change from orthorhombic and hexagonal lateral lipid phase to a liquid phase. No shift of the reflections was detected (Fig. 5).

Contrary to long pulses, short high voltage pulses



(a)



(b)

Fig. 5. (A) SAXS scattering intensity profile as a function of the distance. The control were compared to $60 \times (500 \text{ V} - 1 \text{ ms})$, $20 \times (100 \text{ V} - 160 \text{ ms})$, $20 \times (200 \text{ V} - 80 \text{ ms})$, $5 \times (200 \text{ V} - 160 \text{ ms})$ or $20 \times (200 \text{ V} - 160 \text{ ms})$. (B) WAXS scattering intensity profile as a function of the distance. The control samples were compared to electroperated stratum corneum: $20 \times (200 \text{ V} - 160 \text{ ms})$ (open square) or $5 \times (200 \text{ V} - 160 \text{ ms})$ (open rhomb). Each curve corresponds to the mean of different scattering patterns ($n = 2$ to 4). The curves were vertically shifted (adapted from Refs. [11,39]).

(i.e. $60 \times (500 \text{ V} - 1 \text{ ms})$) only slightly altered the s.c. lamellar phases. The difference observed between both protocols could be attributed to the difference in energy applied and/or to the difference in the amount of charges transferred. It is probable that application of short pulses induced more localised disordering, which is unlikely to be detected by X-ray scattering.

3.6. The ultrastructure of the s.c. by FFEM

As shown by FFEM, high voltage pulses induced a general perturbation of the intercellular lipid material, i.e., a general loss or severe distortion of the lamellarity was observed, and new structures emerged in the intercellular lipid domain, including spherical deformations and network-like structures, confirming the results obtained with SAXS.

Occasionally the s.c. was fractured perpendicular to its surface showing cross sections through alternatingly lipid bilayer regions and corneocytes. These cross fractures revealed no major evidence for changes in the corneocyte ultrastructure induced by electroporation treatment [57] (Fig. 3c).

3.7. TEWL

Electroporation enhances transiently the TEWL in rats *in vivo*. This TEWL value was slightly enhanced as compared to the control site for up to 3 h. These data suggest that even if the skin barrier function is slightly impaired straight after pulsing, the recovery is fast [74].

3.8. Transport pathways

Fluorescence microscopy and gel trapping of fluorescent permeants crossing the skin have demonstrated that molecular transport by high voltage pulses across the skin is highly localized. These localized sites of transport are termed “localized transport regions” (LTR). LTR are usually not associated with skin appendages unless the voltage applied to the skin is lower than 50 V [10,12,14]. The extent of the LTR depends on the electroporation protocol: application of short high voltage pulses induced smaller (typically in the range of 100 μm) but more numerous LTR than a low number of long pulses. The pathways of transport seem to be more paracellular during long pulses and more straight-through the corneocytes during short pulses [60].

The understanding of the formation of the LTR remains to be solved. Theoretical and experimental evidence suggest that LTR could result from the creation of new aqueous pathways by high voltage pulsing (see Refs. [10,77] in this issue for review).

4. Discussion

After reviewing the effect of iontophoresis and electroporation on the s.c. by biophysical techniques, the mechanism of s.c. disorganisation and its repercussion on drug transport and safety issues will be discussed.

4.1. Effect of iontophoresis on the s.c.

The information collected for the s.c. submitted to iontophoresis is summarised in Table 2. Iontophoresis increases the s.c. hydration. The electrical resistance of the skin is decreased [21]. The FFEM observations indicate that at low current densities iontophoresis disorganizes the s.c. only locally, while at higher current densities a general perturbation of the s.c. lipid organisation has been observed [50]. This is confirmed by the results obtained using SAXS, since by using this bulk method a disordering of the lamellar intercellular structure is observed above 0.33 mA/cm² [35]. In addition no change in the lipid lateral phases was observed below 1 mA/cm² (Table 2; [50]) suggesting that the lamellar stacking is more sensitive to iontophoresis than the lateral packing. Persistence of the lipid packing ordering as revealed by WAXS and disappearance of the lamellar ordering in SAXS has previously been observed after azone treatment [61]. These studies revealed that the lamellae, although not properly stacked, were still present. Moreover, it has been reported that the long range lamellar packing tends to be much more sensitive to treatment than the lateral packing. The hydrocarbon chains do not tend to passively fill any volume accessible to them, rather they maintain a nearly identical conformational state [62].

It might be argued that the iontophoretic conditions used in some studies were strong and unlikely to be employed *in vivo*. However, FFEM suggested that electrical perturbations start at discrete sites, possibly highly sensitive sites [23]. These highly sensitive sites might correspond with dislocation sites in the lipid organisation.

Although the primary mechanism of drug permeation enhancement by iontophoresis is an electrophoretic drift, increased skin permeability is often reported postcurrent application *in vitro*. The simi-

Table 2

Summary of the observations made by the different biophysical techniques used to study the s.c. after iontophoresis

Study	Observations	I (mA/cm ²)	Skin	References
Impedance	↓ Resistance			[21,22]
FTIR	= Fluidity of the lipid chain	0.3 and 0.5	R ^a	[35]
	↑ Hydration			
	= Fluidity of the lipid chain	(5 to 30V)	H	[41]
	= Fluidity of the lipid chain	0.2	H	[33,34]
	↑ Hydration			
DTA	= T (T ₂ and T ₃₊₄)			
	↑ Enthalpies	0.33	H	[11]
	= Enthalpies	0.5		
	= Protein conformation	(5 to 30 V)	H	[41]
X-ray	↓ Lamellar ordering	0.3 and 0.5	H	[11]
	= Intralamellar packing			
	↓ Lamellar ordering	> 1.3		
	↓ Intralamellar packing	at 13	H	[23,50]
	↑ Hydration			
FFEM	Disorganisation of the intercellular lipid lamellae, spherical deformations, water pools	1.3 and 13	H	[23,50]
TEWL	↑ Water loss attributed to an increase in hydration	0.1 to 0.4	H	[20,35,79]
Thermoelectrical analysis	↓ 10°C Temperature at which the thermal transition of the electrical properties starts	0.13	H	[23,46]
Fluorescence quenching	↑ Quenching with current density	0.3 or 1	H	[52]

^a R = resistance; H = hydration.

larity of the results summarised in Table 2 with the perturbations observed after application of penetration enhancers indicates that the disorganisation could partly account for the increase in skin permeability observed in drug transport studies.

4.1.1. What is the mechanism of s.c. disorganisation after iontophoresis?

Various authors suggest a possible role of water and/or ions in the permeability increase induced by iontophoresis. Moreover, a heat dissipation could very likely occur while passing an electrical current across a highly resistive tissue. Besides indirect effects, direct electric field effects are also potential mechanisms of s.c. disorganisation.

4.1.1.1. Ions

Experimental support for the fact that alteration in ion concentration and ion type can lead to changes in skin integrity has been given by electrical properties and permeation experiments [19,20,26,31,32,63]. Moreover, it is probable that the displacement of structurally important ions, such as Ca²⁺, could play an important role in the structural perturbation of the s.c. in vivo [64]. The pH of the vehicle was also shown to influence the TEWL values after ion-

tophoresis [65]. The pH is known to be able to affect s.c. lipid organisation [Bouwstra et al., unpublished data]. The negative charge density of the s.c. and the resulting electrostatic barrier that anions must overcome was hypothesised to be at least partly responsible for the skin irritation usually observed at the cathode. Moreover, deprotonation of free fatty acids due to pH influence of the bath medium could be responsible for the disturbance of the bilayer structure observed by the in vitro FFEM study of fully hydrated s.c. [66]. The pK_a of the fatty acids present in ceramide-cholesterol-fatty acid mixtures is around 5–6 [67].

4.1.1.2. Water

Increase in water content was clearly demonstrated by FTIR [33,34] and by WAXS [23,50]. Water is known as an effective penetration enhancer and could therefore (partly) be responsible for the increase in skin permeability observed after current termination [68].

The influence of water on s.c. organisation as revealed by impedance, FTIR, DTA, ESR, X-rays and FFEM is collected in Table 3. Various observations made after iontophoresis (Table 2) could be explained by an increase in water content of the s.c.

Table 3
Effect of an increase in water content in the s.c.

Study	Hydration ^a	Observations	Reference
Impedance	Various levels	↓ Resistance	[16,80]
FTIR	< 40%	= Fluidity of the lipid alkyl chain	[81]
DTA	< 60%	↓ Temperatures	[47,82,7]
		↑ Enthalpies	
	> 60%	Minor changes	
X-ray:			
SAXS	40 < x < 60%	↓ Lamellar ordering	[1,48,49]
WAXS	< 40%	= Intralamellar packing	
FFEM	Fully hydrated ^b	Smooth planes but also rough surfaces and water pools	[66]

^a Water content is defined as % w/w (weight hydrated s.c. – weight dry s.c.)/weight hydrated s.c.

^b Considering that s.c. is capable of taking up to about 300% of its own weight [68], this would correspond to about 70%.

However, the lack of correlation with some observation suggests the participation of other phenomena in the s.c. disorganisation. The drop in skin resistance might be due to an increase in ion concentration in aqueous pathways following iontophoresis [21]. However, the rapid fall in skin impedance observed during application of low intensity electrical current might hardly be explained by the increase in skin hydration and ion content [23]. The elevated ion and water levels may have been facilitated by transport through current-induced structural changes in the s.c. [21]. However, the decrease in intensity of the X-ray diffraction peaks observed after application of high current densities is different from the changes due to an increase in s.c. water content. The latter only occasionally decreased the 4.5 nm peak intensity. In addition FFEM revealed only very local changes in the lipid organisation of fully hydrated s.c. [66], while at high current densities a general lipid disorganisation was detected. Furthermore the slow recovery observed in the X-ray patterns of s.c. submitted to iontophoresis [35] contrasts with the rapid decrease in water content observed notably by FTIR studies [33–35].

The results suggest therefore that the electric field is capable of additional perturbations in s.c. lipid organisation at high current densities (> 0.33 mA/cm²) compared to the lipid perturbations induced by an increased hydration level even though hydration could contribute to an increase in skin permeability.

4.1.1.3. Heat

Since s.c. electrical resistance is higher than in the viable skin, heating by Joule effect should primarily

occur within the s.c. Equations can be used to theoretically estimate the rise in temperature occurring during iontophoresis [22]. It was calculated for example that application of a low voltage current (1 V) for 1 min across skin (10 μm thick and 100 kΩ · cm²) could give rise to an increase in temperature of 0.14°C [22]. These models assume that all electrical energy dissipated is converted into heat in the skin and provide therefore an overestimate of the temperature rise. Hence, the heat induced by iontophoresis seems to be minimal. Moreover, comparing the effects of heating detected by different biophysical techniques summarized in Table 4 on one hand and the perturbations induced by iontophoresis on the other hand, a disordering effect induced by heating seems unlikely.

However, due to the heterogeneity of the s.c. structure and composition, current could flow within localised pathways and dissipation of the energy could result in localised heating underestimated by calculation. The resultant localised perturbations would be unlikely to be detected by FTIR, X-ray or DTA.

4.1.1.4. Direct effects

The direct interaction of the electrical field with the s.c. components is commonly reported as a possible mechanism of s.c. perturbation. Different hypotheses were proposed to explain the direct interaction of electric field with the s.c.

By application of a few volts, Inada et al. stated that electroporation is likely to occur during conventional iontophoresis as when short duration high voltage pulses are involved [24]. However, elec-

Table 4
Effect of heating on the s.c. structure

Study	T (°C)	Observations	Reference
Impedance	< 60°C	↓ Resistance	[20,45]
	60 > x > 75°C	Abrupt decline in resistance	
	> 75°C	Fairly constant	
FTIR	60 > x > 75°C	↑ Fluidity of the lipid alkyl chain	[42]
DTA	40–70°C	Reversible phase transitions	[82]
	85–100°C	Irreversible phase transitions	
X-ray: SAXS	< 60°C	= Lamellar ordering	[43,48]
	> 65/75°C	↓ Lamellar ordering	
WAXS	> 75°C	Lamellar order disappeared	[49]
	45°C	↓ Orthorhombic packing	
	75–95°C	↓ Hexagonal packing	

troporation of lipid bilayers had been theoretically demonstrated when a potential drop of 0.5–1 V is applied across the lipid bilayers [69–71]. Therefore, electroporation of the s.c. is unlikely to occur at the low potentials used in iontophoresis [20]. This phenomenon, if occurring during iontophoresis, could be limited to regions such as appendages.

A possible factor involved in the electrical perturbation of the s.c. structure could be a polarisation of the lipids [23]. Such a mechanism has been held responsible for the observed nonlinear voltage–current relationship in phospholipid membranes [72]. The electric field applied during iontophoresis could induce changes in the s.c. components, forcing them to adopt high energy conformations that facilitate charged polar and ionic species transport, enlarging the preexisting channels and/or creating new ones [21]. Ruddy and Hadzija explained the electrical behaviour of the skin by a reorientation of the lipid bilayer leading to the creation of physically long

pathways but less resistive to electric current [73]. Furthermore, the polarizability of the tissue was shown to depend on the water content. Polarisation phenomenon and water content may be inversely related, explaining the influence of the donor medium on the damages induced by iontophoresis.

4.2. Effect of electroporation on s.c.

The information collected up to now by biophysical analysis of the s.c. submitted to electroporation is shown in Table 5. One striking result is the induction of a general disorganisation of the s.c. by long high voltage pulses. This general perturbation greatly contrasts with the localised transport pathways and with the small fraction of skin area involved in the transport of molecules during application of high voltage pulses [14,60].

No significant perturbation of the s.c. structure could be detected after application of some pulsing

Table 5
Summary of the observations made by the different biophysical techniques used to analyse the s.c. after electroporation; the strong(est) electroporation protocol used for each study is indicated

Study	Observations	Type of protocol	Reference
Impedance	↓ Resistance		[21,53,54,60]
	(up to three orders of magnitude on a time scale of microsecond)		
FTIR	= Fluidity of the lipid alkyl chain	10 × (250 V—400 ms)	[59]
	↑ Hydration		
DTA	= T (T ₂ and T ₃₊₄)	20 × (300 V—120 ms)	[57]
	↓ In enthalpy → disordering		
X-ray	↓ Lamellar ordering	20 × (200 V—160 ms)	[59]
	↓ Intralamellar packing		
FFEM	Spherical deformations, rough surfaces, disorganisation of the lamellae, appearance in a network-like structure	20 × (300 V—120 ms)	[57]

protocols. However, enhancement in drug transport is usually associated with such electrical protocols [6,7,11,55,56]. This discrepancy supports the view that only a small change in fraction area is sufficient to produce a significant increase in s.c. permeability.

The effects induced by electroporation on the s.c. are relatively similar to the disorganisations observed after iontophoresis. A general trend can be drawn. A lipid disordering of the intercellular structures was induced by the strongest electrical protocols, as suggested from the DTA. The disorganisation at high voltage pulses was associated with a perturbation of the lateral lipid packing (WAXS study, [59]) as reported after iontophoresis performed at very high electrical current [23]. Disruptions of the lamellar phases have been observed as well [57]. The lamellar disordering has been supported by the FFEM observations: more extended zones of disorganisation were observed when the number, voltage and/or duration of the pulse were increased. Interestingly, the FFEM images revealed that network structures were present in the s.c. lipid organisation after application of high voltage pulses. These network structures were not observed after application of high current densities, even up to current densities of 13 mA/cm².

The recovery process of the s.c. disorganisation has not been deeply investigated yet. Nevertheless, the reversibility in skin permeability observed in drug permeation experiments and in the TEWL values could indicate a recovery of the barrier perturbations [74].

4.2.1. *What is the mechanism of s.c. disorganisation by electroporation?*

The influence of water, heat dissipation and direct interaction with the electric field will be discussed as possible mechanisms.

4.2.1.1. *Water*

Like during iontophoresis application, changes in ion and water content may play a role in the barrier perturbations induced by high voltage pulsing. An increase in water content was observed but seemed to be less important than during iontophoresis consistent with the short current application [11]. The role of water in the s.c. disorganisation could therefore be less important than during iontophoresis.

4.2.1.2. *Heat*

One of the possible mechanisms which is proposed to explain the perturbation of the s.c. barrier is a heat dissipation phenomenon [75,76]. For short pulse, membrane electroporation has been reported to be a nonthermal phenomenon. Indeed, for an electrical pulse of 100 V (transmembrane voltage) applied during 1 ms, the temperature rise calculated was 0.02°C [22]. Further, the formation of “pores” is supposed to occur very rapidly, before any temperature rise could occur [10,22,75]. However, localised heating may also occur at sites of locally large current density specially with long pulses [10]. Even though heat convection takes place and propagates the heat front across the skin, the Joule heating could be sufficient for melting skin lipids with phase transition around 70°C supporting an important role of temperature rise [10]. High temperature rise might likely produce significant changes in the s.c. structure (Table 4). However, the defects should be spread on a large surface area or should occur at very frequent localisations to be detected by bulk methods. Experimental and theoretical investigations of localized heating by skin electroporation predict that LTR formation could be partly explained by joule heating during pulsing and that temperature rise is relatively small in the localized dissipation region that surrounds the LTR [77].

If data support the important role of localized heating, heat dissipation is not a very rapid mechanism and can therefore hardly be responsible for the rapid initial changes observed in the electrical properties during iontophoresis (less than 10 μs; [20,23]). Moreover, the SAXS pattern of s.c. heated to 75°C and cooled to room temperature showed only slight changes in the diffraction pattern. After heating to 120°C or 90°C and cooling, the X-ray pattern showed a single repeat distance of 13.4 nm [48,49]. These results demonstrate that the SAXS patterns obtained after electroporation deviate from recrystallised s.c. Moreover, the general lamellar disordering observed by X-ray scattering would imply, by assuming that this disordering effect was induced by a heat dissipation, a temperature of more than 65°C which could have occurred locally but not generally [23]. However, WAXS studies revealed that a temperature rise to just above 40°C transforms the orthorhombic lateral phase to an hexagonal lateral

phase [1]. This phase transition was observed in the s.c. submitted to electroporation.

4.2.1.3. Direct effects

The primary interaction of electric field during electroporation seems to be a nonthermal effect [70,71,75]. Theoretical models support the hypothesis of “pore” or aqueous pathways formation [70,71,75–78]. Evidences for skin electroporation are summarized in this special issue [8]. If aqueous pathways are created across the s.c. as across lipid membrane, the pathways are believed to be small (a few nm), sparse ($\leq 0.1\%$) and short-lived (microseconds to seconds). The characterisation of those structures by any form of microscopy is therefore extremely difficult. FFEM was the more adequate technique to detect the presence of long-lived pores after application of high voltage pulses. No pore-like structures were revealed by the micrographs of s.c. submitted to electroporation. Since the pores are believed to be short-lived, they could have disappeared during the sample manipulation. Moreover, only pores which have evolved to larger structures could be large enough to be detected.

If no pores were detected, the general disordering of the lipid bilayers demonstrates that structural changes were induced but contrasts with the LTR of molecules observed during high voltage pulses [13,54,60].

4.3. Comparison of the effects of iontophoresis and electroporation

The comparison of the data on the effects of iontophoresis and/or electroporation collected from different studies is difficult because most studies involve different skin sources, different amount of electrical charges transferred, different amount of energy applied and/or different methods of analysis. However, most of the biophysical studies of the s.c. after iontophoresis and electroporation were, at least partly, performed with similar experimental conditions and allow us to draw conclusions.

As summarized in Tables 2 and 5, both iontophoresis and electroporation induced similar structural changes as demonstrated by different biophysical and ultrastructural techniques. The perturbations involved mainly an increase in water content and

modifications of the lipid bilayers of the s.c. The higher amount of charges transferred or energy applied, the higher the perturbations.

The biophysical studies were supplemented by noninvasive bioengineering methods in vivo. Previous studies had demonstrated that iontophoresis ($< 0.5 \text{ mA/cm}^2$, $< 6 \text{ h}$) induced a reversible and mild increase in LDF (laser Doppler flowmetry) value, an increase in s.c. hydration by ATR-FTIR and a transient but not iontophoresis-induced increase in TEWL value [33,79]. A recent TEWL study combined with chromametry and LDF compared the effect of electroporation and iontophoresis in hairless rats. Fifteen \times (250 V—200 ms) pulses induced an up to 2.2-fold increase in TEWL versus the control site. Fifteen \times (100 V—500 ms) pulses and iontophoresis (0.5 mA/cm^2 , 1 h) induced similar changes. The primary increase in TEWL was associated to an enhancement in skin hydration and alteration in s.c. barrier function. Reversible increase in LDF and chromametry associated with an erythema were also observed [74].

5. Conclusion

This review focuses on the effects induced by iontophoresis or electroporation on the s.c. Hence, the aims were: (1) to contribute to the understanding of the mechanisms of drug transport by these methods; (2) to evaluate the safety issues associated with current application.

Complementary biophysical methods were used to provide a complete picture of the s.c. after iontophoresis or electroporation. Even though the mechanisms of drug transport is believed to be different, i.e., electrophoresis for iontophoresis and creation of new aqueous pathways for electroporation, the effects detected minutes after current application on the s.c. were very similar.

Disorganisation of the s.c. structure has been demonstrated after both iontophoresis and electroporation. The major perturbations observed after the application of continuous current or high voltage pulses were (i) an increase in s.c. hydration; (ii) a disorganisation of the lipid bilayers. Similar perturbations of the s.c. structure were observed following iontophoresis and electroporation. The extent of

the perturbations were dependent on the electrical protocol. The higher the amount of electrical charges transferred, the higher the perturbations. Further, the perturbations were sufficiently expanded to be detected by bulk biophysical methods.

Changes in lipid ultrastructure could be induced by direct electric field effects, heat dissipation and/or by water and ion content alterations. Whether the structural changes observed are primary or secondary phenomena is still under debate. However, evidences from experimental data and theoretical modeling support the idea that structural changes are mainly primary and secondary phenomena for electroporation and iontophoresis respectively. Whether the changes were localized and originated from existing or new aqueous pathways to expand laterally or whether the modifications were induced all over the s.c. remains to be solved.

These perturbations can be associated with an increase in skin permeability explaining partly the enhancement in drug transport. They are also unwanted side effects of current application. Nevertheless, if iontophoresis is generally considered as a safe procedure, further studies are required to study the long term effects of prolonged and repeated applications of high voltage pulsing on the skin. Electrical devices have to be developed to focalize the electric field mainly in the s.c., avoiding therefore unwanted effects on the viable parts of the skin.

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