ELSEVIER

Bioelectrochemistry 57 (2002) 65–72

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Kinetics of the temperature rise within human stratum corneum during electroporation and pulsed high-voltage iontophoresis

Uwe F. Pliquett [1], Gregory T. Martin, James C. Weaver *

Harvard-MIT Division of Health Science and Technology, Massachusetts Institute of Technology, Room 16-316, Cambridge, MA 02139, USA

Received 11 September 2000; received in revised form 14 November 2001; accepted 28 November 2001

Abstract

Electroporation is believed to be a nonthermal phenomenon at the membrane level. However, the effects of associated processes, such as Joule heating, should be considered. Because electroporation of skin, specifically the stratum corneum (SC), occurs at highly localized sites, the heating is expected to conform locally to the sites of electroporation. Significant localized heating was found to be strongly dependent on the voltage and duration of the high-voltage pulses. Specifically, a localized temperature rise was predicted theoretically and confirmed by experiments, with only a small rise (about 17 °C) for short, large pulses (1 ms, 100 V across the SC), but was increased (about 54 °C) for long, large pulses (300 ms, 60 V across the SC). The latter case appears to result in irreversible structural changes like vesicularization of the lipid lattice. These results support the hypothesis that electroporation occurs within the SC and that additional processes, such as localized heating, may be important. © 2002 Published by Elsevier Science B.V.

Keywords: Human skin; Local transport regions; Electroporation; Iontophoresis; Local temperature rise

1. Background

Electrically induced changes in skin have been investigated [1–4] in order to seek information about burning or denaturation of the skin due to high-voltage accidents. Electroporation also involves electrically induced changes in the skin permeability that may be used for transdermal drug delivery without causing significant thermal damage of the tissue.

The skin is a multi-layer system with a 15-μm-thick outer layer, called the stratum corneum (SC). While the SC is the most resistive layer [5,6], its barrier function is interrupted by appendages (hair follicles, sweat ducts), which serve as conducting pathways. A sample of heat-stripped skin with an area of 0.69 cm^2 has a resistance that varies between 50 and 250 kΩ. This resistance is dependent on the density of appendages. Assuming most of the resistance is in the SC and that its thickness (h_{SC}) is about 15 μm, the resistivity of the SC is estimated to be between 0.23 and 1.15 MΩ m. For comparison, the resistivity of 150 mM saline is about 0.72 Ω m and viable dermis is about 150 Ω m [7].

If the skin is subjected to an electric field, it will confine within the stratum corneum. If the voltage across the SC achieves about 60 V, an electric breakdown associated with electroporation is very likely. During an electric breakdown, the resistance of the SC decreases by up to four orders of magnitude within less than a microsecond. This electric breakdown of the skin shows a high localization, where the drop in resistivity occurs in local dissipation region (LDR) [8]. Electroporation is believed to create aqueous pathways through single lipid bilayers, which can yield molecular transport either by local electrophoresis as long as the pulse lasts or by other driving forces such as electroosmosis, pressure gradients or osmotic gradients [10,11]. While the transport of small ionic species (Na^+, Cl^-, Ca^{2+}, etc.) takes place within the LDR, the transport of larger, fluorescent molecules is limited to a spot inside the LDR. This region is called the local transport region (LTR) [8,9] (Fig. 1). The fractional surface area of LDR and LTR depends on the pulsing protocol and ranges from 1% to 30% for LDR and 0.1% to 10% for LTR.

Because of the tremendous drop in resistance of the stratum corneum and the high-voltage applied, the current density achieves as much as 2 A/cm^2 during our experiments (see Materials and methods). Since the current is localized within the LDR, the peak density is even much higher. If we assume no significant heat conduction and consider an

* Corresponding author. Tel.: +1-617-253-4194; fax: +1-617-253-2514.
E-mail address: jim@geldrop.mit.edu (J.C. Weaver).
[1] Present address: Faculty of Chemistry, University of Bielefeld, 33615 Bielefeld, Germany.

1567-5394/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S1567-5394(01)00177-3

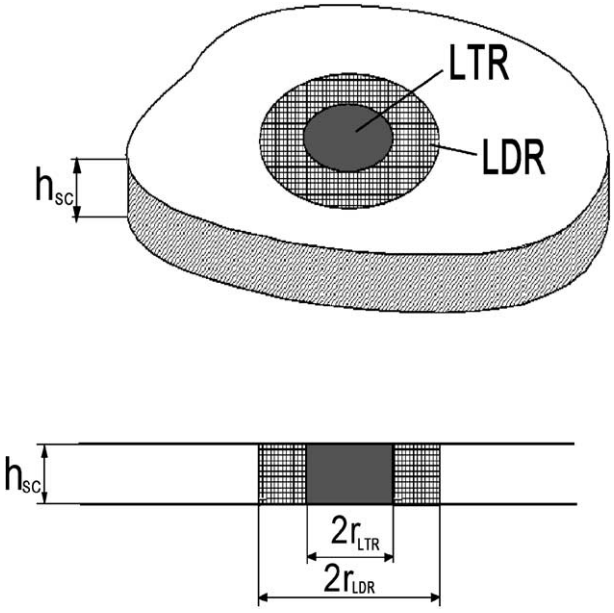

Fig. 1. Schematic of the stratum corneum with local dissipation region (LDR), a localized region (radius = 30–500 μm) where electric energy is dissipated, and which is characterized by a drop in resistance. Due to the current density, a temperature rise during the application of high-voltage pulses occurs. Within the center region of an LDR, we usually found an even more localized region (LTR, $r = 10–300$ μm) involved in molecular transport. An LTR is characterized by the ability to transport molecules up to a few kilograms per mole without visible damage of the SC as seen under a light microscope. Even after recovery, it shows the lowest resistance within the skin surface, i.e. it does not recover the electrical resistance. The LDR were initially identified by the AgCl deposition at a silverplate [8]. In these experiments, the LDR corresponds to the heated area of the skin.

adiabatic temperature rise within an LDR ($U_{skin} = 50$ V, pulse duration = 1 ms), the temperature rise would be an astounding 1500 °C. In reality, the true temperature will depend on a variety of factors such as the current density distribution in the LDR and the heat transport into regions not electrically heated up. This would be mainly due to heat conduction and also to thermal convection through the SC [12].

Thus, the localization of molecular and ionic transport as well as the Joule heating as a function of pulse magnitude and duration is of basic interest. With this in mind, the present study emphasizes the fundamental behavior of the SC during "high-voltage" pulses.

2. Materials and methods

Several types of microscopy, electrical measurements for determining the skin's passive electrical properties, fluorescent molecule flux measurements, and local temperature measurements were used to quantitatively assess the number and size of LTRs created by both short and long "high-voltage" pulses. Because the diameter of LDR ranges from about 50 to 300 μm and the pulse duration ranges typically between 1 and 300 ms, it was not possible to measure the temperature distribution within the LDR in a direct way. However, using an infrared (IR) detector, it was possible to measure the average temperature over an area of 7 mm^2 synchronous with a light microscopy utilizing temperature sensitive dyes to visualize heat propagation.

2.1. Measuring chamber

The body of the chamber had two compartments and was machined from Lucite. It was clamped in an aluminum frame (Fig. 2).

At the lower side of the skin, a temperature-sensitive liquid crystal (TLC), which turns green around 45 °C, was applied (Licristal™, Hallcrest, Glenview, IL). In order to maximize resolution, the experiments were done in a dark room and the minimal illumination necessary to visualize the color change of the Licristal was used. The chamber had a window at the bottom for imaging the skin during pulsing. LEDs (red, green and blue) were used for illumination of the skin. This prevented an artificial heating of the skin from the light source. The top of the skin faced a mesh of thin gold wires (35 μm) which served as anode (+). A thin film of saline was used to maintain good electrical contact between the gold and the skin. However, water, a strong absorbent for long IR, was not allowed to accumulate between the electrode wires. Thus, the interference with the IR detection

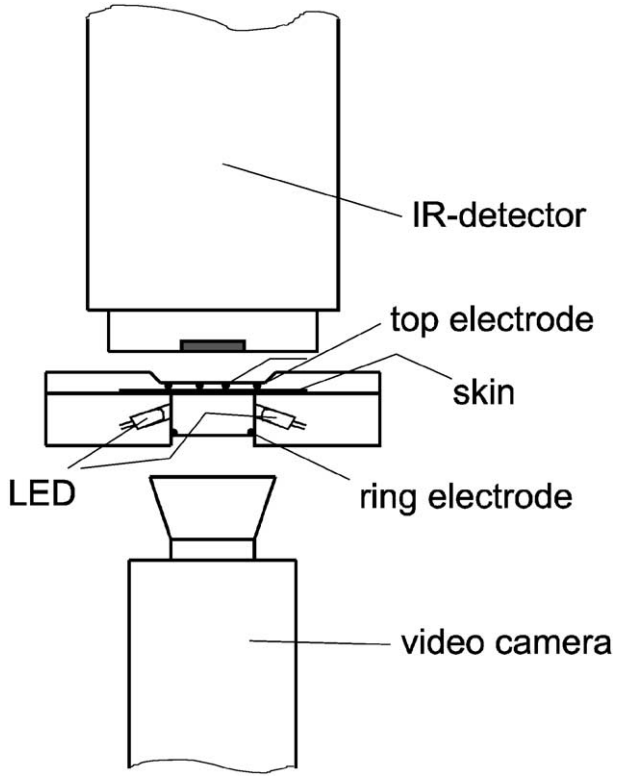

Fig. 2. Apparatus used for simultaneous measurement of temperature and visualization of the surface area due to localized heating.

was minimized. The counter electrode (cathode (−), silver) placed at the chamber bottom was ring-shaped, allowing the observation of the skin specimen. For filling purpose and pressure adjustment, the chamber was designed with flow through facilities.

2.2. Infrared measurement

The photo-resistive detector (HgCdTe) housed in a vacuum insulated dewar, which was filled with liquid nitrogen, maintaining the proper operating temperature for the sensor (Infrared Associates, Stuart, FL). A ZnSe lens focused the detector to a small spot (1.5 mm radius, $A = 7\ mm^2$) at the skin surface. The detector, electrodes and video camera were aligned such that the same area of the skin could be simultaneously visualized and measured with the IR detector.

This detector was specifically chosen for this application because it has a peak sensitivity at 310 K and a very fast time response ($\tau = 10^{-6}$ s). The detector was held 2 mm above the surface of the skin. The signal was obtained by measuring the output voltage resulting from a constant current of 5 mA. This signal was conditioned using a six-stage DC amplifier with a gain of about 800 and a low-pass filter with an upper cutoff frequency of about 2 kHz. The signal was recorded by a digital storage oscilloscope (HP54601A, Hewlett Packard, Palo Alto, CA) and transferred to a PC for further analysis. The IR detector was calibrated with a temperature-controlled black body source from 20 to 71 °C. A linear fit was performed with the amplifier output voltage as a function of temperature. The standard deviation of the calibration was 0.15 °C.

2.3. Visualization of LDR

For visualization of the LDR and subsequent calculation of the surface area, a temperature sensitive dye (temperature sensitive liquid crystal, TLC) was used. The liquid crystal (Licristal, C20-10 TLC) and a binder (AQB-3), obtained from Hallcrest, were mixed and applied to the surface of the SC samples. The samples were placed into the chamber facing the video camera with the Licristal side. The TLC is designed to turn green when 45 °C is reached, which can be seen by the video camera. The video signal was recorded by a VCR for further computer processing.

2.4. Convection measurements

The water transport through the skin was measured using tritiated water. Since water transport during and after pulsing can flow by diffusion and electroosmosis, the experiments were divided into two parts. (1) Both compartments of the penetration chamber (Crown Glass, Somerville, MA) were filled with saline doped with 10 μCi tritiated water introduced at the cathode side. Exponentially decaying pulses of 80 and 105 V with time constants of 100 and 360 ms, respectively, were applied. The voltage refers to the maximum voltage across the skin (exponential decaying). It was calculated using the voltage across an inner pair of electrodes, the resistance of the saline and the total current through the chamber. Since the voltage depends on the skin resistance as well, the given numbers are the average of all the experiments. The voltage applied at the outer electrodes was 100 and 200 V. Ten pulses with 5-s spacing were applied. Immediately after pulsing, the anode side was emptied and the saline was mixed with a scintillation cocktail. The α-activity was measured using a scintillation counter (Wallac Rackbeta Liquid Scintillation Counter Model 1214-005 from Wallac, Turku, Finland). (2) The setup, as in (1) but with the tritiated water in the anode side while the activity in the cathode side, was measured. The experiments were performed at the same time using two chambers for each setup.

2.5. Experimental protocol for temperature measurements

Pulses were applied using a BTX-ECM600 (Genetronix, San Diego, CA). The time between pulses was 1 min in order to reequilibrate the temperature to the ambient temperature. The total current through the chamber was measured as voltage drop across a noninductive 5 Ω resistor. The voltage across the skin was determined by taking into account the resistance of the electrodes and electrolytes as a function of the total current. The measurement chamber was filled with saline such that the skin was pressed against the gold electrode. Care was taken such that only a very thin film of water was at the skin surface in order not to absorb the IR signal.

Heat-stripped SC was used in almost all experiments where the temperature rise and the temperature distribution were measured at the same time.

However, for comparison, full thickness skin was used. In these cases, only the temperature rise or the temperature distribution could be measured.

2.6. Skin preparation

Heat-stripped SC, obtained from human cadaver donors, was used throughout the experiments for simultaneous temperature measurement and visualization. The preparation of the skin is well described in Ref. [13]. For experiments designed to assess possible differences in convection between heat-stripped skin and full thickness skin, we used about 4-mm-thick (uncompressed) preparations of the same sample as the heat-stripped skin. The prepared skin was stored at 94% humidity at 4 °C for further use. Because the behavior of skin changes with time, the specimens were used within a week.

2.7. Ambient temperature during the experiments

Experiments using different pulsing protocols were performed at room temperature (24–26 °C). The ambient temperature was taken into account for the calculation of

the temperature rise. The pattern for temperature distribution was only assessable at the absolute temperature specified by the TLC. The parameter of interest is the temperature difference to the ambient temperature. Thus, the ambient temperature will influence the distribution pattern of temperature what we could not correct. However, by changing the initial temperature by 1 °C, no significant change in the temperature distribution was found.

3. Results

The data collected during the heat distribution visualization and IR measurements together yielded a trace of temperature and current for each single pulse. Usually, three pulses at applied voltages of 100 or 200 V were performed on a single piece of skin.

The pattern of the temperature distribution was recorded by a VCR for subsequent frame by frame analysis. Finally, the frame with the largest heated region was chosen (Fig. 3A), which corresponds approximately with the moment of the largest temperature rise. Therefore, it could serve for synchronizing the video frames with the temperature trace (Fig. 3B).

From the current trace together with the electrical behavior of the chamber, the voltage was calculated across the skin and found it to be 46 ± 14 V for 100 V and 73 ± 22 V for 200 V applied, respectively.

The average temperature rise over the 7 mm^2 IR detector field of view was 7.24 ± 0.73 °C for the 100 V pulses. Taken into account a fractional LDR area of $10.5 \pm 5.7\%$, we estimated an area average temperature rise within the LTR of 68.9 °C. The strong variability in transdermal voltage, seen especially between the first and the subsequent pulses, was caused by the decrease in the skin resistance during the first pulse, which caused a drop in transdermal voltage.

3.1. Short high-voltage pulses

Starting at pulse conditions, resulting in a strong signal, we decreased the pulse duration. The smallest temperature rise signal reliably obtained resulted from a pulse of $U_{skin} = 81$ V and $\tau_{pulse} = 16$ ms. Short high-voltage pulses such as 150 V/1 ms could not be detected with the IR detector nor with the Licristal (TLC).

3.2. Heat distribution with electrodes not attached to the skin

In experiments where all parameters are measured at the same time, we had to compromise between the accuracy of the IR detector temperature measurement and the quality of the acquired image. Another point of uncertainty is the possible confinement of the field at the electrodes, which might lead to the localization of the heat during pulsing. We therefore performed a number of experiments with a

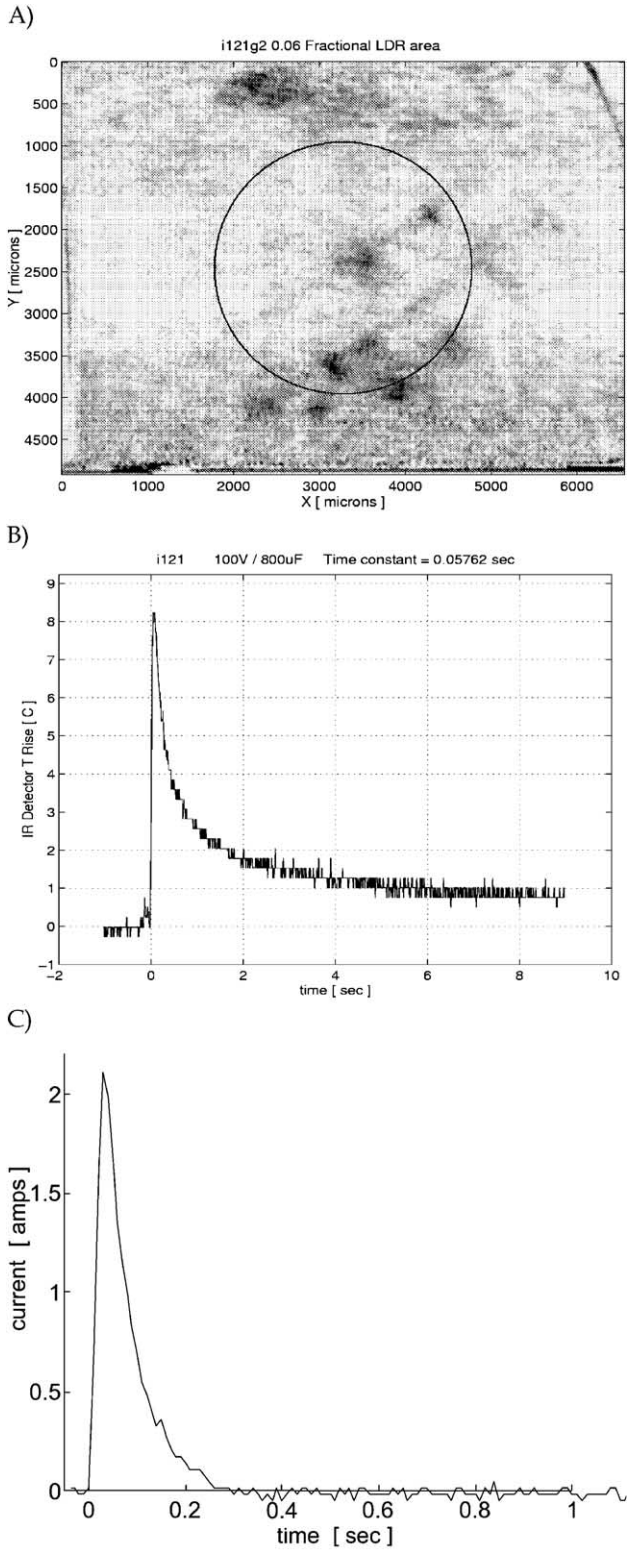

Fig. 3. A complete set of results from one experiment. The pulse parameters were $U_{skin} = 83$ V and $\tau_{pulse} = 95$ ms. (A) The image acquired by the video camera at the time of the highest temperature (frame by frame selection). The ring indicates the area viewed by the IR detector. (B) The temperature rise during the pulse. The starting temperature at this particular experiment was room temperature (24 °C measured). (C) The current through the whole chamber needed for calculation of the voltage across the skin, at the same time scale as the temperature rise.

chamber in which the electrodes were placed far from the skin. To enhance the color contrast with the Licristal, the skin (heat-stripped) was placed on black filter paper. From observation of these images, it was evident that the heat localization was not due to a confinement of the field by the electrodes but appears stochastically across the skin surface, in agreement with observations of LTR/LDR formation [8]. The number of LDRs showed a dependence on the applied voltage, with larger voltages corresponding to more LDRs. By increasing the duration of the pulses, the LDR size increased, but not significantly their number. With the pulsing protocols used, we found between 1 and 10 LDR/cm^2 with a size ranging from 100 to 400 μm. We should emphasize that the measured size of the LDR is determined after the area that turned green because of the transition of the Licristal.

3.3. Heat dissipation vs. energy transport through the skin

We define as the energy dissipated within the skin, the product of voltage across the skin (U_{skin}), current through the entire system and the time duration of the pulse, i.e. ($W = \int_0^\infty U_{SC}(t)I(t)\mathrm{d}t$). Fig. 4 shows the results of average temperature rise in a 7 mm^2 spot for the experiments carried out under observation with the video camera.

The area involved in dissipation ranged from less than 1% to 40%. By dividing the temperature rise by the fractional area of the LDR, we get an estimate of the peak temperature rise within an LDR. For the largest pulses, more than 60 °C temperature increase was found, which means that for our experiments, conducted at room temperature, the phase transition temperature, T_0, of the skin lipids ($T_0 \approx 70$ °C) was exceeded.

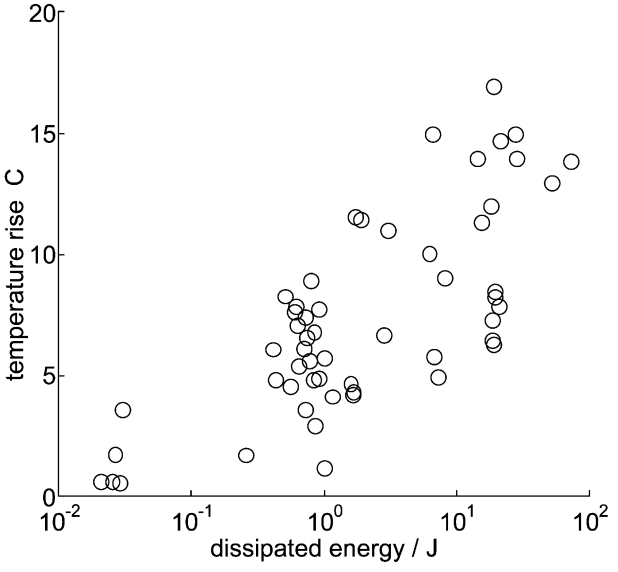

Fig. 4. Temperature rise vs. energy dissipated by the skin. Note that the *x*-scale is logarithmic, i.e. the temperature rise levels off.

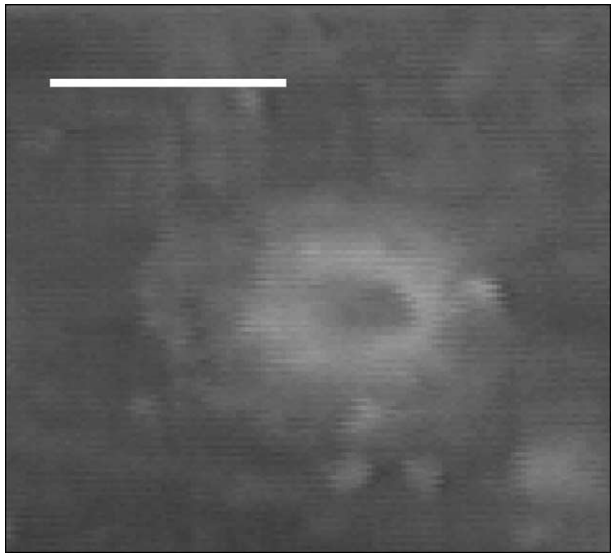

Fig. 5. Snapshot image of an LDR during pulsing (green channel, scale bar 200 μm). In these experiments, no infrared measurements were performed. Therefore, the pulsing electrodes were far from the skin, i.e. the localization of the LDR is not forced by the location of the electrodes. The ring-like appearance occurs because the Licristal turns green only in a small temperature range around 45 °C. At higher temperatures, it becomes colorless.

3.4. Convection during pulsing

The net water transport during 10 pulses ($U_{skin} \approx 60$ V, $\tau = 100$ ms) was directed from the anode to the cathode. The water transport from the anode side was about 100 nl per pulse while the transport into the other direction was about half of this. The transport was not a significant function of the transported charge, which suggests that a considerable transport occurs passively between the pulses. However, it should be clear that the measured water transport is completely through the skin while a considerable water exchange may take place within the hydrated sites of the skin.

3.5. Heat distribution during pulsing

In principle, it was possible to measure all parameters at the same time; however, the quality of visualization of the LTR (contrast and time resolution of the frames) was much better with bright incandescent light and a black background. Here, we can refer only to the part of the kinetics revealed by the apparent green color change of the Licristal. Fig. 5 (green channel) is an example of an LDR, visible as green ring which marks the location at about 45 °C. From the frame by frame analysis, it was found that the heating starts in the very center and expands through the duration of the pulse. During cooling, the ring decreases in diameter until the interior is completely green and then disappears from the outside in. Because of the white appearance of the stratum corneum and the inability to

clearly resolve color information, we could not determine the exact temperature gradient. However, from these kinetics of the LDR formation, we can conclude that the center of the LDR is hotter than the colored ring, which is approximately at 45 °C.

3.6. Heat-stripped skin vs. full thickness skin

To better interpret the results in terms of underlying heat transfer mechanisms, the more realistic model of full thickness skin was chosen. However, only the visualization of the heat distribution or IR measurements could be done at one time. By comparison of the data, we found that the heat pattern as well as the total heating did not show significant differences for full thickness vs. heat-stripped skin.

4. Discussion

It is well accepted that the temperature within an electrolyte rises due to dissipation of electric energy. However, it is not clear to what extent the temperature within a LDR rises during electroporation of skin. A simple estimation using a fixed adiabatic (no heat transfer) cylindrical pore model predicts that the temperature rise would exceed the phase transition (vaporization) of water. Since we observe no evidence of vaporization during electroporation, the temperature rise should not be that high. The values measured in our experiments show clearly that the temperature rise is below the electrolyte phase transition temperature. However, there is still a question to what extent the skin will be altered by the temperature rise. Given the recovery behavior of the skin after electroporation in the medium pulse range, there is a clear difference in the resistive behavior of the skin after heating to less than the water-phase transition temperature.

While the resistance of the skin recovers after high-voltage application (30–98% of initial resistance) depending on the pulse parameters, this behavior was not found if the skin was slowly heated above 70 °C using a water bath, which means that the skin is not only thermally altered. A key feature of high-voltage application is the spontaneous localization of the changes within the SC. If the pulsed skin is put on a silver plate as anode, the distribution of AgCl (brown color) reveals that the interior of LDR has a large electrical conductance even after a long time, i.e. essentially no recovery occurs at these sites after the pulsing. Our working hypothesis is that the propagation of a heat front, i.e. the front of 70 °C creates a highly permeable region with sharp boundaries (Fig. 6).

4.1. Model for electrothermal effect at stratum corneum

The stratum corneum is modeled as "brick wall". The corneocytes are like the bricks held together with "lipid mortar" (Fig. 6A). If a voltage is applied to this structure, transport of charged molecules through existing pathways will occur. However, the pathways (here NOT hair follicles nor sweat ducts) are very small and usually negligible. However, if the field strength achieves the breakdown conditions, the structure rearranges electrically, which is assumed to be similar to membrane electroporation. This yields a very high local current density (thick arrow in Fig. 6B). As soon as the current flows, heat production follows. Electroporation lowers the field strength not only at the pore but also in the surrounding region by up to 20%, depending on the pore size and density. The influence radius, however, is submicrometer-sized, i.e. the vicinity of an existing pore experiences almost the whole field strength. The probability for electroporation rises with temperature. If the immediate vicinity of an LTR is heated up, electroporation occurs due

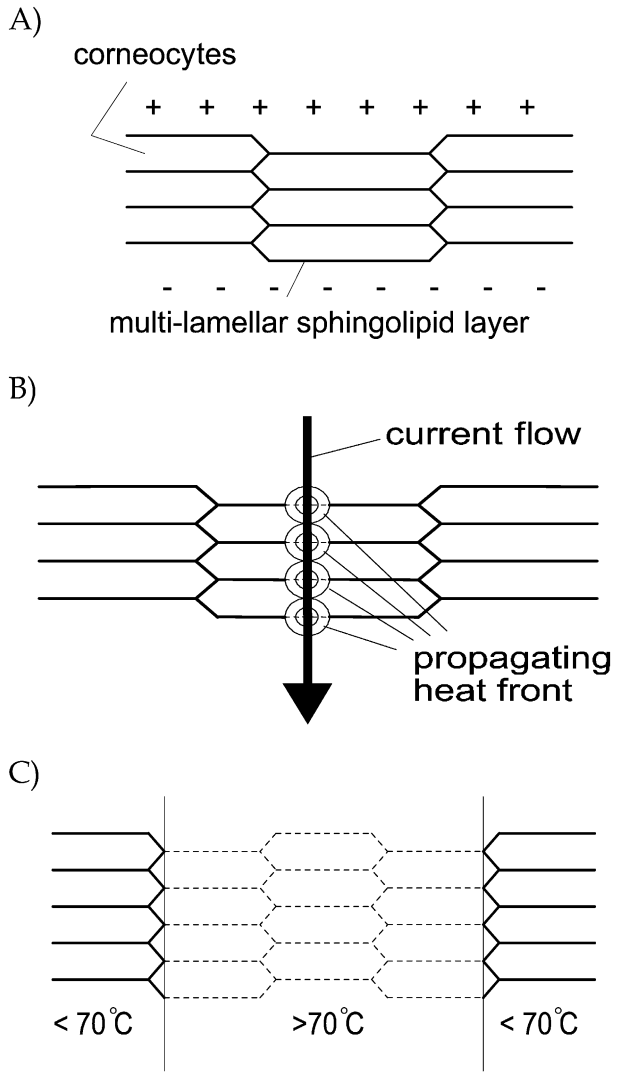

Fig. 6. Model for the electrothermally induced changes at stratum corneum. (A) Stratum corneum before high-voltage pulse, (B) during electric current flow with propagating heat front starting at the center of maximal current density, and (C) partial recovery of the stratum corneum after pulse at sites where the phase transition of the lipid structure was not reached.

to the limited influence radius of existing pores and thus a sufficient high field strength. This induces a propagating heat (Fig. 6B) from the inside to the outside of the local transport region (LDR). It is very likely that in the middle of the LTR, the temperature rises to more than 70 °C, which will yield vesicularization and thus water-rich pathways across the lipid structure. Now, an electrophoretic transport of even larger molecules becomes possible—a part of the LDR converts to an LTR.

By introducing water laterally into the skin structure, water-soluble molecules may spread out laterally. Because the edges of corneocytes may be permeabilized by electroporation or even by the temperature-induced permeability, it seems possible that water-soluble molecules can enter the corneocytes, yielding the characteristic ring-like structure around an LTR [8,9].

With ceasing pulse, the temperature decreases from the outside in. By interpreting images from fluorescence microscopy, it seems that a sharp border exist between the interior of an LTR where after washing, only very little dye was found. This means that pathways in the perpendicular direction are still open (Fig. 6C). Around this center region, a bright ring exists, indicating that the dye is trapped within this region, which means that no perpendicular pathways exist or are closed by recovery and the lateral pathway used by introduction of the dye into the ring region is interrupted. Otherwise, the dye would escape through the same way as it entered. We propose that at the border of the transition region (70 °C), the lipids are melted together, while in the interior, nonrecoverable lipid phase changes, like vesicularization, took place. The region around the LTR trapped the dye because there is no driving force and no high-permeability region. All this is supported by the comparison of the ionic pathways during and after the application of high-voltage pulses. During the HV-pulses, the LDR shows a very high conductance while after pulsing, only the high conductance in the LTR remains. Since the membranes recover after electroporation and do not recover after a temperature rise to more than 70 °C, an LTR involves a region where the temperature reached more than 70 °C. In contrast, an LDR is the region that is electroporated as well, but no temperature rise above 70 °C occurred.

The temperature rise is not a linear function of the energy dissipated in the skin but instead reaches a plateau (Fig. 4, note the logarithmic scale). This means that with larger pulsing parameters, an additional heat transfer mechanism is responsible for the observed behavior. This can be explained by a model using both conduction and convection of heat as well as the phase transition latent heat of the lipids [14].

4.2. Significance and limitations of the measured data

The overall quality of the data is suitable for interpretation. However, some sources of uncertainties should be discussed. The infrared radiation used for the temperature measurement is strongly absorbed by water. Therefore, water on the surface of the skin might cause the measured temperature to be below the actual temperature of the skin during pulsing. However, only a very thin film of saline was allowed on the skin surface to contact the electrodes. Experiments for validating the electrical behavior of the chamber were done with the electrodes touching the saline but not immersed. Therefore, the electrical measurements made allow us to estimate with a reasonable confidence (about 90%, taken into account the uncertainties in the chamber resistance and the standard deviation of 10 independent current measurements) the energy dissipation within the skin or the peak power delivered to the skin.

The best color contrast with the Licristal is achieved on a black surface. This could not be accomplished without changing the properties of the skin. Specifically, if the skin was painted black, it would influence the heat distribution and the electrical properties, but it was found that the TLC-slurry (about 5 μm thick) had no effect on the passive electrical properties of the skin. Without the black background, only monochromatic images could be acquired instead of a color image with information about temperature gradients. Therefore, we did not seek an interpretation of the red and blue components of the image. Another limitation of the TLC is the time resolution, which is on the order of millisecond. However, since the TLC is only at the skin surface, it should heat up before color changes occur. The resulting temperature distribution at the surface yields the size and location of the LDR.

Our data clearly show that there is no large temperature rise ($\approx 10^2$ °C), and from video imaging, we did not find evidence for phase transition of the water within the skin. This means that in the range of pulse parameters we used in these experiments, the temperature levels off. We did not, however, investigate the range of electrical effects associated with heavy injury like burning of the skin. For short pulse duration protocols (50–150 V across the skin, 1-ms duration), we could not detect any temperature rise during pulsing with our IR detector. This has been addressed by theoretical analysis [14].

The issue of biological significance of heating of the stratum corneum for less than a second above the phase transition temperature of sphingolipids (70 °C) is not understood yet. In vivo experiments using hairless rats found no significant skin irritation or damage for short ($\approx$ 1 ms) and long pulses ($>$ 500 ms) [15].

5. Conclusion

Electroporation of skin is associated with a temperature rise within the highly localized dissipation regions (LDR). As the transdermal voltage and pulse time constant increase, the temperature tends to a plateau but does not reach the phase transition of the water. The recovery of the skin's electrical resistance after pulsing cannot be explained as

only a thermal phenomenon. Structural changes persist, leaving small unrecovered central regions with a very high electrical conductivity that is due to combined electrical and thermal effects.

Acknowledgements

This work was supported by NIH Grant ARH4921 and Whitaker Foundation Grant RR10963.

We thank the group of Dr. H.F. Bowman for loaning us the infrared detector. Hallcrest kindly provided the TLC (Licristal).

References

[1] D.B. Bommannan, L. Leung, J.A. Tamada, J. Sharifi, W. Abraham, R.O. Potts, Transdermal delivery of luteinizing hormone releasing hormone: comparison between electroporation and iontophoresis in vitro, Proc. Int. Symp. Controlled Release Bioact. Mater., 1993, pp. 97–98.

[2] R.L. Bronaugh, H.I. Maibach (Eds.), Percutaneous Absorption, Mechanisms–Methodology–Drug Delivery, Marcel Dekker, New York, 1989.

[3] R.R. Burnette, Iontophoresis, in: J. Hadgraft, R.H Guy (Eds.), Transdermal Drug Delivery: Developmental Issues and Research Initiatives, Marcel Dekker, New York, 1989, pp. 247–291.

[4] R.O. Potts, M.L. Francoeur, R.H. Guy, Routes of ionic permeability through mammalian skin, Solid State Ionics 53–56 (1992) 165–169.

[5] P.M. Elias, Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism, J. Controlled Release 15 (1991) 199–208.

[6] P.M. Elias, Structure and function of the stratum corneum permeability barrier, Drug Dev. Res. 13 (1988) 97–105.

[7] U. Pliquett, J.C. Weaver, Electroporation of human skin: simultaneous measurement of changes in the transport of two fluorescent molecules and in the passive electrical properties, Bioelectrochem. Bioenerg. 39 (1996) 1–12.

[8] U. Pliquett, T.E. Zewert, T. Chen, R. Langer, J.C. Weaver, Imaging of fluorescent molecule and small ion transport through human stratum corneum during high-voltage pulsing: localized transport regions are involved, Biophys. Chem. 58 (1996) 185–204.

[9] M.R. Prausnitz, J.A. Gimm, R.H. Guy, R. Langer, J.C. Weaver, C. Cullander, Imaging regions of transport across human stratum corneum during high voltage and low voltage exposures, J. Pharm. Sci. 85 (1996) 1363–1370.

[10] Y.A. Chizmadzhev, V.G. Zarnitsin, J.C. Weaver, R.O. Potts, Mechanism of electroinduced ionic species transport through a multilamellar lipid system, Biophys. J. 68 (1995) 749–765.

[11] J.C. Weaver, Electroporation: a general phenomenon for manipulating cells and tissue, J. Cell. Biochem. (1993) 426–435.

[12] W.H.M. Craane van Hinsberg, J.C. Verhoef, H.E. Junginger, H.E. Bodde, Thermoelectrical analysis of the human skin barrier, Thermochim. Acta 248 (1995) 303–318.

[13] C.L. Gummer, The in vitro evaluation of transdermal delivery, in: J. Hadgraft, R.H. Guy (Eds.), Transdermal Drug Delivery: Development Issues and Research Initiatives, Marcel Dekker, New York, 1989, pp. 177–186.

[14] G.T. Martin, U. Pliquett, J.C. Weaver, Theoretical analysis of localized heating in human skin subjected to high voltage pulses, Bioelectrochemistry 57 (1) (2002) 55–64 (this issue).

[15] R. Vanbever, D. Fouchard, A. Jodoul, N. de Morre, V. Preat, J.-P. Marty, In vivo noninvasive evaluation of hairless rat skin after high-voltage pulse exposure, Skin Pharmacol. 11 (1998) 23–34.