Spatially constrained localized transport regions due to skin electroporation

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Abstract

Rapid, controlled molecular transport across human skin is of great interest for transdermal drug delivery and minimally invasive chemical sensing. Short, high-voltage pulses have been shown previously to create localized transport regions in the skin. Here, we show that these regions can be constrained to occur at specific sites using electrically insulating masks that restrict the field lines. The increase in total ionic and molecular transport per area was comparable to the levels observed in unconstrained electroporation of human skin. Constraining the area of intervention to encompass small areas of interest, a primary feature in the design of microdevices for transdermal drug delivery, can provide the same levels of flux as the unconstrained case.

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1. Introduction

The stratum corneum (SC), the outermost, essentially dead layer of the skin, is the primary barrier to entry of ions and molecules. Electroporation of SC by high-voltage (transdermal voltage, \(U_{\text{skin}} > 50\) V) pulses is believed to create aqueous pathways in skin which facilitate molecular transport \([1-13]\). Previous studies have shown that the transport of small molecules such as fentanyl \([14]\), calcein, sulforhodamine, cascade blue, and lucifer yellow \([15]\) through human skin can be enhanced by over four orders of magnitude with high-voltage pulses \([1,8]\). Transport of larger molecules, such as DNA fragments \([4]\), heparin \([6]\) and peptides \([16]\) using \(U_{\text{skin}} \approx 30-500\) V has been demonstrated as well.

Significantly, the transport of fluorescent molecules and small ions occurs in highly localized regions (local transport regions, LTRs) surrounded by regions that conduct ions but not molecules (local dissipation regions, LDRs) \([4,8,11,17]\). Exponential pulses with \(\tau_{\text{pulse}} = 1\) ms created LTRs with diameters not exceeding 100 \(\mu\)m \([5]\), while longer pulses \((\tau_{\text{pulse}} > 100-400\) ms) produced LTRs with diameters up to 600 \(\mu\)m \([11]\). The number of LTRs per area depends on peak transdermal voltage. Pliquett et al. \([8]\) showed that during high-voltage pulsing, LTRs for both calcein and sulforhodamine were distributed almost randomly over the SC topography. However, no LTRs were found in the valleys (rete pegs) of the SC or near the appendages (sweat ducts and hair...
folicles). Using gel trapping microscopy [4,8], they showed that LDRs are larger than LTRs and that molecular transport occurred through a small area in the center of the LTR. Recent experiments with snake skin (no appendages) confirm that LTRs form in SC for \( U_{\text{skin}} > 50 \text{ V} \) [15].

Transdermal drug delivery research will need to consider development of devices for delivery and chemical sensing. A common requirement in the design of these devices will be minimizing the area of intervention. In some applications, the intervention may have to be focused to a small area of interest. However, the effect of constraining the area of skin exposed to high-voltage (HV) pulses on transdermal flux has not been previously reported.

We show that the location, number and size of LTRs created by HV pulsing can be controlled by constraining the current to predetermined regions of interest. A thin insulating sheet with an array of straight-through microholes was used to concentrate the electric field lines at small areas on the skin. The insulating sheet was placed adjacent to the skin, touching the SC. The conduction of ions and transport of molecules thus occurs primarily through that part of the skin next to the microholes.

### 2. Materials and methods

The skin was secured from either the abdomen, arm, or back of adult human cadavers (NDRI, Philadelphia, PA; Ohio Valley Tissue and Skin Center, Cincinatti, OH). The experiments were conducted on either full thickness skin or heat-stripped skin at 25°C. The skin was cut into 0.75 inch (1.9 cm) diameter circular pieces and was stored at 4°C in a 95% humidity environment. In the case of unconstrained electroporation, the skin was mounted in a side-by-side permeation chamber (Crown Bio Scientific, Somerville, NJ), with a skin exposure area of 0.64 cm² (Fig. 1). The constrained electroporation experiments were performed with a 50-μm thick insulating polyimide sheet placed adjacent to the skin on the SC side. The polyimide sheet contained a 11 × 11 array of microholes, 40–125 μm in diameter, with 300–500 μm center-to-center separation.

High-voltage (HV) pulses were applied using an exponential pulser (Electroporation System 600, BTX Industries, San Diego, CA). A 5-Ω resistor \( (R_{\text{res}}) \) was placed in series with the chamber in order to measure the current through the skin. Silver-wire electrodes (0.7 mm diameter, 3 cm long) were placed adjacent to the microholes.

**Fig. 1.** Skin is mounted in a side-by-side chamber. The 50-μm thick polyimide insulating sheet was perforated with an array of 11 × 11 microholes. The sheets featured microholes, 40–125 μm in diameter and 300–500 μm center-to-center separation. The pulsing electrodes were protected from chemical by-products released during pulsing by encasing the electrodes in polyacrylamide gel and flowing fresh PBS through the electrode assembly [15].
placed in the inner ports of the chamber parallel to skin surface, at a distance of 2.75 cm between them (Fig. 1). The voltage drops across the inner electrodes \( U_{\text{inner}} \) and the 5-Ω series resistor \( U_{\text{res}} \) during pulses were stored in a digital oscilloscope (Hewlett Packard 54601) for subsequent calculations. The transdermal voltage \( U_{\text{skin}} \) was calculated from

\[
U_{\text{skin}} = U_{\text{inner}} - IR_{\text{sal}}
= U_{\text{inner}} - \left( \frac{U_{\text{res}}}{R_{\text{res}}} \right) R_{\text{sal}},
\]

where \( R_{\text{sal}} \) is the resistance of saline solution between the inner electrodes [5,9]. The value of \( R_{\text{sal}} \) was determined by applying a series of pulses with no skin present in the chamber and was found to be approximately 220 Ω.

Skin impedance was measured prior to the start of pulsing, between pulses, and after the end of pulsing. The skin impedance was measured by applying a 100 mV peak-to-peak sinusoidal voltage of 100 Hz, 1 kHz, and 10 kHz between the inner electrodes. An LCR meter (SR715, Stanford Research Systems, Sunnyvale, CA) was used to measure the skin resistance.

As in many previous studies [8,9,15,17], two different water-soluble, charged fluorescent molecules were used in transport measurements: calcein (Molecular Probes, Eugene, OR, charge of \(-4, 623 \text{ g mol}^{-1}\)) and sulforhodamine (Sigma Chemical Co., St. Louis, MO, charge of \(-1, 607 \text{ g mol}^{-1}\)). The donor compartment of the chamber was filled with 1 mM calcein and 1 mM sulforhodamine in phosphate buffered saline (PBS; 150 mM dissolved salts; pH 7.4). The receptor compartment contained PBS. The fluorescence was measured with a spectrophotometer (Fluorolog II F112, Spex Industries, Metuchen, NJ) with excitation and emission wavelengths set at 488 nm/515 nm (calcein) and 586 nm/607 nm (sulforhodamine). In the case of heat-stripped skin, the solution in the receptor compartment was continuously replenished with fresh PBS. Fluorescence measurements were taken starting 10 min prior to pulsing and ending 1 h after the completion of pulsing.

One hour after the completion of pulsing, the skin was removed from the chamber and rinsed with de-ionized water. The skin was mounted on a microscope slide and examined under a fluorescence microscope (Olympus BH-2, Olympus Co., Woodbury, NY). The fluorescent image of the skin was photographed using a camera (Olympus OM2) mounted on top of the microscope.

3. Results and discussion

Polyimide insulating sheets with a 11 × 11 array of microholes were used to constrain the ionic and molecular transport. The microholes in the polyimide sheet cause the field near the access region to each microhole to be nonuniform. The current flowing through a microhole causes a potential drop not only within the microhole, but also in the regions of the electrolyte near the entrance of the microhole. This effect near the microhole can be regarded as resulting from a “spreading resistance”, \( R_s \), which
exists in the bathing electrolyte. Because the polyimide sheet contacts the high resistance stratum corneum on one side, the spreading of field lines at the exit is negligible. Using the expression for a single conducting disk in an insulating surface [18], the total spreading resistance of a single microhole in the array is

\[ R_s = \frac{1}{4 \pi r^2} \sigma_e, \]  

where \( r \) is the radius of each microhole in the array, \( \sigma_e \) is the conductivity of the electrolyte (1.39 S m\(^{-1}\) for phosphate buffered saline used in our experiments). The internal resistance of a microhole is

\[ R_h = \frac{h}{\pi r^2 \sigma_e}, \]  

where \( h \) is the thickness of the polyimide sheet (50 \( \mu \)m). The resistance between inner electrodes with an intervening array of microholes is thus given by

\[ R_{array} = \frac{1}{N_{holes}} \left( \frac{1}{4 \pi r^2 \sigma_e} + \frac{h}{\pi r^2 \sigma_e} \right), \]  

where \( N_{holes} \) is the number of microholes in the array. The resistance as a function of microhole size is shown in Fig. 2.

The insulating sheets were characterized by measuring the current through the polyimide immersed in PBS in response to a series of 250 V, 4 ms pulses. The resistance of the microhole array computed from these measurements is shown in Fig. 2. The measured values are also compared with the theoretical value of resistance. The measured values of resistances for different microhole sizes are within an order of magnitude of the theoretical estimate (Fig. 2). The electrochemical reactions at the measuring electrodes during the 250 V pulses were not taken into account in the theoretical calculations.

Changes in the electrical properties of skin in constrained electroporation are similar to those in unconstrained electroporation. The voltage across skin, \( U_{skin} \), and current through the skin, \( I_{skin} \), show

![Fig. 3. Heat stripped skin: Peak transdermal voltage and peak skin current for different microhole sizes during HV pulsing. The skin was mounted with an adjoining sheet of polyimide with a 11 \times 11\) array of microholes. A series of 250 V, 4 ms pulses, 5 s apart, were applied for 15 min.](Image)
similar behavior in heat stripped skin (Fig. 3) and in full thickness skin (Fig. 4) with and without an insulating mask. The initial magnitude and level of decline $U_{\text{skin}}$ of both heat stripped skin and full thickness skin are similar. This supports the premise that the stratum corneum is the most resistive part of the skin. As with electroporation of unconstrained skin, $U_{\text{skin}}$ drops rapidly over the first few pulses followed by a very gradual decrease over the subsequent pulses. Correspondingly, the current through the skin increases during the first few pulses and tends towards a plateau thereafter. In the case where the skin is constrained by an array of larger microholes (100 $\mu$m diameter), the current is higher than with microholes of smaller size.

According to theoretical predictions, the current through the skin decreases and the transdermal voltage increases with decrease in the size of the microhole. This behavior is seen in the case of full thickness skin (Fig. 4). In the case of heat stripped skin, though skin current (Fig. 3, right) is in agreement, the transdermal voltage (Fig. 3, left) does not follow a clear relationship with the size of the microhole. In the case of full thickness skin, the skin adheres to the insulating sheet better because of the backing of the dermis. The heat stripped skin, however, leaves a conductive layer between the skin and the insulating sheet which alters the measured voltage. Since transdermal voltage is inferred from the measurement of voltage between inner electrodes, it is susceptible to variations caused by imperfect contact between the skin and the polyimide sheet. Also, at higher currents, as in the case of unconstrained case, the voltage drop across the electrode increases due to the production of electrochemical byproducts.

Changes in impedance, measured at different frequencies, are shown in Fig. 5 for constrained electroporation of heat-stripped skin with an array of 65 $\mu$m microholes. The pre-pulse constrained impedance is only three times greater than the impedance in the unconstrained case. This indicates that the masking of the skin by the polyimide sheet is not perfect. As with unconstrained electroporation, skin
impedance decreases rapidly over the first few pulses, and approaches a plateau thereafter. The rapid decrease in skin impedance results from the formation of aqueous pathways in the skin. The decrease in skin impedance is accompanied by an increase in the molecular flux across the skin (Fig. 6), which increases sharply over the first few pulses. The impedance shows a slight increase immediately after the cessation of pulsing, but over the next hour the recovery is incomplete.

Transport of fluorescent molecules through skin constrained by an insulating sheet with an array of microholes is shown in Fig. 6. Both calcein (charge $-4$, Fig. 6, left) and sulforhodamine (charge $-1$, Fig. 6, right) show levels of transport in the same order of magnitude as in the unconstrained case. Only in the case of very small microholes (~40 μm), the transport of both molecules is smaller than for larger microholes. Following an onset time lasting over the first two or three pulses, the calcein flux reached a quasi-stationary state over the next ten to twenty pulses. In contrast, the sulforhodamine flux had a longer onset time and did not reach a quasi-steady state even at the end of 15 min of pulsing. The post-pulse decline of molecular fluxes is also different. The calcein flux decreased rapidly following the cessation of pulsing, while the sulforhodamine flux decreased more slowly. The difference in transport kinetics of the two molecules is, in part, due to their differences in charge and binding affinity. This behavior of molecular fluxes is similar to that observed in the unconstrained case [9,15].

Transport of fluorescent molecules occurs through localized regions in both unconstrained and constrained skin electroporation. The number of LTRs in the unconstrained and constrained cases were counted from the fluorescent micrographs of the skin following pulsing. The cumulative flux per LTR over the duration of pulsing is shown in Table 1. Calcein and sulforhodamine fluxes are of the same order of
Fig. 6. Flux of calcein and sulforhodamine across heat-stripped skin for different sized microholes in polyimide sheet. The fluorescence values are normalized to the area of skin exposed to the voltage pulses. In the unconstrained case, the skin exposure area was 0.64 cm². The square area encompassing the 11 x 11 array with 300 μm center-to-center distance, 0.1 cm², was used as the exposure area in the constrained case.

magnitude in both unconstrained and constrained electroporation. The calcein flux was approximately twice that of the sulforhodamine flux in most cases, except with an array of 40 μm where the calcein and sulforhodamine fluxes were nearly equal.

The flux in the unconstrained case is lower than when constrained by certain size masks (Fig. 6). The flux shown in this figure is normalized to the total area of exposure. It should be noted that in the unconstrained case, most of the flux is confined to the LTRs. If the data shown is normalized to the LTRs, the difference in flux magnitude between different cases is much smaller (Table 1). When the skin transport is limited by the array mask, the LDRs are confined to the size of the masks. This might affect the distribution of aqueous pathways in the LDRs and their transport capacity. This may explain why the transport in the unconstrained case, occurring over a large area, is lower than in the constrained case, when normalized to the exposure area or the area of LTRs.

Transport of calcein, sulforhodamine, and small ions by unconstrained electroporation was shown to

Table 1
Cumulative flux (in Mol) of fluorescent molecules per LTR during 15 min of pulsing in unconstrained and constrained skin electroporation

<table>
<thead>
<tr>
<th>Microhole diameter</th>
<th>Cumulative flux</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcein</td>
<td>Sulforhodamine</td>
<td></td>
</tr>
<tr>
<td>Unconstrained</td>
<td>3 x 10⁻¹¹</td>
<td>2 x 10⁻¹¹</td>
<td></td>
</tr>
<tr>
<td>100 μm</td>
<td>5 x 10⁻¹¹</td>
<td>2 x 10⁻¹¹</td>
<td></td>
</tr>
<tr>
<td>65 μm</td>
<td>4 x 10⁻¹¹</td>
<td>3 x 10⁻¹¹</td>
<td></td>
</tr>
<tr>
<td>55 μm</td>
<td>4 x 10⁻¹¹</td>
<td>2 x 10⁻¹¹</td>
<td></td>
</tr>
<tr>
<td>40 μm</td>
<td>2 x 10⁻¹¹</td>
<td>1 x 10⁻¹¹</td>
<td></td>
</tr>
</tbody>
</table>

The number of LTRs in the unconstrained electroporation, determined from fluorescent micrographs, was 100. In the case of constrained electroporation, the number of microholes contributing to fluorescent transport, 100 to 110 in our experiments, was used as the number of LTRs.
occur through highly localized regions [8,17]. The transport of fluorescent molecules in constrained electroporation is also seen to occur through localized regions in the microholes of the masking array. Localized transport regions (LTRs) of fluorescent molecules in constrained electroporation are compared with unconstrained electroporation in Fig. 7. The constrained electroporation shows skin staining over most, but not all, microholes in the array. The striking observation was that no LTRs were observed away from the microholes. This suggests that the LTRs can be forced to occur only at selected sites in the skin. The central region of the microhole is stained red with a surrounding green fluorescence region, similar to an unconstrained LTR [8,17]. The number of LTRs is, within an order of magnitude, the same in both constrained and unconstrained electroporation.

The pre-pulse skin impedance of human skin (0.64 cm² exposure area) measured at 100 Hz without an adjoining insulating sheet is approximately 30 kΩ. The corresponding impedance of the skin masked by an insulating sheet with a 11 × 11 array is about 100 kΩ (Fig. 5). If the stratum corneum is modeled with a parallel network of a resistor and a capacitor, the skin impedance measured at 100 Hz is close to the DC resistance of the skin. The exposure area (the sum of microhole areas) in the constrained case is approximately 0.02 cm². A simple scaling of impedance by total open area with perfect sealing to the mask would give a skin impedance of around 1 MΩ for the conditions used in Fig. 5. Thus, an order of magnitude discrepancy in measured skin impedance indicates the presence of a PBS-filled gap between the skin and the insulating sheet. In spite of the presence of a layer of conducting solution, the transport of fluorescence material is constrained to the regions of skin aligned with the microholes in the polyimide sheet.

The apparent discrepancy between electrical be-

![Fig. 7. Unconstrained and constrained local transport regions. Top left: unconstrained LTRs in heat-stripped human skin using a standard side-by-side chamber (Fig. 1) with 1 mM calcein and 1 mM sulforhodamine in PBS in the donor compartment. Each LTR has a dark central region (low red fluorescence of sulforhodamine) surrounded by a bright ring (green fluorescence of calcein). Top right: constrained LTRs in heat-stripped skin for same donor solution, created with a 11 × 11 array of 65 μm diameter microholes in a polyimide sheet held next to the skin. Bottom panels: enlarged images of some of the LTRs (scale bar = 100 μm).](image-url)
havior and transport regions may be explained by considering the relative importance of the conducting layer. If the gap between the skin and the polyimide sheet is significantly large, the field lines would diverge on leaving the array to create an equipotential surface on the SC causing unconstrained electroporation with LTRs spread throughout the 0.64 cm² exposed skin area. The pre-pulse impedance in this case would have been greater than 30 kΩ by only 30 Ω (the resistance of a 11 × 11 array of 65 μm microholes filled with saline). The measured value of 100 kΩ suggests, therefore, that the polyimide sheet causes considerable, although imperfect, localization of the field lines at the microhole.

The striking result from this study is that the transport of charged fluorescent molecules is constrained to the areas of skin directly below the microholes in the polyimide sheet, in spite of an imperfect localization of the field. For the magnitude and duration of the pulses used, not all microholes create LTRs, but no LTRs form away from microholes. In addition, the magnitude of average flux in the constrained case is comparable to the case of unconstrained electroporation. Cumulative fluxes per LTR of both calcine and sulforhodamine were of the same order of magnitude in the constrained case as in the unconstrained case. This implies that useful levels of drug transport may be achieved with intervention that involves only a small number of microholes created at predetermined sites.

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References

