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Electroporation-enhanced transdermal drug delivery: Effects of logP, pK_a, solubility and penetration time

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ABSTRACT

Electroporation is an important physical technique to improve drug transdermal delivery, although its mechanism remains unclear. Here, some types of polar drugs, including aspirin, diclofenac sodium, metformin hydrochloride, ibuprofen and zidovudine, were used as the model drugs for the exploration of electroporation mechanisms. Electroporation had great influences on the structure of stratum corneum to improve the cumulative permeability due to the formation of pores maintaining for at least 2 h, depending on the power and time, and then the permeation gradually recovered to the normal value after 12 h. A mathematical model was firstly established to exhibit the relationship between the electroporation-improving cumulative permeation and the physiochemical properties of the model drugs, involving oil-water partition coefficient (logP), dissociation constant (pKa) and solubility (S). Increased cumulative permeation depended on increased S, decreased logP and pKa. Electroporation is an effective physical technique to improve transdermal drug delivery depending on itself and the properties of drugs.

1. Introduction

Transdermal drug delivery is a commonly applied delivery strategy to allow drugs to be transported across the skin into the body (Palmer and DeLouise, 2016; Park et al., 2019; Wiraja et al., 2019). [4] It is a prospective drug delivery strategy that can overcome the limitations of conventional drug delivery systems, including oral and injectable administration (Lee et al., 2018; Li, 2013; Münch et al., 2017). The advantages of this approach lie in the ability to prevent first-pass metabolism effectively, enhance patient compliance, maintain a stable blood concentration, reduce the administration frequency, and minimize side effects (Ita, 2015; Parhi and Swain, 2018; Prausnitz and Langer, 2008).

However, the transdermal delivery dose of drugs is very limited due to the large barrier function of the skin, mainly contributed by the stratum corneum (SC). As the largest organ of the human body, the skin accounts for about 15% of body mass (Dąbrowska et al., 2018). The skin can not only resist the invasion of pathogenic microorganisms, but also protect the body from harmful substances, as well as absorption, feeling, secretion, excretion, regulation of body temperature and metabolism (Lopez et al., 2011). SC of the outer skin acts as a barrier to limit the penetration of substances through the skin due to its dense structure like brick walls (Berkó et al., 2016; Zorec et al., 2015). As a result, lipophilic drugs with molecular weight < 1 kDa are generally required for transdermal absorption (Escobar-Chávez et al., 2009; Schoellhammer et al., 2014). Moreover, most drugs across the skin are very slow, making it difficult to reach the level of effective concentration quickly (Denet et al., 2004; MB et al., 2008). Therefore, how to promote transdermal absorption amount and rate across SC has become the focus of a transdermal drug delivery system.

Chemical penetrators have been developed to improve drug dermal penetration, such as azone (Lane, 2013), cyclodextrins. However, besides limited transdermal penetration enhancement efficiency, some unexpected side effects may simultaneously appear, such as rash, irritation and hypersensitivity. Novel formulations were also used to improve the transdermal absorption, including nanoparticles (Wiraja et al., 2019), liposomes (Franzè et al., 2019; Madhavia et al., 2019), transfersomes (Shamshiri et al., 2019).

Physical penetration technologies can change the skin surface structure directly and reversibly so as to enhance drug transdermal

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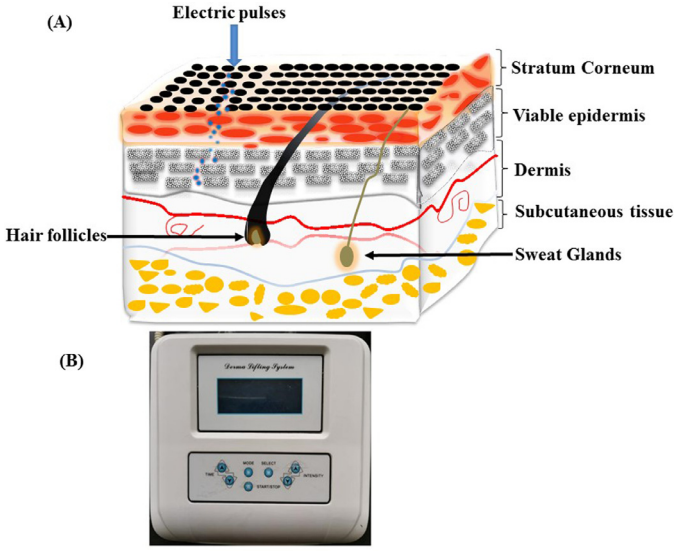

Fig. 1. Scheme of electroporation on skin (A) and the electroporation equipment (B).

absorption (Mitragotri, 2013; Puri et al., 2017; Ulashchik, 2018). Moreover, such technologies are safe, efficient, highly bioavailable and widely used, which is why they are a better choice to promote the transdermal absorption of drugs (Benson and Namjoshi, 2008). These technologies include microneedles (Ita, 2017; Wang et al., 2017), sonophoresis (Nguyen and Banga, 2018; Park et al., 2019), iontophoresis (Liu et al., 2013; Tokumoto et al., 2016), and electroporation (Prausnitz and Langer, 2008; Rizwan et al., 2009).

Electroporation is a physical enhancement technique that uses transient high-pressure pulses for a short time to create a temporary pathway through the skin in order to promote transdermal absorption of drugs (Blagus et al., 2013; Pavselj et al., 2015) (Fig. 1. A). The advantages of electroporation are many: (1) The electroporation parameters can be adjusted to control the transdermal permeation rate and extent. (2) The pores generated after the high-pressure pulse is reversible and the damage is relatively small. (3) Most drugs that can permeate the skin could be improved, such as macromolecules, fat-soluble or water-soluble drugs, or charged molecules (Feng et al., 2017). So far, electroporation has received extensive attention as an effective transdermal technique.

The effect of electroporation on the transdermal absorption was diverse for different drugs with various physicochemical properties. The physical and chemical properties of drugs specifically include the oil-water partition coefficient (logP), dissociation constant (pKa), solubility (S), molecular weight and melting point. The delivered macromolecules amount were much higher by electroporation at mild hyperthermia temperature (Gallo et al., 2002; Murthy et al., 2004b) and the more rapid recovery of the electroporation samples at 4 °C due to a lower supra-threshold voltage. The surfactant effect (Murthy et al., 2004a) meant that sodium dodecylsulfate (SDS) enhanced the transdermal delivery of molecules by electroporation, which was most likely by facilitating the barrier disruption during pulse application and also by prolonging the lifetime of electropores created by the pulse. pH-sensitive postpulse resistance recovery and molecular transport are mainly due to the charge states of epidermal lipids (Murthy et al., 2003). However, there is a lack of systematic research about the influence of electroporation on transdermal absorption of drugs. Questions that need to be answered include how long the transient diffusion pathway on the skin induced by electroporation can last and how deep into the skin drugs can reach with electroporation.

Therefore, this study comprehensively evaluated the effects and mechanism of electroporation on the absorption of drugs across the skin. A mathematical model was established about the relationship between the cumulative permeation amount (Q_n) and the characteristics of drugs, including logP, pKa, S, cumulative penetration time (t). Moreover, it clarified the duration and the transdermal pathway depth of electroporation in the process of promoting drug absorption. This study is intended to provide data for subsequent transdermal or transmucosal research, offer guidance for clinical trials, and make electroporation more accessible.

2. Materials and methods

2.1. Materials

The main drugs and materials used in the study were as follows: Ibuprofen (Wuhan Dahua Weiye Pharmaceutical Chemical Co., Ltd., Wuhan, China); Aspirin (Hubei Xingyin Chemical Co., Ltd. Wuhan, China); Diclofenac sodium (Beijing InnoChem Technology Co., Ltd., Beijing, China); Zidovudine (Shanghai Kangtuo Chemical Co., Ltd., Shanghai, China); Metformin hydrochloride (Shanghai Ruiyong Biotechnology Co., Ltd., Shanghai, China); Doxorubicin hydrochloride (Beijing Inoke Technology Co., Ltd., Beijing, China); Cy-7 (Beijing Fanbo Biochemical Co., Ltd., Beijing, China, maximum excitation wavelength: 745 nm; maximum emission wavelength: 774 nm, MW = 720.94); 4',6-diamidino-2-phenylindole (DAPI) (excitation wavelength: 330-380 nm, emission wavelength: 420 nm); Anti-fluorescence quenching sealer (Servicebio Biochemical Co., Ltd., Wuhan, China); Acetic acid (National Pharmaceutical Group Chemical Reagent Co., Ltd. Beijing, China); Tetrahydrofuran (chromatographic grade, Honeywell, New Jersey, USA); Sodium heptane sulfonate and phosphoric acid (National Pharmaceutical Group Chemical Reagent Co., Ltd. Beijing, China); Acetonitrile and methanol (Thermo Fisher Scientific, Massachusetts, USA).

2.2. Animals

Kunming mice (male, body weight of 24-28 g, SPF grade) were purchased from Beijing Vital River Experimental Animal Technology Co., Ltd (the animal quality certificate number of SCXK (Beijing) 2016-0011). Principles on good laboratory animal care were followed and the animal experiments were conducted in compliance with the Guidelines for the Care and Use of Laboratory Animals in Beijing Institute of Radiation Medicine.

2.3. Transdermal permeation

The mice were sacrificed by dislocation of cervical vertebrae, shaved of abdomen hair, stripped of the abdominal skin, and washed with physiological saline repeatedly. Then, the isolated skin was placed in a plastic wrap and stored in 4 °C until use.

The Franz diffusion cell with an upper chamber of the supplying chamber and a lower chamber of the receiving chamber was used for the transdermal study. The two electrodes of the electroporation equipment (M-222, Ruili Beauty Equipment, Beijing, China) (Fig. 1. B) were applied to the isolated skin before the affected skin was fixed between the two chambers immediately. The SC side of the skin faced the supplying chamber with the dermis side facing the receiving chamber.

Drug solution of 1 mL (10 mg/mL) was in the supply chamber, and PBS (pH 7.4) of 8.6 mL served as a receiving solution. The whole chambers were fixed in the Intelligent Transdermal Tester (TK-24BL, Shanghai Yukai Technology Trade Co., Ltd., Shanghai, China), with the receiving solution stirred at a constant rate of 300 r/min under a constant temperature of 32 °C. After the sample was equilibrated for 15 min, the time was counted. Samples of 0.9 mL were taken from the receiving chamber at 0.5, 1, 2, 4, 8, and 12 h, respectively, and an equal volume of PBS solution (32 °C) was added to the receiving chamber.

The samples at each time point were filtrated through a filter of 0.45 μm, and 20 μL subsequent filtrated solution was injected into the High Performance Liquid Chromatograph (HPLC, Model 1260, Agilent, California, USA) to determine the drug content.

2.4. Effect of electroporation power and time on transdermal absorption of ibuprofen

Ibuprofen is a typical non-steroidal anti-inflammatory drug with antipyretic, analgesic and anti-inflammatory effects (Tombs et al., 2018). Ibuprofen gel or ointment has been available on the market as a topical skin preparation to relieve tissue pains. In this experiment, the effects of the power and duration of electroporation on the transdermal absorption of ibuprofen were investigated. A mathematical model between the cumulative permeation amount (Q_n) and electroporation parameters (power and time) was established.

Transdermal permeation procedures were specified in *Section 2.3*. First, Q_n of the control group without electroporation and the groups with electroporation of different power (0.028 mW, 1.45 mW, 5.51 mW for 10 min) was evaluated. Then, the influence of electroporation with 0.028 mW and 1.45 mW respectively for 5, 10, 20 min on transdermal permeation of ibuprofen was evaluated.

2.5. Relationship between electroporation and transdermal absorption of different drugs

The standards of drug selection included topical use and different physiochemical properties. Aspirin, known as acetylsalicylic acid, is a nonsteroidal anti-inflammatory drug (NSAIDs) that relieves pain, inhibits blood clots and treats febrile conditions such as the flu (Zhu et al., 2015). Zidovudine is an antiviral drug used to treat HIV and AIDS infections (Sharma et al., 2017). Diclofenac sodium is a fast acting NSAIDs with analgesic, anti-inflammatory and antipyretic effects by inhibiting the synthesis of prostaglandin (Rainsford et al., 2008). Metformin hydrochloride is commonly used in diabetes, hypoglycemic and obesity (Cuyàs et al., 2018) (Fig. 2).

These drugs involved different properties, including molecular weight, pKa, logP, solubility in water and melting point, which will determine the transdermal permeation efficiency (Table 3).

Transdermal permeation of drugs with or without electroporation were evaluated as in *Section 2.3* with the electroporation condition of 1.45 mW for 10 min. The cumulative penetration amount was calculated as the followings.

$$Q_n = \frac{[C_n \cdot V + V_0 \sum_{i=1}^{n-1} C_i]}{A}$$

Where Q_n is the cumulative permeation amount (μg/cm^2) at different time points; V_0 is the sampling volume (mL); C_n is the concentration measured at the n-th sampling point (μg/mL); V is the volume of the receiving liquid (mL); C_i is the concentration measured at the i-th sampling point (μg/mL); and A is the effective transdermal area (cm^2).

A Q_n-t release curve was obtained, and the slope of the line represented the steady state of the transdermal absorption rate J_{ss} (μg•cm^{-2}•s^{-1}). The larger the J_{ss} value was, the more easily the drugs penetrated the skin. The ratio of the J_{ss} value of the electroporation group to that of the control group without electroporation was the multiple ratio (also called the infiltration ratio) of the electroporation group.

2.6. The transdermal depth of electroporation on skin

2.6.1. Transdermal depth of doxorubicin hydrochloride (DOX) under electroporation

Mice were fixed and an electroporation probe (1.45 mW) was applied to the abdomen of the mice for 10 min. 20 μl of DOX (10 mg/ml) was applied onto the topical site using a quantitative pipette immediately. After 30 min, the excess DOX on the skin surface was wiped off, the mice were anesthetized and the ventral back skin was taken off from the affected area and stored at -80 °C.

DOX was added on the same part of another mouse and an electroporation probe (1.45 mW) was immediately applied on the abdomen skin for 10 min. The excess DOX on the skin surface was wiped off at 30 min after electroporation, and the skin from the affected area was removed for observation.

Similarly, the blank control without electroporation was prepared as the followings. DOX was put on the abdomen skin and the excess DOX on the skin surface was wiped off after 30 min.

The frozen sections were prepared with the freezing microtome (Cryotome E, Thermo Fisher Scientific, Massachusetts, USA). They were stained with an immunofluorescence - 4, 6-diamino-2-phenyl indole (DAPI) according to the standard protocol. The excitation wavelength of DOX was 495 nm and it demonstrated red under fluorescence. DAPI could bind with DNA inside nuclei and looked blue. Therefore, the transdermal depth could be evaluated with the fluorescence of DOX and DAPI. Finally, the frozen sections were observed under a positive fluorescence microscope (NIKON ECLIPSE C1, Tokyo, Japan).

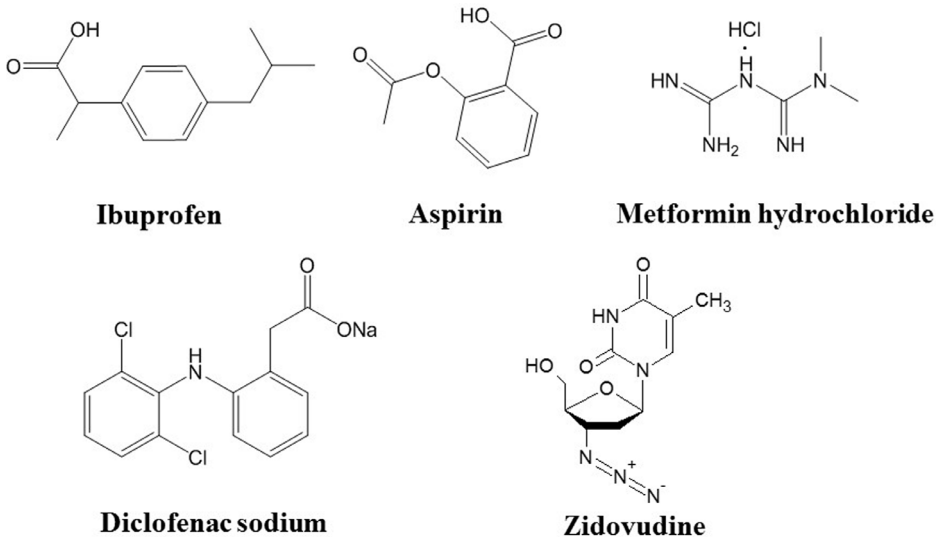

Fig. 2. Chemical structure of ibuprofen, aspirin, metformin hydrochloride, diclofenac sodium, zidovudine.

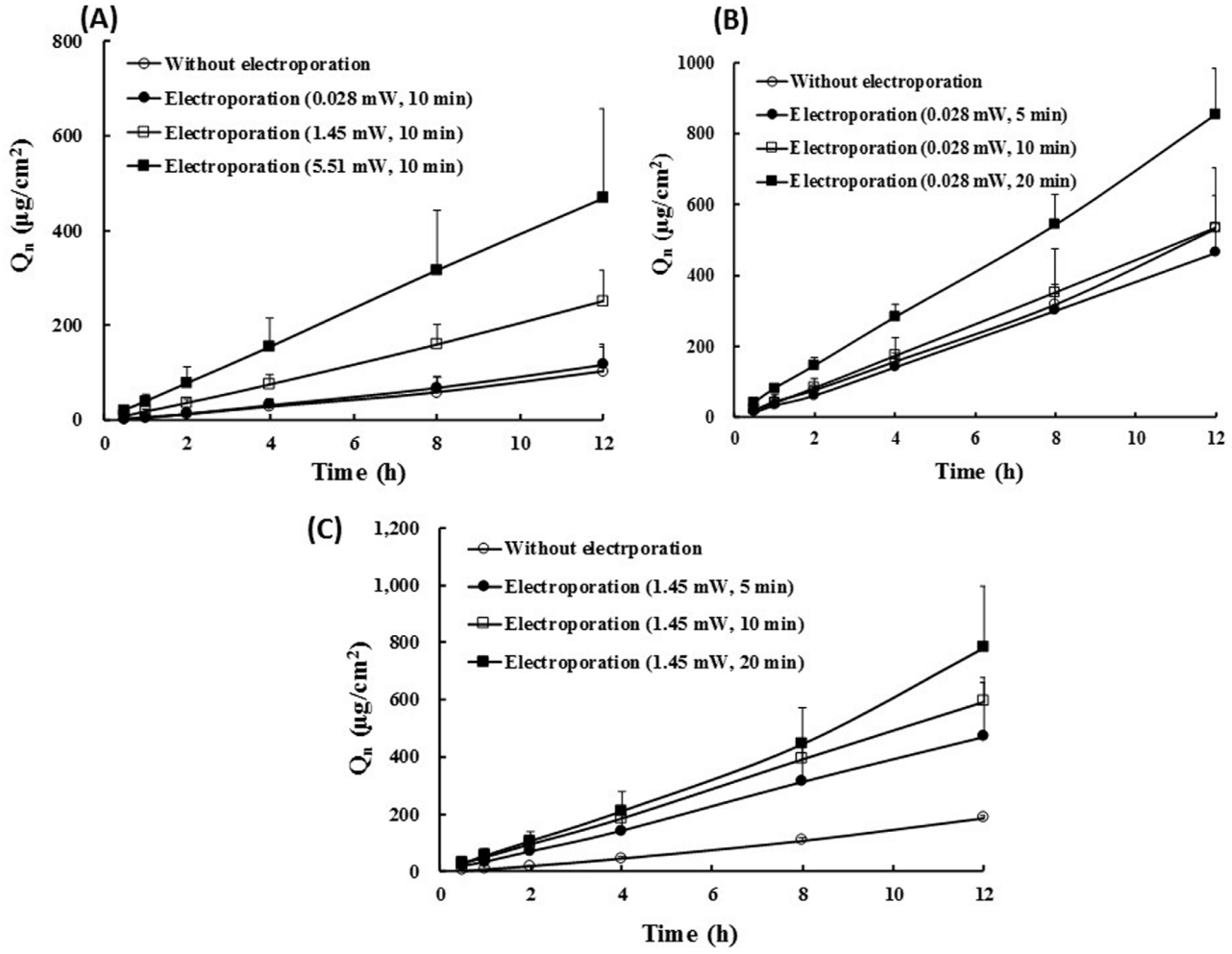

Fig. 3. Effects of electroporation with different parameters on transdermal absorption of ibuprofen. (A) Different power and (B) different time for low power and (C) for high power.

2.6.2. Influence of electroporation on stratum corneum

Mice were anesthetized and fixed before an electroporation probe (1.45 mW) was applied on their abdomen for 10 min. The affected part of the skin with the width of 1 mm was removed at 0, 2 h and fixed with 3% glutaraldehyde immediately. The same part of the skin of the other mouse without electroporation served as the blank control. Ultrathin sections were prepared and the structural changes in the stratum corneum were observed with a transmission electron microscope (TEM) (CM12080-kV, Philips, Amsterdam, Holland).

2.7. Determination of the duration time of electroporation on skin

2.7.1. The duration of electroporation with transdermal depth of DOX

Mice were anesthetized, fixed and unhaired with the ventral dorsal hair. Then an electroporation probe (1.45 mW) was applied to the abdomen of the mice for 10 min. DOX (20 μl, 10 mg/ml) was put on the affected skin at 0, 10, 30, 60, 300 and 720 min after electroporation. Then, the excess DOX on the skin surface was wiped off after 30 min. The affected skin was removed, and the frozen sections were prepared and observed with a fluorescence microscope (BDS200-FL, Chongqing Optec Instrument Co., Ltd., China). The transdermal depth of DOX was evaluated with the fluorescence intensity. The skin of the control group was not treatcontroled with electroporation.

2.7.2. In vivo imaging of mice to evaluate the duration time of electroporation

The mice were anesthetized with intraperitoneal injection of chloral hydrate solution for shaving. Cy7 solution (2 mg/ml, 20 μl) was added to the abdomen of the normal mice, followed by application of an electroporation probe of 1.45 mW for 10 min. Then, the mice were fixed and placed in the dark box of the living animal imaging system (IVIS Luminia II, Caliper, USA) to be observed at 0, 10, 30, 60 and 240 min after electroporation.

2.8. Safety of electroporation by pathological observation of skin structure

Mice were anesthetized, depilated and fixed. An electroporation probe of 1.45 mW was applied on their abdominal skin for 10 min. Then, the mice were sacrificed and the electroporated skin was taken out and fixed with formalin at 30 min after electroporation. The normal skin without electroporation was used as the blank control. Pathological slides of the skin stained with hematoxylin-eosin (H. E.) were observed under a microscope (BDS200-FL, Chongqing Optec Instrument Co., Ltd., China).

3. Results and discussion

3.1. Effects of electroporation parameters on transdermal absorption of ibuprofen

As for the action mechanism of electroporation, different biomembranes are slightly different. A pulse voltage of about 0.5 - 1V that is applied on a simple lipid bilayer membrane can produce defects that will form a local transport region (LTR) after self-assembly due to its

Table 1
Promoting effect of electroporation with different parameters on transdermal absorption of ibuprofen.

Electroporation parameters	Fitting equation	J_{SS} (μg cm^{-2} s^{-1})	Multiples ratio
Blank control (untreated)	Q_n = 8.484t-4.320 (r = 0.9957)	8.484	-
0.028 mW, 10 min	Q_n = 9.924t-6.195 (r=0.9976)	9.924	1.17
1.45 mW, 10 min	Q_n = 20.91t-4.522 (r = 0.9994)	20.91	2.46
5.51 mW, 10 min	Q_n = 39.14t-0.148 (r=0.9999)	39.14	4.61
Low-power	Q_n = 43.84t-9.624 (r = 0.9974)	43.84	-
0.028 mW, 5 min	Q_n = 39.38t-10.41 (r = 0.9996)	39.38	0.90
0.028 mW, 10 min	Q_n = 44.68t-3.195 (r = 0.9990)	44.68	1.02
0.028 mW, 20 min	Q_n = 69.68t+5.842 (r = 0.9993)	69.68	1.59
High-power	Q_n = 15.87t-12.12 (r = 0.9960)	15.87	-
1.45 mW, 5 min	Q_n = 39.93t-9.812 (r = 0.9995)	39.93	2.52
1.45 mW, 10 min	Q_n = 49.47t-4.722 (r = 0.9997)	49.47	3.12
1.45 mW, 20 min	Q_n = 63.99t-22.80 (r = 0.9949)	63.99	4.03

Note: The control groups are without electroporation.

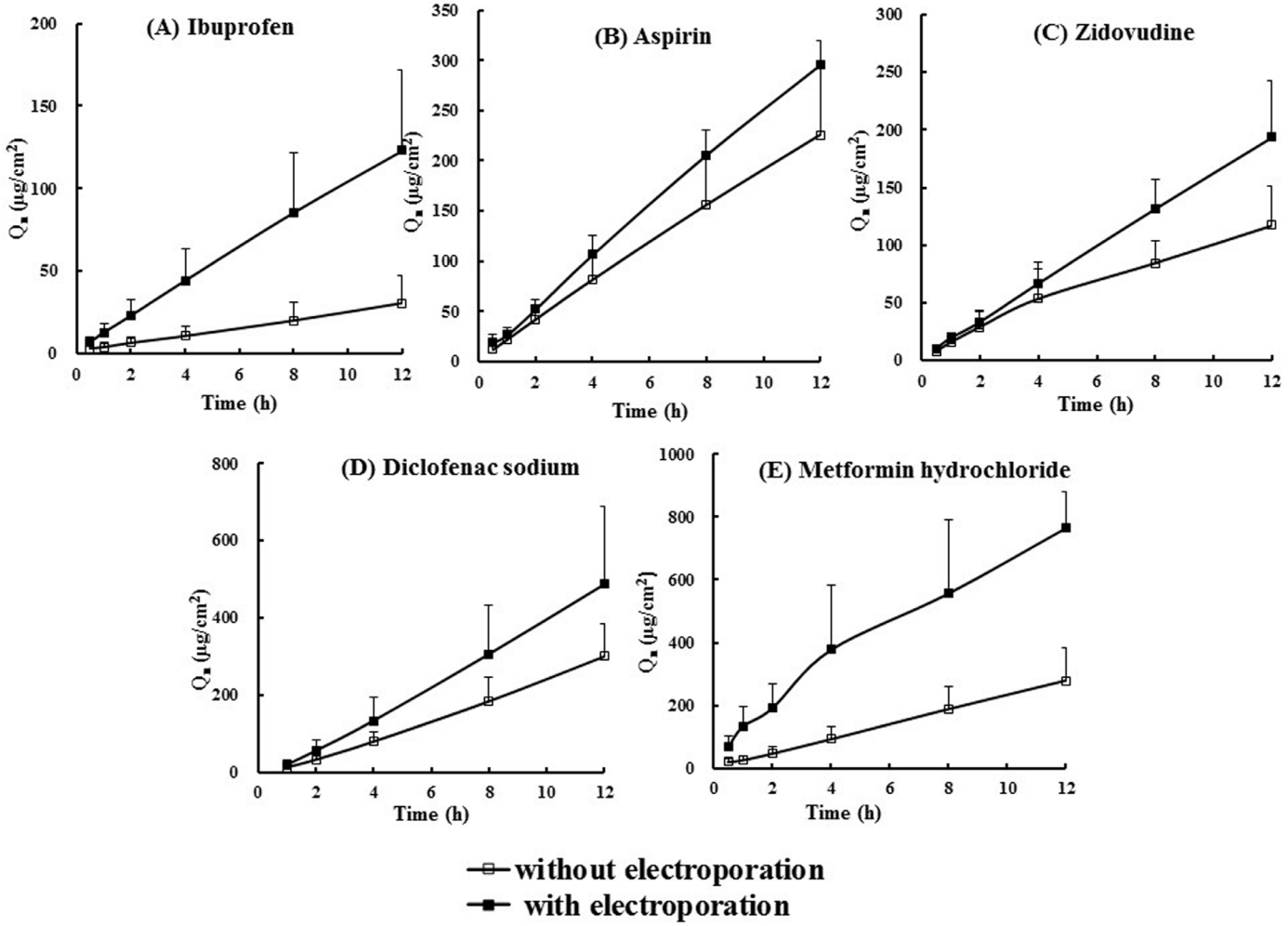

Fig. 4. Effects of electroporation on drugs penetration, (A) ibuprofen, (B) aspirin, (C) zidovudine, (D) diclofenac sodium and (E) metformin hydrochloride.

own fluidity, thermal motion and electrostatic free energy. LTR could form hydrophilic pores in the skin, thereby reducing the cell membrane barrier and promoting rapid drug permeation through the membrane (Dujardin et al., 2002). However, the stratum corneum is composed of approximately 100 layers of continuous cells. Only when a short pulse of high voltage (50-100 V) and a long pulse of low voltage (about 20 V) are applied on the skin surface can the stratum corneum form a transient pore (Riviere et al., 1995; Tokumoto et al., 2016). When the pulse voltage is terminated and the topical skin cools down, the stratum corneum does not rearrange itself immediately into the previous multilayer system, and therefore some hydrated areas are form (F et al., 2002). These are the channels that promote drug delivery after the pulse is terminated. It is generally believed that the improved transdermal absorption mechanism of electroporation is the high-voltage pulsed electric field that could disturb the ordered structure of the stratum corneum, resulting in reversible permeability pores (Denet et al., 2004).

The power and the action time of electroporation are two important factors to improve transdermal drug permeation. The cumulative permeability of ibuprofen increased with the electroporation power

Table 2
Transdermal penetration efficiency of drugs with or without electroporation.

Drugs	logP	pKa	Solubility (water, mg/mL)	Molecular		
weight	Melting Point (°C)	Drug cumulative amount ($\mu g \cdot cm^{-2}$) vs. t (min)	J_{ss} ($\mu g \cdot cm^{-2} \cdot s^{-1}$)	Enhanced ratio		
Aspirin	1.74	3.58	1.87	180.16	135	-: Q_n = 18.61t+4.408 (r = 0.9996)
+:Q_n = 24.45t+5.612 (r = 0.9995)	18.61					
24.45	1.31					
Diclofenac sodium	4.086	-0.74	0.001	318.136	288-290	-: Q_n = 26.32t-20.73 (r = 0.9988)
+:Q_n = 42.79t-31.22 (r = 0.9995)	26.32					
42.79	1.63					
Zidovudine	0.003	9.11	10.597	267.24	106-112	-: Q_n = 9.370t+8.267 (r = 0.9940)
+:Q_n = 16.01t+2.336 (r = 0.9999)	9.370					
16.01	1.71					
Metformin hydrochloride	-0.819	10.59	100.049	129.166	220-225	-: Q_n = 22.68t+4.081 (r = 0.9996)
+:Q_n = 59.22t+77.39 (r = 0.9916)	22.68					
59.22	2.61					
Ibuprofen	3.646	4.54	0.1	206.28	75-77	-: Q_n = 2.380t+1.742 (r = 0.9993)
+:Q_n = 10.14t+2.812 (r = 0.9997)	2.380					
10.14	4.26					

Note: -, representing the group without electroporation; +, representing the group with electroporation

Table 3
Coefficients and parameters of the regression equation for different drugs.

Variables	Regression coefficients	Standard error	Degrees of freedom	t	P
Constant term	82.70	40.42	25	2.05	0.0514
logP	-27.80	9.337	25	-2.98	0.0064
pKa	-20.78	4.283	25	-4.85	<.0001
Solubility (S)	1.977	0.268	25	7.39	<.0001
Group (G)	79.05	14.22	25	5.56	<.0001
Cumulative penetration time (t)	23.13	1.715	149	13.49	<.0001

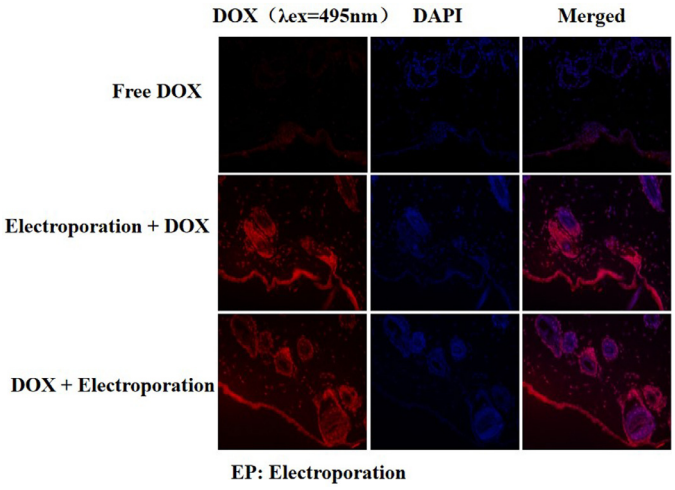

Fig. 5. Effects of electroporation on transdermal absorption.

(Fig. 3A, Table 1). The J_{ss} of electroporation with 0.028, 1.45, 5.51 mW was 1.17 times, 2.46 times and 4.61 times as high as that without electroporation, respectively.

Action time was also important for transdermal delivery. The transdermal delivery of ibuprofen could not be promoted in a short time (Fig. 3B, Table 1). J_{ss} of 5, 10, and 20 min with 0.028 mW was 0.90, 1.02, and 1.59 times as high as that without electroporation, respectively. When the power was too small, sufficient action time could also promote permeation.

In comparison, high power electroporation was more effective for transdermal permeation. J_{ss} with the power of 1.45 mW for 5, 10, 20 min was 2.52, 3.12, and 4.03 times as high as the J_{ss} without electroporation (Fig. 3C, Table 1), indicating that action power is more important than time for transdermal permeation.

3.2. Relationship between drugs with different logP, pKa, solubility and electroporation

Physiochemical characteristics of drugs are significant for transdermal permeation, and its relationships with electroporation parameters are not clear yet, such as logP, molecular weight, pKa, solubility and melting point.

The regression equations of ibuprofen, diclofenac sodium, zidovudine, metformin hydrochloride and ibuprofen with or without electroporation were obtained (Fig. 4). The enhanced ratios of electroporation to non-electroporation were 1.31, 1.63, 1.71, 2.61 and 4.26, respectively (Table 2). Overall, electroporation promoted the absorption of all the five drugs and the varied enhanced ratio was related to the different drugs.

In order to explore the relationship between the characteristics of drugs and electroporation, the regression of the prediction model was obtained with covariance analysis (Table 3). In details, the cumulative

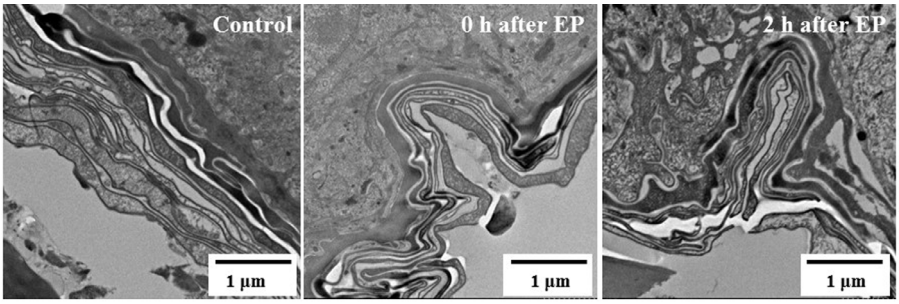

Fig. 6. Morphology of skin stratum corneum structure after electroporation.

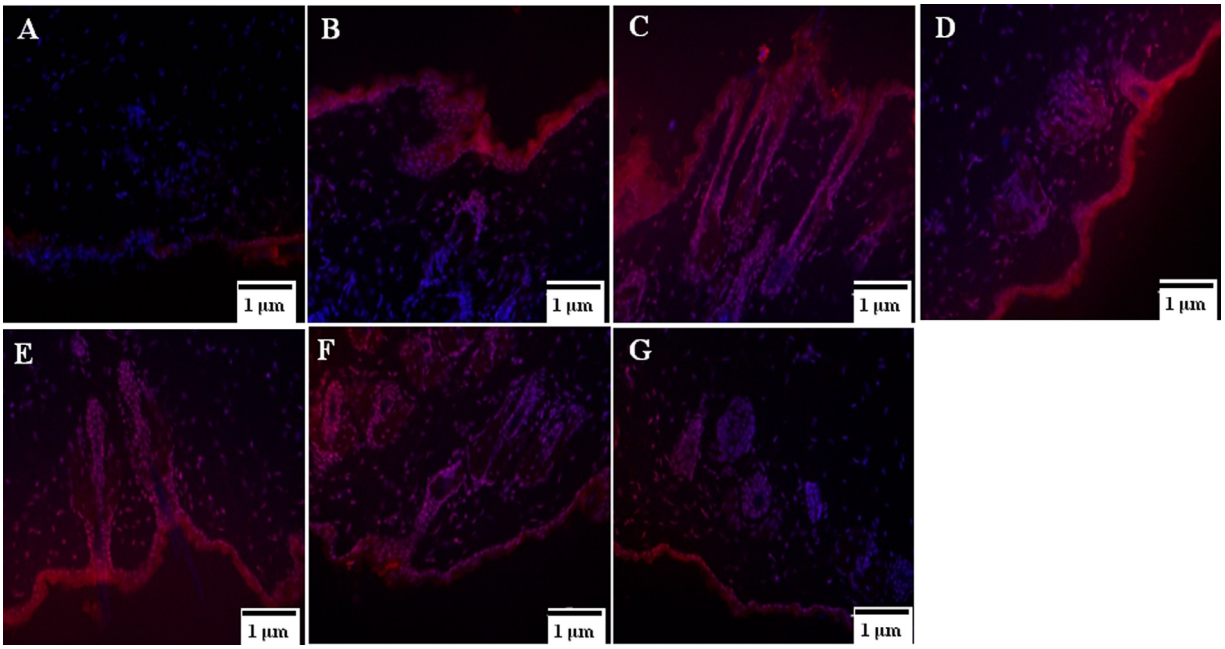

Fig. 7. Transdermal absorption of doxorubicin hydrochloride at different time points after electroporation, control (A), 0 min (B), 10 min (C), 30 min (D), 1 h (E), 5 h (F) and 12 h (G).

permeation amount (Q_n) and oil-water partition coefficient (logP), dissociation constant (pKa), solubility (S), groups (G) and cumulative permeation time (t) were established, Q_n = -27.7974logP - 20.7802pKa + 1.9767S + 79.0504G + 2 3.1271t + 82.6960. G = 0, 1 represented without or with electroporation. Molecular weight and melting point were not included into the simulated model in the end, indicating that the effect of electroporation was independent of molecular weight and melting point possibly due to the transient pore formation. The statistically significant properties on the penetration enhancement with electroporation was logP, pKa, and S. It demonstrated that the effects of electroporation on transdermal penetration might not depend on molecular weight and melting point of drugs. This model could help determine whether a particular drug is appropriate for transdermal absorption with electroporation according to its logP, pKa, and S. The establishment of mathematical models provides data for prediction of transdermal efficiency with or without electroporation, improves experimental efficiency and reduces financial consumption.

3.3. Mechanism of electroporation to improve transdermal permeation

DOX is fluorescent with a maximum excitation wavelength of 495 nm. DAPI is a fluorescent dye that penetrates the cell membrane and binds to DNA in the nucleus with a maximum excitation wavelength of 340 nm. Therefore, the effect of electroporation on transdermal absorption could be evaluated by the transdermal depth represented by fluorescence intensity of DOX and DAPI.

DOX was diffused passively and distributed in the outermost stratum corneum without electroporation. When electroporation was applied, there was very strong red fluorescence on the epidermal layer (Fig. 5), suggesting that DOX penetrated deep into the epidermis. The increased permeation amount with electroporation indicated electroporation could promote transdermal absorption of DOX. The sequence of electroporation and DOX solution on the skin was not much different. This indicated that the permeation effect was mainly dependent on the transient pores formed by electroporation. This effect did not disappear immediately and would last for a while.

The control group was the normal mouse skin that was not treated. The stratum corneum of normal mice without electroporation displayed lamellar with clear structure and neat arrangement (Fig. 6). After electroporation, there were obvious pores in the stratum corneum of the mice, and some of the lamellar structures broke. At 2 h after electroporation, the pores did not disappear yet, indicating that pores in the stratum corneum with electroporation could be maintained for at least 2 h.

The duration of electroporation was investigated using the autofluorescence properties of DOX with red and the nuclear staining of DAPI with blue.

The fluorescence intensity of DOX added to the pore-forming part at

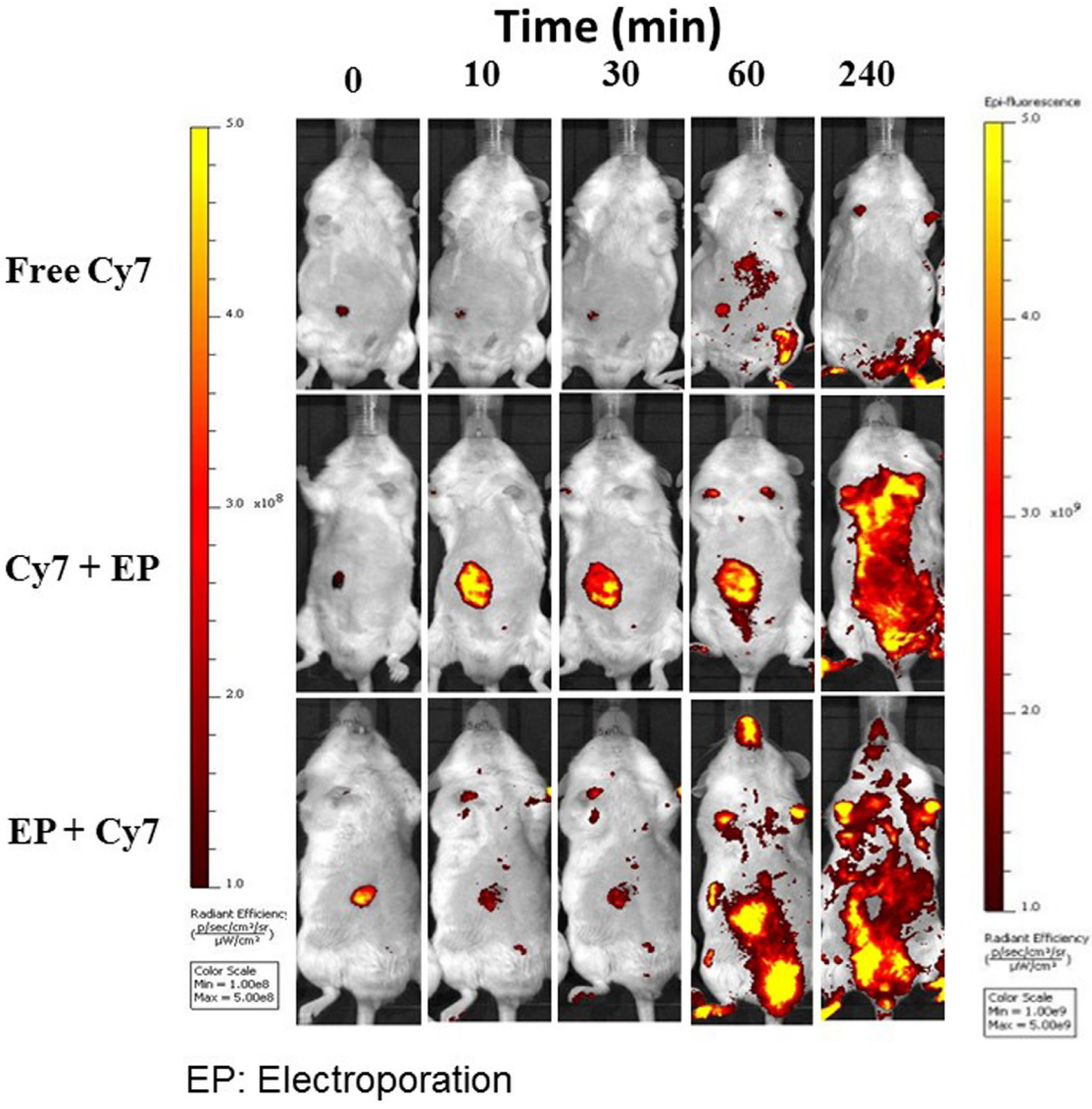

Fig. 8. Near-infrared fluorescence imaging of mice at different time points after electroporation.

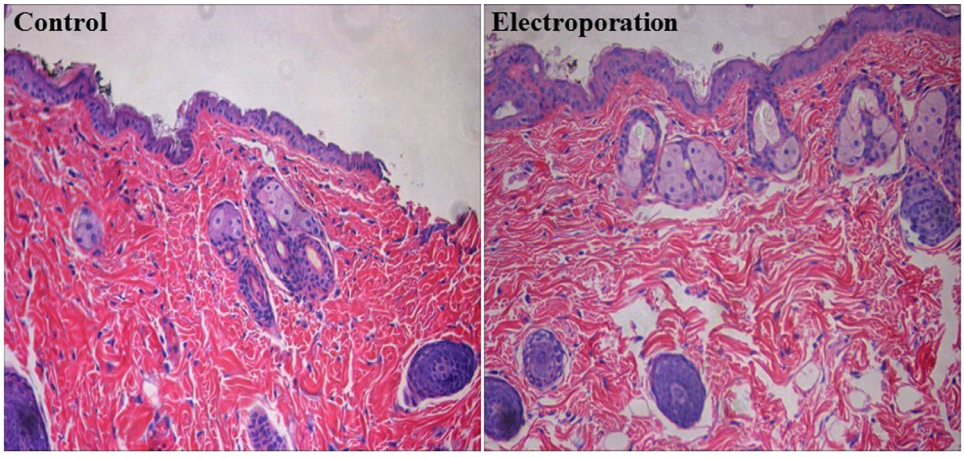

Fig. 9. Histopathological examination of mice skin (H. E. × 200).

different time points after perforation was higher than that of the non-porous group, increased after electroporation and began to decrease at 5 h. The lowest intensity appeared at 12 h (Fig. 7). The permeation effect produced by electroporation could not be restored immediately, but was gradually recovered and lasted for at least 12 h.

In vivo imaging uses bioluminescence and fluorescence to monitor cellular activity and genetic behavior in living organisms. Cy7 is a fluorescent dye with high fluorescence intensity and stability and is suitable for imaging in living small animals. The fluorescence intensity of the electroporation group was stronger than that of the normal group without electroporation (Fig. 8), and this was with no relation to the sequence of electroporation and drugs. The fluorescence intensity of the mice with electroporation increased with time, and was distributed throughout the body at 4 h after electroporation. The transdermal amount and the fluorescence intensity increased after electroporation, which proved electroporation could improve transdermal absorption directly.

The influence of electroporation (1.45 mW, 10 min) on skin structure is striking. In the normal skin, the structure was tight and integrated, and the epidermal cells were closely packed (Fig. 9). The skin structure was loose with increased interstitial and epidermal cracks after electroporation action. Electroporation altered skin structure rather than disrupt it, increased skin permeability, and finally enhanced transdermal drug delivery.

Electroporation could work alone or in conjunction with other techniques to improve transdermal absorption (Banga et al., 1999; Yang et al., 2018). Two surfactants, Tween 80 and sodium lauryl sulfate, were used simultaneously with electroporation to improve the transdermal absorption of the lipophilic drug - piroxicam. The transdermal rate of piroxicam increased by 30-50 times finally (Jia et al., 2005). The effects of simultaneous iontophoresis and electroporation on transdermal delivery of insulin were studied. Insulin was capable of little skin penetration when iontophoresis alone was applied. But electroporation increased transdermal insulin levels in plasma. Moreover, insulin plasma levels increased obviously when electroporation and iontophoresis were used together (Tokumoto et al., 2006). Similar results were obtained for transdermal delivery of 5-fluorouracil (5-FU) with electroporation and iontophoresis. Electroporation significantly increased the penetration of 5-FU compared to passive diffusion. The combination of two penetration-enhancing techniques resulted in a much higher transdermal penetration of 5-FU than alone (Fang et al., 2004).

4. Conclusion

With the rapid development of transdermal drug delivery systems, barriers of stratum corneum have become a big challenge. Electroporation has been expanded and applied to transgenic engineering, cell fusion, wound healing, membrane protein electroporation, cancer therapy, gene therapy, and DNA vaccination (Ibrahim et al., 2016; Kichaev et al., 2013). In drug delivery fields, electroporation provides an amazing route for improved transdermal absorption of drugs.

This study focused on the regularity and the mechanism of electroporation promoting the transdermal absorption of drugs with different physiochemical characteristics. Electroporation could produce reversible channels or pores in stratum corneum of the skin, and the permeation effect could be maintained for more than 12 h. These findings can facilitate the optimization of electroporation parameters for transdermal absorption of different drugs. It will provide reference and comparison for transdermal permeation with high efficiency.

Author statement

Xiao Chen: Methodology and investigation.
Lin Zhu: Part of the experiments, data analysis, writing and original draft preparation.
Ruiteng Li: Part of the experiments.
Lulu Pang: Part of the experiments.
Siqing Zhu: Part of the experiments.
Jinqiu Ma: Mathematics simulation.
Lina Du: Design of the experiments, writing, reviewing and editing.

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References

Banga, A., Bose, S., Ghosh, T., 1999. Iontophoresis and electroporation: comparisons and contrasts. Int. J. Pharm. 179, 1–19.
Benson, H., Namjoshi, S., 2008. Proteins and peptides: strategies for delivery to and across the skin. J. Pharm. Sci. 97, 3591–3610.
Berkó, S., Szűcs, K.F., Balázs, B., Csányi, E., 2016. Electroporation-delivered transdermal neostigmine in rats: equivalent action to intravenous administration. Drug Des. Devel Ther. 10, 1695–1701.
Blagus, T., Markelc, B., Cemazar, M., Kosjek, T., 2013. In vivo real-time monitoring system of electroporation mediated control of transdermal and topical drug delivery. J. Controlled Release 172, 862–871.
Cuyàs, E., Fernández-Arroyo, S., Verdura, S., García, R.Á.-F., Stursa, J., Werner, L., Blanco-González, E., 2018. Metformin regulates global DNA methylation via mitochondrial one-carbon metabolism. Oncogene 37, 963–970.
Dąbrowska, A.K., Spano, F., Derler, S., Adlhart, C., Spencer, N.D., Ross, R.M., 2018. The relationship between skin function, barrier properties, and body-dependent factors. Skin Res. Technol. 24, 165–174.
Denet, A.-R., Vanbever, R., Préat, V., 2004. Skin electroporation for transdermal and topical delivery. Adv. Drug Deliv. Rev. 56, 659–674.
Dujardin, N., Staes, E., Kalia, Y., 2002. In vivo assessment of skin electroporation using square wave pulses. J. Controlled Release 79, 219–227.
Escobar-Chávez, J.J., Bonilla-Martínez, D., Villegas-González, M.A., Revilla-Vázquez, A.L., 2009. Electroporation as an efficient physical enhancer for skin drug delivery. J. Clin. Pharmacol. 49, 1262–1283.
F, P.U., T, M.G., C, W.J., 2002. Kinetics of the temperature rise within human stratum corneum during electroporation and pulsed high-voltage iontophoresis. Bioelectrochemistry 65, 65–72.
Fang, J., Hung, C., Fang, Y., Chan, T., 2004. Transdermal iontophoresis of 5-fluorouracil combined with electroporation and laser treatment. Int. J. Pharm. 270, 241–249.
Feng, S., Zhu, L., Huang, Z., Wang, H., Li, H., 2017. Controlled release of optimized electroporation enhances the transdermal efficiency of sinomenine hydrochloride for treating arthritis in vitro and in clinic. Drug Des. Devel. Ther. 11, 1737–1752.
Franzè, S., Musazzi, U.M., Minghetti, P., Cilurzo, F., 2019. Drug-in-micelles-in-liposomes (DiMiL) systems as a novel approach to prevent drug leakage from deformable liposomes. Eur. J. Pharm. Sci. 130, 27–35.
Gallo, S.A., Sen, A., Hensen, M.L., Hui, S.W., 2002. Temperature-dependent electrical and ultrastructural characterizations of porcine skin upon electroporation. Biophys J. 82, 109–119.
Ibrahim, A., Yin-Ku, L., Jia-You, F., 2016. Noninvasive approach for enhancing small interfering RNA delivery percutaneously. Expert Opin. Drug Deliv. 13, 265–280.
Ita, K., 2015. Transdermal delivery of drugs with microneedles-potential and challenges. Pharmaceutics 7, 90–105.
Ita, K., 2017. Dissolving microneedles for transdermal drug delivery: advances and challenges. Biomed. Pharmacother. 93, 1116–1127.
Jia, Z., Jiang, G., Lin, Y., 2005. Transdermal delivery of piroxicam by surfactant mediated electroporation. Tsinghua Sci. Technol. 10, 542–547.
Kichaev, G., Mendoza, J.M., Amante, D., Smith, T.R.F., 2013. Electroporation mediated DNA vaccination directly to a mucosal surface results in improved immune responses. Hum. Vac. Immunother. 9, 2041–2048.
Lane, M.E., 2013. Skin penetration enhancers. Int. J. Pharm. 447, 12–21.
Lee, H., Song, C., Baik, S., Kima, D., Hyeon, T., Kima, D.-H., 2018. Device-assisted transdermal drug delivery. Adv. Drug Deliv. Rev. 127, 35–45.
Li, K., 2013. Passive and iontophoretic transport through the skin polar pathway. Skin Pharmacol. Physiol. 26, 243–253.
Liu, K.-C., Green, C.R., Alany, R.G., Rupenthal, I.D., 2013. Synergistic effect of chemical penetration enhancer and iontophoresis on transappendageal transport of oligodeoxynucleotides. Int. J. Pharm. 441, 687–692.
Lopez, R., Seto, J., Blankschtein, D., Langer, R., 2011. Enhancing the transdermal delivery of rigid nanoparticles using the simultaneous application of ultrasound and sodium lauryl sulfate. Biomaterials 32, 933–941.
Madhavia, N., Sudhakara, B., Reddyb, S., Ratnaa, V., 2019. Design by optimization and comparative evaluation of vesicular gels of etodolac for transdermal delivery. Drug Dev. Ind. Pharm. 45, 611–628.
MB, B., MJ, T., GP, M., FK, A., 2008. Transdermal drug delivery systems: skin perturbation devices. Drug Deliv. Syst. 37, 119–139.
Mitragotri, S., 2013. Devices for overcoming biological barriers: the use of physical forces to disrupt the barriers. Adv. Drug Deliv. Rev. 65, 100–103.
Münch, S., Wohlrab, J., Neubert, R., 2017. Dermal and transdermal delivery of

pharmaceutically relevant macromolecules. Eur. J. Pharm. Biopharm. 119, 235–242.
Murthy, S.N., Sen, A., Hui, S.W., 2004a. Surfactant-enhanced transdermal delivery by electroporation. J. Control Release 98, 307–315.
Murthy, S.N., Sen, A., Zhao, Y.-L., Hui, S.W., 2003. pH influences the postpulse permeability state of skin after electroporation. J. Control Release 93, 49–57.
Murthy, S.N., Sen, A., Zhao, Y.-L., Hui, S.W., 2004b. Temperature influences the post-electroporation permeability state of the skin. J. Pharm. Sci. 93, 908–915.
Nguyen, H.X., Banga, A.K., 2018. Electrically and ultrasonically enhanced transdermal delivery of methotrexate. Pharmaceutics 10, 117–134.
Palmer, B., DeLouise, L., 2016. Nanoparticle-enabled transdermal drug delivery systems for enhanced dose control and tissue targeting. Molecules 21, 1719–1736.
Parhi, R., Swain, S., 2018. Transdermal evaporation drug delivery system: concept to commercial products. Adv. Pharm. Bull. 8, 535–550.
Park, J., Lee, H., Lim, G.-S., Kim, N., 2019. Enhanced transdermal drug delivery by sonophoresis and simultaneous application of sonophoresis and iontophoresis. AAPS Pharm. Sci. Tech. 20, 96–103.
Pavselj, N., Zorec, B., Miklavcic, D., Becker, S., 2015. Experimental factors to be considered in electroporation-mediated transdermal diffusion experiments. J. Biomech. Eng. 137, 124501–124508.
Prausnitz, M., Langer, R., 2008. Transdermal drug delivery. Nat. Biotech. 26, 1261–1268.
Puri, A., Murnane, K.S., Blough, B.E., Banga, A.K., 2017. Effects of chemical and physical enhancement techniques on transdermal delivery of 3-fluoroamphetamine hydrochloride. Int. J. Pharm. 528, 452–462.
Rainsford, K.D., Kean, W.F., Ehrlich, G.E., 2008. Review of the pharmaceutical properties and clinical effects of the topical NSAID formulation, diclofenac epolamine. Curr. Med. Res. Opin. 24, 2967–2992.
Riviere, J., Riviere, N.M., Rogers, R., 1995. Pulsatile transdermal delivery of LHRH using electroporation: drug delivery and skin toxieology. J. Controlled Release 36, 229–233.
Rizwan, M., Aqil, M., Talegaonkar, S., Azeem, A., Sultana, Y., Ali, A., 2009. Enhanced transdermal drug delivery techniques: an extensive review of patents. Recent Pat. Drug Deliv. Formul. 3, 105–124.
Schoellhammer, C., Blankschtein, D., Langer, R., 2014. Skin permeabilization for transdermal drug delivery: recent advances and future prospects. Expert Opin. Drug Deliv. 11, 393–407.
Shamshiri, M.K., Momtazi-Borojeni, A.A., Shahraky, M.K., Rahimi, F., 2019. Lecithin soybean phospholipid nano-transfersomes as potential carriers for transdermal delivery of the human growth hormone. J. Cell Biochem. 120, 9023–9033.
Sharma, V.K., Mamontov, E., Ohl, M., Tyagi, M., 2017. Incorporation of aspirin modulates the dynamical and phase behavior of the phospholipid membrane. Phys. Chem. Chem. Phys. 19, 2514–2524.
Tokumoto, S., Higo, N., iSugibayashi, K., 2006. Effect of electroporation and pH on the iontophoretic transdermal delivery of human insulin. Int. J. Pharm. 326, 13–19.
Tokumoto, S., Higo, N., Todo, H., Sugibayashi, K., 2016. Effect of combination of low-frequency sonophoresis or electroporation with iontophoresis on the mannitol flux or electroosmosis through excised skin. Biol. Pharm. Bull. 39, 1206–1210.
Tombs, E.L., Nikolaou, V., Nurumbetov, G., Haddleton, D.M., 2018. Transdermal delivery of ibuprofen utilizing a novel solvent-free pressure-sensitive adhesive (PSA): TEPI® technology. J. Pharm. Innov. 13, 48–57.
Ulashchik, V.S., 2018. The physical and chemical properties of the skin and the action of therapeutic physical factors. Vopr. Kurortol. Fizioter. Lech. Fiz. Kult. 95, 4–13.
Wang, M., Hu, L., Xu, C., 2017. Recent advances in the design of polymeric microneedles for transdermal drug delivery and biosensing. Lab. Chip. 17, 1373–1387.
Wiraja, C., Zhu, Y., Lio, D.C.S., Yeo, D.C., 2019. Framework nucleic acids as programmable carrier for transdermal drug delivery. Nat. Commun. 10, 1147–1159.
Yang, G., Zhang, Y., Gu, Z., 2018. Punching and electroporation for enhanced transdermal drug delivery. Theranostics 8, 3688–3690.
Zhu, Y., Fu, J., Shurlknight, K.L., Soroka, D.N., Hu, Y., Chen, X., Sang, S., 2015. Novel resveratrol-based aspirin prodrugs: synthesis, metabolism, and anticancer activity. J. Med. Chem. 58, 6494–6506.
Zorec, B., Jelenc, J., Miklavcic, D., Pavšelj, N., 2015. Ultrasound and electric pulses for transdermal drug delivery enhancement: Ex vivo assessment of methods with in vivo oriented experimental protocols. Int. J. Pharm. 490, 65–73.