Numerical analysis and animal study of noninvasive handheld electroporation delivery device for skin superficial lesion treatment

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\textbf{ABSTRACT}

\textbf{Introduction:} This study aims to investigate the feasibility of a noninvasive handheld electroporation pulses delivery device (EPDD) for electroporation-based treatment (EBT) of skin superficial lesions through numerical analysis and animal study.

\textbf{Methods:} Finite element analysis was performed to investigate the performance of the EPDD. The electric field, temperature, EI and TI were calculated under pulse voltages of 600, 800, and 1000 V. A mouse subcutaneous tumor model was established to evaluate the performance of the EPDD through histopathology and survival analyses.

\textbf{Results:} The electrical field strength increased from 151 (600 V) to 252 V/cm (1000 V) in the skin and from 1302 (600 V) to 2171 V/cm (1000 V) in the tumor. The volume of EI grew and reached a plateau at the 165th pulse, whereas the maximum volume of EI increased with higher voltage. The growth tendency of TI differed between groups, and it was higher in the high-voltage group (HVG) than in the low-voltage group. Histopathological analysis showed that the depth and range of the ablation area could be controlled by adjusting pulse voltage. Survival analysis showed that the survival of the HVG was better than that of the low-voltage and the control group (p < 0.01).

\textbf{Conclusions:} The results demonstrate that the EPDD is feasible, safe, and effective for skin EBT. The volume of EP tissue injury can be controlled by adjusting the pulse voltage, pulse number, and other parameters. The proposed noninvasive handheld EPDD can be a potential therapeutic tool for EBT of superficial skin lesions in the future.

\textbf{INTRODUCTION}

Electroporation-based treatment (EBT) is an emerging focal therapeutic technique based on the biological effect of electroporation pulses (EPs) [1]. Pulsed electrical fields can destroy the balance of transmembrane potential of cell membrane and disrupt the cell homeostasis [2–5]. Since several nanoscale pores are formed on cell membrane during the process, the cell permeability increases, which promotes the exchanges of molecules between cytoplasm and external environment [6]. In this context, EPs can be used in biotechnology and chemotherapy to deliver genes, molecules, and drugs to cells via gene electrotransfer, electro-assisted transdermal drug delivery, and electrochemotherapy (ECT), respectively [1,7]. If the electrical field strength is larger than the threshold (500–1000 V/cm) for non-thermal irreversible electroporation (IRE), the cell could be killed by EPs, this approach can be used for a type of tissue ablation known as pulsed field ablation [6,8].

Usually, EPs take the form of square waves with a pulse width on the order of tens to hundreds of microseconds [1]. Since the duration of an EP is ultra-short, the heat generated during the process can be quickly diffused or absorbed by the tissue. Therefore, the cumulative heat during EBT is negligible when the electrical injury (EI) is achieved with no irreversible thermal injury (TI) delivered to the tissue [9]. EPs can promote cell permeability or inactivate malignant cells in situ rapidly, while sparing tissue scaffolds such as the extracellular matrix, vascular wall, and nerve fiber [10]. These advantages of EP expanded the application of EBT, especially for heat-sensitive structures which are usually out-of-bound areas for thermal-based treatments (e.g., radiofrequency ablation, microwave ablation, etc.).

Skin disease is a class of common diseases, and originates from the intradermal or subcutaneous tissue. In particular, superficial skin lesions are the most common skin diseases, that are usually initial in the basal membrane, and include
malignant melanoma and nevi [11]. However, surgical resec-
tion, laser, chemotherapy, radiation, and other physical thera-
pies are the most common methods used for the treatment of
skin disease, which may destroy the integrity of the skin
and lead to skin defect post treatment [12]. The non-thermal
advantages of EBT makes it an attractive candidate for the
treatment of skin superficial lesions, which can preserve the
tissue scaffold of the skin. Moreover, EBT has been dem-
onstrated to rejuvenate the skin and promote drug delivery in
cosmetic surgery [13,14]. Therefore, the non-thermal EBT has
been treated as an emerging focal treatment method for
skin superficial lesions, because the thermal-based treat-
ments may lead to irreversible TI to the skin, which are not
well suited for clinical practice because scars, defects, ulcers
may occur [15]. Moreover, ECT has been used for treatment
of skin cancer and keloids by Cliniporator System [16,17].

The electric field distribution directly determines the out-
comes of EBT. Pulse parameters, tissue properties, and the
electrode configurations are the key factors that affect elec-
tric field distribution [18]. The electrode configuration
includes structure, materials, quantity, treatment approach,
and distribution configuration, which can be optimized
according to the anatomy of the lesion. The needle-shaped
electrode, which consists of a series of two types of needles,
an anode (high voltage) and a cathode (ground), is the most
common type used in EBT. The electric field distribution of a
needle-shaped electrode appears as a double sphere, in
which the high voltage area is concentrated near the elec-
trode needle [19]. The electrical field strength between the
two electrodes decreased with increasing electrode distance.
The electric field distribution can be controlled by adjusting
the distance of the two electrodes or the pulse voltage.
However, the main disadvantage of the needle electrode is
that it needs to invade the tissue, which causes unnecessary
wounds due to bleeding or tumor spreading. Plate-type elec-
trodes consist of a couple of round or rectangle plate elec-
trodes [9]. The electric field distribution of plate-type elec-
trode evenly distributed between the electrodes, which
may be adjusted by shape (area), distance, and pulse voltage
[18]. However, the plate-type electrodes must be clamped to
the skin during treatment, which may result in an increase in
electrode distance, causing higher voltage and increased risk
of TI, thereby making it unsuitable for skin superficial lesion
treatment. An ideal skin ablation electrode should not caus-
ing any mechanical injury when placed on the skin surface.
To ensure this, the electric field distribution should have suf-
cient depth and area of expansion for skin superficial
lesions to avoid compressing, deforming, or stretching
the skin.

Thus, in this study, a noninvasive handheld electropor-
ation pulses delivery device (EPDD) was designed and fabri-
cated for EBT of skin superficial lesions. The novel noninvasive handheld EPDD can achieve sufficient and
adjustable depth and volume for focal treatment. The aim
of this study was to investigate the electrical and thermal inju-
ries of the EPDD for EBT of skin superficial lesions by numer-
ical analysis, and evaluate the performance of the EPDD
through animal study.

Materials and methods

Electrode configuration and prototype of EPDD

As shown in Figure 1(A), the electrode configuration of the
EPDD is a type of concentric electrode, which comprises a
round anode (high voltage, 5 mm in diameter), and a ring-
shaped cathode (ground, 2 mm in width). The EPDD (Figure
1(C,D)) contains handgrip, connector at the end, and medical
grade stainless steel electrode at the head. The ergonomic
handgrip is cylindrical with a handle as the body and a steer-
ing knuckle as the neck. The connector is at the tail end of
handgrip and is connected to the EP generator through cables.

Model for computational simulation

As shown in Figure 1(B), a 3D geometric model of the skin,
tumor (light blue area), and electrode was established in
COMSOL Multiphysics V5.6 for Windows (COMSOL,
Stockholm, Sweden). The tissue model is a cuboid with size
25 × 25 × 5 mm consisting of two parts: the skin and the
tumor. The tumor was modeled as a hemi-ellipsoid with size
5 × 5 × 3 mm; the skin comprises the rest of the model. The
depth of the tumor is approximately 3 mm from the skin
surface. In order to simplify the calculation and optimize
the geometric model, the skin layer on the tumor surface was
omitted. A convergence test of the mesh model was per-
formed to ensure validity and stability of the simulation.

Numerical analysis

According to the initial parameters shown in Table 1, the
electric field distribution, temperature distribution (TD), EI
and TI were calculated by the finite element analysis
method, which was used to solve the numerical models and
performed by COMSOL Multiphysics based on our previous
study [18]. The governing equations and the lethal threshold
of electrical field strength for electroporation, was set as
596 V/cm in skin and 479 V/cm in tumor (see supplementary
files). If the value of electrical field strength was greater than
the threshold, it was defined as effective electric field. The
volume of the effective electric field, EI, TI, average tempera-
ture, and electrical field strength in each domain were calcu-
lated. The relative volume ratio (RVR) was defined as the ratio
of the valid volume of these indicators to the total vol-
ume of the domain. The ratio was calculated separately
for the EI and TI of the entire tissue and each domain. Typically,
the parameters of EP were set as 100 μs in duration, 1 Hz in
frequency, and 200 in pulse number. The pulse voltage was
set as 600, 800, 1000 V for parametric sweep methods. The
anode electrode was set as having a positive potential and
the cathode set as ground (0 V). All remaining boundaries
were treated as being electrically insulating. The initial tem-
perature of the tissue and artery was set to 310.15 K. The
internal boundaries between the tissue and electrode were
defined as thermally continuous, and their external bounda-
ries were defined as adiabatic to completely confine the heat
generated during electroporation within the tissue, depending on the upper limits of calculating Joule heating.

Animal study

Cell culture and tumor model

To investigate the efficacy of the EPDD, mouse B16F10 cell lines, purchased from Procell (Procell Life Science & Technology Co., Ltd., Wuhan, Hubei, China), and 52 six-week-old (body weight ~18–20 g) male C57BL/6J mice, purchased from the Experimental Animal Center of Xi’an Jiaotong University, were prepared for an in vivo tumor ablation experiment. The cells were cultivated in the medium at normal cell incubator setting (37°C, 5% CO₂). The back of each mouse was injected with 0.1 ml of 2 × 10⁷ cell/ml B16F10 melanoma cell suspension to establish a subcutaneous tumor model. The tumor volume was calculated by the formula \[ V = 0.52 \times D_1 \times D_2^2 \] (\(D_1\): long diameter, \(D_2\): short diameter) [20], and was measured with a caliper every 2 days. When the tumor volume reached 100 mm³, the tumor model was treated as successfully established. The experimental protocol was approved by the Institutional Animal Care and Use Committee (No. XJTU2020-287). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution.

IRE procedure

The IRE procedure was performed to evaluate the performance of the EPDD in accordance with the numerical analysis, in which the EP parameter was set as 600 and 1000 V in pulse voltage, 100 μs in duration, and 150 pulse number. Mice were anesthetized with an intraperitoneal injection of isoflurane (0.6 mL/kg) (RWD, Shenzhen, China) to maintain muscle relaxation. All the mice were randomly divided into three groups: control (\(n = 15\), no treatment), low-voltage group (LVG, \(n = 19\), 600 V, 150 pulses), and high-voltage group (HVG, \(n = 18\), 1000 V, 150 pulses). Three mice in each group were selected for histopathology analysis and only once treatment was performed before being killed. The rest of the mice were used for survival analysis, in which the treatments were performed once every 24 h for 3 days. Scab formation on the skin post IRE could have caused deviation in the measurement of tumor diameter; thus, animal survival was selected as the assessment indicator of this study. The animals were killed when the tumor volume reached
Survival analyses were performed using Kaplan–Meier methods. All statistical analysis was performed with GraphPad Prism 9.0 for Windows (GraphPad Software Inc., La Jolla, CA, USA), and \( p < 0.01 \) was considered statistically significant.

**Histology**

To evaluate the outcomes of the EPDD on different pulse conditions in acute injury stage, histology analyses were performed post IRE treatment. The tissue samples were fixed in 10% formalin, embedded in paraffin, and cut into 5 \( \mu \)m sections for histopathological analyses. Sections were stained with hematoxylin and eosin (H&E) (G1005, Servicebio, Wuhan, China) and terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) (In Situ Cell Death Detection Kit, Roche, Basel, Switzerland) in accordance with the manufacturer’s instructions. Tissue sections were observed and photographed with a microscope (BX53, Olympus, Japan).

**Results**

**Numerical analysis**

**Electric fields distribution of electrode**

The electric field distribution in the tissue under the conditions of 600, 800, and 1000 V in \( y-z \) section view, exhibits a substantially hemi-elliptical shape (Figure 2(A)). The high electrical field strength area (greater than 2000 V/cm), shown in red, is mainly distributed in the closest area between the two electrodes. The distribution of the effective electric field increased between the tumor and the skin with increasing pulse voltage. In particular, the increase of depth means a high coverage rate in tumor. The differences in the electrical properties of the skin and tumor result in unevenness and discontinuity in the electric field distribution at the junction of the skin and tumor. Table 2 shows the volume of effective electric field and average electrical field strength in skin and tumor at 600, 800, and 1000 V. Determining the dynamic conductivity of skin and tumor is limited to operability in practical measurement. Thus, the conductivity of skin and tumor were modeled in this study as two constants, which were obtained from the study of Pliquett et al. [21]. Therefore, the volume of effective electric field and average electrical field strength remained unchanged under same pulse voltage. The average electrical field strength for the tumor and surrounding skin showed a significant gap, in which the value in the tumor was much higher. With increasing pulse voltage, the volume of effective electric field increased from 208 (600 V) to 386 mm\(^3\) (1000 V) in the skin, but remained unchanged at 39 mm\(^3\) in the tumor, which is

![Figure 2](image_url)

**Table 2.** Simulation results of skin and tumor.

| Volume of EEF (mm\(^3\)) | Average EFS (V/cm) |
|---------------------------|--------------------|
| **Skin**                  | **Tumor**          |
| 600 V                     | 208                | 39                 | 151               | 1302              |
| 800 V                     | 301                | 39                 | 202               | 1737              |
| 1000 V                    | 386                | 39                 | 252               | 2171              |

**Electric field distribution and growth curve of performance indicator of the noninvasive handheld electroporation pulse delivery device in different levels of pulse voltage.** (A) The electric field distribution under the condition of 600, 800, and 1000 V in \( y-z \) section view; (B) Relative volume ratio of electrical and thermal injuries in tumor; (C) Relative volume ratio of electrical and thermal injuries in skin; (D) Average temperature in tissue; (E) Volume of electrical injury in skin and tumor; (F) The volume of thermal injury in skin and tumor.
exactly equal to the volume of effective electric field within the tumor.

**Estimated electrical and thermal injury**

Figure 2(B,C) show the growth curve of the RVR of the electrical and thermal injuries in the tumor and the skin. The pulse number by the occurrence of EI or TI is an important parameter because it indicates the beginning of effective treatment. In tumor, the pulse number by the occurrence of EI is 17 (600 V), but is 1 for 800 and 1000 V. EI of the tumor increased by 3.25–4.83 times from the 100th to the 150th pulse at 600–1000 V.

The tendency of skin EI was present as two phases: growth phase and plateau phase. The EI occurred instantly after the action of electroporation for 600–1000 V, but showed only slow growth. The end point of the growth phase was the same for different pulse voltages, which occurred at pulse number 165. The duration of the growth phase was determined by the pulse duration of EP and threshold of effective electric field for electroporation.

The growth tendency of TI rose continuously. TI occurred at pulse number 139 (600 V), 39 (800 V), and 20 (1000 V) in the skin, and at 61 (800 V) and 32 (1000 V) in the tumor. There was no TI in the tumor at 600 V, but TI rose sharply when temperature exceeded a threshold value (43°C), and then reached a maximum in the tumor. Compared to TI, the growth tendency of EI was more regular.

Figure 2(D) shows the average temperature for different levels of pulse voltage in the tissue, skin, and tumor. The average temperature in tissue increased with the increase in pulse number. The average temperature in tumor was higher than that of the entire tissue and skin. Furthermore, the average temperature in the tissue was higher at 1000 V than at 800 and 600 V. By way of taking a typical point in time as an example, when the pulse number reached 200 (the end of the treatment), the tumor, skin, and tissue temperatures were 55.5, 40.3, and 40.5 °C, respectively, at 600 V.

Figure 2(E,F) show the volume of EI and TI. The growth tendency of the injury volume is consistent with RVR. The maximum volume of EI in the skin was 208 (600 V), 301 (800 V), and 386 (1000 V) mm³, whereas it was 13 (600 V), 25 (800 V), and 31 (1000 V) mm³ in the tumor. The maximum volume of TI in the skin was 0.4 (600 V), 82 (800 V), and 296 (1000 V) mm³, whereas it was 0 (600 V), 39 (800 V), and 39 (1000 V) mm³ in the tumor.

**Distribution of electrical and thermal injury**

Figure 3 shows the 3D distribution of effective electric field, temperature, EI, and TI for different levels of pulse voltage at 50, 100, 150, and 200 in pulse number. The light blue represents the area of effective electric field, which was discoid and increased with the increasing pulse voltage and remained unchanged with the increasing pulse number. The light orange represents the area where the tissue temperature was >43 °C, which increased from the center of anode toward the cathode with increasing pulse number and pulse voltage. The light yellow represents the area where the probability of EI was >99% having the same discoid shape as effective electric field and increased with the increasing pulse number and reached the maximum volume at a certain pulse. The maximum volume of EI was the same as that of effective electric field. The light red color represents the area where the probability of TI was >99%, which increased with increasing pulse number and pulse voltage.

**Histopathological analysis**

Figure 4 shows the histopathological analysis of tissue samples. The left panel shows the images from H&E staining, and the right panel shows the images from TUNEL. In Figures 4(A,B), the upper layer of skin is light in color and the cells were sparsely distributed where the tissue was affected by the IRE, and the shape of ablated area was approximately the same as electric field distribution. The depth of injury area increased with increasing pulse voltage. For TUNEL, the dark brown marked cells, which represent apoptotic cells in the experimental group. The quantity and areas of TUNEL positive cells was larger in the HVG than in the LVG.
Survival analysis

Figure 5 shows the appearance changes of subcutaneous tumor of HVG by EPDD from day 0 to day 18. The subcutaneous tumor, which can be treated as a phantom of skin superficial lesion was circled in red, was ablated by IRE within 18 days. The scab was gradually formed from day 0 post IRE to day 4, and its area expanded to the surrounding skin until day 8. The repair process started gradually, and the skin was repaired from day 10 to day 14. The ablated area was approximately the same with the surrounding skin on day 18. No irreversible tissue injury occurred after the treatment.

Figure 6 shows the Kaplan–Meier curve of the animal post IRE by EPDD; significant differences among the three groups ($p < 0.0001$) were observed. The survival rate of the animals on day 14 post IRE was 8.33% (Control), 43.75% (LVG), and 73.33% (HVG), while it was 0% (Control), 0% (LVG), and 26.67% (HVG) on day 28. The survival rate of the HVG was significantly higher than that of the other groups, and that
of the LVG was significantly higher than that of the control group but lower than that of the HVG. The animals in the LVG may die from tumor recurrence.

**Discussion**

The parameters including pulses number, pulse width, applied voltage, and frequency were set up in COMSOL for numerical analysis. Furthermore, the range of electric field distribution determined by the shape of the EPDD was presented through simulation before experimentation. The simulation results provided a reference for parameter selection for use in the animal experiments. In addition, the histopathological analysis and results of antitumor effect supported the reliability of the simulation. The simulation and the experimentation results can be constantly optimized through several rounds of update and interaction, so that the EPDD and simulation can meet clinical expectations. A noninvasive handheld EPDD for EBT of skin was designed, fabricated and evaluated by numerical analysis and animal study. The characteristics of electric field distribution, TD, EI, and TI of the EPDD were preliminarily investigated; it was found that they can be adjusted by pulse voltage and electrode configuration.

The needle-shaped electrodes array is the most common type used in bioelectronic treatment \[22\]. The needle-shaped electrodes are easy for fabrication, disinfection, and operation. Its most important advantage is that the depth of treatment can be simply controlled by adjusting the length of exposed electrode in tissue \[23\]. However, puncturing the tissue is inevitable, and increasing the electrode length may cause bleeding, infection, and even tumor spread. The plate-type electrode is another common type of electrode configuration used in EBT, and has enough surface area that can form a uniform electric field distribution \[24\]. Therefore, it is the ideal option for EBT, because the electric field distribution is exactly the same in every area between the electrodes. However, plate-type electrodes are not suitable for biomedical application in humans due to the lack of suitable approach for operation. The tweezer-style electrode, which is a special type of plate-type electrode, can be used for thin tissue or in small animals only \[25\]. In this study, a noninvasive electrode configuration was created by placing two plate-type electrodes in the same plane, lowering chances of mechanical injury. The discoid electric field distribution of the electrode configuration is large enough for skin superficial lesions. The noninvasive handhold EPDD can achieve effective EI in the target area without mechanical injury, thus it is feasible for EBT in skin treatment.

As mentioned previously, the electric field distribution and EI caused by the noninvasive handhold EPDD were discoid with calculable thickness and diameter. Moreover, the electric field distribution can be optimized by adjusting the electrode geometry and pulse voltage. Therefore, this type of electrode configuration is suitable for certain skin superficial lesions, such as melanoma, or metastasis tumor \[16\]. In particular, this noninvasive electrode configuration is also valuable for trans-dermal drug delivery for skin care and beauty treatment. Li et al. \[26\] have demonstrated that IRE approach could effectively stimulate keratinocyte proliferation, extracellular matrix synthesis, and angiogenesis in an aged rat model, which rejuvenates the aged skin. Zasada et al. \[16\] have confirmed the positive effect of antiaging effect of L-ascorbic acid in combination with EBT.

Usually, TI needs to be avoided during classical EBT \[27\]. The growth of TI differs for different levels of pulse voltage. The higher the pulse voltage, the earlier TI occurs. The time window for non-thermal EBT is complete EI with no or lowest TI, which can be estimated by numerical analysis before treatment \[18,27\]. Moreover, increasing the times of EBT and
decreasing total pulse number in one set of burst also can avoid the unnecessary TI. The difference in tissue properties caused the significant differences in electric field distribution and temperature distribution [25]. The electrical conductivity of stratum corneum and skin is lower than that of the tumor, thus there is distinct distortion in the junctional zone in electric field distribution [21]. Although the results need further evaluation through experiments, it is possible to optimize the electrode configuration and pulse parameters to well adapt to particular skin lesions.

There are some limitations to this study. First, the geometry parameters of electrode configuration are additional determinant factors for electric field distribution besides pulse voltage, and their effect was not analyzed in this study. Second, the thickness of skin is significantly different in various parts of human body, thus the results of this study may not be suitable for all conditions. Despite these, this study has demonstrated the efficacy of the noninvasive handheld EPDD. Future studies should focus on the effect of geometry parameters and thickness of skin on the electric field distribution and outcomes of EBT. Finally, the animal model used in this study was subcutaneous tumor in mice, and its volume was relatively smaller than the estimated injury volume of noninvasive handheld EPDD. Therefore, future studies should be performed on large animals and even humans to further evaluate the performance of the noninvasive handheld EPDD.

Conclusions

This study preliminarily demonstrated by numerical analysis and animal study that the electrode configuration of the proposed noninvasive handheld EPDD is feasible, safe, and effective for EBT of skin superficial lesions. The volume of EP tissue injury can be controlled by adjusting the pulse parameters. This noninvasive handheld EPDD can be a potential therapeutic tool for the treatment of skin superficial lesions in the future.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by: 1. National Natural Science Foundation of China [Grant No. 61772782]; 2. The Fundamental Research Funds for the Central Universities [Grant No. JXH012019060 and JXH012020022]; 3. The Scientific Development Funding of the First Affiliated Hospital of Xi’an Jiaotong University [2020QN-08]; 4. The Basic Natural Science Research Project of Shaanxi Province [2021JQ-401]. The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

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