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IGBT-Based Pulsed Electric Fields Generator for Disinfection: Design and *In Vitro* Studies on *Pseudomonas aeruginosa*

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Abstract—Irreversible electroporation of cell membrane with pulsed electric fields is an emerging physical method for disinfection that aims to reduce the doses and volumes of used antibiotics for wound healing. Here we report on the design of the IGBT-based pulsed electric field generator that enabled eradication of multidrug resistant *Pseudomonas aeruginosa* PAO1 on the gel. Using a concentric electric configuration we determined that the lower threshold of the electric field required to kill *P. aeruginosa* PAO1 was 89.28 ± 12.89 V mm^{-1}, when 200 square pulses of 300 μs duration are delivered at 3 Hz. These parameters disinfected 38.14 ± 0.79 mm^2 area around the single needle electrode. This study provides a step towards the design of equipment required for multidrug-resistant bacteria disinfection in patients with pulsed electric fields.

Keywords—Multidrug-resistant bacteria, IGBT, Pulsed electric field, Electroporation, *Pseudomonas aeruginosa* PAO1.

INTRODUCTION

Nosocomial infection is complicated and harmful health effect caused by biological indoor air pollutants in the hospital environment.[10] Preventable complications associated with health care in the United States estimated to cost of $88 billion per year.[10] In addition, a 2007 study by Aon suggests that Nosocomial accounted for 12.2% of the healthcare facilities total legal liability costs.[10] The illness from nosocomial infections can cause damage to all who take place in a hospital environment but it's especially dangerous for people with the low function of the immune system such as people with burn wounds.[49] Burns is one of the most common and devastating forms of trauma. Patients with serious thermal injury, in fact, armor-less against all kinds of viral or bacterial infections and can easily get species. Data from the National Center for Injury Prevention and Control in the United States show that approximately 2 million fires reported each year, which result in 1.2 million people with burn injuries. Moderate to severe burn injuries requiring hospitalization account for approximately 100,000 of these cases, and about 5000 patients die each year from burn-related complications such as infection.[8]

Infection remains as a primary cause of delayed healing and infection in both acute and chronic wounds.[6] Moreover, bacteria can successfully form biofilms on medical devices and implants, leading to additional infection concern. Currently, the local wound infection is addressed by early surgical debridement and skin grafting,[6] topical and prophylactic antibiotics,[6] an enzymatic detachment of biofilms,[25] immunoprophylaxis, and immunotherapy,[60] photodynamic therapy,[22] hyperbaric oxygen therapy,[6] or vacuum-assisted wound closure.[6] However, in many cases, especially with the emergence of multidrug-resistant strains of bacterias,[34] these methods are not efficient and therefore additional means of disinfecting wounds are clearly needed. To address the problem, we recently proposed to use pulsed electric fields (PEF) technology for wounds and surgical mesh disinfection for drug-resistant strains.[16]

PEF is an emerging medical technology currently used for tissue ablation by irreversible electroporation,[11] cancer treatment by electrochemotherapy[38] and gene electrotransfer.[35] The effect of PEF on cells can be explained by the induced change in biological

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membrane permeability, a phenomenon known as electroporation.[27,33] Current consensus describes electroporation as the formation of aqueous pores in the lipid bilayer that enable molecular transport.[27,55,63] The theory of aqueous pore formation, based on thermodynamics, describes the formation of aqueous pores as started by penetration of water molecules into the lipid bilayer of the membrane, which leads to reorientation of the adjacent lipids with their polar headgroups towards these water molecules.[63] The chemical thermodynamic concept for membrane electroporation was recently critically revisited.[33]

In wound healing, PEF has been used in experimental models for skin rejuvenation,[18] scar treatment[20] and genetic engineering to enhance expression of healing enhancing factors.[66] Based on previous exhaustive work on bacteria inactivation in food systems, we proposed to use PEF for wound and implant disinfection.[16] Application of NanoKnife, a clinically available device for tissue irreversible electroporation, was recently shown to effectively eradicate microorganisms.[26]

A key element for the development of PEF protocols for disinfection is a pulse generator, which can provide sufficient flexibility to allow for determining the critical protocol parameters such as the minimum required electric field strength, pulse number, duration, and frequency, that are necessary for developing technologies and technical systems for wound disinfection.[4,48] Several designs have been proposed for the laboratory scale systems in the previous studies.[24,36,41,42,47,48,51,52,56] The drawbacks of the available commercial systems[42,47] are their costs, limitation on supplied current and voltage, and inability to modify hardware when needed.

The goal of this work is to design and develop laboratory scale device, which will allow gaining the data that are necessary for clinical systems for wounds disinfection with PEF. The developed prototype is designed to determine the required electric field strength (E_c), pulse duration (t_P), the number of current pulses acting on the biomass (N) and of pulse repetition rate (f) for disinfection. The system performance was optimized for a model multidrug-resistant organism *Pseudomonas aeruginosa* PAO1, commonly found in burn wounds,[59] on the gel. Concentric electrode system allowed for a single-step determination of the critical electric fields required for complete bacteria inactivation.[5,13]

MATERIALS AND METHODS

Inoculum

Pseudomonas aeruginosa PAO1, kindly provided by Prof. Eliora Ron, taken from − 80 °C storage, was refreshed first on electroporation-low salt media (ELS) solid agar at 25 °C overnight. The used ELS media composition was as follows: 0.1 mg mL^{-1} NaCl (Merck, Israel), 0.01 g mL^{-1} Bacto-tryptone (Academia, Israel), 0.005 g mL^{-1} Yeast extract (BD-extract of autolyzed yeast, Israel), 0.015 g mL^{-1} Agar (Bacteriological Agar-Academia, Israel), 0.5 mg mL^{-1} Glucose –D + (Sigma-Aldrich, Israel), 0.0239 g mL^{-1}. HEPES buffer (HEPES 100G-H buffer-Sigma-Aldrich, Israel). The reagents were dissolved in the double distilled water and autoclaved (instrument) for 30 min in 121 °C. Each plate was filled with 10 ml of ELS media. After the refresh of the bacteria on the solid ELS agar, we prepared a liquid starter, using a single colony cultured in 2 mL of liquid ELS (same composition as solid but with no addition of agar) at 32 °C and 150 rpm for 8 h. One hundred μL of liquid starter with OD of 0.1925–0.195 (measured with Tecan infinite M200 PRO with 600 nm wave) and pH of 7 were spread on solid ELS agar with Dregalski stick and cultivated at 32 °C for 8 h before electroporation experiments.

PEF System

Concentric ring electroporation as described by Fernand *et al.*[13] was used. The concentric electrode design creates a gradient of the electric field from the center outwards to the periphery, allowing for screening the effect of multiple electric fields in a single experiment.[5] The local electric field strength at each point is described using Eq. (1) as follows:

$$E(r, V) = \frac{\Delta V}{r \cdot \ln\left(\frac{R2}{R1}\right)}, \quad (1)$$

where E (V mm^{-1}) is the field strength, r is the distance from the center of the central electrode, ΔV (V) is the potential difference between the central and peripheral electrodes. $R1$ (mm) is the radius of the inner electrode and $R2$ (mm) is the radius of the outer electrode. In this study, $R1$ was 0.75 mm and $R2$ was 11.95 mm. Currents were measured using a PicoScope 4224 Oscilloscope with a Pico Current Clamp (60A AC/DC). The voltage was measured with PicoScope TA044 70 MHz 7000 V differential oscilloscope probe 100:1/1000:1. Currents and voltages were analyzed with Pico Scope 6 software (Pico Technologies Inc., UK).

Imaging and Measuring with Image-J Software

The digital image of each experiment was captured by Binocular (Leica M420) and analyzed by Image-J (ver. 1.6.0, NIH). The disinfected area with a radius, r_c

(mm), the radius from the center where no bacteria growth was observed, was measured at least at 4 different points. The average of measured radii was taken and used for the calculation of threshold of the electric field, E_c, required for complete eradication of bacteria as follows:

$$E_c(N, t_p, T) = \frac{\Delta V}{r_c \ln\left(\frac{R2}{R1}\right)} \quad (2)$$

where N is the number of pulses, T is the interval between pulses.

The energy delivered for a single treatment was calculated as in Eq. (3)

$$J = V \cdot t_p \cdot N \cdot I, \quad (3)$$

where J (Joule) is the invested energy, V is the measured voltage (V), t_p (s) is the duration of the pulse, N is the number of pulses and I (A) is the measured current.

Statistical Analysis

Microsoft Excel 2016 (Microsoft, WA) and GraphPad Prism 5 (GraphPad Software, CA) were used to analyze data. The pairwise comparison was done using Student's t test, one tail. Multiple groups were compared using 1 way ANOVA multi-variant (Newman-Keuls Multiple comparison test).

RESULTS

IGBT-Based Pulse Generator for Pulsed Electric Field Inactivation of Bacteria

A custom made pulsed electric field generator was developed for disinfection protocols development. The generator provides at a maximum voltage of 1000 V and current of 160 A at the 5 Ohm load. The maximum pulse duration, the number of pulses and pulse frequencies are limited by the permissible heating of the IGBT transistors. In our system, described below, for 5 Ohm load and 1 Hz pulse repetition rate the maximum pulse duration is 100 μs. The dependence of the power dissipated on transistors on the magnitude of the current is quadratic. Therefore, a current reduction of two times allows increasing the duration of pulses or the frequency of their repetition by a factor of four.

The designed high voltage pulsed fields generator provides the following functions: (1) electrical connection to external electrodes; (2) application of pulsed fields to the external variable impedance; (3) regulation of the voltage value, duration, and frequency of high voltage pulses within the specified limits during in the microcontroller controlled application mode; (4) regulation of the high voltage value, number and duration of pulses in the testing mode; (5) limiting the charging current of the energy storage capacitors; (6) limiting the pulse currents for safety; (7) measurement of the voltage drop across the energy storage capacitors for each discharge pulse to allow for calculation of the current and energy values for the electroporation treatment.

The functional circuit diagram of the developed pulsed generator is shown in Fig. 1. The main functional nodes of the system include: (1) energy storage capacitor (ESC) with a capacity of 50 μF for voltage 1.25 kV; (2) high-voltage source of charge of energy storage capacitors; (3) parallel-connected high-voltage switches for pulsed discharge of ESCs; (3) driver of high-voltage switch with electrical circuits of control of transistors gates and own power supply; (4) high-power current-limiting resistors; (5) circuit node for manual control of high-voltage switch and high-voltage–power supply in testing mode; (6) microcontroller for controlling the process of PEF treatment, calculating the current at the treated biomass, and transferring the results of calculations to the computer for writing to the experiment file; (7) low-voltage–power supply for control circuits and fans of the device. The device is connected by a USB interface to a computer for input the experiment parameters in the microcontroller, displaying the current state of the process and record the received data in the experiment file.

Parallel-connected high-voltage switches for the pulsed discharge of ESC on electroporated biomass, driver of the high-voltage switch with electrical circuits of control of transistors gates and own power supply and a block of high-power current-limiting resistors are structurally integrated into the switching module. In accordance with the output parameters for the developed device, a pair of IGBT transistors IXYN120N120C3 (IXYS, CA) with parameters of 1200 volts, 120 A are chosen for the application of PEF bacteria inactivation. The choice of the driver, required to control the gates of the switching transistors, is determined by the minimum voltage and current of both IGBT gates of the transistors. Gate Driver Optocoupler FOD3184 (Fairchild, CA) was selected for this device.

The high sensitivity of the IGBT to short-circuits was addressed by the sequential inclusion of IGBT transistors current-limiting resistors with low inductance. DC–DC converter ITB0515S for voltage 5/15 V with high-voltage insulation between primary and secondary voltage circuits was used for power supply of driver and gate circuits of IGBT transistors. Current-limiting resistors must be connected to the emitters of each of the transistors. Their resistance is

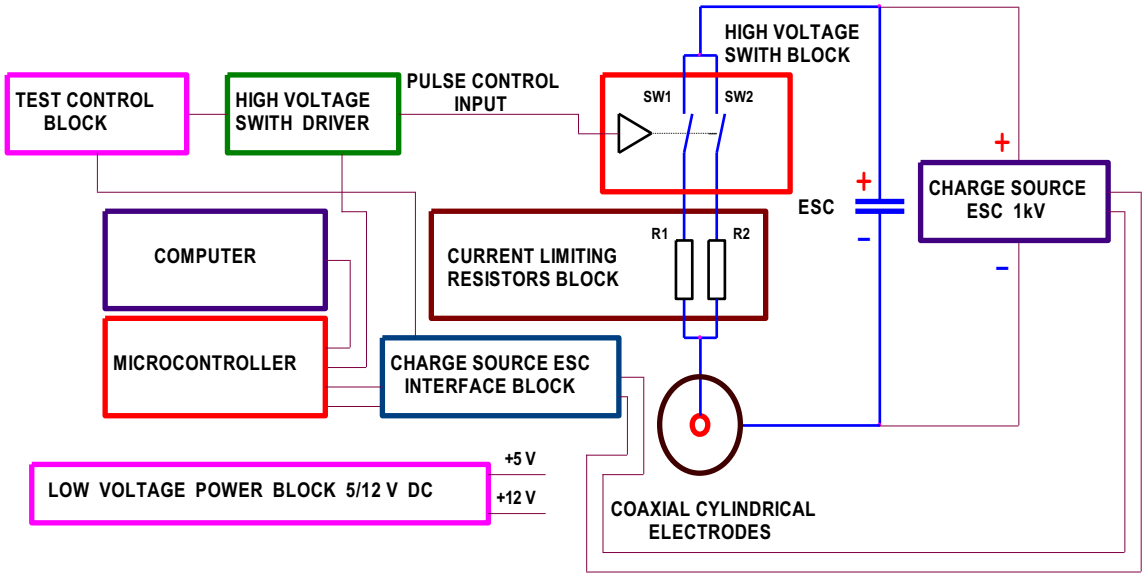

FIGURE 1. Schematic design of the functional units of the pulsed electric field generator.

determined by the value of a single permissible current pulse. For the transistor IXYN120N120C3, it is equal to $I_{CM} = 700$ A. Therefore, the resistance of the current-limiting resistance (R_I) in the circuit of each of the IGBT transistors is calculated according to Ohm's law as $R_l = U_m/I_{CM}$ or 1.429 Ohm for this system, where U_m (V) is the maximum allowed operation voltage. The required dissipation power (P_R) of the current-limiting resistance is determined on the basis of the maximum pulse current of each of the transistors ($I = 80$ A), the current limiting resistance ($R_l = 1.5$ Ohm, the closest available resistor), the pulse duration ($t_i = 100$ μs) and the pulse repetition rate ($F = 10$ Hz):

$$P_R = I^2 \cdot R_l \cdot t_i \cdot F = 9.6 \text{ W} \quad (4)$$

A matrix of $4 \times 2 = 8$ resistors of the type RR02-3 OHM-2W with a total resistance of 1.5 Ohms and a total power dissipation $P_R = 2 \times 8 = 16$ W was assembled. These are low-inductance metal-film resistors. The total power dissipation of two such matrices is $P_R = 2 \times 16 = 32$ W, and their inclusion in parallel branches determines the resistance $R_L = R_l/2 = 0.75$ Ohm. Then, with a pulse duration of $t_i = 100$ μs and a pulse current of $I = 160$ A, the permissible pulse repetition rate of the device will be

$$F = P_R/I^2 \cdot R_L \cdot t_i = 16.666 \text{ Hz} \quad (5)$$

At the maximum pulse current, $I = 160$ A, the resistance of the discharge circuit must be at least $R = 1000/160 = 6.25$ Ohm. From this resistance R, $R_L = 0.75$ Ohm is the resistance of current limitation. Therefore the minimum efficiency of the device will be

$$\eta = (1 - (R_L/R)) \times 100\% = (1-(0.75/6.25)) \times 100\% = 88\% \quad (6)$$

The universal charging source CCM1KW (Spellman, NY) was selected and used in the device is designed for the process of controlled ESC charge. The device provides an interface to measure the voltage on the charged capacitor. The voltages from charging sources were used as an input for the microcontroller for recording and for the control panel voltmeter for visual control. Using the current shape, voltage measurement on the ESC and internal resistance of the power supply, it is possible to calculate both the applied energy. One of the limitations of this specific study was the use of the current probe with a declared by a manufacturer bandwidth of 20 Hz. However, our studies on external resistors showed the measured current values correspond to the predicted values. Nerveless, for studies where the high resolution of voltage (current) front and back fronts are needed, a more sensitive current probe is needed. The complete developed experimental setup is shown in Fig. 2.

The safety during the experiments is provided by placing the samples in a closed high-voltage chamber with high-voltage blocking and discharging of the energy storage capacitor when the chamber is opened. In abnormal situations during the experiments, it is possible to stop the pulse series with the software by clicking on the "STOP" button or directly by switching the "TEST/WORK" switch on the front panel to the "TEST" position. There are also options for stopping the experiment in emergency situations by turning off

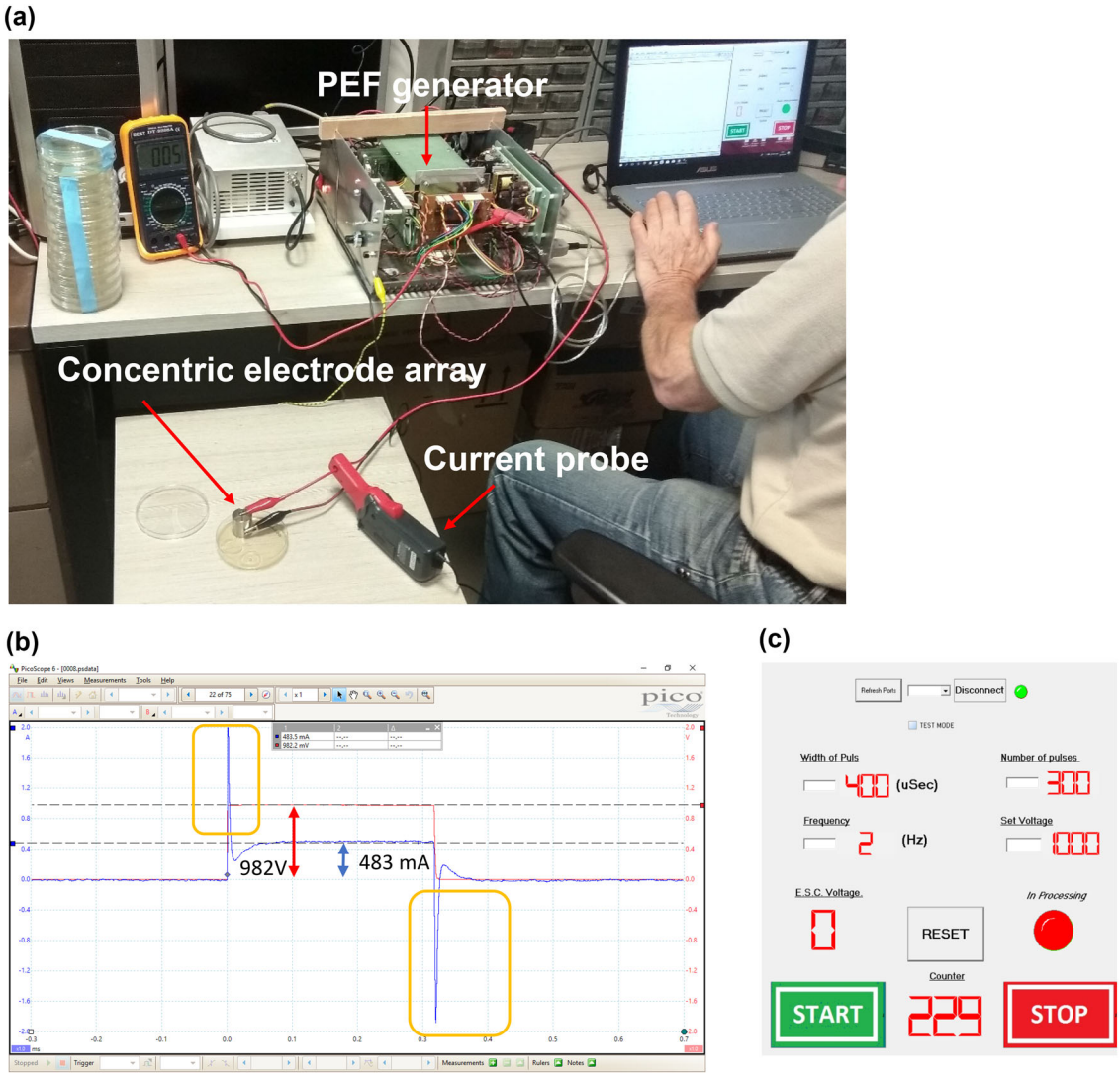

FIGURE 2. (a) Digital image of the experimental setup. (b) The current and voltages shape of the single pulse. Spikes, caused by capacitance and inductance are highlighted. (c) Control software screen snapshot.

the high-voltage charge source of the energy storage capacitor by pressing the "STOP HV" button or by completely turning off the device with its general switch.

Determination of PEF Parameters for P. aeruginosa PAO1 Disinfection

To demonstrate the applicability of the device for disinfection, we investigated the effects of various parameters (Table 1) as delivered by this device on *P. aeruginosa* PAO1. Using a concentric electrode design we determined the E_c for each experiment (Table 1, Fig. 3a).

We found the lowest electric field required for complete eradication of bacteria was 89.28 ± 12.89 V mm^{-1} when 200 pulses with a duration of 300 μs were applied at 3 Hz (Table 1, Fig. 3a). This is equivalent to the 3.48 ± 0.50 mm, Area of 34.36 mm^2. The completely disinfected area which can be achieved with a single central needle (Figs. 4a and 4b, Table 2).

For the same pulse duration at a constant frequency, increasing the number of pulses significantly reduced the E_c (Fig. 3b) and increased the disinfected area (Fig. 4b, Table 2). For pulses with a duration of 200 μs, increasing the number of pulses from 100 to 150 reduced the E_c from 310.36 ± 21.14 to 274.89 ± 19.92 V mm^{-1} (p val 0.007 in Table 3) and increased A from 9.75 to 11.02 mm^2 (p val 0.007). Further increase in the number of pulses from 150 to 200, reduced the E_c to 237.06 ± 12.06 V mm^{-1} (p val 0.000) and increased A to 12.74 mm^2 (p val 0.000). For pulses with a duration of 250 μs, increasing the number of pulses from 100 to 150 reduced the E_c from

TABLE 1. List of PEF parameters and the E_c for square shape pulse wave form. Reported data followed the recommendation in Cemazar *et al.*[7] and Raso *et al.*[46] $n = 6$ for each experimental point.

Experiment number	Voltage (V)	Pulse duration (μs)	Number of pulses	Pulse Repetition Frequency (Hz)	Total energy input (Joule)	E_c (V mm^{-1})
1	1000	200	100	2	17	310.36 ± 21.14
2	1000	250	100	2	24.79	282.78 ± 19.29
3	1000	300	100	2	31.68	267.83 ± 23.44
4	1000	200	150	2	26.7	274.89 ± 19.92
5	1000	250	150	2	35.5	218.04 ± 22.33
6	1000	300	150	2	46.27	173.07 ± 19.43
7	1000	200	200	2	40	237.06 ± 12.06
8	1000	250	200	2	52.08	168.30 ± 28.52
9	1000	300	200	2	58.8	122.91 ± 14.06
10	1000	200	100	3	18.63	297.64 ± 7.03
11	1000	250	150	3	35.5	184.57 ± 17.25
12	1000	300	200	3	74	89.28 ± 12.89
13	1000	300	200	1	51.87	180.34 ± 30.95
14	1000	300	200	4	66.47	82.12 ± 12.46

282.78 ± 19.29 to 218.04 ± 22.33 V mm^{-1} (p val 0.000) and increased A from 10.71 to 13.94 mm^2 (p val 0.000). Further increase in the number of pulses from 150 to 200, reduced the E_c to 168.30 ± 28.52 V mm^{-1} (p val 0.004) and increased A to 18.29 mm^2 (p val 0.004). For pulses with a duration of 300 μs, increasing the number of pulses from 100 to 150 reduced the E_c from 267.83 ± 23.44 to 173.07 ± 19.43 V mm^{-1} (p val 0.000) and increased A from 11.33 to 17.59 mm^2 (p val 0.000). Further increase of the number of pulses from 150 to 200, reduced the E_c to 122.91 ± 14.06 V mm^{-1} (p val 0.000) and increased A to 24.79 mm^2.

For the same number of pulses at a constant frequency, increasing the pulse duration we show that for 100 pulses group, increasing duration from 200 to 250 μs reduced the E_c from 310.36 ± 21.14 to 282.78 ± 19.29 V mm^{-1} (p val 0.020) and increased A from 9.75 to 10.71 mm^2 (p val 0.020). Further increase of the pulse duration from 250 to 300 μs reduced the E_c to 267.83 ± 23.44 V mm^{-1} but was not significant (p val 0.128), with A of 11.33 mm^2. For a group with 150 pulses, increasing duration from 200 to 250 μs reduced the E_c from 274.89 ± 19.92 to 218.04 ± 22.33 V mm^{-1} (p val 0.000) and increased A from 11.02 to 13.94 mm^2 (p val 0.000). Further increase of the pulse duration from 250 to 300 μs reduced the E_c to 173.07 ± 19.43 V mm^{-1} (p val 0.002) with A of 17.59 mm^2. For a group with 200 pulses increasing duration from 200 to 250 μs reduced the E_c from 237.06 ± 12.06 to 168.30 ± 28.52 V mm^{-1} (p val 0.000) and increased A from 12.74 to 18.29 mm^2 (p val 0.000). Further increase of the pulse duration from 250 to 300 μs reduced the E_c to 122.91 ± 14.06 V mm^{-1} (p val 0.003), with A of 24.79 mm^2.

For the same number of pulses (300) and pulse duration (200 μs) the frequency of pulse repetition affected the E_c (Fig. 3c, Table 3). Increasing the frequency from 1 to 2 Hz decreased the E_c from 180.34 ± 30.95 V mm^{-1} to 120.91 ± 14.06 V mm^{-1} (p val 0.001). The additional increase from 2 to 3 Hz further decreased the E_c to 89.28 ± 12.89 (p val 0.001). The increase of the frequency from 3 to 4 Hz did not change the E_c significantly (p val 0.175). The reports in the literature on the impact of pulse repetition frequency on electroporation field threshold are contradicting as some studies reported increased effects with the frequency increase,[29,37,61,62] others showed no effect of the frequency[31,44,45] and the third group of studies showed increased effects with a decrease of the frequency.[30,39] Such a broad spectrum of opposing impacts of pulse repetition frequencies probably implies that additional factors, such as pH, temperature, salinity, osmotic pressure, and others,[17] affect the impact of frequency change on electroporation field threshold. Clinically, increasing of pulse frequency repetition was shown beneficial as it decreases the pain and muscle contractions.[3,44]

DISCUSSION

Pulsed electric fields is a non-thermal, chemical-free technology with multiple applications in medicine and biotechnology.[67] Currently, there are very few controllable and flexible laboratory devices available, which could provide sufficient voltages and currents for wounds disinfection optimization. Therefore, the goal of this work is to design and develop laboratory scale device, which will allow gaining the data that are necessary for clinical systems for wounds disinfection with PEF. A major component in PEF devices is a high voltage pulsed electric field generator. A basic element of the pulsed electric field generator is a high-voltage switch. The choice of a switch is determined by the

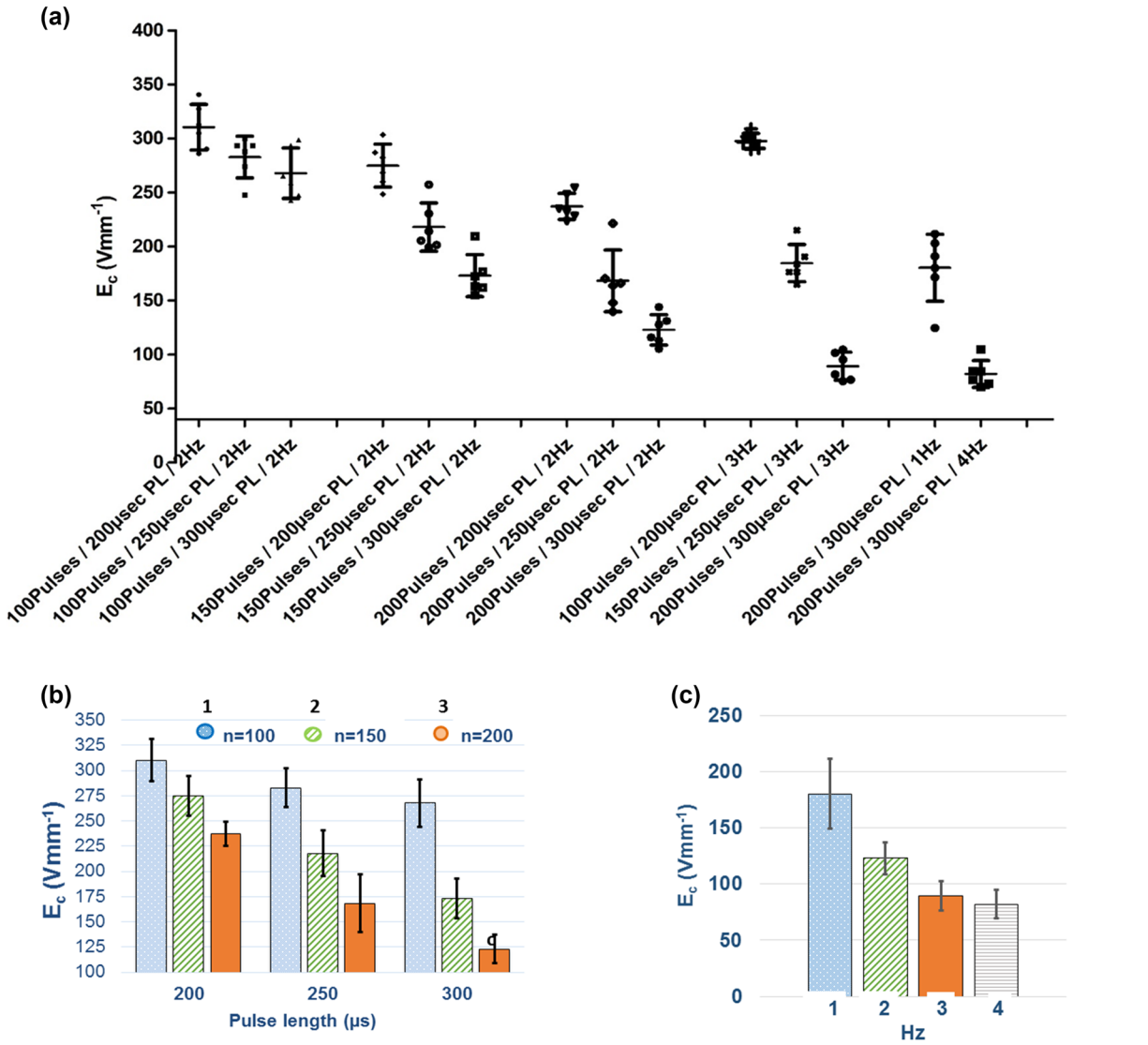

FIGURE 3. The threshold of the electric field strength (E_c) required for complete inactivation of *Pseudomonas aeruginosa* PAO1. (a) Data from all performed experiments. (b) The dependence of E_c on the pulse duration. (c) The dependence of E_c on the frequency of pulse delivery for 200 pulsed with 300 μs pulse duration. For each point $n = 6$.

maximum voltage and impulse current. Preliminary analysis of the maximum rated voltage of high-voltage elements in impulse devices should be at least 20% higher than the working voltage. This is related to the impulse resonance excess of the voltage (spikes), which occurs in the operating mode.

In many of the existing high-voltage pulse generators, which are used for electroporation, gas-discharge and semiconductors devices are used for switching. Advantages of the gas-discharges elements are high working voltage and resistance to current overloads up to short circuits. A significant disadvantage of these elements is the impossibility of flexible (operative) control of the pulse duration and frequency of their repetition. Devices built on gas-discharge devices have large dimensions and weight.

On contrary, semiconductor high-voltage–power elements have more opportunities for controlling the process of pulse formation of different durations, as well as the frequency of their repetition. These include, in particular, IGBT transistors[51] used in the system developed here. In comparison with gas-discharge devices, IGBT transistors have lower operating voltages. As in most laboratory experiments, as well for most of the medical applications, including wound healing, PEF treatment does not require a voltage above 1000 volts, then the use of IGBT transistors is preferable.

The shortcomings of IGBT transistors include their low resistance to short-circuit currents. With a possible

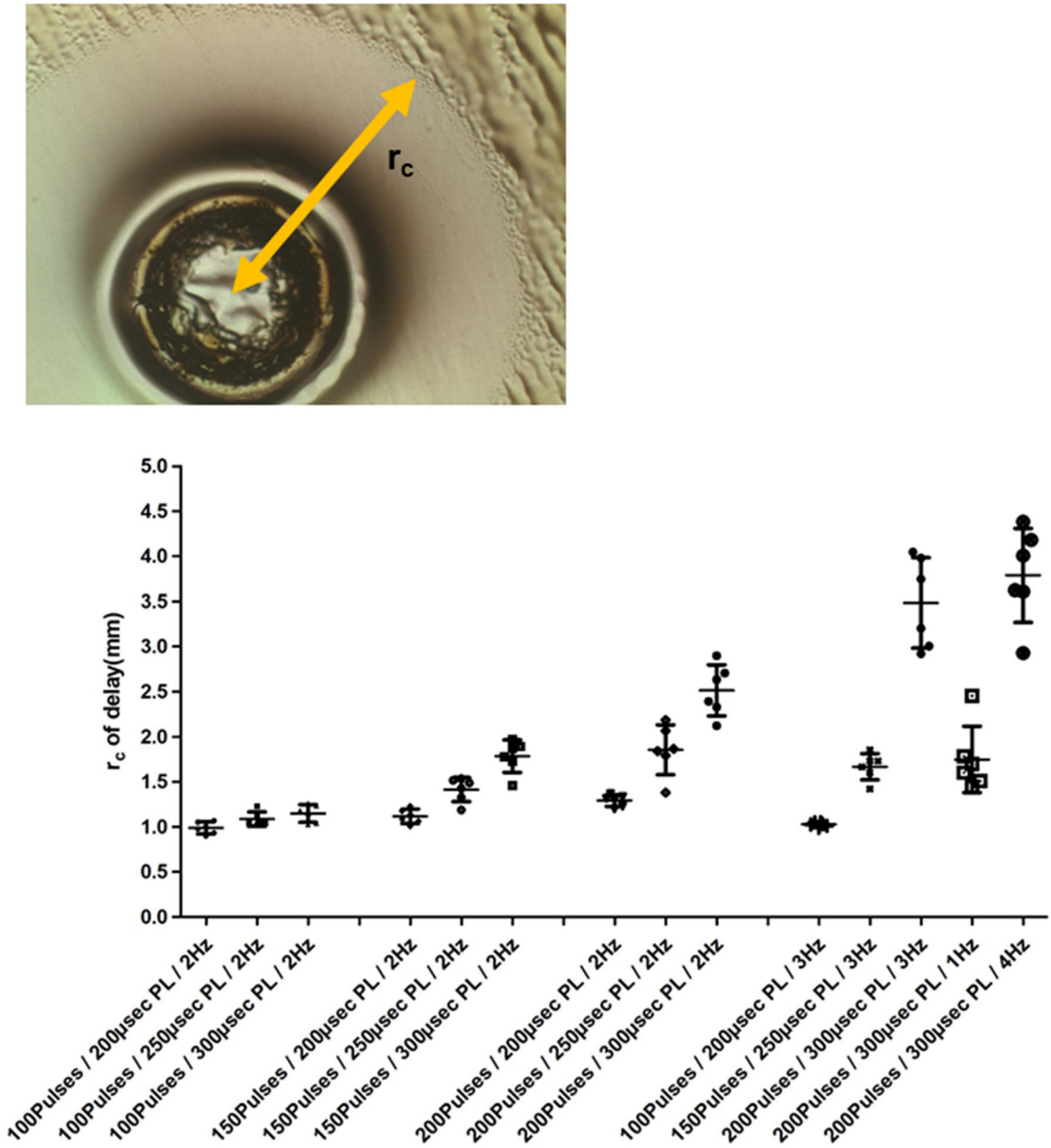

FIGURE 4. Disinfected area by pulsed electric field delivered with a single needle. Top panel: digital image of a sample gel that shows the disinfected area. Bottom panel: The dependence of the disinfected area on the applied pulsed electric field parameters.

electrical breakdown of tissue during its electroporation, a short-circuit current might occur. Such a short current can destroy the transistor. Therefore, it is necessary to select as switching elements the transistors with as large a permissible pulse current. In addition, active or passive current limiting devices are needed to protect the used switching elements from failure. The simplest and most reliable in performance and working is passive current limiting. It is implanted in the developed in this work device by sequential inclusion in the circuit of emitters of IGBT transistors current-limiting resistors with low inductance.

The developed IGBT based pulsed generator which provides the output PEF: Voltage range (0–1000 V), currents (0–160 A) on 5 Ohm load, was tested for inactivation *in vitro* of a clinically relevant multidrug resistant *Pseudomonas aeruginosa* PAO1 using a set of con-centric electrodes. One of the major challenges, we faced in this work was the appearance of current spikes (Fig. 2b) during the application of electric fields. The nature of these spikes is most probably related to the whole circuit capacitance and inductivity, which also changes within and between the experiments. The major problem with spikes is the limited ability, to this

end, to control and predict their behavior. Spikes lead to cell exposure to high amplitude, but very short pulsed electric fields during the duration of a planned pulse. This can affect the threshold of the electroporation, by causing structural changes in the membrane.[23] Previous reports on electric fields burst[53,54] probably is the closest to this phenomenon that are controlled. The authors showed that burst reduces the irreversible electroporation threshold.

Application of PEF on *P. aeruginosa* PAO1 with concentric electrode array allowed for a rapid, single-step determination of the critical electric field, required for complete eradication of the bacteria for given number of pulses, pulse duration, and frequency. The major advantage of the used methods is the elimination of the need for multiple experiments, required to determine the electric field strength required for bacteria inactivation.[13] We show that in the tested range of parameters, increasing the number of pulsed, pulse duration and frequency reduces the critical electric field threshold (Fig. 3). This reduction of the critical electric field threshold with an increase of pulse number and duration is consistent with multiple previous studies on other types of bacteria inactivation, especially in food systems.[1,43,58,65] One of the parameters that were not tested in this work is the size of the central electrode. A previous theoretical study suggested that increasing the contact surface between electrodes and tissue increases the current and thus reduces the critical electroporation field threshold.[9] However, an experimental study showed no difference in ablated areas between 16G/9 cm core biopsy needles with a 2 cm throw length and clinically used 19G monopolar IRE probes.[64] Additional work is needed to optimize the design of the electrodes that can be applied to large infected surfaces.

However, increasing number of pulses, pulse duration, and frequency could also induce additional to irreversible electroporation bacteria inactivation mechanisms, such as electrolysis and generation of various free radicals, which could kill the bacteria alone or in combination with electric fields.[50] Previous studies on bacteria inactivation with PEF that the most energy efficiency strategy is to reduce the number of pulses and to increase the applied electric field strength.[1] Future studies should address the effectiveness and mechanisms of bacteria inactivation where PEF is used alone or in combination with PEF modulated chemical or thermal impacts on cells.

Previous works on inactivation of *Pseudomonas fluorescens* in solution showed that at field strengths > 25 kV cm^{-1}, pulse duration 2 μs number of pulses > 10 and pulse period > 2 s, differences of the waveform, which were affected by the distance between electrodes impacted the most the level of inactivation.[23] Furthermore, switching to bipolarity, allowed the reduction of the critical electric field to 10 kV cm^{-1}.[23]

TABLE 2. Area of the bacteria complete inactivation due to the different electroporation parameters.

Experiment number	R_c (mm)	A (mm^2)
1	0.99	9.75
2	1.09	10.71
3	1.15	11.33
4	1.12	11.02
5	1.41	13.94
6	1.78	17.59
7	1.29	12.74
8	1.86	18.29
9	2.51	24.79
10	1.03	10.13
11	1.67	16.45
12	3.48	34.36
13	1.74	17.23
14	3.79	37.36

TABLE 3. *T* test for experimental groups comparisons.

Experiment	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2	0.020													
3	0.004	0.128												
4	0.007	0.251	0.293											
5	0.000	0.000	0.002	0.000										
6	0.000	0.000	0.000	0.000	0.002									
7	0.000	0.000	0.008	0.000	0.048	0.000								
8	0.000	0.000	0.000	0.000	0.004	0.371	0.000							
9	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.003						
10	0.096	0.053	0.007	0.012	0.000	0.000	0.000	0.000	0.000					
11	0.000	0.000	0.000	0.000	0.008	0.152	0.000	0.130	0.000	0.000				
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000			
13	0.000	0.000	0.000	0.000	0.018	0.318	0.001	0.250	0.001	0.000	0.388	0.000		
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.175	0	

An interesting additional approach for prevention of *Pseudomonas* biofilm formation prevention and inactivation is the application of low voltage, high frequency pulsed electric fields. Different from our approach, this method uses fields at tens of V cm^{-1} delivered at kHz to MHz frequencies.[2,12,14,40] This approach, however, requires much longer time.

Previous *in vivo* work on burns disinfection showed the feasibility for the use of PEF alone in small animals models.[15] Furthermore, we showed a full regeneration of normal rat skin ablated by PEF.[19] However, applications of the PEF technology for human wound healing will require additional safety studies as PEF will affect both bacteria and host cells locally and modulate the immune system response. Rapid release of a large number of bacteria intracellular molecules, such as DNA could lead to innate immune system response,[28] which could lead to secondary damages due to strong inflammation.[32]

Previous clinical trials which assessed irreversible electroporation ablation safety in humans showed the procedure is safe, especially when pulses are electrocardiographically synchronized.[57] In addition, the data on the short and long-term response of human skin to the electric fields with strengths sufficient to inactivate the bacteria are needed. Importantly, previous work on *Pseudomonas putida* inactivation by PEF in hospital wastewater showed no acquired resistance of *Pseudomonas putida* to PEF up to 80 generations, when surviving bacteria were exposed again to the PEF.[21]

To summarize, pulsed electric field wound disinfection is an emerging technology for multiple biomedical engineering applications such as wound healing, tumor ablation, and genetic therapy. Yet, the available today for research devices are expensive and have little flexibility in functionalities. In this work, we developed an IGBT based pulsed generator which provides maximum 1000 V, currents 160 A, on 5 Ohm load. The generator was used for inactivation *in vitro* of a clinically relevant multidrug resistant *Pseudomonas aeruginosa* PAO1. We showed that increasing the pulse duration, pulse number reduced the threshold of the electric field that is required for bacteria deactivation and increased the disinfected by a needle electrodes areas.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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