AC Cell Electroporation Based on Asymmetrical Waveform

M. Folprecht¹, D. Cervinka¹, and V. Novotna¹

¹Brno University of Technology, Czech Republic

E-mail: Martin.Folprecht@vut.cz, cervinka@vut.cz, novotnav@vut.cz

Abstract—This paper discusses an application of different waveforms during a cell electroporation process. Latest scientific papers emphasize advantages of asymmetrical waveform applied to tissue. Higher effect of the electroporation process is reached and muscle contractions are less significant. Most high-voltage generators are based on a controlled discharge of storage capacitors. We present a different solution of the generator, where a power part contains a pulse transformer.

Keywords—Asymmetrical waveform, capacitor discharge, cell electroporation, DC-AC inverter, high frequency, MOSFET transistor, pulse transformer.

1. INTRODUCTION

Cell electroporation is a relatively new, less invasive method of a tissue ablation, which is based on the application of short high-voltage pulses. It can be used for treatment of some diseases, e.g., cancer, cardiac arrhythmia, etc. Older DC electroporation requires unipolar voltage pulses, while modern and more advantageous AC electroporation uses bursts of bipolar pulses. Lower risk of arrhythmia, lower muscle contractions and non-existent electrolysis are the main advantages of this method [1]. Latest studies refer about the use of asymmetrical waveform during this process, that can bring higher effect compared to more common symmetrical waveform. High-voltage generators developed at FEEC BUT contain pulse transformer in their power part. The aim of this paper is to show, that asymmetrical voltage can be produced by generator with step-up pulse transformer.

2. COMMONLY APPLIED WAVEFORMS

Different waveforms can be used for the electroporation process. Some examples are shown in Fig. 1. Exponential pulses (A) characterized by voltage peak $V_m$ and time constant $\tau$ are suitable for inserting of DNA molecules into the cell. The voltage peak in units of kV provides a permeabilization of a cell wall and a low-voltage tail transfers appropriate molecules [2]. These pulses can be up to units of ms long. Square and rectangular pulses are mainly used in clinical practice, because it is easy to control an amount of delivered energy and to reproduce a setup of the equipment [3]. These waveforms are suitable for DC and AC electroporation. In the case of AC electroporation, pulses in individual burst can have different duty cycle. For example BUT AC generator [4] produces bipolar pulses with permanent duty cycle 0.5 (B). In publication [5], authors applied pulses with lower duty cycle (C). Higher electroporation effect can be reached, when asymmetrical pulses are used.

Figure 1: Examples of commonly used waveforms.

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3. ADVANTAGES OF ASYMMETRICAL PULSES

Positive voltage pulse causes an electric intensity with the same orientation as the unipolar pulse during DC electroporation. According to study [6], creation of nanopores in the cell wall depends strongly non-linearly on a membrane voltage. For this reason, it is very convenient to apply higher electric intensity for a shorter time than lower intensity for a longer time. And moreover, a positive value of electric intensity should be higher than a value of intensity in the opposite direction. As the result, positive pulse should be higher and shorter than the negative to reach higher electroporation effect. Area of positive and negative current pulses caused by asymmetrical voltage must be equal to achieve a zero DC component. DC component is undesired due to electrolysis, which leads to creation of gaseous bubbles, that can endanger a patient. Authors of mentioned study observed, that asymmetrical pulses had higher electroporation effect than symmetrical pulses with the same energy transferred during one period at the same frequency. Authors also claimed, that asymmetrical pulses transferred more energy than symmetrical pulses with the same charge. An example of symmetrical and asymmetrical waveform is drawn in Fig. 2 [6].

![Figure 2: Symmetrical and asymmetrical waveform][1]

All labeled charges are the same. Symmetrical pulses (A) have the same lengths $\frac{1}{2}T$ and amplitudes $I_A = -I_A$. Positive asymmetrical pulse has the length of $\frac{1}{4}T$ and amplitude $I_B = 2I_A$. Negative pulse must then have the length of $\frac{3}{4}T$ and amplitude $-I_B = \frac{3}{2}I_A$ so that the negative charge $-Q_B$ is the same as the positive charge $Q_B$. If symmetrical pulses (A) transfer energy $W$ during one period $T$, then asymmetrical pulses (B) transfer energy $\frac{3}{4}W$ during the same time. In order to be able to compare electroporation effect of both waveforms, it is necessary that the same energy or the same charge is transferred during one period $T$. Authors used symmetrical and asymmetrical waveform with frequency of 167 kHz. Symmetrical pulses had amplitudes $\pm 600$ V, while asymmetrical pulses had positive amplitude $+544$ V and negative amplitude $-221$ V. In both cases, 10 bursts with a length of 2 ms were applied and a space between bursts was 400 ms. A total energy $10 \times 20$ J was delivered, but asymmetrical pulses transferred during single period $T$ only 92% of charge of symmetrical pulses. As the result, lesions caused by asymmetrical waveform were deeper than lesions caused by symmetrical pulses [6].

4. USE OF TRANSISTORS IN HV GENERATORS

Transistors are very suitable for high-voltage sources of square and rectangular pulses. These generators have low weight and dimensions. They can be constructed from common parts, so they are relatively cheap and it is possible to develop the generator with a wider range of parameters than is commercially available. The use of power transistors is limited by their maximum drain to source voltage, drain current and especially by their switching times. For this reason, power transistors are not suitable for ns and ps pulses generating. But this claim may not be valid in the near future, because the parameters of power transistors are improving rapidly [2].

There are two main groups of electroporation generators: devices with HV storage capacitors and generators with step-up pulse transformer. The first group is more common, typical example is a commercial NanoKnife device proposed for DC electroporation. Power part of these generators is simple, because voltage pulses are created by the main transistor switch, which connects storage capacitors with application electrodes for a certain time. Rise and fall times of pulses are affected practically only by switching time of the transistor switch. Some generators contain additional transistor,
that grounds the output terminal and cuts off the fall time [7]. But there is a certain risk of pulse with 
uncontrolled length, when the main transistor switch is short-circuited. For this reason, the generator 
must be equipped with some safety switch, that disconnects the patient in the case of the main switch 
malfunction. Safety requirements also lead to increase of demands on control circuits [2]. Storage 
capacitors are charged from rectified AC mains through voltage regulator, namely isolated DC-DC 
converter, where the pulse transformer provides a galvanic isolation of the patient. Typical block diagram 
of HV generator with storage capacitors can be seen in Fig. 3. Marx generators, where spark gaps are 
replaced by power transistors, can be also included in this group. This topology produces unipolar pulses 
suitable for DC electroporation. For AC method, a pair of generators is necessary [8].

![Figure 3: Typical block diagram of generator with storage capacitors.](image)

DC-DC converter or DC-AC inverter with step-up pulse transformer is another solution of generators 
for electroporation. The transformer provides the galvanic isolation of the patient from AC mains and its 
turns ratio determines maximum output voltage. Storage capacitors are placed into a DC bus at the power 
part. Low-voltage electrolytic capacitors also consume less space than high-voltage film capacitors in 
generators without step-up transformer, if we consider the same amount of stored energy. Mentioned risk 
of uncontrolled discharge of capacitors is completely eliminated here, because when the power transistor 
fails, DC bus is short-circuited. This is the main feature of all generators developed at FEEC BUT. The 
main disadvantage of this solution is a leakage inductance of the transformer, which makes rise and fall 
times of pulses longer compared to devices with storage capacitors. Parasitic capacitance of windings 
is another problem. But excellent security for the patient can be seen as the main advantage in terms 
of future certification. Typical block diagram of generator with step-up pulse transformer is shown in 
Fig. 4. Secondary HV rectifier in dashed block is used only in generators for DC electroporation [9].

![Figure 4: Typical block diagram of generator with pulse transformer.](image)

5. GENERATOR OF ASYMMETRICAL WAVEFORM WITH PULSE TRANSFORMER

Pulse transformer is a common part of DC-DC switch mode supplies or DC-AC inverters. At these 
devices, symmetrical pulses are usually transformed. But transformer is also able to transform 
asymmetrical waveform, when certain conditions are met. Flux linkage $\Psi$ (magnetic flux in core $\Phi$) 
is a time integral from primary voltage $v_1$ (1) [10].

$$\Psi(t) = N_1 \Phi(t) = \int v_1(t) \, dt$$

(1)

$N_1$ is a number of primary turns. Primary voltage pulses $v_1$ are rectangular. Then flux linkage $\Psi$, 
magnetic flux $\Phi$ and also magnetizing current $I_p$ have triangular shape. To avoid a saturation of the 
transformer core, the value of integral from positive pulse $+V_1$ must be equal to the value of integral from 
negative pulse $-V_2$. If we consider, that the pulse period $T$ consists of positive pulse $t_1$ and negative pulse 
time, then areas of both pulses must be equal (2) [10].

$$V_1 \, t_1 = -V_2 \, t_2$$

(2)
If this condition is met, primary voltage $v_1$ does not contain a DC component, which would cause the saturation of the core. Power part of the generator requires two independent DC buses with different voltages $V_1$ and $V_2$ (see Fig. 5). Each DC bus is created from electrolytic capacitors, which are charged from rectified AC mains through voltage regulator. Primary voltage $v_1$ is positive, when both transistors $T_1$ and $T_4$ are switched on and negative, when transistors $T_2$ and $T_3$ in opposite diagonal are switched on. Secondary voltage $v_2$ has the same shape as primary voltage $v_1$, but its amplitudes are different with turns ratio $\frac{N_2}{N_1}$. Primary current $i_1$ is a sum of secondary current $i_2$ transformed to primary side and magnetizing current $i_{\mu}$. Capacitor $C_1$ eliminates the DC magnetization of the transformer, when the DC component of primary voltage $v_1$ occurs. Snubber $R_2$, $C_2$ is connected to the secondary winding [10].

Load is represented by resistor $R$, but real tissue has a resistive-capacitive character. Charging of capacitive component of the load causes undesired current peaks and reaction of an overcurrent protection. For this reason, variable inductors $L_3$ and $L_4$ are connected between secondary winding and output terminals. These inductors suppress capacitive current peaks and also limit capacitive current, which flows from the mains through the parasitic capacitance of the transformer and body of the patient to the ground. Because the load resistance is changed during the electroporation process and depends on many factors, such as area of electrodes, tissue conductivity, etc, inductors are variable. They can also be totally short-circuited [4].

![Figure 5: Circuit diagram of DC-AC inverter.](image)

Both voltage regulators in DC buses must be controlled by control unit with microprocessor. In terms of dynamics, it is faster to change times $t_1$ and $t_2$ than amplitudes $V_1$ and $V_2$. For this reason, the user sets pulse frequency $f$ (pulse period $T$), positive amplitude $V_1$ and length of the positive pulse $t_1$. Then the processor unit calculates remaining values $V_2$ and $t_2$. Both voltages $V_1$ and $V_2$ are permanently measured and the processor must be able to modify the time $t_2$ immediately, when some voltage deviation occurs. Only then the zero DC component of primary voltage $v_1$ is achieved. Primary current $i_1$ is sensed to protect power transistors $T_1$–$T_4$. When it exceeds its maximum value, power part is disabled. It is obvious, that demands on the processor unit are high. The user can also set the length of the burst, the number of bursts and the space between them. All significant values will be displayed on touch screen. New generator will be designed as a compact portable device, which will be able to be used in various research institutes.

6. CONCLUSION

This paper is focused on possible solution of HV electroporation generator, which is able to produce asymmetrical waveform. Application of asymmetrical voltage pulses is the latest trend at research of AC electroporation. Asymmetrical pulses have higher effect than symmetrical pulses with the same energy. Most of HV generators are based on controlled discharge of storage capacitors into the tissue. This solution is simple, but there is a certain risk of uncontrolled discharge of the capacitor, when the transistor switch is short-circuited. We present different generator, where pulse transformer is used and where mentioned risk is eliminated. Pulse transformer can transform asymmetrical voltage pulses, when the integral from positive voltage is the same as the integral from the negative voltage. This requirement places the great demands on the control circuits, but excellent safety for the patient outweighs this disadvantage and facilitates any future certification.
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