Review

Concepts and Capabilities of In-House Built Nanosecond Pulsed Electric Field (nsPEF) Generators for Electroporation: State of Art

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Abstract: Electroporation is a pulsed electric field triggered phenomenon of cell permeabilization, which is extensively used in biomedical and biotechnological context. There is a growing scientific demand for high-voltage and/or high-frequency pulse generators for electroporation of cells (electroporators). In the scope of this article we have reviewed the basic topologies of nanosecond pulsed electric field (nsPEF) generators for electroporation and the parametric capabilities of various in-house built devices, which were introduced in the last two decades. Classification of more than 60 various nsPEF generators was performed and pulse forming characteristics (pulse shape, voltage, duration and repetition frequency) were listed and compared. Lastly, the trends in the development of the electroporation technology were discussed.

Keywords: electroporation; electroporators; nanosecond pulsed electric fields; nsPEF; pulsed power devices; high frequency; MOSFET; capacitor discharge; Blumlein; high-voltage generators

1. Introduction

High intensity pulsed electric fields (PEF) can trigger increased permeability of biological cells to exogenous molecules to which the cells were initially impermeable [1]. PEF polarizes the cell membrane, which causes reorientation of lipids and formation of pores [2,3], thus increasing molecular transport across the cell membrane [4,5]. In case of reversible electroporation (depends on pulse parameters), the cell membrane is then resealed [6]. As a result, there is a variety of electroporation applications, which include cancer treatment [7], gene delivery [8], food processing [9], biorefinery [10] and many other [11,12]. In each case, various PEF parameters are required, which establishes a challenge in generator design in terms of universality. The straightforward distribution of applications in the pulse amplitude–duration space is summarized in Figure 1 [13–19].

It can be seen that the majority of applications lie in the micro-millisecond range. Indeed, the range of longer but lower amplitude pulses dominated the field for decades, however, in the recent years the number of works focusing shorter (nanosecond) pulses increased significantly. The reason lies in the limitations of conventional microsecond range methodologies such as bioimpedance-dependent field distribution [20], Joule heating [21], muscle contractions [22], electrical breakdown [23] and oxidative stress [24]. Nanosecond pulses cannot solve all the problems completely, however, in many cases the negative factors are diminished. However, state-of-art pulsed power electronics are required for generators to form pulses up to tens of kV and hundreds of amperes in the nanosecond range. Therefore, the price and engineering complexity of such generators is high, nevertheless, the trends in applications of nanosecond PEF make it viable (Figure 2).

Figure 1. Generalized representation of pulse parameters for different electroporation applications.

Figure 2. The trends in the number of publications on the topics of nanosecond electroporation according to Clarivate Analytics Web of Science (25 May 2020).

As it can be seen in Figure 2, there is a definitive rise in publications in the last decade, which partially is a consequence of better availability of pulse forming switches on the market. Development of laboratory grade generators for electroporation has intensified with the development of better semiconductors (i.e., silicon-carbide technology) [25]. Nevertheless, the number of papers focusing nanosecond pulse generators for electroporation is still low. A similar trend is observed in the frontier of short microsecond or sub-microsecond pulses using high frequency bursts (Figure 3), which possibly is a natural transition step from long micro-millisecond to the nanosecond range.

For the optimal effectiveness of the treatment many electroporation parameters must be adjusted, i.e., the pulse amplitude and duration, repetition frequency, waveform and number of pulses. As a result, universality of electroporators is important when a study of new phenomena and biological effects of PEF are established.

Figure 3. The trends in the number of publications on the topics of high frequency electroporation according to Clarivate Analytics Web of Science (25 May 2020).

There is high diversity of techniques used for the development of the PEF generators for electroporation applications. Some are well commercialized and a first comprehensive list of commercially available electroporators has been collected in 2004 [26], which was later updated in 2010 [15], 2017 [27] and 2019 [28]. It can be seen, that commercial approach had followed the most common demands of electroporation application. As a result, most of the commercial electroporators were developed with the pulse amplitude up to few kV and duration from tens of μ s to seconds. In comparison, the selection of commercial high-voltage nanosecond pulsed electric field (nsPEF) electroporators is significantly lower [27,28].

Additionally, it is common, that the commercial devices have a limited range of available pulse durations and repetition frequencies, thus the device applicability is non-flexible. This is one of the main reasons, why the commercial electroporators are also not perfectly suited for electroporation research applications, where a wide parameters selection is desired. To sum up, there is a growing scientific demand for nanosecond and high-frequency electroporators, which can, but are not limited, to deliver sub-microsecond range adjustable energy pulses. To cover the demand, the researchers develop in-house electroporators [29–33]. While the up to date review of commercial available PEF generators is available [27], there is no (at our knowledge) extensive overview of in-house built nanosecond and high-frequency electroporators. Currently, the works are mainly focused on the review of the general topologies used for generator design [28,30,34–40].

In the scope of this article, we reviewed the scientific papers reporting the developed nsPEF generators for electroporation in the last two decades. In addition, this article includes a summary and comparison of techniques used for the development of the PEF generator and the classification of generators is proposed based on the pulse forming circuit design. In total more than 60 in-house built nsPEF generators for electroporation applications were found, classified and compared.

2. Basic Generator Topologies

For electroporation research it is essential to have a pulse generator suitable to deliver controllable energy to biological tissue via application of repetitive electrical pulses with the predefined pulse waveform, voltage amplitude and pulse widths. The pulse forming circuits of the electroporator can be based on relatively simple circuits, which are discussed in the following works [15,26–28,34,38–42] and summarized in this section. The energy delivery can be provided by capacitor, inductor or transmission lines with the control of the switching element. These circuits can be enhanced by the application of

modular approach, application of transformers, diodes or even by merging two concepts into a hybrid one. In this section, the common designs of pulse forming circuits used for electroporation application are presented and discussed.

2.1. Pulse Forming Using Capacitor Discharge Circuits

A direct capacitor discharge pulse forming circuit concept is one of the most common, simple and oldest concepts for PEF pulse forming [15,43]. It is based on the transfer of energy stored in the capacitors into the load throughout well-defined voltage pulses. The pulse delivery is normally controlled with a semiconductor switch. Depending on the switch type or driving mode, the waveform is either rectangular (using metal–oxide–semiconductor field-effect transistors (MOSFETs) and insulated-gate bipolar transistors (IGBTs) [44]) or exponential decay wave (older concept typically used with thyristors [45]). In case of exponential decay pulses, the duration of the pulse (decay time) is defined by RC parameters of the discharge circuit, while in the case of rectangular pulses the parameters are mostly limited by the switch capabilities and the discharge capacitor value. The principle pulse forming circuit of capacitor discharge pulse generator is represented in Figure 4.

Figure 4. The principle circuit of a direct capacitor discharge pulse generator.

The pulse generator is composed of a variable high-voltage power supply V , a discharge capacitor C , a switch SW and optionally a resistor R . The direct capacitor discharge topology benefits from a simple and inexpensive design [38,43]. However, to ensure the rectangular wave delivery and limiting the pulse amplitude droop, a high capacity (μF to mF range) capacitor bank is required and the switch must withstand the full voltage amplitude. It limits the selection of available switches in case of high-voltage builds. In such a case, several series switches must be used, which results in increased complexity of the generator due to challenges in switch synchronization.

To overcome the limitations of the direct capacitor discharge concept, a more complex modular circuit topology can be used [46]. It provides voltage distribution between several switches and gives additional flexibility for the output pulse shape and amplitude. A simple example of a modular structure is presented in Figure 5.

The circuit includes several galvanically isolated high-voltage power supplies $V_1, 2, \dots, n$. Each is controlled individually and can be set to a different amplitude. The voltages of the individual circuits contribute to the generation of a single output pulse at any time. The output voltage will be the sum of the voltages from separate stages, so adjusting the output voltage is possible. However, many stages are required to have enough output voltage levels, which consequently increases the cost of the device [38,39].

The Marx generator is an example of a modular circuit, however, due to high applicability is often separated as an independent topology. Originally it was developed to test power grid high-voltage components and provide a capability to produce high-voltage pulses using a low-voltage DC supply. This topology has been adopted for PEF generation and widely used in electroporation applications [30,31]. The simplified circuit is represented in Figure 6.

Figure 5. The principle circuit of modular direct capacitor discharge pulse generator.

Figure 6. The circuit of pulse generator based on Marx topology.

The full Marx topology is based on separate stages/cells, where each stage has an energy storing capacitor C , a resistor R and a switch SW . The stages are charged in parallel from a direct current power supply V and subsequently connected in series with a load when the switches are triggered. In case of spark gaps the capacitors are fully discharged (decaying pulse), however the current and voltage flexibilities are high. For such a design, the pulse repetition rates are frequently in the order of several Hz, but the total charging voltage can reach tens or even hundreds of kV [47–49]. The voltage levels between 50 and 100 kV are most commonly used in industrial application [50–52]. In case when the semiconductor switches are used, a partial discharge of the capacitors can be achieved. The output voltages are frequently determined by the switch breakdown voltage and the number of stages, however, devices in the range of 1–6 kV are common [31,39,53].

In case the bipolar pulses are required to optimize the electroporation applications (e.g., food products treatment) [54,55], the direct capacitor discharge pulse generator concept can be enhanced with half-bridge or full-bridge circuit [56] as presented in Figure 7.

In both cases the generator can produce positive and/or negative high-voltage pulses. However, the half bridge topology has limitations in operation with capacitive-type of loads, hence full-bridge concept is more common. The increase of the number of the switches in a full-bridge concept brings additional operational flexibility similarly to the direct capacitor discharge topology. Moreover, to enhance the pulse control and scalability several half-bridge or full-bridge pulse forming circuits can be stacked in a modular approach [30,32,57]. Additionally, Marx generators can be arranged in a bridge configuration to enable the generation of bipolar pulses [30,31,58].

Nevertheless, independently on the topologies described above the capacitor discharge circuits are limited by switching dynamics of semiconductor devices, thus the minimal pulse durations are typically in the sub-microsecond range. In order to form shorter pulses (i.e., less than 100 ns) other electroporator topologies are required.

Figure 7. The conceptual circuit of bipolar half-bridge (a) and full-bridge (b) direct capacitor discharge pulse generator.

2.2. Pulse Forming Circuit Topology Based on Transmission Lines

The other group of pulse forming circuits is based on the charge and discharge of the transmission lines. This is a common type of circuit topology for generation of high-voltage pulses with less than 100 ns duration [39,59]. As illustrated in Figure 8, the pulse forming circuit is composed of coaxial cable, which is used as a conductor of length l and charged through a resistor R to a voltage V . The transmission line discharge and pulse forming are controlled by the switch SW .

Figure 8. The conceptual circuit of a transmission line pulse generator.

In order to form rectangular pulses the impedance of the load must match the characteristic impedance of the transmission line. However, load matching requirement by design limits the generator's compatibility with dynamic loads since the impedance of the transmission line is dependent on the parameters of the cable.

The pulse amplitude of a single transmission line generator is limited to the half of the power supply voltage, while two or more lines must be used to match the pulse amplitude voltage to the power supply voltage (Figure 9). This type of configuration is known as a Blumlein transmission line pulse generator. Blumlein pulse generator is one of the most popular designs for the generation of rectangular high-voltage nsPEF pulses [60–62]. The generator is based on the two transmission lines of identical length l . Both transmission lines are electrically connected at one end to a load and discharged by closing the switch. If the load is mismatched (i.e., the load resistance is larger or smaller than the transmission line impedance), reflections of the voltage step at the load and at the open end of the transmission line will lead to a strain of consecutive decaying pulses [42,63,64]. Hence, to generate rectangular wave pulse without any pulse reflections, the impedance of the load must be twice the impedance of the transmission line. This is not always an easy task to achieve, since the electrical impedance of biological tissue is often unknown, can vary from sample to sample and can be even dynamic during the pulse delivery [38,65].

Figure 9. The conceptual circuit of a Blumlein pulse generator.

Lastly, the control of pulse duration in a Blumlein circuit is non-flexible. It is defined by the length of the transmission line and the dielectric between the conductors, while the switch-off time determines the rise time of the generated voltage pulse. When the switch is closed, a voltage pulse is generated across the load for the time it takes the voltage step to propagate along the transmission line [42]. The switching element must withstand full high-voltage, which can be a challenge. On the other hand, in case of a spark gap switch, the output amplitudes can reach tens or hundreds of kV and a few kA.

Lastly, the Blumlein generators are large dimension devices and have high demands for the electrical components. The usual concepts have a relatively short lifetime and operate in low repetition rate, while a rectangular wave pulse is typically delivered with a jitter [38,63]. However, with the latest modifications, Blumlein generators can now generate also a high-frequency (range of up to few MHz) output pulses with variable duration, amplitude and even polarity [38,60,63,66].

2.3. The Inductive Energy Discharge Pulse Generators

The third group of pulse generators is based on inductive energy discharge circuits, which transfer energy stored in the magnetic fields of coils into well-defined voltage pulses. The circuit concept is shown in Figure 10.

Figure 10. The conceptual circuit of an inductive energy discharge pulse generator.

The generator uses an AC power supply, a low voltage capacitor bank C for a primary electric energy storage and an inductor for secondary magnetic energy storage. When the switch SW1 is closed, the electric energy is discharged from the capacitor bank C into the inductor L. Hence, during the first quarter period of the sinusoidal AC-current, the magnetic energy stored in the inductor increases and the electric energy stored in the capacitor bank decreases. At the peak of sinusoidal current, the magnetic energy stored in the inductor reaches its maximum. At this point, the opening switch SW2 is activated and the current is abruptly interrupted influencing high rate of change of the magnetic flux (dB/dt). Such a transient process induces voltage in the inductor, which is further discharged by a spark gap through the load [67].

The inductive energy discharge pulse generator has many drawbacks and is not used for electroporation research. Instead, improved circuits are applied (i.e., the diode opening switch (DOS)

topology). This is a commonly used concept for generation of high voltage (several kV) pulses in the nanosecond range [29]. The diode opening switch circuit is presented in Figure 11.

Figure 11. The circuit of a diode opening switch generator.

The diode opening switch circuit is based on the DC voltage power supply V , which charges the capacitor bank $C1$ through the resistor R . When the capacitor is fully charged, the switch SW is closed and the energy in the capacitor $C1$ starts circulating in the resonant network ($C1$, $C2$, $L1$ and $L2$). After completing half of the period, the resonant network starts pumping the current through the diodes in the reverse direction. Ideally, the diode abruptly stops conducting and commutates the $L2$ current into the load. Therefore, this type of circuit is based on the saved energy transfer from inductor $L2$ to the load with a good repeatability [29].

Beside the application of diodes, it is also common that the inductive energy discharge pulse generator can be improved with the step-up pulse transformer. In this case the amplitude of the voltage pulse did not affect the opening switch [39]. A simplified transformer-based pulse forming circuit is presented in Figure 12.

Figure 12. The conceptual circuit of a transformers-based pulse generator.

Despite the advantages, the transformer-based pulse generator also has several issues, like core saturation, reset time after pulse delivery and distorted pulse shape because of the parasitic circuit elements. All these issues must be considered during the design of the pulse forming circuit [39]. On the other hand, the transformer based topology can be combined with the modular approach discussed before providing additional flexibility and pulse control [68].

3. Overview

3.1. Advantages and Disadvantages of the Typical Electroporator Concepts

The presented concepts of pulse generators demonstrate the diversity of techniques used for high-voltage pulse generation suitable for electroporation applications. All the techniques are summarized in the Figure 13.

Figure 13. The diversity of pulse generators topologies for electroporation applications.

Based on the energy storage, the pulse generators can be divided into three main groups: direct capacitor discharge, transmission line discharge and inductive storage discharge. Each has advantages and disadvantages, which are summarized in the Table 1.

Further, the in-house built nsPEF generators were analyzed and classified based on the switching type. The review focused only on nsPEF pulse generators, which were reported specifically for the application in the field of electroporation. We have included only the references where the reported duration of the pulse is in the sub-microsecond range (below 1 μ s). In total 63 nsPEF generators for electroporation matched the requirements and are listed in Table 2. The devices are grouped based on the pulse forming circuit with the following parameters reported: pulse form, pulse duration, maximum pulse amplitude, pulse repetition frequency, switch type, switch model and additional remarks on the pulse form and topology in case the divergence from the usual performance is noticed.

Table 1. The comparison between the topologies of pulse generators used for electroporation.

Concept		Advantage	Disadvantage	
Direct Capacitor	Non-Modular	Unipolar	Simple and inexpensive construction for systems up to 1 kV; Very flexible pulse shape control in the sub-microsecond–millisecond range; Can operate in a high frequency range.	High-voltage supply required; Amplitude droop during the pulse; High capacity capacitor banks are required for rectangular wave delivery into high loads; Switch must withstand full voltage amplitude or complex synchronization circuits are required in case of array of switches; Not suitable for sub-100 ns pulses.
		Bipolar	Positive and/or negative high-voltage pulses; Highest pulse forming flexibility; Capability to use asymmetrical pulses; Specific electrotransfer mechanisms can be triggered.	Switch synchronization is needed; Complex control systems; Limited voltage handling capability; Not suitable for sub-100 ns pulses.
	Modular	Applicable with low voltage switches and voltage supplies; Wide flexibility of pulse parameters; Arbitrary signal shape; Easy to achieve high currents; Can operate in high frequency range.	Limited amplitude resolution; Complex control system; Switch synchronization is needed; Not suitable for sub-100 ns pulses.	
	Marx Generator	Applicable with low voltage switches and voltage supplies; High voltages up to hundreds of kV; High currents; Can generate sub-100 ns pulses.	Bulky structure; Voltage droop is common when high loads are used; Limited high frequency capability; Electrode degradation in case of spark-gaps.	
Transmission line	Blumlein	Simple design; Commonly used for short pulse generation (sub-100 ns); High-voltages and currents; Can be used for bipolar pulses	Load impedance matching requirement; Pulse width inflexibility (limited to transmission line); Relatively short lifetime; Most of the usual concepts operate in low repetition rate; Big dimensions of the generator.	
Inductive Storage	Resonant Circuit	High energy density pulsing can be ensured.	Not applicable for electroporation directly; Parasitic parameters affect the waveform; Switch synchronization is needed; Complex control system.	
	Diode Opening Switch	High energy density; Accessible electrical components; Variable load impedance; Commonly used for short pulse generation (sub-100 ns); Fast repetition frequency.	Complicated design; Low output power; Switch synchronization is needed; Complex control system; Complex switching and poor control of pulse durations.	
	Transformer based	High pulse amplitude; Applicable with low voltage switches; Flexible pulse amplitude.	Transient processes affect pulse waveform; Core saturation and reset after pulse.	

Table 2. List of in-house developed nanosecond pulsed electric field (nsPEF) generators with their and pulse parameters for electroporation application.

Circuit	Reference	Pulse Form	Pulse Polarity	Pulse Duration	Maximum Amplitude	Repetition Frequency	Switch	Switch Model	Pulse Form and Topology Remarks
Blumlein-type	2000 [73]	Gaussian	Unipolar	8 ns	30 kV	-	Spark gap	-	Pressurized spark gap
	2003 [40]	Gaussian	Unipolar	3–15 ns	>10 kV	-	Spark gap	-	Distorted pulse shape
	2006 [64]	Rectangular	Unipolar	10 ns	40 kV	-	Spark gap	-	Distorted pulse shape
	2006 [64]	Rectangular	Unipolar	10–300 ns	1 kV	0–50 MHz	MOSFET	DE375-102N12A	-
	2007 [48]	Gaussian	Unipolar	50 ns	65 kV	10 Hz	Spark gap	-	Distorted pulse shape
	2008 [74]	Rectangular	Unipolar	8–300 ns	1 kV	-	MOSFET	DE275-102N06A	-
	2009 [66]	Rectangular	Unipolar and bipolar	20, 50, 75, 150, and 230 ns	<0.3 kV	0–1.1 MHz	MOSFET	DE275-102N06A	High pulse amplitude droop
	2011 [75]	-	-	40–200 ns	1 kV	0–100 kHz	-	-	Pulse shape not specified
	2013 [63]	Gaussian	Unipolar and bipolar	10, 20, 60 ns	2 kV	-	Transistor	HTS-UF	Distorted pulse shape
	2014 [76]	Gaussian	Bipolar	2 ns	650 kV	-	Oil switch	-	Hybrid with resonant
	2016 [60]	Rectangular	Unipolar	50–100 ns	2 kV	0–(~3) kHz	MOSFET	DE475-102N21A	Distorted pulse shape
	2016 [61]	Rectangular	Unipolar	100 ns	1.7 kV	0–(~3) kHz	MOSFET	-	Distorted pulse shape; Modular circuit
	2017 [62]	Gaussian	Unipolar	20 ns	2.5 kV	0–10 kHz	MOSFET	DE475-102N21A	-
	2018 [77]	Gaussian	Unipolar and bipolar	30 ns	10 kV	0–200 kHz	MOSFET	DE475-102N21A	Modular
	2019 [78]	Rectangular	Unipolar	30 ns	4 kV	1 kHz	IGBT and spark gap	IRG4PH50K	Distorted pulse shape; With transformer
	2020 [79]	Rectangular	Unipolar	5 ns	0.5 kV	0–10 MHz	MOSFET	IXZ631DF12N100	Fixed pulse duration;

Table 2. Cont.

Circuit	Reference	Pulse Form	Pulse Polarity	Pulse Duration	Maximum Amplitude	Repetition Frequency	Switch	Switch Model	Pulse Form and Topology Remarks
Transmission line	2003 [40]	Gaussian	Unipolar	150 ns	12 kV	-	Spark gap	-	Distorted pulse shape
	2010 [80]	Rectangular	Bipolar	2 ns	1.6 kV	0–10 Hz	PCSS ¹	-	Distorted pulse shape and laser triggering
	2013 [81]	Rectangular	Bipolar	10, 40, 60, 92 ns	12.5 kV	-	Spark gap	-	Laser triggering
	2018 [36]	Rectangular	Bipolar	10, 60, 300 ns	10 kV	-	Spark gap	-	Distorted pulse shape; Hybrid with resonant circuit
	2019 [59]	Rectangular	Unipolar	120, 160, 200, 300, 400 ns	10 kV	-	Spark gap	-	Distorted pulse shape
Direct capacitor discharge	2001 [49]	Gaussian	Unipolar	2 ns	2.6 kV	-	Spark gap	-	Distorted pulse shape
	2003 [40]	Rectangular	Unipolar	12 ns	1 kV	-	MOSFET	DE275-501N16A	Distorted pulse shape
	2004 [82]	Rectangular	Unipolar	75 ns to 10 ms	0.400 kV	600 kHz	MOSFET	IXYSRF DE275-501N16A	-
	2004 [83]	Exponential	Unipolar	100 ns to 100 μ s	0.3 kV	0–2 kHz	IGBT	-	-
	2004 [84]	Exponential	Unipolar	-	3.4 kV	0–1 kHz	BJTs	ZTX415	Pulse rise time to 2 ns
	2012 [85]	Gaussian	Bipolar	2.5 ns	1.66 kV	-	Optoelectronic	-	-
	2014 [71]	Rectangular	Unipolar	200 ns to 5 μ s	8 kV	0–30 Hz	MOSFET	HTS 91-12	-
	2015 [86]	Rectangular	Unipolar	38 ns to 7 μ s	0.5 kV	-	MOSFET	IRF740	-
	2016 [87]	Rectangular	Unipolar	100 ns to 1 ms	3 kV	0–1 MHz	MOSFET	C2M0080120D	-
	2019 [72]	Rectangular	Unipolar	80 ns to 1 μ s	1.4 kV	0–50 Hz	MOSFET	C2M1000170D	-
Marx-bank/Modular	2019 [88]	Rectangular	Unipolar	80 ns	0.5 kV	-	MOSFET	-	-
	2001 [49]	Gaussian	Unipolar	6 ns	6 kV	-	Spark gap and MOSFET	40N160	Distorted pulse shape
	2007 [48]	Gaussian	Unipolar	200 ns	6 kV	-	Spark gap	-	Single pulse
	2007 [89]	Gaussian	Unipolar	1.3 ns	1.1 kV	0–200 kHz	Diode opening	SOT-23 Zetex FMMT417	-
	2008 [47]	Gaussian	Unipolar	135–220 ps	20–120 kV	0–15 Hz	Peaking	-	-

Table 2. Cont.

Circuit	Reference	Pulse Form	Pulse Polarity	Pulse Duration	Maximum Amplitude	Repetition Frequency	Switch	Switch Model	Pulse Form and Topology Remarks
Marx-bank/Modular (continue)	2011 [90]	Rectangular	Bipolar	100 ns	1 kV	1 kHz	MOSFET and JFET ²	-	Hybrid with Blumlein; Distorted pulse shape
	2012 [91]	Rectangular	Unipolar	200 ns to 1 µs	8 kV	0–1 kHz	MOSFET	-	-
	2013 [92]	Rectangular	Bipolar	300 ns to 10 µs	4 kV	0–40 kHz	IGBT	IRGPS60B120KDP	-
	2015 [93]	Gaussian	Unipolar	600 ps	31.2 kV	-	Spark gap	-	Distorted pulse shape
	2016 [94]	Rectangular	Unipolar	100 ns to 1 µs	8 kV	0–1 kHz	MOSFET	C2M0080120D	-
	2016 [95]	Gaussian	Unipolar	620 ps	1 kV	10 kHz	Avalanche transistors	FMMT417	Hybrid with microstrip transmission line
	2016 [96]	Rectangular	Unipolar	200 ns to 100 µs	10 kV	0–1 kHz	MOSFET	C2M0280120D	-
	2016 [97]	Rectangular	Bipolar	100 ns to 1 µs	3 kV	0–1 kHz	MOSFET	C2M0080120D	Voltage droop
	2016 [98]	Rectangular	Unipolar and bipolar	100 ns to 100 µs	3 kV	0–2 MHz	MOSFET	C2M0080120D	-
	2017 [99]	Gaussian	Unipolar	300 ps	1.6 kV	0–10 kHz	Avalanche transistors	FMMT417	Marx with gradient transmission
	2017 [58]	Rectangular	Bipolar	100 ns to 1 µs	3 kV	1 kHz	MOSFET	C2M0080120D	-
	2017 [100]	Gaussian	Unipolar	400 ns to 20 µs	6 kV	0–100 MHz	IGBT	IXYK 120N120C	-
	2018 [101]	Gaussian	Unipolar	350 ps	3.1 kV	0–10 kHz	Avalanche transistors	FMMT417	-
	2018 [102]	Rectangular	Bipolar	200 ns to 1 µs	2 kV	0–1 kHz	MOSFET	-	-
	2019 [69]	Rectangular	Bipolar	500 ns to 1 ms	15 kV	10 kHz	MOSFET	C2M0160120D	-
	2019 [29]	Gaussian	Unipolar and bipolar	8 ns	6 kV	0–3.5 kHz	MOSFET	IXDD609SI and C2M0025120D	Fixed pulse duration
	2019 [103]	Rectangular	Bipolar	500 ns to 10 s	5 kV	0–0.5 MHz	MOSFET	-	-
	2020 [104]	Rectangular	Bipolar	500 ns to 5 µs	10 kV	0–0.5 MHz	MOSFET	C2M0080120	-
Inductive storage	2020 [70]	Rectangular	Unipolar	200 ns to 1 µs	15.3 kV	0–10 kHz	MOSFET	C3M0065090J	Integrated with DOS circuit
	2005 [105]	Gaussian	Unipolar and bipolar	3.5 ns	1.2 kV	0–100 kHz	MOSFET	APT10035JLL	DOS
	2007 [106]	Gaussian	Unipolar	20 ns	4.5 kV	20 Hz	IGBT	CM300HA-12H	Distorted pulse shape; DOS with transformers
	2007 [106]	Gaussian	Unipolar	5 ns	7.5 kV	20 Hz	MOSFET	APT10035	-
	2009 [107]	Gaussian	Unipolar	5 ns	4.4 kV	0–3 MHz	MOSFET	-	Resonant circuit
	2009 [107]	Gaussian	Unipolar	2.6 ns	1 kV	0–3 MHz	MOSFET	-	Resonant circuit
	2010 [108]	Gaussian	Unipolar	50 ns	30 kV	0–0.5 kHz	Magnetic	-	-
	2012 [109]	Gaussian	Unipolar	50 ns	1 kV	-	MOSFET	APT37M100L	DOS
	2019 [110]	Rectangular	Unipolar	23 ns	8.2 kV	-	MOSFET	C3M0120090J	Hybrid with Blumlein

¹ PCSS – Photoconductive Semiconductor. ² JFET – Junction Field Effect Transistor.

3.2. Classification of Available In-House Built Generators for nsPEF Electroporation

It was identified, that the traditional transmission line (including Blumlein-type) pulse forming circuits are no longer dominating in the nsPEF electroporation applications. The progress of semiconductor technologies enabled the development of cost effective, small-size and flexible sub-microsecond generators based on direct capacitor discharge topology. The Marx generator topology with new ultra-fast semiconductor switches forms a new leading technology trend now. In addition, there were few successful attempts to develop resonant-circuit nsPEF generators, which at the end were not followed by other researchers. Unpopularity of this topology is driven by the limited pulse duration flexibility and resulting Gaussian pulse shape.

All topologies demonstrate the possibility to produce high-voltage pulses, however, the transmission line (including Blumlein-type) topologies, which use spark gap switches, demonstrate a possibility to produce very high amplitude (peak voltages exceeding 10 kV) nsPEF rectangular wave pulses. Yet there is complexity associated with these designs and recent developments of Marx-bank [69,70] and other direct capacitor discharge circuits [71] indicate the growing availability of the off-the-shelf components fulfilling the high-voltage pulse delivery requirements. It is expected that the transmission line topologies will be pushed out of the sub-microsecond range due to a lack of flexibility, requirement of impedance matching and distorted waveforms. These drawbacks are not associated with the reported direct capacitor discharge circuits (including Marx-bank). However, the sub-100 ns pulses in the range of tens of kV are still yet hardly achievable by the direct capacitor discharge technology.

Indeed, for the high-voltage sub nsPEF pulse delivery, the switch performance and characteristics are crucial. The spark gap switches are fast and cost-effective for nsPEF generation, but these switches have a short lifetime due to electrode erosion, poor pulse duration control and can be frequently associated with turn-on jitter [36]. In contrast, the semiconductor switches offer high flexibility of pulse control, but are more constrained by high-voltage or current withstand limits as well as switch opening and closing times. A series connected switches can solve high-voltage limitation issues, but this increases the stray inductance, which is making the circuit slower.

One of the latest comparison between typical MOSFET, IGBT and bipolar junction transistor (BJT) switches was made in 2019 [72]. It was demonstrated that all three could ensure the breakdown voltage (collector–emitter or drain–source voltage) higher than 1 kV. However, the BJT switches, which among all would be the most cost-effective option, are slower than MOSFETs or IGBTs and cannot fulfill the nsPEF pulse forming requirements. It was demonstrated that only the high power MOSFETs can form pulses within the 100–300 ns range with the transition times (rise and fall time) faster than 100 ns [72]. More than a half of reported nsPEF electroporators (Table 2) use the MOSFET switch as a main pulse forming component.

The review indicates that the direct capacitor discharge topology is taking a lead in the nsPEF applications when the sub-microsecond pulse is required. In addition, the MOSFET switches are now the main technology to produce high-voltage nsPEF pulses in various circuit topologies. The spark gap switches are still applicable in case of a very high-voltage amplitude or ultra-short pulse duration (a few nanoseconds) [64]. Other types of switches (like photoconductive semiconductor, optoelectronic switch or even IGBT) are rarely applicable even if they also provide a capability to deliver ultra-short pulses. In all cases, the transient processes are triggered during the switch turn-on and turn-off, which negatively influence the pulse parameters. The proper compensation circuits must be applied to ensure system protection and the precise pulse waveform with constant pulse rise and fall times independently on the load/electrodes type [111].

4. Conclusions

The interest in shorter and higher intensity PEF pulses for electroporation is increasing, which leads to the growing demand for the nsPEF electroporators. Different techniques to design the pulse forming circuits for the nsPEF electroporators exist. Traditionally transmission line circuit topologies

were used, however, the recent development of SiC MOSFETs have resulted in the new wave of advanced direct capacitor discharge nsPEF electroporators with adjustable pulse parameters.

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