

Article

A SiC Photo-Conductive Switch-Based Pulse Generator with Nanoseconds and High Voltage for Liver Cancer Cells Ablation Therapy

Haocheng Yin , Zeyu Zhang, Yapeng Liu, Yutian Wang *, Hui Guo and Yuming Zhang

Key Laboratory of Wide Band-Gap Semiconductor Materials and Devices, School of Microelectronics, Xidian University, Xi'an 710071, China; hcyin@xidian.edu.cn (H.Y.); 22111213866@stu.xidian.edu.cn (Z.Z.)

* Correspondence: ytwang@xidian.edu.cn

Abstract: Electroporation ablation, as an innovative cancer treatment, not only preserves the structure and function of affected organs but also significantly reduces surgical risks, offers patients a safer and more effective therapeutic option, and demonstrates immense potential in the field of oncology. This paper presents the innovative design of a high-voltage nanosecond pulse generator triggered by a silicon carbide (SiC) photoconductive switch. The generator is capable of stably outputting adjustable voltages ranging from 10 kV to 15 kV, with pulse widths precisely controlled between 10 and 15 nanoseconds, and an operating frequency adjustable from 1 Hz to 10 Hz. This device enables instant activation and deactivation of the pulse generator during ablation, enhancing the efficiency of strong electric field applications and preventing overtreatment due to delayed shutdown. This paper introduces the structure and basic principles of this novel SiC photoconductive switch-triggered pulse device and reports on the impact of device-related pulse parameters on the ablation effect of hepatocellular carcinoma cells through cell experiments. Under optimal ablation parameters, the CCK8 results show that the number of viable cells is only 0.7% of that in the untreated control group after 12 h of subculture following ablation. These findings hold significant importance for expanding the application areas of SiC devices.



Citation: Yin, H.; Zhang, Z.; Liu, Y.; Wang, Y.; Guo, H.; Zhang, Y. A SiC Photo-Conductive Switch-Based Pulse Generator with Nanoseconds and High Voltage for Liver Cancer Cells Ablation Therapy. *Electronics* **2024**, *13*, 4816. <https://doi.org/10.3390/electronics13234816>

Academic Editor: Pedro J. Villegas

Received: 13 October 2024

Revised: 18 November 2024

Accepted: 29 November 2024

Published: 6 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Keywords: pulse power generator (PPG); high voltage; nanoseconds; electroporation; tumor cell ablation

1. Introduction

Cancer, medically defined as a malignant tumor, originates from abnormal changes in cellular genetic material. These changes drive cells to divide and proliferate uncontrollably and irregularly, ultimately forming masses that invade adjacent tissues and may even spread to other parts of the body through the bloodstream or lymphatic system. Currently, the primary treatments for cancer include surgical therapy [1], radiotherapy [2], chemotherapy [3], immunotherapy [4], and targeted therapy [5], among others [6]. While these traditional therapies have shown high effectiveness in eliminating cancer cells, they also cause significant functional damage to the human body. Surgical therapy, often regarded as a direct and effective means, is costly and carries certain risks such as infection and bleeding, without guaranteeing a completely trauma-free procedure. Furthermore, patients face risks of complications such as infection, bleeding, and pulmonary embolism after surgery, all of which increase patients' suffering and treatment burden. Moreover, surgery may sometimes fail to remove the tumor completely due to its location or size, leaving the possibility for recurrence or regrowth [7–9]. Chemotherapy, as another common treatment, also has side effects that cannot be ignored. While chemotherapy drugs kill cancer cells, they also damage normal cells, triggering a series of side effects such as nausea, vomiting, hair loss, and decreased white blood cell count, all of which severely affect the quality of patients' lives. In more severe cases, excessive chemotherapy may even shorten

the patient's lifespan [10,11]. Additionally, since certain types of tumors are not sensitive to chemotherapy drugs, forcibly administering chemotherapy may yield minimal results [12]. Radiotherapy differs from surgery and chemotherapy. Although modern radiotherapy techniques have achieved considerable precision, it is still difficult to completely avoid radiation damage to surrounding healthy tissues and lead to a series of side effects such as skin inflammation, nausea, vomiting, and fatigue. Furthermore, radiotherapy has a relatively long treatment cycle, slow onset of efficacy, and a high recurrence rate [13,14].

With advancements in technology, ablation therapy has gradually attracted the attention of researchers. Ablation methods, primarily including radiofrequency ablation (RFA), cryoablation, microwave ablation, laser ablation, and ultrasound ablation, significantly reduce patients' suffering during cancer treatment by directly destroying local cancer cells or tissues, demonstrating considerable application potential [15]. However, these traditional ablation methods still face numerous challenges in clinical applications. RFA, though effective, involves a relatively lengthy ablation process, and the expansion effect during tumor necrosis may lead to residual cancer cells. Meanwhile, in some cases, increased vascular endothelial growth factor (VEGF) can inhibit drug efficacy, thereby increasing the risk of tumor recurrence [16,17]. Cryoablation is limited by its high cost and time-consuming procedure, and multi-needle ablation may lead to a significant reduction in platelets, increasing the risk of bleeding [18,19]. Microwave ablation currently faces issues with imperfect equipment, resulting in unstable treatment effects [20]. Laser ablation may cause local temperatures to rise excessively during treatment, damaging surrounding healthy tissues and nerves, leading to complications. Furthermore, the effectiveness of laser ablation is closely related to needle placement, making it difficult to operate [21,22]. As for ultrasound ablation, accurate lesion localization can sometimes be challenged due to factors such as echo, bone obstruction, and lung air, resulting in a longer treatment duration. Additionally, there is a possibility of missing the target when treating irregular tumor tissues [23].

With the continuous progress of science and technology, researchers have gained a deeper understanding of the electromagnetic effects on biological cells, leading to the development of an innovative ablation technique—pulsed electric field ablation (PEFA). This technique widely utilizes high-voltage electric pulses to act on the phospholipid bilayer of cell membranes, generating transmembrane potentials and forming unstable potential differences, thereby inducing irreversible permeabilizing damage to the cell membrane and ultimately leading to cell apoptosis [24]. Specifically, this ablation method relies mainly on high-voltage direct current pulses with pulse widths precisely controlled between 5 and 100 microseconds. PEFA technology has been successfully applied in clinical practice for the ablation of liver and pancreatic tumors, and it has also shown great potential in the treatment of arrhythmias such as atrial fibrillation [25,26]. Compared with traditional thermal and cold ablation techniques, PEFA exhibits higher targeting precision, more precise action on target tissues, significantly reduced complications, and a substantially shortened ablation time [27]. With continuous technological iterations and upgrades, PEFA has evolved from millisecond pulses to microsecond pulses and further to nanosecond pulses, bringing about broader application prospects and more possibilities to this technology [28,29]. Looking ahead, PEFA is expected to play a significant role in various medical fields such as tumor ablation and arrhythmia treatment, providing patients with more and better treatment options.

Compared with cold and thermal ablation, PEFA has several notable advantages. Firstly, in terms of safety, as a non-thermal ablation method, electric pulse ablation almost eliminates the risk of thermal-related injuries. It demonstrates high selectivity for surrounding tissues such as the phrenic nerve and esophagus, avoiding damage to these normal tissues and significantly reducing the risk of damaging normal tissues during surgery [16,30,31]. Secondly, PEFA also excels in ablation efficiency. By using a circular multi-electrode ablation catheter, this technology simplifies the surgical process, enabling the entire procedure to be completed through a single catheter [32]. Its multi-point dis-

charge feature allows each discharge to occur in just 2–4 s, significantly shortening the ablation time and improving surgical efficiency compared to methods like RFA and cryoablation. Finally, in terms of applicability, PEFA shows broad potential. Since its mechanism of action does not depend on specific tumor types, it may be applicable to various types of tumors, providing a wider range of options for tumor treatment [33]. In summary, PEFA has clear advantages over cold and thermal ablation in terms of safety, efficiency, and applicability; it is expected to become one of the crucial means for future tumor treatment.

Currently, PEFA devices commonly use traditional semiconductor power devices as pulse switches to emit treatment pulses. However, these devices typically operate at kilovolt-level pulse source voltages during treatment, and semiconductor power devices struggle to achieve nanosecond-level rapid shut-off at such high voltages [34]. This limitation can easily lead to ablation beyond the expected range or overtreatment during high-precision surgeries, particularly in areas where thermal ablation is contraindicated, such as the brain, liver, and blood vessels, where overtreatment may cause unnecessary organ damage to patients [35]. Additionally, the rising edge slope (dv/dt) of the treatment pulses output by traditional semiconductor power devices such as pulse switches is relatively gentle, approximately between 100 V/ns and 300 V/ns, which is insufficient to generate instantaneous strong electric fields within cells to achieve cell perforation. Although increasing the rising edge slope by paralleling multiple semiconductor power devices is possible, it also increases the width of the treatment pulses, leading to additional thermal accumulation during treatment and increasing the risk of thermal damage to healthy tissues. To overcome this challenge, this paper reports on a novel high-power pulse ablation device with a maximum voltage of 15 kV, a frequency of 10 Hz, and a pulse width of only 5 ns. This pulse source uses a SiC photoconductive switch for control and has been tested for pulse ablation on A549 cells to study the ablation effect of the high-voltage pulse source on liver cancer cells. This innovation provides strong support for the further development of PEFA technology.

2. Equipment Set Up and Material

2.1. Pulse Equipment Set Up

Unlike the square wave pulses used in traditional electro-pulse ablation, the pulse waveform employed in our study, as shown in Figure 1c, is presented in the form of a steep pulse. Traditional square wave pulses switch rapidly between high and low voltage levels, with a stable waveform characterized by clear rising and falling edges, as well as constant pulse width and frequency. The stable waveform of square wave pulses ensures uniform electric field distribution and a consistent duration of action, thus offering advantages such as good stability, high controllability, and consistent ablation effects. In contrast to square waves, steep pulses have a sharp waveform, reaching a high peak voltage in a short time and quickly falling back. They are characterized by concentrated energy and a short duration of action, resulting in a more significant electroporation effect on cell membranes compared to square waves. At the same time, steep pulse ablation offers advantages such as high efficiency, precision, and safety. Its rapid rising and falling edges ensure the quick action and disappearance of the electric field, reducing damage to normal tissues. However, there is relatively little research on ablation using steep pulses currently, which also makes our research meaningful.

To generate nanosecond high-voltage pulses, a photoconductive pulse-triggering circuit based on SiC material is used in this experiment. 4H-SiC-doped V as the material for PCSS in cancer therapy has several advantages. Firstly, SiC does not contain any harmful components like GaAs. Secondly, no avalanche effect has been found in SiC so far. Therefore, its photoelectric linear characteristics are easily triggered by laser control. Finally, due to the ability of a single SiC photoconductive switch to operate at 15 KV for a long time, the trigger circuit is simple. The circuit consists of a pulsed high-voltage power supply (Wiswan DE70P6), a high-voltage capacitor, a SiC photoconductive switch, and a load module, as shown in Figure 1.

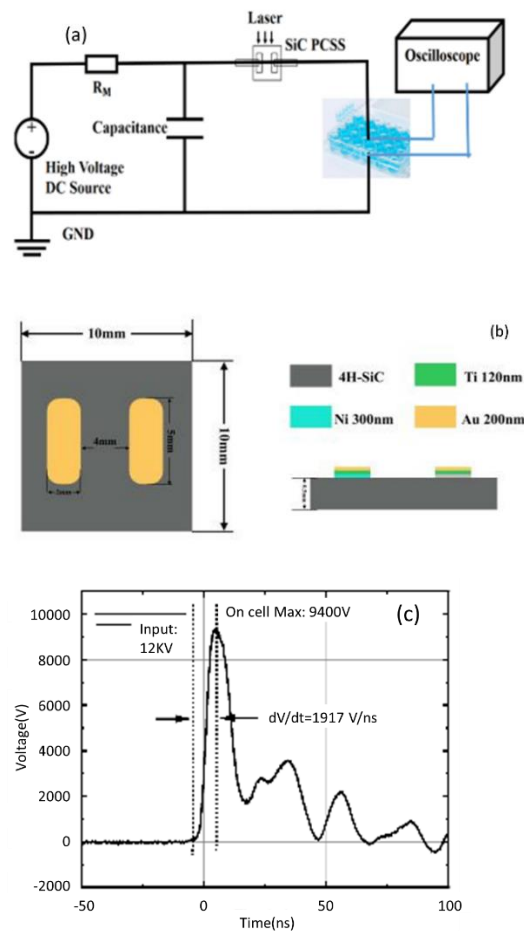


Figure 1. (a) Schematic diagram of a pulse system based on photoconductive switches for cancer treatment. (b) The photoconductive switch used in this experiment. (c) The load waveform collected on the cell under 12 KV input voltage.

The entire pulse system consists of two circuits. Firstly, the charging circuit is composed of a high-voltage power supply, a charging resistor, and a charging capacitor. The charging circuit is composed of the same capacitor PCSS and a cell plate as the discharge circuit, where the cell plate can be equivalently regarded as a load resistor. Before applying the pulse voltage, we tested the resistance value of the cells with a multimeter, which was approximately at the level of 100K ohms.

The VCSI 4H-SiC substrates used for fabricated PCSS in this experiment were provided by SICC Company Ltd.1. (Jinan, China), with a thickness of 500 μm and a size of 10×10 mm. We first cleaned the SiC material using the microelectronics standard cleaning process and then deposited a layer of nickel metal according to the electrode shape in Figure 1, with a thickness of 300 nm. Then, it is annealed at temperature 1100 $^{\circ}\text{C}$ for 60 s under nitrogen conditions. Finally, we covered the nickel metal with titanium and gold, with thicknesses of 120 nm and 200 nm, respectively.

When there is no strong laser triggering PCSS, the resistance of PCSS is around 10^{10} ohms, so the discharge circuit is not connected. During operation, the charging circuit charges the capacitor through a high-voltage source and charging resistor and then uses a 532 nm laser to touch the PCSS. Due to the generation of the 10^{17} cm^{-3} concentration of photo-generated carriers in PCSS under the action of laser pulses, the switching resistance decreases from 10^{10} ohms to 30 ohms within 1–3 ns, and the capacitor discharges instantaneously to generate high-voltage pulses. High-voltage pulses are applied to the tip of the electrode needle by linking it to the cell plate wall, generating an electric field strength of 12 kV/cm. The electrodes space 4 mm, ensuring that the switching material

can withstand high voltages of up to 20 kV to deliver a stronger nanosecond pulse. A Q-switched Nd: YAG laser form is used as the optical trigger source, whose full width at half maximum is 10 ns at a wavelength of 532 nm. The load side is a cell plate filled with cells for this experiment.

During operation, the high voltage originally charged the high-voltage capacitor. After the capacitor was full, the pulsed laser triggered the photoconductive switch to instantaneously discharge the capacitor. For a standard 50 ohm capacitor, a quasi-Gaussian pulse with a pulse width of about 15 nanoseconds was generated from 1 kV to 12 kV. Meanwhile, for the subsequent study of the equivalent resistance model of the cell, we connected a 2G bandwidth oscilloscope (Siglent SDS6204 H10 Pro, Shenzhen, China) through a high-voltage probe (P6039A of Pintech, Guangzhou, China) and connected it to both ends of the cell plate to record the pulse waveforms of the cell plate under the high-voltage electricity.

2.2. Human Pulmonary Carcinoma Cell Preparation

The procedure for preparing hepatocellular carcinoma cells for the experiments follows the standard protocols [28] and has been adaptively adjusted according to the laboratory conditions and actual needs, as detailed below. For cell cryopreservation, a combination of Dulbecco's Modified Eagle Medium (DMSO, sourced from ThermoFisher Scientific, Waltham, MA, USA) and a serum-free cell cryopreservation solution (FBS, obtained from Merck KGaA, Darmstadt, Germany) is utilized. To formulate the cell culture medium, Dulbecco's Modified Eagle Medium (DMEM, also from ThermoFisher Scientific, USA), FBS, and a penicillin/streptomycin solution (1000 U/mL, ThermoFisher Scientific, USA) are employed. The human pulmonary carcinoma cells (A549 cells) are maintained in the State Key Laboratory of Oncology at the Air Force Medical University. Preparation of the cell culture medium entails mixing DMEM, adjusting its pH to a range of 7.2 to 7.4, with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution. The cells are cultured in a Midi 40 carbon dioxide incubator (Thermo, USA), maintained at 37 °C, with a 5% CO₂ concentration and 80–90% humidity. Every two days, the medium is replaced to promote optimal cell growth. Subculturing is performed when cell density reaches 80%–90%, involving cell detachment with a 0.25% trypsin solution, followed by the addition of an appropriate volume of fresh culture medium to achieve uniform cell dispersion for subculturing. Upon reaching 80–90% cell density again, viable cells are chosen for cryopreservation. All cell preparations follow the standard procedure for cell suspension preparation [36]. These cells are mixed with a solution comprising 10% DMSO and 90% FBS, transferred to cryopreservation tubes, securely sealed, and stored in liquid nitrogen for future applications. Prior to use, the cells are thawed in a 37 °C water bath.

2.3. Cell Experiment Set Up

In the cell experiments, a specific volume (400 microliters) of cell suspension containing approximately 2.5×10^5 cells, which was selected based on the standard references [37,38], was dispensed into each well of a 48-well plate. Sterile needle electrodes, which are coated with an insulating material except at the tip, are then obtained. These electrodes are inserted into the wells of the cell culture plate with a predetermined spacing, as dictated by the experimental protocol. In the cell experiment, we initially used the electrode cups recommended in the relevant literature. However, we observed that the pulse output waveform was altered when using these electrode cups. This phenomenon could be attributed to the fact that the electrode cups themselves act as capacitors. Due to the charging and discharging characteristics of capacitors, the rising and falling edges of the pulse become more gradual, leading to the distortion of the pulse waveform. After consulting the literature [39], we decided to adopt a method of directly inserting the electrode needles into the culture dishes. Prior to the experiment, the workbench and its surroundings were thoroughly disinfected to preclude any contamination that might skew the experimental outcomes. Following pulse ablation, the cells' physical attributes were

examined using a transmission microscope (MSD530, Murzider, Dongguan, China). For all cell experiments, electrode cups with a 1 mm gap were utilized, sourced from ThermoFisher Scientific (USA). The CCK8 dye employed for the cell proliferation assessment was procured from Selleck (Houston, TX, USA). In the CCK8 assay, the cells are permitted to proliferate for a duration of 12 h before being analyzed using a Varioskan LUX microplate reader, Shanghai, China. After this period, a precise volume (400 μ L) of CCK-8 reagent is added to each well, with meticulous care taken to prevent bubble formation. The culture plate is then gently agitated to ensure homogeneous mixing of the solution. The absorbance value of each well is subsequently measured at a wavelength of 450 nm using a microplate reader. Statistical analysis is conducted on the recorded absorbance values to discern differences among various treatment groups and to compute cell viability or survival rates. In the experimental process, the final experimental results are obtained by calculating the average value. Specifically, 30 groups of cells are selected for each parameter ablation experiment, with each group originating from the same batch of cultured liver cancer cells. After the experiment, when calculating the cell survival rate, the three groups with the highest values and the three groups with the lowest values were excluded. The average survival rate of the remaining 24 groups of cells was then calculated, which is considered to be the final cell survival rate after ablation for that parameter. The same calculation method was applied to the control group.

3. Results and Analysis

3.1. Voltage Effect

Studies have shown that enhancing the electric field intensity can significantly improve the ablation effect on cancer cells; when a specific threshold is reached, it can ensure the complete ablation of cells within the targeted ablation area. However, this does not imply that the electric field intensity can be increased indefinitely. When the electric field intensity reaches a saturation level, further increases not only fail to enhance the ablation effect but may also trigger a series of adverse effects, such as expanding the scope of tissue damage and inducing cardiac arrhythmias. Therefore, during the administration of electroporation ablation therapy, precise control of the electric field intensity is essential to ensure both effective ablation and the avoidance of unnecessary risks and side effects due to exceeding the saturation value. Given this, it is necessary to further verify experiments whose voltage settings can achieve the threshold voltage required for effective ablation when using our self-developed pulse generator.

In the test of electric field intensity on ablation effect, we maintained constant conditions of a frequency of 10 Hz, 300 pulses, and an electrode spacing of 1 cm. By adjusting the voltage across the electrodes, we varied the electric field intensity from 10 kV/cm to 12 kV/cm for ablation testing. During the experiment, we used a temperature gun for real-time monitoring and recorded an indoor temperature of 25.2 °C and an initial cell sample temperature of 24.8 °C. The waveform of the applied voltage on the cell under 12 KV is referred to in Figure 1c.

After the ablation treatment, we first observed the morphological changes in the cells using an optical microscope, and the related images are displayed in Figure 2. Subsequently, 0.1 mL of DMEM culture medium was added to each well, and the 48-well cell culture plate was placed in a CO₂ incubator. After 12 h of incubation, we conducted a CCK8 cell viability assay, and the experimental results are shown in Figure 3b.

Throughout the experiment, we focused our observations using an optical microscope on the central region between the electrodes. When a voltage of 10 kV was applied, no significant cell shrinkage was observed. As the ablation electric field intensity gradually increased from 10 kV/cm to 12 kV/cm, the number of shrunken cells around the electrodes significantly increased, reaching 68%. This phenomenon is clearly demonstrated in the red-boxed area in Figure 2b. Meanwhile, during the ablation process, the temperature remained relatively stable; compared to 10 kV/cm, the temperature after ablation only rose by 0.6 °C even at a field intensity of 12 kV/cm. The average temperature change per

second for each group during the experiment is shown in Figure 3a. Confirming the fact that minimal temperature increase has almost no impact on cell viability, the entire ablation process is not dominated by thermal effects, and the final ablation effect should be mainly attributed to the action of the pulsed electric field.

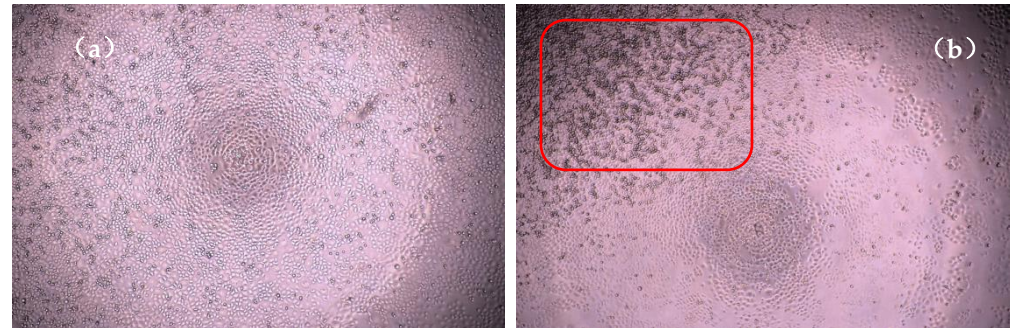


Figure 2. Optical microscope images after ablation at different pulsed electric field intensities: (a) field intensity of 10 kV/cm, 10 Hz, 30 s; (b) field intensity of 12 kV/cm, 10 Hz, 30 s.

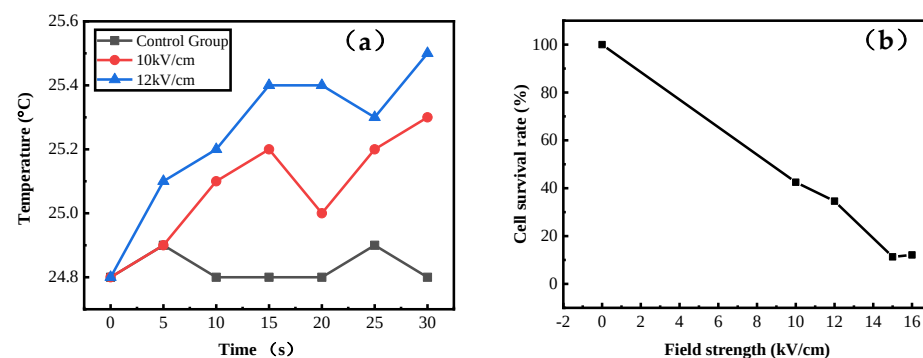


Figure 3. Graphs show temperature change over time and cell survival rate after ablation experiment. (a) Black squares represent the control group, red represents 10 kV/cm, and blue represents 12 kV/cm; (b) CCK8 results of cells after ablation at different electric field intensities and subsequent subculture for 12 h.

Although optical microscope images show that cells did not exhibit extensive shrinkage at a field intensity of 10 kV, this does not imply that the ablation effect is ineffective. The fundamental reason for this is that pulsed electric fields can induce permanent pores in the cell membrane, thereby enhancing its permeability and causing intracellular substances to leak out through osmosis. This process ultimately leads to cellular energy depletion, disrupts normal cellular physiological functions, and triggers cell death. It is noteworthy that even after experiencing permanent electroporation, cells may still maintain a certain degree of structural integrity, making it difficult to accurately determine cell death solely through an optical microscope observation. To more precisely quantify the ablation effect on cells more precisely, we employed the CCK8 cell viability assay. In the CCK8 test, we maintained consistent experimental parameters and performed the test in electrode cups. After the ablation procedure, we promptly disinfected the surface of the electrode cups in a biosafety laboratory without affecting the samples and then transferred the cells to a 48-well plate and added an appropriate amount of culture medium. After 12 h of incubation, we introduced the CCK8 reagent and used the Varioskan LUX microplate reader to conduct detailed analysis and detection of the CCK8 values. The survival rates of the cells in each group were rigorously calculated based on Equation (1), as follows:

$$R = \frac{Z_{CG} - Z_n}{Z_{CG}} \quad (1)$$

where Z_{CG} represents the CCK8 value of the control group after 12 h; Z_n represents the CCK8 value of the test group after 12 h. The CCK8 experimental results are depicted in Figure 3. As the voltage was increased from 10 kV to 12 kV, the survival rate of the cells cultured in Petri dishes for 12 h significantly decreased from 42.5% to 24.6% compared to the control group. Previously, after ablation at a field intensity of 12 kV, the proportion of cell shrinkage observed through an optical microscope was approximately 68%. Assuming that shrunk cells are dead cells, the proportion of cells maintaining normal morphology after ablation should be around 30%. This proportion is highly consistent with the 34.6% survival rate observed 12 h after ablation at 12 kV/cm. We speculate that the reason for this phenomenon may be the reduction in the number of viable cells after ablation, which indirectly affects the number of viable cells after 12 h of culture. Furthermore, the CCK8 results also reveal that during the ablation process, there is a positive correlation between the electric field intensity and the number of liver cancer cell deaths. However, this relationship is not linear between 10 kV/cm and 15 kV/cm. When the applied frequency and the number of pulses are kept constant at 10 Hz and 300, respectively, a significant enhancement in the ablation effect can be achieved even with a slight increase in electric field intensity once it exceeds 10 kV/cm. This finding provides an important scientific basis for optimizing the parameters of electric field ablation therapy.

Existing research indicates that cells predominantly die through cellular apoptosis under nanosecond pulses [40,41]. When using our pulse generator, cells shrink and die within just a few seconds of the ablation process, a phenomenon that can even be directly observed under a microscope. This phenomenon is caused by strong electric field stimulation. When we reduce the electric field intensity to below 10 kV/cm, the ablation effect diminishes, and the cell shrinkage phenomenon almost disappears. However, the ablation effect is still lower than that achieved with square wave pulses. This phenomenon may be related to the differences between square wave pulses and steep pulses. When using high-voltage steep pulses, the electric field reaches its peak rapidly and then quickly falls back. Higher voltages result in stronger penetration effects, and steep pulse electric fields are more likely to penetrate deep into cells and affect mitochondria, which aligns with existing reports that the ablation effect is dependent on mitochondria [42]. When the electric field intensity is lower, the duration for which the pulse electric field reaches the threshold on cancer cells becomes the dominant factor. At this point, the ablation effect is primarily influenced by the electroporation of the cell membrane. Therefore, when using our pulse device, a higher electric field intensity is required to achieve the desired effect. However, high electric field intensities pose a higher risk to device stability. Thanks to the advantages of SiC photoconductive switches, our device excels in terms of both safety and precise control compared to other high-voltage steep pulse devices. This is also a significant advantage of our self-developed pulse power device.

To further explore the threshold of electric field intensity's impact on ablation efficiency, we gradually increased the electric field intensity to 15 kV/cm and 16 kV/cm. As other experimental parameters remain unchanged, when the electric field intensity was increased to 15 kV/cm, the cell survival rate decreased to 11.3% after 12 h of culture. However, when the electric field intensity increased to 16 kV/cm, the cell survival rate decreased to 12.1%. Considering that inherent differences between samples in biological experiments may introduce experimental errors, we can reasonably infer that when the electric field intensity reaches 15 kV/cm, the ablation effect begins to saturate. In other words, the effect of pulsed electric fields on cells reaches a plateau at 15 kV/cm, and thereafter, even if the electric field intensity continues to increase, the ablation efficiency is no longer significantly enhanced. This discovery holds significant practical significance, as continuously and indefinitely increasing the electric field intensity typically brings unnecessary risks and side effects, such as tissue damage. Therefore, our research results indicate that under specific experimental conditions, there is an optimal range of electric field intensities that can ensure ablation efficiency while minimizing potential risks and side effects. This finding

provides an important scientific basis and reference for the clinical application of electric field ablation technology.

3.2. Frequency Effect

Traditional high-frequency pulse generators face numerous challenges when pursuing a high voltage output. A reduction in voltage often necessitates a narrowing of the treatment area to compensate for insufficient electric field intensity. Furthermore, when voltage is significantly increased, ensuring precise shut-off of the pulse generator power supply becomes another technical bottleneck, which is crucial for guaranteeing treatment safety. By addressing these challenges, we have independently developed a novel pulse generator that not only stably provides voltages up to 15 kV, ensuring a wide treatment range, but also integrates photoconductive switching technology to achieve fine control over the pulse generator's on-off operation, effectively avoiding the risk of overtreatment due to shut-off delay. Additionally, pulse devices typically face limitations in their stable operating frequency range when outputting high voltages. Even if attempts are made to increase the frequency, maintaining pulse stability and consistency become difficult. Particularly in the low-frequency region (commonly defined as less than 100 Hz), research on the ablation effects of electrical pulses is insufficient, yet this is precisely the operating frequency band of our novel pulse generator. Therefore, there is an urgent need to delve deeper into the ablation mechanisms of low-frequency electrical pulses.

To systematically assess the impact of frequency on ablation effectiveness, we designed a series of experiments where the number of pulses and electric field intensity were kept constant at 300 pulses and 10 kV/cm, respectively, while the frequency was adjusted to observe changes in ablation effectiveness. The experimental design rigorously followed the framework of previous studies on voltage impact to ensure the comparability and accuracy of the results. The trend of temperature changes during ablation is shown in Figure 4a, providing important data for analyzing ablation efficiency. After ablation, the CCK8 assay was used to evaluate cell survival after 12 h of culture, with the results presented in Figure 4b. These data directly reflect the biological effects of pulse ablation at different frequencies. In this study, we used the ratio of viable cells after 12 h of culture post-ablation as a measure of pulse ablation efficiency, and through scientific analysis of this key indicator, we aim to deeply reveal the underlying mechanisms of low-frequency electrical pulse ablation, providing a solid theoretical foundation for optimizing treatment protocols and expanding clinical applications.

The experimental results indicate that when the frequency is increased from 1 Hz to 5 Hz, the cell survival rate after 12 h of ablation improves from 82.3% to 72.9%. As the frequency continues to rise to 10 Hz, while other electrical pulse parameters remain unchanged, the ablation effectiveness increases to 57.5%. Comparing these results with previous studies on voltage impact, it is found that under the same parameters, the ablation rate is 58.6%, which is essentially consistent with the 57.5% ablation rate at 10 Hz. When the frequency is doubled from 5 Hz to 10 Hz, the ablation efficiency increases from 27.1% to 57.5%, representing a nearly 50% improvement. In contrast, in previous experiments, when the voltage increased from 10 kV/cm to 12 kV/cm, the ablation efficiency rose from 56.5% to 75.4%, an increase of approximately 30%. These findings suggest that when using our self-developed pulse generator for cell ablation, as the applied frequency falls within the low-frequency range, the ablation effect correspondingly improves with increasing frequency. However, the ablation effect at 10 Hz is still lower than that achieved with an electric field of 12.5 kV/cm. From this perspective, when the frequency and voltage are increased by the same ratio, electric field intensity has a more promotive effect on cell ablation than frequency.

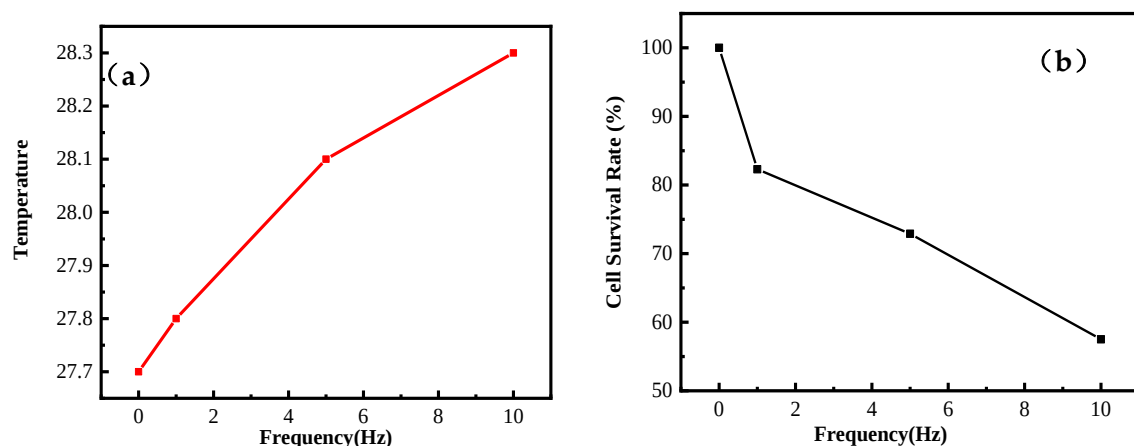


Figure 4. Temperature variation over time during the experiment and cell viability after 12 h. (a) Final temperature after ablation at different frequencies; (b) CCK8 results of cells after 12 h of subculture following ablation at different frequencies.

Previous studies have shown that pulse electric field intensity has a greater impact on cell ablation than pulse frequency [28,37,38]. The magnitude of electric field intensity directly determines the electric field's ability to penetrate cell membranes and its driving force on charges within tissues. During ablation, the level of voltage directly influences the degree of cell damage and the ablation range caused by the electric field. Frequency, on the other hand, primarily affects the changing rate of the electric field during ablation. Although frequency also has an impact, its influence is relatively smaller within the low-frequency range. The experimental data reveal that as the frequency gradually increased from 1 Hz to 5 Hz, the cell survival rate after 12 h of ablation significantly decreased, dropping from 82.3% to 72.9%. Further increasing the frequency to 10 Hz resulted in a substantial decrease in the survival rate to 41.7%, while keeping other electrical pulse parameters constant. By comparing these results with previous studies on voltage impact, it is found that under the same parameter settings, the cell survival rate is 41.7%, which is almost identical to the 42.5% survival rate at 10 Hz in previous studies. Notably, when the frequency is doubled from 5 Hz to 10 Hz, the survival rate decreases by nearly 50% (from 72.9% to 41.7%), which is a much larger improvement than the approximate 30% decrease in the survival rate (from 42.5% to 34.6%) observed in previous experiments when the voltage was increased from 10 kV/cm to 12 kV/cm. These results strongly demonstrate that using our self-developed low-frequency pulse generator for cell ablation shows a positive correlation with increasing frequency within the low-frequency range. However, it should be noted that even at 10 Hz, the ablation effect is slightly weaker than that achieved with an electric field intensity of 12.5 kV/cm. This indicates that, when the frequency and voltage are increased by the same ratio, electric field intensity (directly determined by voltage) has a more significant promotive effect on cell ablation than frequency.

Electric field intensity, as a direct reflection of voltage, directly determines the electric field's ability to penetrate cell membranes and drive charges within tissues. During ablation, a higher voltage means that the electric field can disrupt cell structures and expand the ablation range more effectively. In contrast, frequency mainly affects the changing rate of the electric field. Within the low-frequency range, although frequency has a certain impact on ablation effectiveness, its role is relatively weaker. Meanwhile, the potential optimization space of frequency should not be overlooked, especially when exploring possible nonlinear effects or synergistic interactions at specific frequencies. In summary, by fine-tuning the combination of voltage and frequency, it is possible to achieve more efficient and precise cell ablation effects. On the other hand, during the experiment, the temperature showed an increasing trend. At frequencies of 1 Hz, 5 Hz, and 10 Hz, the temperature rises by 0.1 °C, 0.4 °C, and 0.6 °C, respectively, throughout the ablation process, as shown in Figure 4b. As the frequency increases, the rate of temperature rises also accelerates. However, this change

is relatively gradual and small, with almost no thermal energy generated throughout the process. This phenomenon also indicates that it is the electric field, rather than heat, that plays a direct and primary role in the ablation of A549 cells during the process.

3.3. Pulse Number Effect

In electroporation ablation technology, the number of pulses plays a crucial role, with its influence rooted in the complex interaction mechanism between electrical pulses and cells. When cells are exposed to a pulsed electric field, subtle changes occur in the phospholipid bilayer structure of the cell membrane, resulting in the formation of nanoscale pores. This process is known as electroporation. When the electric field intensity is maintained within a specific range, these pores can self-recover, manifesting as reversible electroporation (RE). However, if the electric field intensity exceeds a certain threshold, the pores in the cell membrane will undergo irreversible changes, leading to cell death. This is referred to as irreversible electroporation (IRE). As the number of pulses increases, the effect of IRE becomes more pronounced. This enhanced effect stems from the accumulation of pulse numbers, meaning that more energy is delivered to the cell membrane, resulting in an increased number of pores that are harder to recover from, thereby increasing the probability of cell death. It is worth noting; however, that this enhancement is not unlimited and there is a saturation limit. When the number of pulses increases to a certain value, the enhancement trend of the IRE effect tends to plateau, with no significant further improvement. This implies that, upon reaching the saturation point, whether increasing the number of pulses, adjusting the voltage, or changing the frequency, the cumulative effect will stabilize. Currently, there is relatively little research on electroporation ablation in the low-frequency range, with most studies focusing on the field of square wave medium-to high-frequency pulses. Therefore, combining our newly developed pulse generator to study the impact of pulse number on cancer cell ablation effects is expected to provide more comprehensive and in-depth scientific support for the optimization and practical application of electroporation ablation technology.

To specifically investigate the influence of pulse number on the effect of electroporation ablation, we designed a series of carefully controlled experiments where the frequency and electric field intensity were consistently maintained at 10 Hz and 15 kV/cm, respectively. These parameters were based on the optimal ablation conditions determined in previous studies. To comprehensively assess the role of pulse number, we selected four different pulse number levels for testing: 0 (as a control group), 300, 600, and 900. This experimental design aimed to precisely determine the optimal pulse parameters by comparing the ablation effects at different pulse numbers. The experimental results are shown in Figure 5. Under the conditions of a frequency of 10 Hz and an electric field intensity of 15 kV/cm, when the pulse number was 300, the cell viability reflected by the CCK8 value after 12 h of culture post-ablation reached 9.7%. This result is highly consistent with previous research data of 11.3%, validating the stability and reliability of the experiment. Furthermore, when the pulse number increased to 600 and 900, the cell viability significantly decreased to 1.3% and 0.7%, respectively, indicating that the ablation effect gradually strengthened with the increase in pulse number, as shown in Figure 5b.

It is particularly noteworthy that when the pulse number increased from 300 to 600, there was a significant reduction in cell viability from 9.7% to 1.3%, indicating a strong effect. In contrast to similar PEF ablation studies, the results of one of the studies indicated that following the completion of pulsed electric field (PEF) treatment, an electrochemical assessment of the ablation of colorectal liver metastases revealed that 23 out of 27 metastases achieved complete remission; on the other hand, Dr. Jing Wang found that the proportion of cell apoptosis could reach around 80% in a study on the ablation of human oral squamous cell carcinoma using nanosecond pulsed electric fields. Combined with the results of other similar studies, the ablation efficiency is about 80–95% [39,43–45]. In contrast, our study showed that only 0.7% of the cells in the treatment group survived within 12 h after the ablation. This figure indicates an ablation efficiency exceeding 90%, a result attributed to the

high-voltage pulse generator we employed. However, when the pulse number continued to increase from 600 to 900, although the ablation effect still increased, the magnitude of the increase was relatively small, with cell viability only decreasing from 1.3% to 0.7%. This phenomenon suggests that after the pulse number increases to a certain level, the enhancement trend of the ablation effect begins to plateau, possibly reaching a saturation point. On the other hand, in studies examining the effects of electrical pulses on cells, there have been previous reports using almost the same pulse parameters as ours, yet the ablation effect on the cells was significantly weaker compared to our results [46]. By comparing each of the pulse parameters used in both studies, it was found that the pulse width provided by our pulse source was only 10 ns, while the other study used a pulse width of 300 ns. This significant difference in pulse width is a crucial factor contributing to the disparity in ablation outcomes. Existing research has shown that narrower pulse widths result in better penetration of the pulse during cell ablation [40,47,48]. Therefore, our pulses are more likely to penetrate deeply into the cells and act on the intracellular organelles, leading to more effective cell destruction and consequently a more pronounced ablation effect. In summary, this experiment systematically studied and revealed the significant impact of pulse number on the effect of electroporation ablation and initially explored its potential saturation effect. Future research should further investigate the changes in the ablation effect over a wider range of pulse numbers and how to optimize pulse parameters to achieve more efficient and safer electroporation ablation therapy.

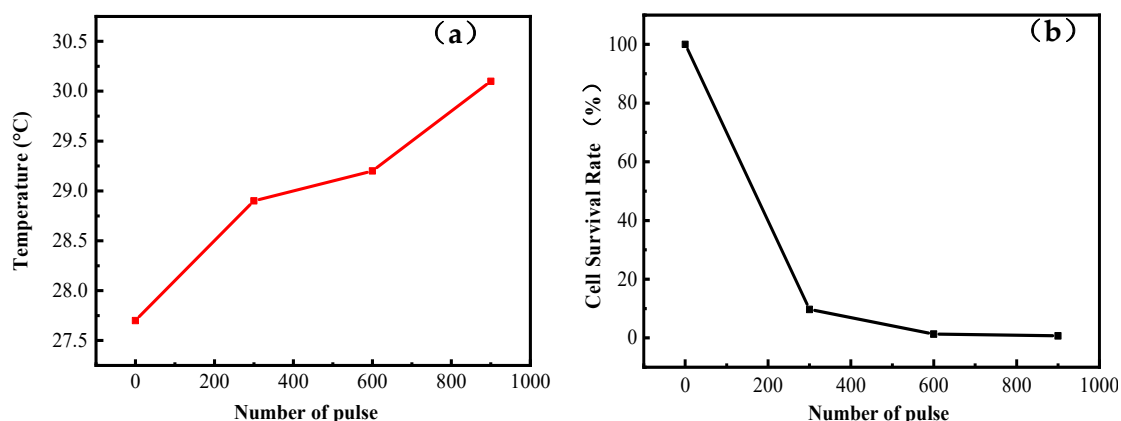


Figure 5. Temperature variation over time during the experiment and cell viability under ablation with different pulse numbers. (a) Temperature changes in the samples after ablation with different pulse numbers; (b) cell viability after 12 h of subculture following ablation with different pulse numbers.

In conclusion, during the process of the electroporation ablation of liver cancer cells using our newly developed pulse generator, the ablation effect is positively correlated with the cumulative effect of the applied number of electrical pulses. At the same time, when the number of electrical pulses reaches 900, further increasing the pulse number no longer has a significant impact on the final ablation effect. We refer to this as the “cumulative saturation effect of pulse number”. This saturation differs from the saturation caused by increasing the electric field intensity. When treatment saturation is reached due to the electric field intensity, increasing the electric field will expand the ablation area, but the ablation effect within the original area between the electrodes does not significantly improve. On the other hand, the saturation caused by increasing the pulse number results in the number and size of pores on the cells reaching saturation and stabilization, without significantly affecting the ablation range.

4. Conclusions

This paper reports on a novel pulse generator, which is based on a silicon carbide (SiC) photoconductive switch. Through cell ablation experiments, we investigated the impact of various pulse parameters on the final ablation effect when using this device for liver cancer cell ablation. The pulse generator can reach a maximum voltage of 15 kV and a maximum frequency of 10 Hz, with the number of pulses flexibly adjustable according to demand. In our cell ablation studies, we found that within the low-frequency range (<10 Hz), voltage has a greater influence on the ablation of liver cancer cells than frequency. Furthermore, optimal ablation effects were achieved when the electric field intensity was set to 15 kV/cm, the frequency to 10 Hz, and the number of pulses to 900. Under these parameters, the cell survival rate after 12 h of subculture following ablation was only 0.7% of that of the control group under the same conditions, indicating a high level of ablation efficiency compared to similar studies. On the other hand, during the ablation process, the temperature change near the ablation probe was less than three centigrade, demonstrating that this device generates minimal heat during operation and thus exhibiting high medical safety. This research is significant for broadening the application fields of SiC devices and actively promotes the exploration of SiC devices in interdisciplinary applications.

Author Contributions: Conceptualization, Y.W. and H.G.; Methodology, Y.W. and Y.Z.; Software, Z.Z.; Validation, H.Y., Y.L. and Y.W.; Formal analysis, Z.Z.; Investigation, H.Y., Z.Z. and Y.L.; Resources, Y.W., H.G. and Y.Z.; Writing—original draft, H.Y.; Writing—review & editing, H.Y. and Z.Z.; Project administration, H.Y. and H.G.; Funding acquisition, Y.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tsuji, W.; Takuwa, H. Surgical treatment for stage IV breast cancer: A single-center, retrospective study. *Asian J. Surg.* **2020**, *43*, 713–715. [[CrossRef](#)] [[PubMed](#)]
2. Shirai, K.; Aoki, S.; Endo, M.; Takahashi, Y.; Fukuda, Y.; Akahane, K.; Musha, A.; Sato, H.; Wakatsuki, M.; Ishikawa, H.; et al. Recent developments in the field of radiotherapy for the management of lung cancer. *Jpn. J. Radiol.* **2024**. [[CrossRef](#)] [[PubMed](#)]
3. Domagała-Haduch, M.; Gorzelak-Magiera, A.; Michalecki, Ł.; Gisterek-Grocholska, I. Radiochemotherapy in Pancreatic Cancer. *Curr. Oncol.* **2024**, *31*, 3291–3300. [[CrossRef](#)]
4. Ferroni, C.; Varchi, G. Editorial: Small molecules and smart drug delivery systems for combination cancer immunotherapy. *Front. Chem.* **2024**, *12*, 1412624. [[CrossRef](#)] [[PubMed](#)]
5. Huang, J.H.; Guo, W.; Liu, Z. Discussion on gemcitabine combined with targeted drugs in the treatment of pancreatic cancer. *World J. Gastroenterol.* **2023**, *29*, 579–581. [[CrossRef](#)] [[PubMed](#)]
6. Huimin, Z.; Xueting, W.; Qi, Q.; Lingxin, F.; Xue, Y.; Zhuang, Y.; Jing, W. Multiple Primary Lung Cancers with ALK Rearrangement: A Case Report and Literature Review. *Front. Oncol.* **2022**, *12*, 897451. [[CrossRef](#)]
7. Wyld, L.; Audisio, R.A.; Poston, G.J. The evolution of cancer surgery and future perspectives. *Nat. Rev. Clin. Oncol.* **2015**, *12*, 115–124. [[CrossRef](#)]
8. Riis, M. Modern surgical treatment of breast cancer. *Ann. Med. Surg.* **2020**, *56*, 95–107. [[CrossRef](#)]
9. Raoof, S.; Clarke, C.A.; Hubbell, E.; Chang, E.T.; Cusack, J. Surgical resection as a predictor of cancer-specific survival by stage at diagnosis and cancer type, United States, 2006–2015. *Cancer Epidemiol.* **2023**, *84*, 102357. [[CrossRef](#)] [[PubMed](#)]
10. Mollaei, M.; Hassan, Z.M.; Khorshidi, F.; Langroudi, L. Chemotherapeutic drugs: Cell death- and resistance-related signaling pathways. Are they really as smart as the tumor cells? *Transl. Oncol.* **2021**, *14*, 101056. [[CrossRef](#)]
11. Brianna; Lee, S.H. Chemotherapy: How to reduce its adverse effects while maintaining the potency? *Med. Oncol.* **2023**, *40*, 88. [[CrossRef](#)]
12. Tilsed, C.M.; Fisher, S.A.; Nowak, A.K.; Lake, R.A.; Lesterhuis, W.J. Cancer chemotherapy: Insights into cellular and tumor microenvironmental mechanisms of action. *Front. Oncol.* **2022**, *12*, 960317. [[CrossRef](#)]
13. Chandra, R.A.; Keane, F.K.; Voncken, F.E.M.; Thomas, C.R., Jr. Contemporary radiotherapy: Present and future. *Lancet* **2021**, *398*, 171–184. [[CrossRef](#)]
14. Abdel-Wahab, M.; Gondhowiardjo, S.S.; Rosa, A.A.; Lievens, Y.; El-Haj, N.; Rubio, J.A.P.; Ben Prajogi, G.; Helgadottir, H.; Zubizarreta, E.; Meghzifene, A.; et al. Global Radiotherapy: Current Status and Future Directions—White Paper. *JCO Glob. Oncol.* **2021**, *7*, 827–842. [[CrossRef](#)]

15. Strand, N.H.; Hagedorn, J.M.; Dunn, T.; Johnson, B.; Abd-Elseyed, A.; Covington, S.; Freeman, J.; Dawodu, A.; Maloney, J. Advances in radiofrequency ablation: Mechanism of action and technology. *Ann. Palliat. Med.* **2024**, *13*, 1028–1034. [\[CrossRef\]](#)
16. Gómez-Barea, M.; García-Sánchez, T.; Ivorra, A. A computational comparison of radiofrequency and pulsed field ablation in terms of lesion morphology in the cardiac chamber. *Sci. Rep.* **2022**, *12*, 16144. [\[CrossRef\]](#)
17. Liu, Z.-L.; Chen, H.-H.; Zheng, L.-L.; Sun, L.-P.; Shi, L. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal Transduct. Target. Ther.* **2023**, *8*, 198. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Wu, C.; Li, X.; Lv, Z.; Chen, Q.; Lou, Y.; Mao, W.; Zhou, X. Second-generation cryoballoon versus contact force radiofrequency ablation for atrial fibrillation: An updated meta-analysis of evidence from randomized controlled trials. *Sci. Rep.* **2021**, *11*, 17907. [\[CrossRef\]](#)
19. Thai, J.N.; Sevrakov, A.B.; Ward, R.C.; Monticciolo, D.L. Cryoablation Therapy for Early-Stage Breast Cancer: Evidence and Rationale. *J. Breast. Imaging* **2023**, *5*, 646–657. [\[CrossRef\]](#) [\[PubMed\]](#)
20. De Vita, E.; De Tommasi, F.; Massaroni, C.; Iadicco, A.; Faiella, E.; Carassiti, M.; Grasso, R.F.; Schena, E.; Campopiano, S. Investigation of the Heat Sink Effect During Microwave Ablation in Hepatic Tissue: Experimental and Numerical Analysis. *IEEE Sens. J.* **2021**, *21*, 22743–22751. [\[CrossRef\]](#)
21. Fan, Y.; Xu, L.; Liu, S.; Li, J.; Xia, J.; Qin, X.; Li, Y.; Gao, T.; Tang, X. The State-of-the-Art and Perspectives of Laser Ablation for Tumor Treatment. *Cyborg Bionic Syst.* **2024**, *5*, 0062. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Zhang, L.; Zhou, W.; Zhou, J.Q.; Shi, Q.; Rago, T.; Gambelunghe, G.; Zou, D.Z.; Gu, J.; Lu, M.; Chen, F.; et al. Expert consensus on the use of laser ablation for papillary thyroid microcarcinoma. *Int. J. Hyperth.* **2022**, *39*, 1254–1263. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Kok, E.N.D.; Eppenga, R.; Kuhlmann, K.F.D.; Groen, H.C.; van Veen, R.; van Dieren, J.M.; de Wijkerslooth, T.R.; van Leerdam, M.; Lambregts, D.M.J.; Heerink, W.J.; et al. Accurate surgical navigation with real-time tumor tracking in cancer surgery. *NPJ Precis. Onc.* **2020**, *4*, 8. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Geboers, B.; Scheffer, H.J.; Graybill, P.M.; Ruarus, A.H.; Nieuwenhuizen, S.; Puijk, R.S.; van den Tol, P.M.; Davalos, R.V.; Rubinsky, B.; de Gruijl, T.D.; et al. High-Voltage Electrical Pulses in Oncology: Irreversible Electroporation, Electrochemotherapy, Gene Electroporation, Electroporation, and Electroimmunotherapy. *Radiology* **2020**, *295*, 254–272. [\[CrossRef\]](#)
25. Maan, A.; Koruth, J. Pulsed Field Ablation: A New Paradigm for Catheter Ablation of Arrhythmias. *Curr. Cardiol. Rep.* **2022**, *24*, 103–108. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Shaheen, N.; Shaheen, A.; Ramadan, A.; Nashwan, A.J. Efficacy and safety of novel pulsed field ablation (PFA) technique for atrial fibrillation: A systematic review and meta-analysis. *Health Sci. Rep.* **2023**, *6*, 1079. [\[CrossRef\]](#)
27. Reddy, V.Y.; Gerstenfeld, E.P.; Natale, A.; Whang, W.; Cuoco, F.A.; Patel, C.; Mountantonakis, S.E.; Gibson, D.N.; Harding, J.D.; Ellis, C.R.; et al. ADVENT Investigators. Pulsed Field or Conventional Thermal Ablation for Paroxysmal Atrial Fibrillation. *N. Engl. J. Med.* **2023**, *389*, 1660–1671. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Gudvangen, E.; Kim, V.; Novickij, V.; Battista, F.; Pakhomov, A.G. Electroporation and cell killing by milli- to nanosecond pulses and avoiding neuromuscular stimulation in cancer ablation. *Sci. Rep.* **2022**, *12*, 1763. [\[CrossRef\]](#)
29. Ezzeddine, F.M.; Asirvatham, S.J.; Nguyen, D.T. Pulsed Field Ablation: A Comprehensive Update. *J. Clin. Med.* **2024**, *13*, 5191. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Zang, L.; Gu, K.; Zhou, T.; Si, P.; Ji, X.; Zhang, H.; Yan, S.; Wu, X. Investigate the relationship between pulsed field ablation parameters and ablation outcomes. *J. Interv. Card. Electrophysiol.* **2024**. [\[CrossRef\]](#)
31. Palmeri, N.O.; Alyesh, D.; Keith, M.; Greenhaw, E.; Erickson, C.; Choe, W.; Sundaram, S. Pulsed-field ablation for atrial fibrillation without the use of fluoroscopy. *J. Interv. Card. Electrophysiol.* **2024**. [\[CrossRef\]](#)
32. Verma, A.; Haines, D.E.; Boersma, L.V.; Sood, N.; Natale, A.; Marchlinski, F.E.; Calkins, H.; Sanders, P.; Packer, D.L.; Kuck, K.H.; et al. PULSED AF Investigators. Pulsed Field Ablation for the Treatment of Atrial Fibrillation: PULSED AF Pivotal Trial. *Circulation* **2023**, *147*, 1422–1432. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Alshahat, M.A.; Elgenedy, M.A.; Aboushady, A.A.; Williams, M.T.S. Cancer Treatment: An Overview of Pulsed Electric Field Utilization and Generation. *Appl. Sci.* **2023**, *13*, 10029. [\[CrossRef\]](#)
34. Lutz, J.; Scheuermann, U.; Schlangenotto, H.; De Doncker, R. Power Semiconductor Devices—Key Components for Efficient Electrical Energy Conversion Systems. In *Semiconductor Power Devices*; Springer: Cham, Switzerland, 2018. [\[CrossRef\]](#)
35. Zhang, Y.; Udrea, F.; Wang, H. Multidimensional device architectures for efficient power electronics. *Nat. Electron.* **2022**, *5*, 723–734. [\[CrossRef\]](#)
36. Pakhomova, O.N.; Gregory, B.W.; Semenov, I.; Pakhomov, A.G. Two Modes of Cell Death Caused by Exposure to Nanosecond Pulsed Electric Field. *PLoS ONE* **2013**, *8*, e70278. [\[CrossRef\]](#)
37. Kielbik, A.; Szlasa, W.; Novickij, V.; Szewczyk, A.; Maciejewska, M.; Saczko, J.; Kulbacka, J. Effects of high-frequency nanosecond pulses on prostate cancer cells. *Sci. Rep.* **2024**, *11*, 15835. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Gianulis, E.C.; Lee, J.; Jiang, C.; Xiao, S.; Ibey, B.L.; Pakhomov, A.G. Electroporation of mammalian cells by nanosecond electric field oscillations and its inhibition by the electric field reversal. *Sci. Rep.* **2015**, *5*, 13818. [\[CrossRef\]](#)
39. Wang, J.; Guo, J.; Wu, S.; Feng, H.; Sun, S.; Pan, J.; Zhang, J.; Beebe, S.J. Synergistic Effects of Nanosecond Pulsed Electric Fields Combined with Low Concentration of Gemcitabine on Human Oral Squamous Cell Carcinoma In Vitro. *PLoS ONE* **2012**, *7*, e43213. [\[CrossRef\]](#)

40. Wang, Y.; Yin, S.; Zhou, Y.; Zhou, W.; Chen, T.; Wu, Q.; Zhou, L.; Zheng, S. Dual-function of Baicalin in nsPEFs-treated Hepatocytes and Hepatocellular Carcinoma cells for Different Death Pathway and Mitochondrial Response. *Int. J. Med. Sci.* **2019**, *16*, 1271–1282. [[CrossRef](#)]
41. Liu, Z.G.; Chen, X.H.; Yu, Z.J.; Lv, J.; Ren, Z.G. Recent progress in pulsed electric field ablation for liver cancer. *World J. Gastroenterol.* **2020**, *26*, 3421–3431. [[CrossRef](#)] [[PubMed](#)]
42. He, L.; Xiao, D.; Feng, J.; Yao, C.; Tang, L. Induction of apoptosis of liver cancer cells by nanosecond pulsed electric fields (nsPEFs). *Med. Oncol.* **2017**, *34*, 24. [[CrossRef](#)]
43. Sano, M.B.; Arena, C.B.; Dewitt, M.R.; Saur, D.; Davalos, R.V. In-vitro bipolar nano- and microsecond electro-pulse bursts for irreversible electroporation therapies. *Bioelectrochemistry* **2014**, *100*, 69–79. [[CrossRef](#)]
44. Munavalli, G.S.; Zelickson, B.D.; Selim, M.M.; Kilmer, S.L.; Rohrer, T.E.; Newman, J.; Jauregui, L.; Knape, W.A.; Ebberts, E.; Uecker, D.; et al. Safety and Efficacy of Nanosecond Pulsed Electric Field Treatment of Sebaceous Gland Hyperplasia. *Dermatol. Surg.* **2019**, *46*, 803–809. [[CrossRef](#)]
45. Edhemovic, I.; Brecelj, E.; Gasljevic, G.; Music, M.M.; Gorjup, V.; Mali, B.; Jarm, T.; Kos, B.; Pavliha, D.; Kuzmanov, B.G.; et al. Intraoperative electrochemotherapy of colorectal liver metastases. *Surg. Oncol.* **2014**, *110*, 320–327. [[CrossRef](#)] [[PubMed](#)]
46. Buescher, E.S.; Schoenbach, K.H. Effects of submicrosecond, high intensity pulsed electric fields on living cells-intracellular electromanipulation. *IEEE Trans. Dielectr. Electr. Insul.* **2003**, *10*, 788–794. [[CrossRef](#)]
47. Miller, L.; Leor, J.; Rubinsky, B. Cancer Cells Ablation with Irreversible Electroporation. *Technol. Cancer Res. Treat.* **2016**, *4*, 699–705. [[CrossRef](#)] [[PubMed](#)]
48. Reberšek, M.; Miklavčič, D. Concepts of Electroporation Pulse Generation and Overview of Electric Pulse Generators for Cell and Tissue Electroporation. In *Advanced Electroporation Techniques in Biology and Medicine*; CRC Press: Boca Raton, FL, USA, 2010.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.