Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

Development and Preliminary Validation of a Wearable Tumor Treating Fields Device for Breast Cancer Therapy

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Introduction: Tumor treating fields (TTFields) is a novel non-invasive tumor treatment method that can inhibit the growth of a variety of tumors with few side effects. However, the devices used for TTFields therapy are relatively scarce and has great limitations in scientific research and clinical application scenarios.

Objective: The objective of this study is to address the challenge posed by the large size of TTFields therapeutic devices, which adversely affect patient mobility, quality of life, and treatment adherence. Furthermore, this study aims to explore the potential of TTFields as a therapeutic approach for breast cancer treatment, evaluating its efficacy and feasibility in extracranial applications.

Methods: In this study, we designed a wearable device called TTF-Backpack integrates a stimulator for generating alternating electric field (0~250 kHz, 0~40 mA). In addition, we planned circuit experiments to evaluate the electrical performance of the device and validated its efficacy by establishing rat models of breast cancer syngeneic graft. The models were treated with alternating electric fields (frequency 250 kHz, intensity 1–1.5 V/cm) for 24 hours daily over 7 days.

Results: The maximum output voltage of TTF-Backpack is approximately 55V with the frequency bandwidth of 0~250 kHz, and its current can reach 40 mA. Furthermore, animal experiment results indicated that TTF-Backpack can inhibit the progression of breast cancer. Analysis of tumor tissue sections demonstrated that the mRNA levels of ki67, the mRNA and protein expression levels of the pro-apoptotic gene Bax upregulated, while the protein expression level of the anti-apoptotic marker Bcl2 downregulated significantly.

Conclusion: This study demonstrates the feasibility and preliminary efficacy of a novel wearable TTFields device for breast cancer therapy.

Keywords: tumor treating fields, breast cancer, wearable devices, non invasive, physical therapy

Introduction

Breast cancer remains one of the most common malignancies among women worldwide, with an increasing incidence rate.[1] Despite significant advances in early detection and treatment, traditional therapeutic approaches, including surgery, chemotherapy, and radiotherapy, are often associated with substantial physical and emotional burdens.[2] Radical mastectomy, for instance, involves extensive tissue resection, which can lead to significant disfigurement, severe emotional distress, and a compromised quality of life.[3,4] These challenges have driven the development of breast-conserving therapies aimed at minimizing treatment-related trauma while achieving effective tumor control.[5] Among these, non-invasive treatment strategies have garnered particular interest due to their potential to control tumors effectively while minimizing adverse impacts on patients.[6]

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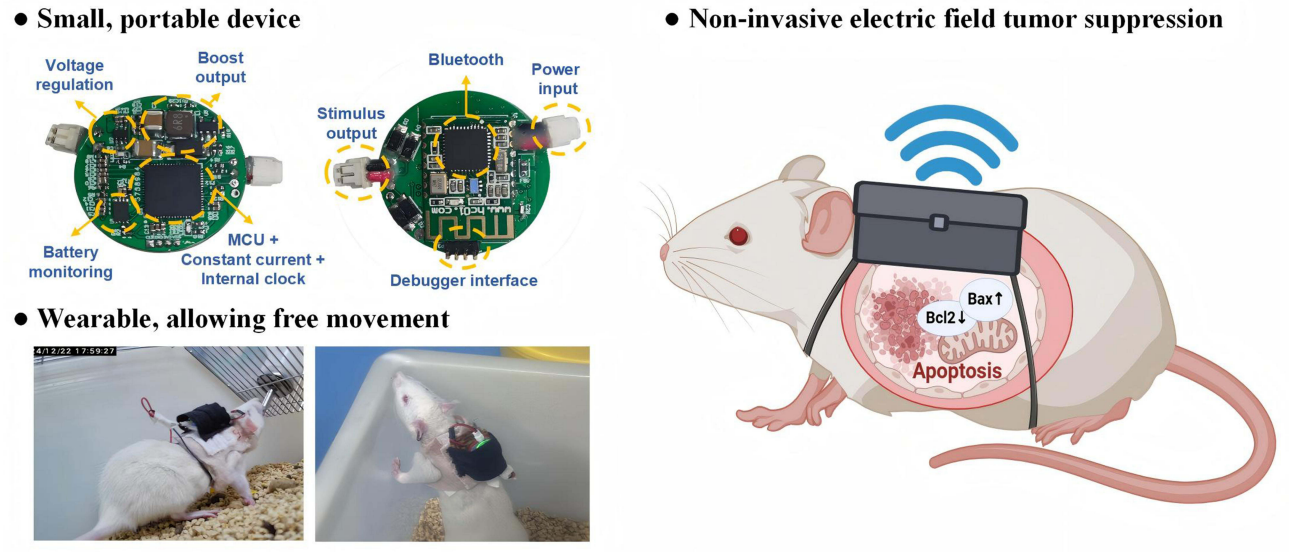
Graphical Abstract



Tumor Treating Fields (TTFields) represent an innovative, non-invasive therapeutic strategy that applies low-intensity (1–3 V/cm), intermediate-frequency (100–300 kHz) alternating electric fields to disrupt the replication and proliferation of tumor cells.[7] The US Food and Drug Administration (FDA) has approved TTFields for the treatment of recurrent or newly diagnosed glioblastoma (GBM), malignant pleural mesothelioma (MPM) and metastatic non-small cell lung cancer (NSCLC).[8] During TTFields therapy, insulated arrays are closely attached to the tumor site, delivering continuous alternating electric fields via a portable field generator. The efficacy and safety of TTFields for newly diagnosed and recurrent GBM patients have been demonstrated in Phase III clinical trials, notably EF-11 and EF-14.[9,10] In addition, the efficacy of TTFields in malignant pleural mesothelioma[11,12] and metastatic non-small cell lung cancer[13,14] has also been verified. Consequently, TTFields have become a standard treatment for GBM, MPM and NSCLC patients and are now being investigated in preclinical studies and clinical trials for their potential to treat other solid tumors such as liver cancer,[15] ovarian cancer[16] and pancreatic cancer.[17] Given its minimal side effects and potential to complement existing treatment modalities,[18] TTFields holds promise as a novel cancer treatment strategy, particularly for patients seeking effective disease control while preserving their breast.[19]

Although TTFields have demonstrated significant therapeutic efficacy in clinical trials, their underlying mechanisms remain incompletely understood. The typical mechanism is to interfere with the mitosis of tumor cells, but has little effect on normal cells.[20] This selective effect is primarily attributed to the distinct biophysical and mitotic properties of tumor cells. Malignant cells frequently exhibit abnormally high proliferation rates and prolonged mitosis,[21,22] during which they are particularly vulnerable to the disruptive effects of alternating electric fields on crucial processes such as mitotic spindle assembly and organelle positioning.[20] Furthermore, the altered morphology and elevated electrical conductivity of tumor cells enhance the energy absorption and focusing of TTFields.[23] In contrast, most non-dividing or slowly dividing normal cells remain largely unaffected due to their lower metabolic activity and more stable cytoskeletal architecture. The therapeutic efficacy of TTFields is influenced by tumor geometry and location.[24] Preclinical and clinical evidence suggests that TTFields are most effective in treating small- to medium-sized tumors, as the electric field intensity can be maintained within the therapeutic range (1–3 V/cm).

Importantly, the rationale for combining TTFields with systemic agents is strongly supported by clinical evidence, such as the EF-14 trial establishing TTFields with temozolomide as a standard for glioblastoma, and the LUNAR trial demonstrating survival benefits when combining TTFields with immunotherapy or chemotherapy in lung cancer. This evidence underpins the potential of integrating our wearable TTFields device into multimodal strategies for breast cancer.

It is hypothesized that TTFields disrupt the movement and assembly of critical proteins and protein complexes, such as β-tubulin and the mitotic spindle, within tumor cells, leading to aberrant mitosis and cell death.[25] Emerging evidence suggests that TTFields may also activate anti-tumor immune responses within the tumor microenvironment, inhibiting tumor progression and proliferation.[26–28] Furthermore, TTFields have been shown to suppress cell invasion and migration, induce apoptosis and autophagy, and modulate immune function.[29–31] These effects may also alter the permeability of key biological barriers, such as the nuclear envelope, cell membrane, and blood-brain barrier.[32,33]

In addition, conventional TTFields devices are bulky and heavy, limiting their feasibility for wearable applications. The devices currently used for TTFields delivery, such as Optune® (Novocure), consist of a portable field generator, battery packs, and insulated transducer arrays that are applied directly to the patient's skin over the tumor region. These devices have demonstrated efficacy in prolonging progression-free survival and overall survival in GBM patients.[9] Despite these successes, the current generation of TTFields device are relatively large and cumbersome, which can restrict patient mobility and daily activities. The device weight and external battery requirements can lead to discomfort and reduced adherence to treatment protocols, particularly for long-term usage. Novocure are designed for cranial applications, such as glioblastoma treatment, where the arrays are optimized for skull-mounted configurations. Translating this technology to treat extracranial tumors, such as breast cancers, poses significant challenges due to anatomical differences and the need for customized array designs.

In this study, we developed a lightweight, compact, and wearable TTFields device, specifically designed for breast cancer therapy, named TTF-Backpack. Additionally, we conducted animal experiments to validate its therapeutic efficacy and investigate its effects on tumor cell proliferation and apoptosis. The findings from this study aim to offer enhanced convenience and therapeutic potential for patients with breast cancer.

Methods and Materials

Device Performance Evaluation

The performance evaluation of TTF-Backpack involves multiple aspects of testing and analysis to ensure the safety and effectiveness of the equipment. The following are commonly used performance indicators for electric field therapy devices. First, the electric field strength generated by the device should be measured to determine whether it meets the design requirements and safety standards. Second, the stability of the electric field strength of the device during long-term operation should be evaluated to ensure that there will be no fluctuations during use or that the fluctuations are within an acceptable range. Third, the electric field frequency and waveform of the device are tested to ensure accurate output to meet the needs of various application scenarios. Fourth, safety performance tests, including overload protection, short circuit protection, and electrode detachment detection, are performed to ensure that the device can safely stop working under abnormal conditions.

In this study, we designed the following experiment to verify the performance of the stimulator.

Performance Parameters

1. The maximum range of stimulator output current and voltage.
2. Stability of stimulator current output.
3. Response to stimulator parameter settings.

Verification Method

1. The output terminal of the stimulator was connected to an adjustable resistor, and the output current was set to 1 mA, 20 mA, and 40 mA respectively. An Agilent DSO3062A oscilloscope (Agilent Technologies, Inc., Santa Clara, CA, USA) was employed to measure the output waveform and document the amplitude that corresponds to each resistance value, after which the resistance-voltage characteristic curve was plotted to systematically evaluate the load-carrying capacity of the stimulator.
2. The output terminal of the stimulator was connected to a 250 Ω resistor load, the frequency was set to 250 kHz, and the current amplitude was adjusted to 6 mA, 12 mA, 24 mA, and 36 mA respectively. The stimulator

continued to work for 12 hours, the output waveform was measured with an oscilloscope and the voltage amplitude was recorded every ten minutes at the same time, then a mean standard deviation bar graph was drawn. In addition, the output voltage can be detected by the stimulator and uploaded to the monitoring terminal to analyze whether there was a drop in the continuous voltage.

3. The output terminal of the stimulator was connected to a 250 Ω resistor load, then adjust the set current amplitude, measure the output waveform with an oscilloscope and record the voltage amplitude to plot the current setting value and the measured value curve. With the same circuit configuration maintained, a fixed amplitude was set and the preset frequency was tuned, after which an Agilent N9320B spectrum analyzer (Agilent Technologies, Inc., Santa Clara, CA, USA) was employed to document the actual measured frequency. Subsequently, the characteristic curve of preset frequency versus actual measured frequency was plotted, based on which the stimulator's current and frequency response characteristics were quantitatively assessed through the deviation of the fitting curve.

Animal Validation

To validate the effect of the aforementioned TTF-backpack device on breast cancer progression, animal models of breast cancer syngeneic graft were established, and the TTF-backpack device was applied to the animal tumor models.

Tumor Cell Lines

Fluorescent enzyme-expressing breast cancer cell line (MADB-106) was purchased from Shanghai Fuheng Biotechnology Co., Ltd. in 2024. MADB-106 cells were cultured in DMEM medium (HAKATA) containing L-glutamine, supplemented with 10% fetal bovine serum (FBS, HAKATA), and 1% penicillin/streptomycin in vitro. The cells were cultured in a humidified incubator at 37 °C with 5% CO_2. Cell passaging was performed every 2 days, and after thawing, the cells were passaged three to four times before being transplanted into rats. Prior to subcutaneous injection, MADB-106 cells were harvested and resuspended in DMEM medium to a final concentration of 1×10^7 cells/mL.

Rats Models

Ten specific pathogen-free (SPF) Sprague-Dawley (SD) rats (female, 190–200 g) were obtained from Hangzhou Qizhen Experimental Animal Technology Co., Ltd., housed at 22 ± 1 °C and 50–60% relative humidity with a 12 hours light-dark cycle. For the breast cancer syngeneic graft models, 1×10^7 MADB-106 cells were subcutaneously injected into the back of SD rats. On the 14th day after tumor injection, the caliper was used to measure the length and width of the tumors, and the tumor volume was calculated using the formula $L\times W^2/2$. Rats were grouped based on tumor volumes into two groups, each group consisting of 5 rats, ensuring that the average tumor volume was similar within each group. On day 14 post-cell injection, the mean tumor volume was 8.6 ± 3.2 mm^3 in the control group and 8.0 ± 2.3 mm^3 in the TTFields-treated group (unpaired *t*-test, $p > 0.05$), confirming no significant baseline difference. This tumor size is consistent with early-stage breast cancer in rodent models, aligning with the study's focus on evaluating primary tumor growth inhibition. All rats were treated with hair removal cream for back and abdominal hair removal as pre-treatment.

Treatment Method

Alternating electric fields treatment was initiated for all breast cancer models after grouping. Each rat wore a therapeutic vest, allowing free movement during the treatment period. Alternating electric fields with strengths of 1–1.5 V/cm were used for treatment group with a frequency of 250 kHz. The control group was treated with the vest, but no electric fields were applied. The treatment was administered for 24 hours daily, lasting for 7 days. The tumor volume was measured daily during treatment. At the end of the 7 days treatment, rats were euthanized. The volume and weight of tumor tissues were recorded. This animal study was approved by the lab of animal experimental ethical inspection of Dr.Can biotechnology (Zhejiang) Co., Ltd. (DRK-2024112000). The animal experiments were conducted in compliance with the ARRIVE guidelines and in accordance with the UK Animals (Scientific Procedures) Act, 1986, and associated

guidelines, the EU Directive 2010/63/EU on the protection of animals used for scientific purposes, and the National Research Council's Guide for the Care and Use of Laboratory Animals. Euthanasia was performed when the tumor diameter reached or exceeded 2 mm in animals.

Dose Analysis by TTFields Simulation

We simulated alternating electric fields in a module rat by a finite element simulation software (Sim4Life),[34] which is a 3D human tissue medical electromagnetic simulation software platform. A model rat called Small Male Rat V1-x is close to the experimental rat in this software, which weighs 198 g and its height is 185 mm. The stimulation electrodes were placed on the back and abdomen, and alternating current with a frequency of 250 kHz, the voltage amplitude of 1.5 V, 3 V, 6 V and 9 V were output respectively. Based on the analysis of the electric field simulation results of rats, the stimulator current value corresponding to the voltage amplitude of the electric field intensity greater than 1 V/cm at 5 mm below the skin was selected as the experimental parameter.

Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR)

Tumor tissue was homogenized and lysed with TRIzol reagent. After standing for 5 minutes, 0.2 volumes of chloroform were added, and the mixture was vortexed and incubated for an additional 5 minutes. The sample was then centrifuged at 12,000 × g for 15 minutes at 4 °C, and the upper aqueous phase was collected. To the collected aqueous phase, an equal volume of isopropanol was added, mixed, and allowed to stand for 10 minutes. The sample was centrifuged at 12,000 × g for 10 minutes at 4 °C, and the supernatant was discarded. The RNA pellet was washed with 75% ethanol (prepared with DEPC-treated water), centrifuged at 7,500 × g for 5 minutes at 4 °C, and the supernatant was discarded. After air-drying, the RNA pellet was dissolved in DEPC-treated water. The RNA concentration was measured using a NanoDrop 300 spectrophotometer (Nano-300, Hangzhou Allsheng Instruments Co., Ltd., China). The RNA was stored at −80 °C.

The reverse transcription reaction was carried out using Evo M-MLV Reverse Transcriptase Master Mix (AG11706, Accurate Biology, China) following the manufacturer's instructions. Reverse transcription was performed on a Mini Flex 96 Gradient PCR System (MP60901, Monad, China) with the following program: 37 °C for 15 minutes, followed by 85 °C for 5 seconds. The synthesized cDNA was stored at −20 °C.

Primers were synthesized by Zhejiang Youkang Biotechnology Co., Ltd. (primer sequences are listed in Table 1). RT-qPCR was performed using the SYBR Green Pro Taq HS PreMix (AG11701, Accurate Biology, China) according to the manufacturer's instructions. PCR amplification was carried out on a Roche LightCycler® 480II PCR system. The thermal cycling conditions were as follows: an initial denaturation at 95 °C for 30 seconds, followed by 40 cycles of 5 seconds at 95 °C for denaturation, 30 seconds at 60 °C for annealing. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference gene. Data were analyzed using the 2-ΔΔCt method, and all experiments were performed in triplicate.

Table 1 Primer Sequences for RT-qPCR

Primer name	Sequence (5'-3')
GAPDH-F	AGGTCGGTGTGAACGGATTTG
GAPDH-R	GGGGTCGTTGATGGCAACA
ki67-F	ATCATTGACCGCTCCTTTAGGT
ki67-R	GCTCGCCTTGATGGTTCCT
Bcl2-F	GCTACCGTCGTGACTTCGC
Bcl2-R	CCCCACCGAACTCAAAGAAGG
Bax-F	AGACAGGGGCCTTTTTGCTAC
Bax-R	AATTCGCCGGAGACACTCG

Statistical Analysis

Data were presented as mean ± standard deviation (SD). Statistical significance was calculated using GraphPad Prism 8 software, with the specific tests used mentioned in figure legends. Differences were considered to be statistically profoundly for p values of < 0.05 and were indicated as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Results

TTF-Backpack Design and Implementation

The TTF-Backpack was successfully developed as a wireless, wearable, rechargeable alternating electric field therapy device with low power consumption. As illustrated in Figure 1A, the device features a compact stimulator with programmable waveform generation capabilities, including sine wave, sinusoidal envelope wave, rectangular wave,

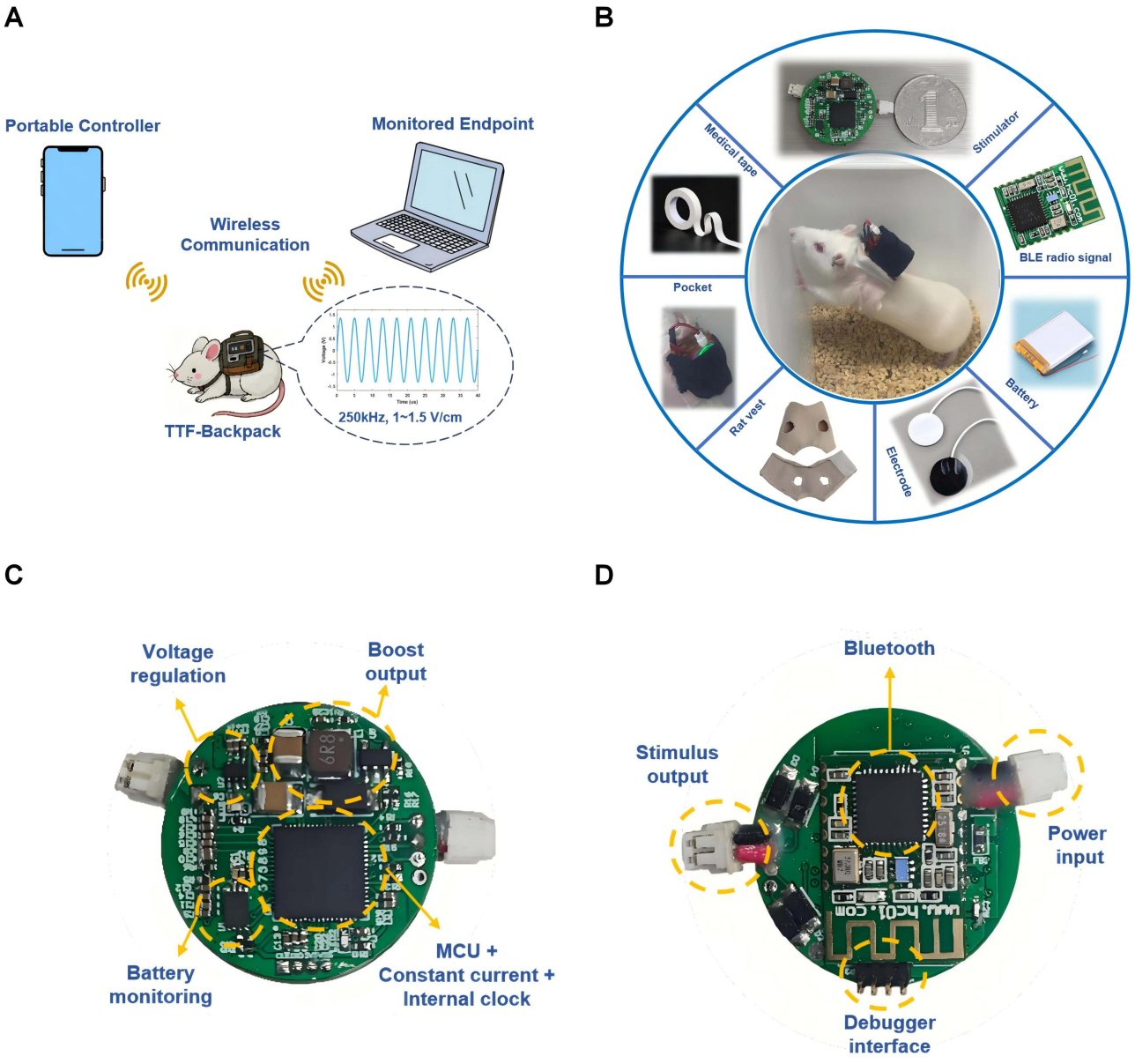


Figure 1 The overall structure of the TTF-Backpack and the distribution of the printed circuit board of the stimulator. (**A**) Application scenarios of rats TTF-Backpack. Rats can move freely with TTF-Backpack and communicate wirelessly with the portable controller or the monitored endpoint. (**B**) The composition of TTF-Backpack. It consists of a flexible fabric vest with a pocket, electrodes with conductive gel, a stimulator, a BLE radio signal transceiver, a rechargeable battery and the medical tape used to assist in fixing the electrodes. (**C**) The top structures of the stimulator. The main circuit is located on the top layer. (**D**) The bottom structures of the stimulator. The communication unit and external interfaces are distributed on the bottom layer.

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and triangle wave outputs. The device boasts an output frequency bandwidth spanning 0–250 kHz, with a single-channel maximum output current capable of reaching 40 mA and a peak output voltage of ±55 V—parameters that are complemented by an actual operating power of approximately 0.4 W. For the current breast tumor inhibition study in rat models, optimal parameters of 250 kHz and 6 mA were selected based on preliminary testing.

The device's mechanical design effectively addressed animal wearability challenges through several key features: a custom-fitted vest secured electrodes to both dorsal and abdominal regions, while a detachable pocket housed the stimulator unit. Strategic placement of electrode lead wires prevented interference from rat limbs, ensuring stable operation during normal activity. This design proved highly robust in continuous 24-hour wear tests, maintaining full functionality over 7-day periods without vest detachment or wire damage.

System implementation incorporated multiple advanced technical components (Figure 1B): a precision stimulator, carbon fiber gel electrodes with custom interfaces, and a Bluetooth Low Energy (BLE) wireless transceiver for real-time parameter monitoring. The electrodes featured carbon fiber conductors and were reinforced with medical tape for enhanced skin adhesion. A specially designed vest with integrated pocket allowed comfortable device carriage while preventing animal interference, as confirmed during experimental validation.

Power management innovations included a compact, high-density lithium battery (3.7 V, 1200 mAh, 30×25×4 mm) providing 12 hours of continuous operation. The stimulator's printed circuit board achieved remarkable miniaturization (25 mm diameter) while integrating multiple functions: an ARM-based System-on-Chip (ENS001-A2), 3.3V regulation, 55V boost conversion, and comprehensive battery management. To address signal integrity challenges, an external boost circuit was implemented, successfully mitigating crosstalk between power switching and stimulation signals.

The complete system demonstrated excellent stimulation performance across the full 0–250 kHz frequency range. Real-time monitoring capabilities, enabled by a CC2541 BLE SoC with 2.4 GHz antenna, provided continuous data transmission of contact impedance, temperature, power status, and supply voltage to paired computing devices (Figure 1C and D). This comprehensive monitoring system, combined with wireless parameter adjustment through dedicated software, established a complete closed-loop treatment platform for preclinical tumor therapy studies.

Performance Verification of TTF-Backpack

Figure 2A illustrates the maximum voltage and current generated by the stimulator are approximately 55 V and 40 mA respectively. At the same time, the current output is 40mA with a load capacity of 1.35 kΩ, while the current is 1mA and can drive a load of 55 kΩ. Figure 2B describes the voltage amplitude and standard deviation across a 250 Ω resistance load at currents of 6 mA, 12 mA, 24 mA and 36 mA. The standard deviation values are all less than 0.5, indicating the current output stability of the stimulator meets the requirements. Figure 2C and D depict the scatter plots of current and frequency control responses. Both of the scatter fitting curves are very close to the baseline, indicating the parameters setting control response meet the requirements. It should be noted that the horizontal and vertical axes of Figure 2D are expressed in logarithms of frequency to more clearly depict the frequency response.

Simulated Electric Fields Intensities Delivered to Rats

Figures 3A and B shows that the simulated electric fields intensities are greater than 1 V/cm at the position 5 mm below the electrodes, which meets the requirements of superficial breast cancer inhibition experiment. Therefore, we selected the corresponding parameters of the stimulator: 6 mA, 250 kHz for the rat breast cancer inhibition experiment. In addition, because of the maximum stimulator current can reach 40 mA and 6 mA does not reach one-sixth of the upper limit, we also simulated the electric field intensities distribution in rats with the application of approximately 12 mA, 24 mA and 36 mA, as shown in Figure 3C–E . The simulation results show that even when the device outputs the maximum current, the electric field intensities stimulated in the rat body meets the safety requirements according to previous studies. It is worth noting that the electric field intensities in the rat's abdominal cavity can be greater than 1V/cm, while under the skin it is still within 3V/cm when the stimulator output current reaches 36mA, which can be used for subsequent research on the effect of electric field therapy for cancer in other internal organs.

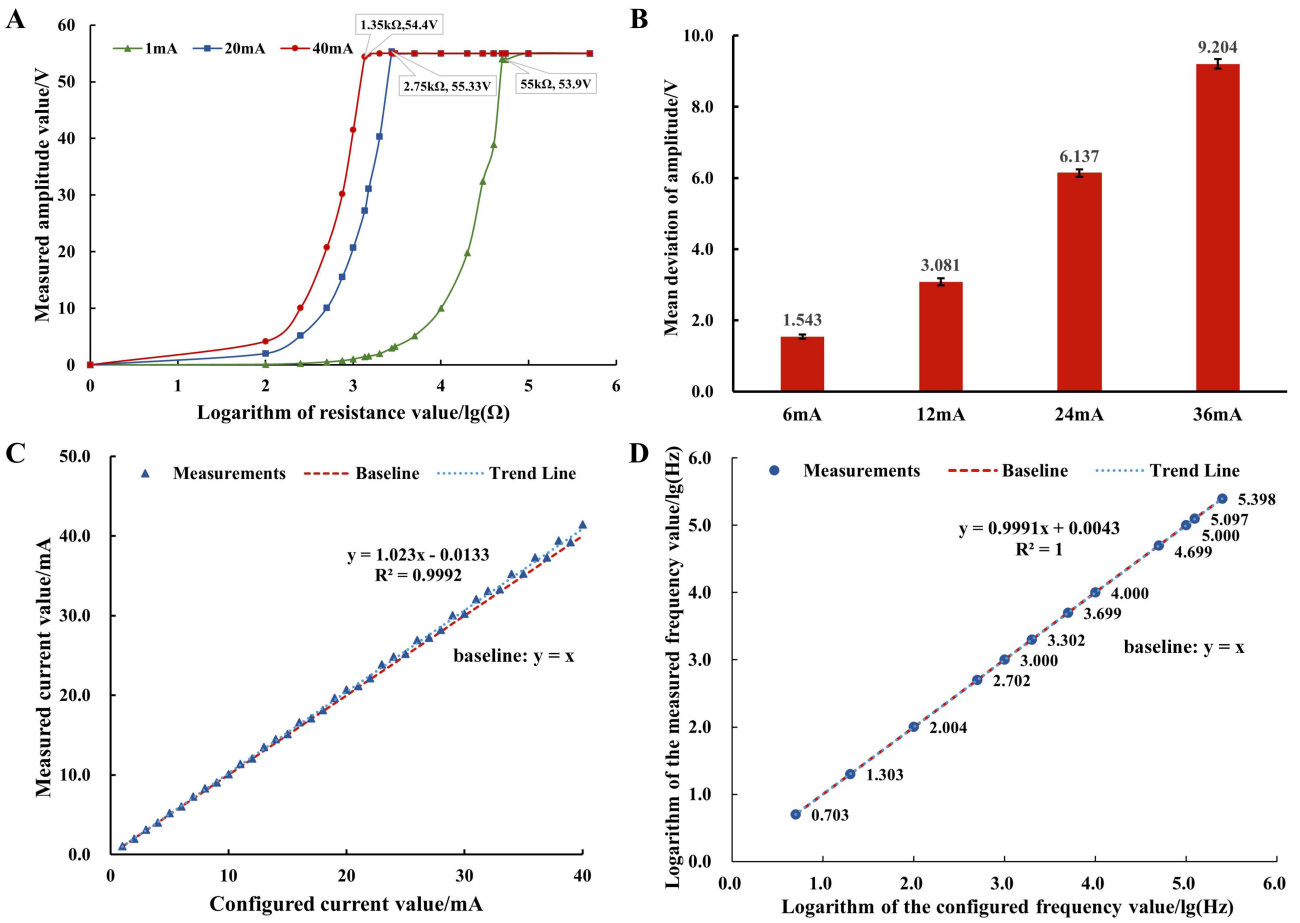

Figure 2 Results of stimulator performance verification. (**A**) Maximum voltage and current test of the stimulator, as well as the load capacity curve under different currents. (**B**) Histogram of mean and standard deviation of stimulator stability test at different currents. (**C**) Scatter plot of configured current and actual measured output current. (**D**) Logarithmic scatter plot of configured frequency and actual measured frequency.

TTF-Backpack Suppressed Breast Cancer Progression

On the 14th day after subcutaneous injection of tumors, SD breast cancer syngeneic graft models were randomly divided into two groups according to tumor volumes as control group. Then, the models were treated with alternating electric fields (frequency 250 kHz, intensity 1–1.5 V/cm) for 24 hours daily over 7 days (Figure 4A). The results indicated that alternating electric fields could considerably inhibit the progression of breast cancer (Figure 4B and C). These findings were confirmed by the fold change in the tumor volume for each rat (Figure 4D). At the end of the treatment, the weight of tumors was measured. The tumor weight of each group further affirmed the significant effect of alternating electric fields in inhibiting breast cancer progression in rats (Figure 4E).

Device Functionality During Animal Activities

Figure 5 illustrates a rat wearing the TTF-Backpack device while engaging in normal behaviors, including free movement, grooming, and drinking. The images confirm that the device does not impede the animal's mobility or disrupt its natural activities. These observations validate the practicality and non-invasive design of the TTF-Backpack as a wearable therapeutic tool for tumor-treating field (TTFields) applications. The device remains securely attached without restricting the rat's movement, supporting its suitability for long-term use in preclinical studies.

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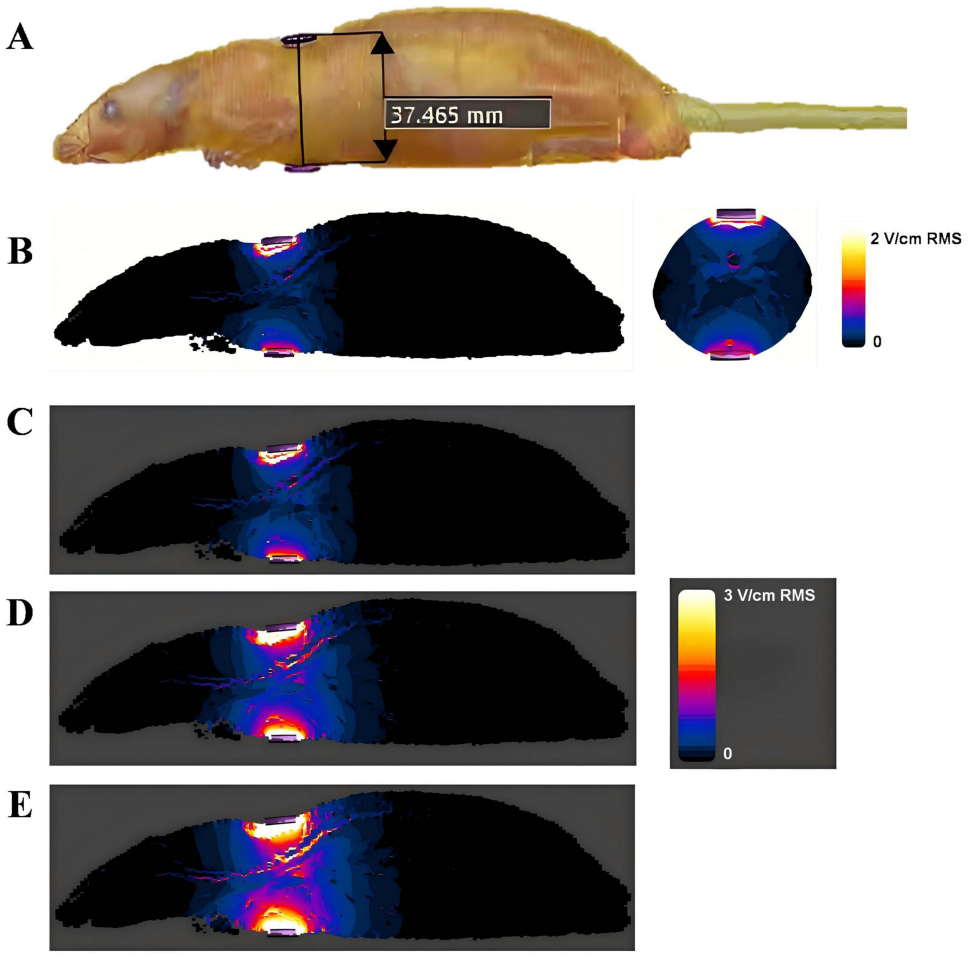

Figure 3 Simulations of EF intensities in the rat torso with a frequency of 250 kHz and a voltage amplitude of 1.5 V, 3 V, 6 V and 9 V. (**A**) 3D illustration of a model rat called Small Male Rat V1-x with electrodes placed on its back and abdomen. The stimulation electrodes were placed on the back and abdomen. (**B**) The transverse and longitudinal cross-sections of the simulated electric fields distribution with frequency of 250kHz and voltage amplitude of 1.5V, the corresponding stimulator current is approximately 6mA. (**C–E**) The transverse cross-sections of the simulated electric fields distribution with frequency of 250kHz and voltage amplitude of 3V, 6V and 9V, the corresponding current is approximately 12mA, 24mA and 36mA, respectively.

TTF-Backpack Regulated the Expression Levels of mRNA and Protein of Proliferation and Apoptosis-Related Genes in Tumor Tissue

At the end of the treatment, mRNA levels of the proliferation marker ki67 and apoptosis markers Bax, Bcl2, as well as their corresponding protein expression levels, were assessed in tumor tissues. The results demonstrated that alternating electric fields upregulated the mRNA levels of ki67 in the tumor tissues (Figure 6A). Furthermore, alternating electric fields increased the mRNA and protein expression levels of the pro-apoptotic gene Bax, while downregulating the protein expression level of the anti-apoptotic marker Bcl2 (Figure 6B and C). In summary, alternating electric fields inhibit tumor cell proliferation and promote tumor cell apoptosis.

Discussion

In this study, we developed, TTF-Backpack, a lightweight, wearable TTFields device tailored for breast cancer therapy and conducted preliminary validation through animal experiments. The results demonstrate the feasibility and therapeutic potential of this device in inhibiting tumor progression and inducing apoptosis in breast cancer cells.

Our device represents a significant advancement in TTFields technology, addressing key limitations of conventional systems. By prioritizing miniaturization, wearability, and adaptability to breast cancer-specific anatomy, the device offers the potential for broader clinical application. This study serves as a proof-of-concept for this integrated wearable

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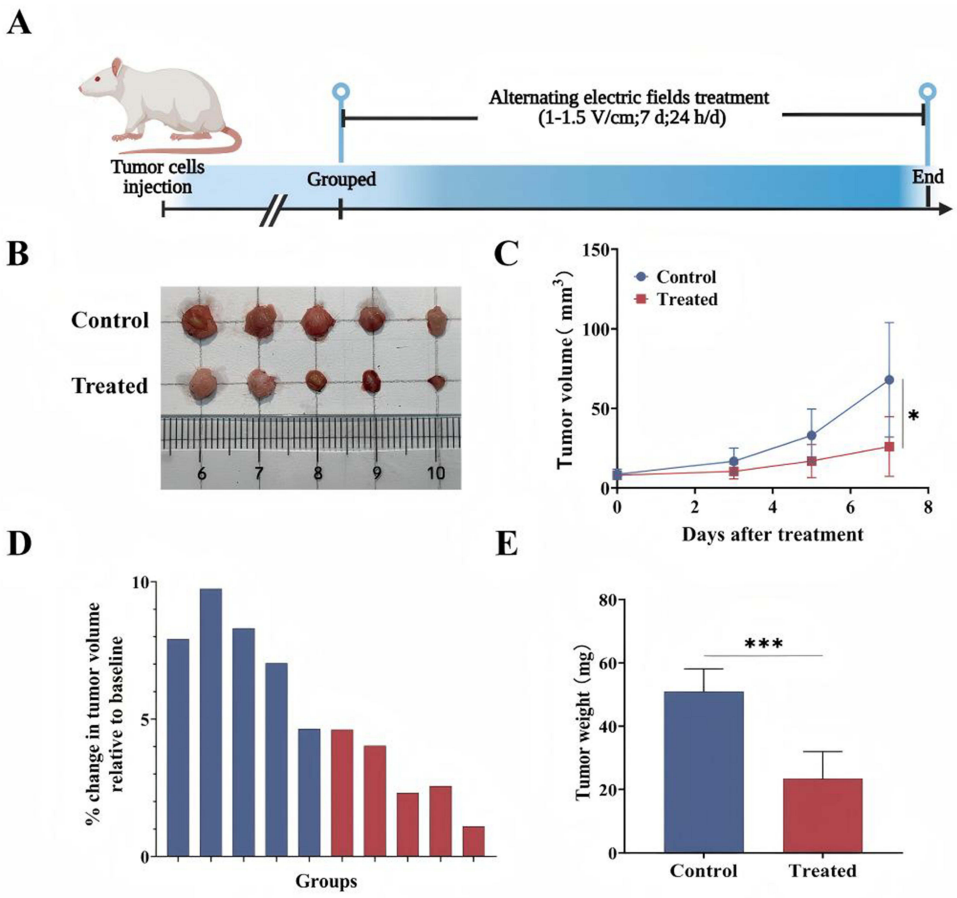

Figure 4 Alternating electric fields suppressed breast cancer progression. (**A**) Experimental design of alternating electric fields treatment for breast cancer models. (**B**) Imaging of breast cancer from rat treated with alternating electric fields for 7 days (n=5). (**C**) Tumor growth curves with mean tumor volumes ± SEM (n=5). A repeated-measures ANOVA was used. (**D**) The individual changes in tumor growth before and after treatment. (**E**) Tumor weight of rat in different experimental groups after alternating electric fields treatment. Kruskal–Wallis test was used. * $p < 0.05$, *** $p < 0.001$.

platform, with its primary novelty lying in the successful development and preliminary biological validation of the TTF-Backpack system. Its lightweight design and flexible electrode arrays not only improve patient comfort but also promote treatment adherence, which is critical for achieving optimal therapeutic outcomes in TTFields therapy. Achieving consistent electric field distribution across the irregularly shaped breast tissue remains a technical challenge. Future work should explore advanced electrode designs, including patient-specific configurations, to maximize therapeutic outcomes while minimizing side effects. In addition, the stimulator can be configured to generated various of waveforms such as sine wave, sinusoidal envelope wave, rectangular wave, and triangle wave for supporting intermediate frequency physiotherapy, conventional TENS, muscle rehabilitation and implantable stimulation.

Previous studies have also demonstrated that alternating electric fields can inhibit the proliferation and progression of breast cancer cells in vitro.[19,35] However, there is currently a lack of relevant in vivo experimental evidence. This study shows that non-invasive alternating electric fields significantly suppressed the progression of breast tumors in vivo without affecting the activity of the animals. We acknowledge that the current study was designed to evaluate the monotherapeutic effect of our TTF-Backpack device over a short-term period, and as such, it does not include a direct comparison with standard-of-care therapies or long-term survival data. The observed tumor growth inhibition and favorable safety profile establish a foundational basis for future studies that will include these critical comparisons. In addition, this study provides a wearable treatment device that can be used for the treatment of surface tumors such as skin and breast cancer. According to the electric field simulation results, the wearable TTFields device could be adapted for other extracranial tumors, such as lung or abdominal cancers. Comparative studies across tumor types could help identify the most suitable indications for this technology.

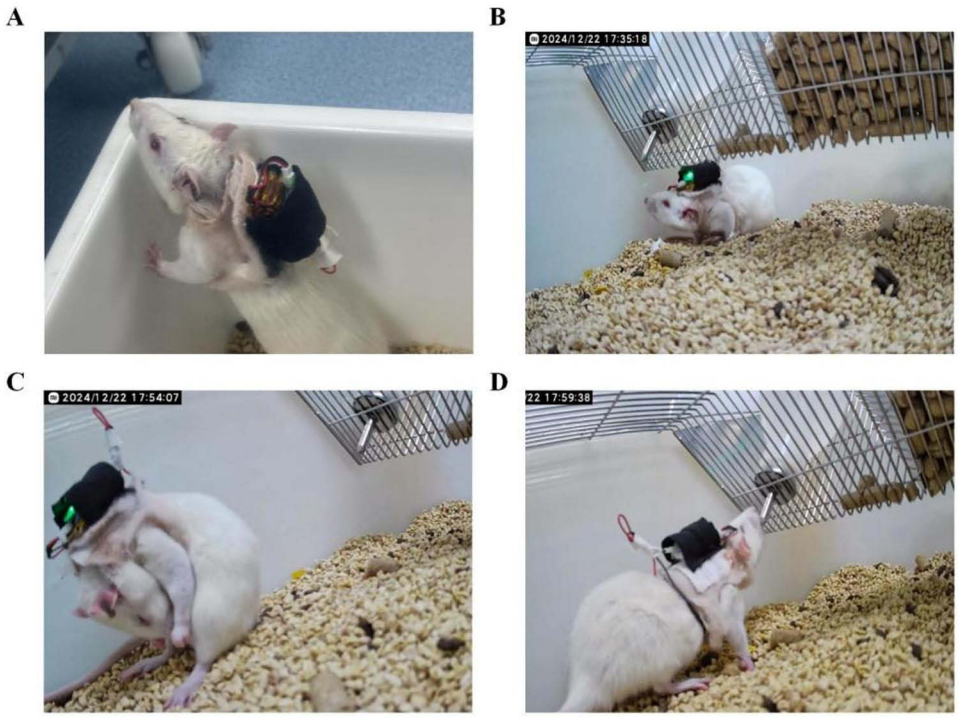

Figure 5 Device Functionality During Animal Activities. The rat wearing the TTF-Backpack device while performing normal activities, including free movement (**A** and **B**), grooming (**C**), and drinking (**D**).

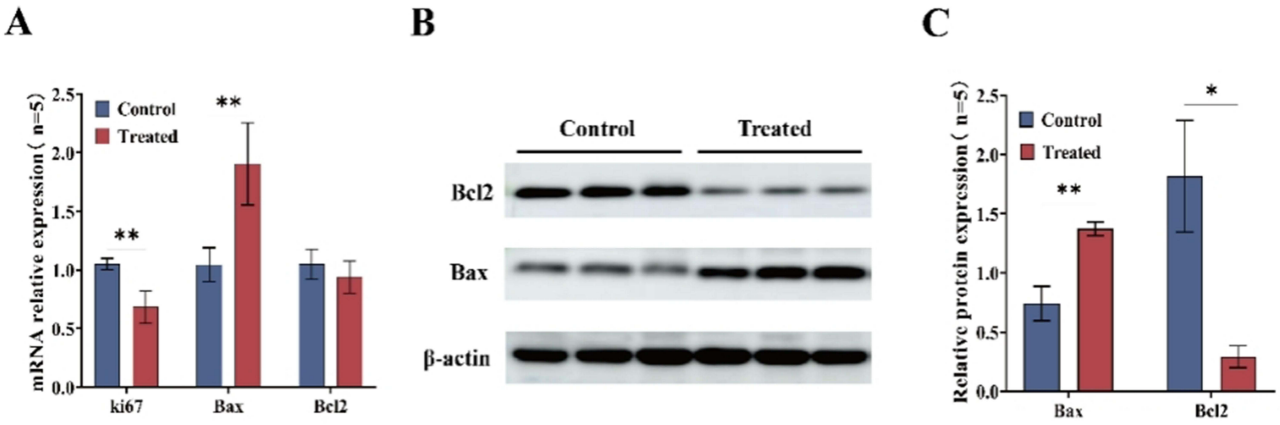

Figure 6 Alternating electric fields regulated the expression levels of mRNA and protein of proliferation and apoptosis-Related Genes in Tumor Tissue. (**A**) mRNA Levels of ki67, Bax, and Bcl2 in Tumor Tissue (n=5). (**B**) Protein expression levels of Bax and Bcl2 in tumor tissue. (**C**) Statistical analysis of protein expression levels of Bax and Bcl2 in tumor tissue. Kruskal–Wallis test was performed. * $p < 0.05$, ** $p < 0.01$.

In order to investigate the mechanisms underlying the inhibitory effect of alternating electric fields on breast cancer, we assessed the mRNA and protein expression levels of key indicators related to proliferation and apoptosis in tumor tissues after treatment. Our results showed that alternating electric fields upregulated the mRNA and protein expression levels of Ki67 and Bax, while downregulating the mRNA expression level of Bcl2. These findings suggest that alternating electric fields may inhibit tumor progression in vivo by suppressing tumor cell proliferation and promoting tumor cell apoptosis. Similar results have been reported in previous studies using different tumor models.[36–38] However, the exact mechanisms through which alternating electric fields suppress tumor proliferation and promote tumor apoptosis still require further investigation.

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Our study primarily focused on short-term outcomes. Long-term studies are needed to evaluate potential side effects,[39] such as skin irritation, thermal effects, or tissue damage, and to confirm sustained anti-tumor efficacy.[40] Although TTFields are known to disrupt mitotic processes, the precise mechanisms underlying their effects on breast cancer cells, particularly their potential immunomodulatory role, require further elucidation. Advanced molecular studies and in vivo imaging could provide deeper insights into these mechanisms.

Conclusion

This study demonstrates the feasibility and preliminary efficacy of a novel wearable TTFields device for breast cancer therapy, highlighting its potential to offer a non-invasive, breast-conserving treatment alternative.

Ethical Approval and Consent to Participate

All animal experiments were conducted in accordance with the guidelines and regulations for the care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) of Dr.Can biotechnology (Zhejiang) Co., Ltd. (Protocol Number: DRK-2024112000). The ethical review was conducted by this independent committee at the facility where the experiments were performed, ensuring adherence to all applicable guidelines. All procedures were performed in compliance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

Animals were group-housed in a specific pathogen-free (SPF) facility under a controlled 12-hour light/dark cycle, with ad libitum access to autoclaved food and water. Environmental conditions including temperature (22±2 °C) and humidity (50±10%) were maintained consistently.

Prior to TTFields treatment, depilation of the dorsal and abdominal areas was performed on the rats. For the depilation procedure and during the subsequent replacement of the wearable devices, Rats were anesthetized by placing them in a sealed induction chamber connected to a gas anesthesia system. Anesthesia was induced with 2-4% isoflurane delivered in oxygen at a flow rate of 0.5-0.7 L/min. Each anesthesia session lasted 3-5 minutes to ensure adequate sedation and immobility while minimizing discomfort.

At the experimental endpoints, euthanasia was performed by carbon dioxide asphyxiation with a displacement rate of 30-70% of the chamber volume per minute. All euthanasia procedures strictly adhered to the AVMA guidelines to minimize animal suffering.

Efforts were made to minimize the number of animals used and to reduce their suffering through a comprehensive monitoring scheme. Throughout the device-wearing period, rats were closely monitored at least twice daily for key welfare indicators: device-related skin condition was inspected for signs such as redness, erosion, or hair loss, with topical application of a non-irritating barrier cream like medical-grade petroleum jelly in cases of mild redness; pain-related behaviors including hunched posture, reduced locomotion, or vocalization upon touch were assessed, and a rescue dose of sustained-release buprenorphine was administered subcutaneously at 0.1 mg/kg if such discomfort persisted for over 24 hours, as per the IACUC-approved protocol; predefined humane endpoints, including severe skin ulceration, weight loss exceeding 15%, or persistent self-mutilation despite analgesia, were strictly enforced, with any affected animal euthanized immediately. The study was designed and reported in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines to ensure transparency and reproducibility.

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Disclosure

The authors declare that they have no competing interests related to this study.

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