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Shu Xiao  
*Old Dominion University*, sxiao@odu.edu

Ryo Yamada

Carol Zhou  
*Old Dominion University*, czhou@odu.edu

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Quadrupoles for Remote Electrostimulation Incorporating Bipolar Cancellation

Shu Xiao, PhD,1,2 Ryo Yamada, MS,3 and Carol Zhou, BS1

Abstract

Introduction: A method that utilizes nanosecond bipolar cancellation (BPC) near a quadrupole electrodes to suppress a biological response but cancels the distal BPC at the quadrupole center, i.e., cancellation of cancellation (CANCAN), may allow for a remote focused stimulation at the quadrupole center.

Objectives: The primary object of this study was to outline the requirement of the CANCANC implementation and select an effective quadrupole configuration.

Results: We have studied three quadrupole electrode configurations, a rod quadrupole, a plate quadrupole (Plate-Q), and a resistor quadrupole. The pulse shapes of electric fields include monophasic pulses, cancellation pulses, and additive pulses. The Plate-Q appears the best for CANCANC as it shows the highest percentage of cancellation pulses among all pulse shapes, allowing for the best spatial focus.

Conclusion: For the region of interest characterized in the Plate-Q configuration, the maximum magnitude of bipolar field is twice as that of the unipolar field, which allows for the CANCANC demonstration that involves membrane electropermeabilization.

Keywords: bipolar cancellation, nanosecond pulses, quadrupole, remote stimulation, electric fields

Introduction

Nanosecond electric pulses (nsEPs) as an effective stimulus for bioresponses have been widely studied, and the emerging clinical applications are found in cancer therapy, treatment of skin lesions, and cardiac defibrillation.1–4 The responses, such as cell viability, membrane permeabilization, and intracellular calcium release, can be reduced or cancelled by subsequently applying a phase of reversed polarity.5–9 The phenomenon was termed “bipolar cancellation” (BPC) and has been shown in both the single-pulse condition and the repetitive-pulse condition. The amplitude of the reversed phase determines the efficiency of BPC.6 The reversed phase can have the same pulse duration (symmetric cancellation) or different duration from the first phase (asymmetric cancellation). Early BPC results showed that with symmetric-amplitude bipolar 60 and 300 ns, the survival and intracellular calcium activation responses were significantly reduced compared with those from monophasic pulses of the same amplitude,6 which were conducted on CHO cells and U937 cells with the peak electric field 30 kV/cm. In another study,5 which used multiple endpoint markers comprising Calcium Green 1, FM1–43, propidium iodide, and FITC-Annexin V, responses to a symmetric 600 ns BP vsEPs were attenuated compared with those for the unipolar pulses, which had twice the phase width and thus overall energy. The electric fields were in the range of 3–24 kV/cm. The BPC was also observed for 900 ns BP pulses with lower electric fields 12 kV/cm.10 Recently, 2 ns pulses were shown to cause BPC similar to that observed with pulse durations from 60 to 900 ns.11 The effectiveness of the cancellation of molecular transport of the fluorescent dyes Yo-Pro-1 and calcine is greater when the amplitude of the second phase of the pulse is 30% of the amplitude of the first phase (170 kV/cm) (compared with equal amplitude for both phases). A sixfold difference in fluorescence intensity was shown.

In fact, BPC does not require the opposite phase to be applied immediately after the first phase.12 What makes BPC remarkable is that a cancellation still is attainable even when the reversed pulses are delayed by as long as 10–100 µs (delayed BPC). However, BPC does not work in all ranges of electric fields. In the case wherein the first phase is very high,
applying a reversed pulse will not cancel the first response. In other words, high field intensity, multiphasic nsEPs, may be equally or more efficient than monophasic pulses. BPC is also dependent on the pulse duration. There is evidence suggesting that BPC is not strictly limited to nsEPs and was also observed in microsecond pulses.13

BPC was postulated to be caused by several reasons6,14–16: assisted membrane discharge, reversal of a two-step chemical process, transport of charged species suppressed by electric field reversal, and reduction of the low frequency component in bipolar pulses. Although the mechanisms of BPC remain to be investigated, we can deliberately utilize BPC near the electrodes to suppress the response but cancel the distal BPC away from the electrodes, that is, cancellation of cancellation (CANCAN),17 to retain the effect.

A linear quadrupole electrode configuration with four electrodes in a row was studied for CANCAN.17 By evaluating the YO-PRO-1 dye uptake in CHO-K1 cells along the dipole line that has a one-dimensional electric field, an enhancement of the dye uptake due to CANCAN was observed in the quadrupole center. It should be noted that CANCAN does not focus the electric field at the quadrupole center or increase the cell response there, which has been demonstrated to some degree by rotating electric field orientation between sets of pulses.18,19 Rather, CANCAN suppresses the bipolar cell response near the electrodes and retains the unipolar cell response at the center created by one dipole of the quadrupole.

The linear quadrupole is an inefficient electrode configuration for CANCAN as it does not utilize the high fields between the dipole electrodes, but relies on the fields outside the dipoles, so the field rapidly decreases from the electrode to the quadrupole center. As a result, BPC was not strong enough to suppress the cell response near the electrodes. In this study, we investigated square quadrupoles that offer more advantages than the linear quadrupole. First, the square quadrupole center is shifted toward the dipole electrode gap and its electric field is higher. Second, the square quadrupole arrangement increases the exposure area and makes it more suitable for practical applications. Third, the square quadrupole has more electrodes to affect the field at the quadrupole center, thus adding more flexibility and controllability to the implementation of CANCN. We have investigated three types of quadrupoles, a rod quadrupole (Rod-Q), a plate quadrupole (Plate-Q), and a resistor quadrupole (Resistor-Q). We selected a region of interest (ROI), a circle with a diameter of 12 mm in the quadrupole that can be used for in vitro CANCN. We used a time domain three-dimensional electromagnetic solver, computer simulation technology, to simulate the electric field distributions. We selected the Plate-Q configuration as our proposed exposure system for future studies based on the criterion that it produces the highest percentage of cancellation pulses (PCPs) in the ROI. An experimental study on a scaled-up Plate-Q was conducted to characterize the electric field distribution.

**A Generic Quadrupole Electrode Configuration for CANCN**

Biological responses to a stimulus can be very complex and nonlinear. However, the response to nanosecond pulses such as membrane permeabilization, intracellular calcium activation, or cell viability can be illustrated by a sigmodal curve, which comprises the responses below a threshold level, a linear region, and above a saturated level.17,20,21 For the three pulse conditions, monophasic, biphasic, and triphasic, each having the same amplitude in Phase 1 ($\Phi_1$) and 50% of $\Phi_1$ in Phase 2 ($\Phi_2=0.5\Phi_1$) that causes the best BPC, one hypothetical scenario is plotted in Figure 1a. Below the threshold field $E_{thr}$, no response can be observed. Above $E_{thr}$, the cell response exhibits a linear increase until the electric field rises to $E_{sat}$. Above $E_{sat}$, the response reaches the maximum (saturated) even if the field is further increased. $E_{thr}$, $E_{sat}$, and the slope of the linear response can all be waveform dependent. It is in the linear region between $E_{thr}$ and $E_{sat}$ that the response is sensitive to the change of electric field or change of the pulse shape. Because of BPC, a unipolar pulse always produces a greater response in the linear region than the other pulse shapes. This is indicated by a field margin $\Delta E$ that shows to create the same response (50% of the maximum response) how large the difference in the electric field is between a monophasic pulse ($E_{mon}$) and a biphasic or a multiphasic pulse ($E_{mul}$). In principle, $\Delta E$ should be large to qualify for CANCN near the dash circle 1. However, a better electric field range for CANCN can be found in the low electric field region (the dash circle 2), in which only monophasic pulses can cause cell responses, whereas triphasic or biphasic pulses are too small. It is noted that Figure 1a only shows a hypothetical scenario, which serves to illustrate that knowing $E_{thr}$, $E_{sat}$, and $\Delta E$ of a particular endpoint response is critical to CANCN. In reality though, the threshold field, $E_{thr}$, may be equal for all three pulse shapes. Therefore, the low-field CANCN is not applicable. Nevertheless, the linear region for CANCN exists for a broad range of cell responses.

To implement CANCN, a triphasic pulse is applied to the first dipole and a delayed biphasic pulse is applied to the second dipole (Fig. 1b). In Phase 1, the field orientation and magnitude should be as uniform as possible. This is to ensure the field at the quadrupole center to be above $E_{thr}$ and the field near the electrodes to be as low as possible (optimally, $<E_{sat}$). In Phase 2, the dipolar field changes to a quadrupole field, as the second dipole is energized. The quadrupole field should also exhibit a small field gradient. The quadrupole field of $\Phi_2$ established by both dipoles should be opposite to that of $\Phi_1$ established only by the first dipole, in order for BPC to occur in most areas except the quadrupole center. In fact, though the fields of $\Phi_2$ and $\Phi_1$ to a large extent may be opposite to each other, some fields can be in the same direction, such as those in the intergap region of the second dipole. In Phase 3, a quadrupole field, with the field direction opposite to $\Phi_2$, is always established. This is guaranteed by alternating the polarities of the dipoles from $\Phi_2$ to $\Phi_3$, which is a quadrupole–quadrupole cancellation, unlike the dipole–quadrupole cancellation from $\Phi_1$ to $\Phi_2$.

**Quadrupole Configurations**

Quadrupoles are used as electromagnetic traps for charged and neutral particles, where four hyperbolically shaped electrodes produce a transverse field increasing exponentially from the center.22 In the negative dielectrophoresis, various quadrupole profiles are used for trapping cells at the quadrupole center.23 These quadrupoles deliberately create an electric field gradient that is needed for generating trapping
forces. Here in our application, a quadrupole with a uniform electric field is desired for CANCAN. Three quadrupole options were chosen for CANCAN: Rod-Q, Plate-Q, and Resistor-Q (Fig. 2). Rod-Q consists of four rods of identical diameter (12.6 mm) and length (15 mm) and they are placed at the four corners of a square. With a large ratio of the rod diameter over the gap distance, the field gradient can be controlled to a small degree. Plate-Q has four plates that form the four sides of a square (square side: 16 mm; plate dimension: 0.5 mm × 12 mm × 9 mm). It evolves from a parallel electrode gap that is often used for the in vitro uniform field exposure. Resistor-Q has four plate resistors (0.5 mm × 20 mm × 16 mm) forming a closed square (side length: 17 mm) with four conductors at the square corners. The resistor plate has conductivity 0.1 S/m. Resistor-Q establishes equal-potential lines that are parallel between the two active diagonal electrodes, which create a uniform electric field.\(^{22,24}\) Note that the dimensions of the quadrupoles were chosen so that they produced the same \(\Phi 1\) field at the quadrupole center for the same range of voltages applied to the quadrupoles. Since they will be used for the in vitro study to demonstrate the feasibility of CANCAN, cells will be placed at the quadrupole bottom. Only Rod-Q with small radius electrodes can be used \(\text{in vivo}\) when electrode penetration is needed. The other configurations can only be applied on tissue superficially.

Figure 3 shows the electric field distributions created by the three quadrupoles. The field is 50 V/m at the quadrupole centers, but becomes much larger (>150 V/m) near the electrodes as indicated by the scale bar. Rather than comparing the electric fields everywhere, we focused on an ROI near the quadrupole center. The ROI is a circle with a radius of 6 mm and its center coincides with the quadrupole center. If CANCAN works, the biological response at the ROI center...
should be the strongest and decreases away from the ROI center because of BPC. However, the fields near the electrodes but outside the ROI become much stronger ($E > E_{sat}$ in Fig. 1) and will cause saturated bipolar responses, which is not suitable for CANCAN.

On the ROI circumference ($R = 6\text{ mm}$), 12 points spaced apart with equal azimuthal angle ($30^\circ$) were selected for comparison of their fields (P1–P12 in Fig. 3). Their $\Phi_1$ field magnitudes after normalization to that at the quadrupole center are shown in Fig. 4. The field distribution of Resistor-Q is the most uniform as indicated by a small variation (0.75–1.25), whereas both Rod-Q and Plate-Q exhibit a larger variation (0.5–3), although Plate-Q seems slightly more uniform than Rod-Q. If selecting a quadrupole is solely based on the field homogeneity, Resistor-Q appears to be the winner.

Figure 3 also shows the quadrupole center is field free for $\Phi_2$ (and expectedly for $\Phi_3$) and thus a monophasic pulse can be created for CANCAN. What is not easily seen is whether $\Phi_2$ cancels $\Phi_1$, as we attempted to reverse the field polarities by a quadrupole to “cancel” the fields of a dipolar field. Some fields in $\Phi_2$ are opposite to those of $\Phi_1$ (cancellation pulses), which is desirable for BPC. However, some fields in $\Phi_2$ remain in the same directions as in $\Phi_1$ (typically found near the Dipole 2 gap), so they constitute a longer pulse (additive pulses). This pulse shape is generally not instrumental for BPC although we can still rely on the less efficient asymmetric cancellation, in which the phase durations are not necessarily equal. In the cancellation and additive pulses, the $\Phi_1$ voltage can be smaller than that of $\Phi_2$. Besides the cancellation and additive pulses, the monophasic pulse only having $\Phi_1$ is mostly found near the quadrupole center and is needed for CANCAN.

To evaluate the fractions of the three pulse types among all pulse shapes, we selected 24 points evenly distributed on three concentric circles ($R=2$, 4, and 6 mm, Fig. 3) and spaced with an azimuthal angle of $15^\circ$. For each point, we examined the pulse shape of $x$ and $y$ components among a total of 144 components. A summary of the percentages of monophasic pulses (PMPs), the PCPs, and the percentage of additive pulses (PAPs) is given in Table 1. Plate-Q showed the least PMP (6%), which is beneficial for a small spatial focus in CANCAN. Interestingly, Plate-Q also showed the largest PCP (72%) and the smallest PAP (22%). They all are
complementary to CANCAN. Resistor-Q, although it produces a very uniform field distribution, does not produce a low PMP and a large PCP, lending itself less appealing for a sharply focused CANCAN. We then concluded that Plate-Q is the best configuration among the three quadrupoles we have considered.

Pulsing Strategy for Plate-Q

Although Plate-Q appears to show a good focus for CANCAN, its field gradient is still large (between $1 + 2.0$ and $1 - 0.5$). To lower the gradient, we can modify the pulses applied to the quadruple. In general, there are two pulse conditions and their difference lies in $\Phi 1$: (1) the common mode (Fig. 5a); only Dipole 1 is powered with a voltage $V_0$; (2) the differential mode (Fig. 5b); Dipole 1 is powered with $0.5V_0$ and Dipole 2 is powered with $-0.5V_0$. Both pulse conditions produce the same field at the quadrupole center, but the differential mode is better than the common mode in that it produces lower fields near the active electrode of Dipole 1, although the field near the active electrode of Dipole 2 is higher. This is shown in Figure 5c. The fields at Points 6, 7, and 8 (near the active electrode of Dipole 1) are reduced from 2.5 to 1.5, but the fields at Points 1, 2, and 12 (near the active electrode of Dipole 2) are increased from 0.5 to 1.5, so the field distribution generally becomes more uniform.

Characterization of Electric Fields of Plate-Q

To demonstrate the pulse shapes needed for the implementation of CANCAN, we chose Plate-Q as the electrodes for future experiments based on the consideration that a large PCP in the ROI could result in a satisfactory biological cancellation and a spatially focused stimulation. A plate-Q with four aluminum plates with a length of 56 mm was fabricated. This was a quadrupole scaled up to a larger dimension than that used in the simulation (length: 12 mm) so it can accommodate a field probe (Fig. 6a, b). Plate-Q was immersed in saline solution. The pulses to power Plate-Q are shown in Figure 6c and were generated by a multiphasic

<table>
<thead>
<tr>
<th>PMP</th>
<th>PCP</th>
<th>PAP</th>
</tr>
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<tbody>
<tr>
<td>Rod-Q</td>
<td>11%</td>
<td>46%</td>
</tr>
<tr>
<td>Plate-Q</td>
<td>6%</td>
<td>72%</td>
</tr>
<tr>
<td>Resistor-Q</td>
<td>20%</td>
<td>40%</td>
</tr>
</tbody>
</table>

PAPs, percentage of additive pulses; PCPs, percentage of cancellation pulses; Plate-Q, plate quadrupole; PMPs, percentages of monophasic pulses; Resistor-Q, resistor quadrupole; Rod-Q, rod quadrupole.

FIG. 5. In $\Phi 1$, the Plate-Q configuration can be powered either by a common-mode voltage (a) or a differential voltage (b). The differential voltage is preferred as it creates the same field at the center while homogenizes the fields in other locations. The $\Phi 1$ fields of the selected points on the circumference of the ROI are shown in (c) for the common mode (Plate-Q:C) and the differential mode (Plate-Q:D).
As discussed previously, in the ROI, the pulse shapes of the electric fields in general have three types: monophasic pulses, cancellation pulses, and additive pulses. The cancellation type, which is multiphasic, alternates its polarity from phase to phase (Fig. 7a). The monophasic pulse was measured at the quadrupole center (Fig. 7b). The additive type has a $F_2$ that shares the same polarity as $F_1$, although $F_3$’s polarity is reversed (Fig. 7c). Of all the measured field components ($E_x$ and $E_y$) in the ROI in Figure 6b, the PMP is 1/16, the PAP is 5/16, and the PCP is 7/16 (Fig. 8). Although we only measured the fields at a limited number of points, the distribution of the pulse shapes generally agrees with the simulation result.

**Discussions**

A quadrupole designed for an effective CANCAN should produce three conditions: (1) a bipolar pulse shape near the electrodes and a monophasic pulse shape near the quadrupole center and (2) the PCP to be as high as possible in the ROI to maximize the BPC efficiency. The PMP should be as low as possible to ensure a good spatial focus to stimulate biological responses. The PAP should be as low as possible because the additive pulses are generally not as efficient in suppressing the cell response as the cancellation pulses and (3) the field in all phases to be as uniform as possible. A small field gradient from the electrodes to the quadrupole center is needed for

**FIG. 6.** A Plate-Q configuration was fabricated with aluminum plates as electrodes and it covers an area of $\sim 56 \text{ mm} \times 56 \text{ mm}$ (a). At the selected points of an ROI (50 mm in diameter), the field was measured (b). The pulses to power the electrodes are shown in (c).

**FIG. 7.** The pulses in the ROI typically include a cancellation pulse (a), a monophasic pulse (b), and an additive pulse (c). For the cancellation pulse, the phases alternate their polarities. For the additive pulse, $\Phi_2$ and $\Phi_1$ have the same polarity, but $\Phi_3$ polarity is reversed.
CANCAN. However, a quadrupole configuration may not meet all three conditions simultaneously. For example, Resistor-Q has a uniform electric field, much better than Rod-Q and Plate-Q, but its PAP is higher. When selecting the best configuration, we prioritized the PCP to be the first, the PMP the second, and the field homogeneity the third. Inside the ROI, provided the field is in the range of \( E_{\text{min}} < E < E_{\text{max}} \), it is expected a high PCP will yield a decent focus at the quadrupole center, at which a monophasic pulse is always produced. Thus, Plate-Q is a good candidate for CANCAN.

In the selection of ROI, we purposely excluded the region proximal to the electrodes. This is because the fields near the electrodes can be much larger than that at the quadrupole center (>3 times). It can be seen that the conventional metallic electrodes always produce a large field gradient, but using resistors or semiconductors as a quadrupole boundary to mitigate the difference in conductivity (as in Resistor-Q) can smooth out the field distribution, which warrants more investigation. For CANCAN to work in the entire quadrupole region, there must be a significant biological difference between the unipolar and bipolar responses, that is, the field margin, \( \Delta E \) in Fig. 1, should be as large as possible and be comparable to the field gradient.

In our next step to validate the CANCAN modality, there are at least three endpoints. First, the electroporative damage of cell membranes will be assessed. As the poration caused by the monophasic pulses \( E_{\text{mon}} \) measured by Yo-Pro-1 uptake is three- to fourfold as that caused by the multiphasic pulses with the same amplitude in \( \Phi_1 \), it makes \( \Delta E \) at least twice as \( E_{\text{mon}} \). In the ROIs considered in our simulation and experiment, we showed the maximum field is 1.5–2 times that at the quadrupole center, which, therefore, allows us to demonstrate the in vitro CANCAN. Yo-Pro-1 uptake is a reliable and well-accepted method for measurement of electroporation (especially nanoporation). Mostly importantly, the fluorescence emitted after the Yo-Pro-1 dye binding to the DNA of cells stays relatively stable for a long time, which can be minutes or even longer, making the method suitable for observing the entire quadrupole region under a fluorescence stereo microscope, such as Olympus SZX16 (Olympus America, Hamden, CT). Second, cytosolic \( \text{Ca}^{2+} \) activation, a much more sensitive endpoint that requires lower electric fields, which shows 5- to 20-fold difference by monophasic pulses and bipolar pulses,\(^6\) can be another option. However, it has a shortcoming in that the calcium dynamic is time sensitive and reaches the peak in a dozen of seconds before it is regulated by the intracellular calcium signaling. This time limitation makes the calcium fluorescence only observable in a small region and not throughout the entire quadrupole region. Recently, \( \text{Ba}^{2+} \) was shown to work as well as \( \text{Ca}^{2+} \) to be a sensitive nanoporation marker, and can avoid being pumped out from the cytosol, thus \( \text{Ba}^{2+} \) can be a stable quantitative candidate over \( \text{Ca}^{2+} \).\(^{27}\) Third, monophasic and biphasic, 300 ns, showed a 10-fold difference in stimulating nerves. Reducing the pulse duration to 10 ns even predicts a 100-fold difference.\(^{17,28}\) Nerve stimulation thus becomes another high-potential endpoint. Certainly, CANCAN modality is not limited to the three endpoints and it can be applicable to many more that await to be discovered.

Regarding the pulse conditions for CANCAN, there were pieces of evidence suggesting that reducing pulse duration to sub-100 ns range can significantly increase \( \Delta E \).\(^{11,29}\) Applying pulses at high repetition rate up to several megahertz (pulse compression) can also increase \( \Delta E \).\(^{13,30}\) We also note that rotating active electrodes between multiple pulses helps abate the effect caused by a large electric field gradient. As rotating electrodes alone could help to achieve the highest effect of electroporation in the center of the quadrupoles, CANCAN incorporating electrode rotation may achieve a more focused effect at the center and less effect near the electrodes than those otherwise with the fixed electrode connection. These ideas certainly warrant further investigations.

Conclusions

BPC is an interesting phenomenon that warrants studies not just on the mechanisms but also on its utility. A quadrupole configuration can be used to incorporate BPC to create bipolar responses near the quadrupole electrodes and unipolar responses at the quadrupole center, thus allowing for a remote and focused stimulation (CANCAN). We have considered three electrode configurations, a Rod-Q, a Plate-Q, and a Resistor-Q. The simulation results show that the Plate-Q exhibits the highest PCP and the least PMP, thus it potentially could generate the best focused stimulation. The

![FIG. 8. The electric fields (a: \( E_x \) and b: \( E_y \)) on the selected points in the ROI are shown in Fig. 6b.](image-url)
experiment showed that the maximum electric field in the ROI was twice the field at the quadrupole center, thus permitting CANCANCAN in electropermeabilization. Although there may be other quadrupole configurations that can yield a smaller field gradient (less than twofold), the ability of spatial focusing should be examined.

Authors’ Contributions

S.X. designed the study and wrote the article. R.Y. and C.Z. constructed the electrodes and performed the experiments. All authors have reviewed and approved the article before submission. The article has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Shu Xiao, PhD
Frank Reidy Research Center for Bioelectrics
Old Dominion University
Norfolk, VA 23508
USA
E-mail: sxiao@odu.edu