Effects of cell morphology and electrical properties on electric field and dielectrophoretic force generated in cell exposed to tumor-treating field

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Abstract. Tumor-treating field (TTF) therapy has been receiving attention as a new minimally invasive treatment for cancer. In this treatment, an alternating electric field applied to target cells inhibits cell division by a dielectrophoretic force acting on chromosomes and microtubules. Previous studies have shown that an extremely weak electric field of only a few volts per centimeter interferes with chromosome migration and spindle formation. However, the therapeutic range of TTF treatment is still limited. To increase the efficacy of the treatment, the electric field to the target cells must be optimized. Therefore, the aim of this study was to investigate the effect of the electric field frequency, cell morphology, and electrical properties on the outcome of TTF treatment. Three-dimensional finite element models of a sphere and dividing cell were developed, and the distributions of the electric field and dielectrophoretic force were calculated.

1. Introduction
The tumor-treating field (TTF) is an alternating electric field with a frequency of several hundred kilohertz and an intensity of a few volts per centimeter. A distorted electric field penetrating into an hourglass-shaped mitotic cell produces a dielectrophoretic force acting on chromosomes and microtubules, thereby inhibiting cell division [1, 2]. Because the TTF does not adversely affect quiescent and interphase cells, it has garnered significant attention as a new minimally invasive treatment that targets only dividing cancer cells. After clinical trials had been performed, TTF therapy received FDA approval for the treatment of glioblastoma, which is the most aggressive type of cancer with an extremely poor prognosis [3]. In this therapy, glioblastoma regression could be achieved by applying a weak external electric field via electrode pads in contact with the head of a patient. However, the outcome is still limited; the median overall survival of TTF-treated patients was 14 months, which was only 3 months longer than that of patients without TTF treatment.

A cell can be considered as a capacitor composed of an insulating cell membrane; therefore, a low-frequency electric field cannot penetrate the cell. On the contrary, the cell becomes transparent to the electric field at high frequencies. An electric field of several hundred kilohertz penetrates the cell and shows a non-uniform distribution in a mitotic cell with an hourglass shape. In this case, two forces will act on polar molecules in the cell: the electrical force caused by the external electric field and the dielectrophoretic force induced by the concentration of the electric field. These forces interfere with
the normal alignment of intracellular molecules and consequently disrupt cell division. To maximize such an effect and obtain a successful outcome from TTF therapy, the settings of the applied electric field must be optimized by considering the effects of cell morphology and electrical properties on the intracellular distribution of the electric field.

The direction and timing of cell division in somatic cells cannot be determined in advance. Therefore, a TTF cannot be applied from the optimum direction at a time that is synchronized with cell division. Meanwhile, Xenopus eggs undergo the first division (oval division) approximately 90 min after fertilization to transform into a two-cell embryo, and 30 min later, it divides in the plane perpendicular to the plane of the first division to develop into a four-cell embryo [4]. Hence, the direction and timing of cell division can be predicted experimentally. In addition, Xenopus eggs are approximately 1.2 mm in diameter, which is 60 times larger than that of somatic cells, and can be easily observed under a stereomicroscope. To exploit these advantages, we investigated the effect of the TTF using Xenopus eggs as a model cell. In this study, the effects of the applied-voltage frequency, cell morphology, and electrical properties on the intracellular distribution of the electric field and dielectrophoretic force were investigated via numerical simulation using a finite element model.

2. Models and analysis
Two finite element models were created: one for a spherical cell (cell diameter: \( d \); cell membrane thickness: \( \delta \)) and another for a cell undergoing mitosis (figure 1). The mitotic model was designed from two spheres separated by 0.8\( d \) between their centers. Each cell model was placed in the center of a square analytical domain with one side length of \( w \), yielding a two-dimensional axisymmetric model with its center as a rotation axis.

![Analytical model and coordinate system for calculating electric field.](image)

The alternating potentials of \( V_1 = V_0 \sin(2\pi f_a c t) \) and \( V_2 = -V_0 \sin(2\pi f_a c t) \) were applied to the upper and lower surfaces of the analytical domain, where \( V_0 \) is the maximum voltage [V], \( f_a c \) the frequency [Hz], and \( t \) the time [s]. The other boundaries were regarded as electrically insulated. The electrical conductivities of the intracellular fluid, cell membrane, and extracellular medium are denoted as \( \sigma_i, \sigma_m, \) and \( \sigma_e \), respectively, and the corresponding relative permittivities are \( \varepsilon_i, \varepsilon_m, \) and \( \varepsilon_e \), respectively.

The distribution of the electric potential in the analytical domain was obtained by solving the following equation for the conservation of charge:

\[
\nabla \cdot \mathbf{J} + \frac{\partial \rho}{\partial t} = 0
\]  

(1)
where \( f \) is the current density \([A/m^2]\) and \( \rho \) is the charge density \([C/m^3]\).

For the finite element analysis, COMSOL Multiphysics software (v. 5.3a, COMSOL AB, Stockholm, Sweden) with an AC/DC module was used. Because the cell membrane was extremely thin compared with the size of other regions, it was regarded as the boundary condition of the contact impedance. The analytical domain with a length of \( w = 20 \, \text{mm} \) in figure 1 was partitioned into triangular elements, and an alternating potential with an amplitude of \( V_0 = 2 \, \text{V} \) was applied to the top and bottom surfaces of the analysis domain. The electrical properties used in the analysis are listed in table 1. The values shown in bold in the table, i.e., cell diameter \( d = 1 \, \text{mm} \); cell membrane thickness \( \delta = 10 \, \text{nm} \); electrical conductivities \( \sigma_l = 0.3 \, \text{S/m}, \sigma_m = 3 \times 10^{-7} \, \text{S/m}, \) and \( \sigma_e = 1.2 \, \text{S/m} \); and relative permittivities \( \varepsilon_l = 72.3, \varepsilon_m = 5, \) and \( \varepsilon_e = 72.3 \) were set as base values.

In the analysis, the effective electric field \( E_{rms} \, [\text{V/cm}] \) and the dielectrophoretic force \( F_{dp} \) were calculated by changing the frequency \( f_{ac} \) in the range of 10 kHz to 1 GHz. The base values in table 1 were used for the size and electrical properties of the cells. Assuming that a tubulin dimer as an object under the dielectrophoretic force, \( F_{dp} \) is proportional to the squared electric field gradient \( \nabla (E_{rms})^2 \, [\text{V^2/m^3}] \).

\[
|F_{dp}| \propto |\nabla (E_{rms})^2| \tag{2}
\]

Therefore, the distribution of \( F_{dp} \) was evaluated from that of \( \nabla (E_{rms})^2 \). Subsequently, the effects of the cell size and electrical properties on \( E_{rms} \) and \( \nabla (E_{rms})^2 \) were analyzed by changing these parameters in the range shown in table 1.

**Table 1.** Model parameters.

| Parameters                                                                 | Values to be analyzed* |
|---------------------------------------------------------------------------|------------------------|
| Cell diameter, \( d \, (\text{mm}) \)                                      | 0.02, 0.1, 0.2, 1, 2   |
| Thickness of the cell membrane, \( \delta \, (\text{nm}) \)                | 5, 10, 50, 100, 500    |
| Electrical conductivity of the intracellular fluid, \( \sigma_l \, (\text{S/m}) \) | 0.1, 0.3, 0.5, 1.2, 3  |
| Electrical conductivity of the cell membrane, \( \sigma_m \, (\text{S/m}) \) | \( 10^{-8}, 3 \times 10^{-7}, 10^{-6} \) |
| Electrical conductivity of the extracellular medium, \( \sigma_e \, (\text{S/m}) \) | 0.1, 0.9, 1.2, 1.5, 3  |
| Relative permittivity of the intracellular fluid, \( \varepsilon_l \)     | 65, 72.3, 80           |
| Relative permittivity of the cell membrane, \( \varepsilon_m \)           | 1, 2.5, 5, 7.5, 10     |
| Relative permittivity of the extracellular medium, \( \varepsilon_e \)     | 65, 72.3, 80           |

*Number in bold represents each base value.

3. Results

Figure 2 shows the effect of frequency on the distribution of the effective electric field \( E_{rms} \) and the squared electric field gradient \( \nabla (E_{rms})^2 \), where \( E_{rms} \) was normalized by \( E_{rms(\text{ext})} \), which is the magnitude of the electric field applied between the upper and lower surfaces of the analytical domain. In the spherical model, the distribution of the electric field was uniform in the cell and its magnitude increased in a frequency-dependent manner. At 1 GHz, the cell became transparent to the electric field, and the magnitudes of the electric fields inside and outside the cells were identical. Because of the uniformity of the electric field, no dielectrophoretic force was observed in the spherical cell. Meanwhile, the mitotic model demonstrated a concentrated electric field at the cell neck at a frequency of 1–10 kHz. Corresponding to the concentration of the electric field, a higher \( \nabla (E_{rms})^2 \) was observed, i.e., a high dielectrophoretic force was expected in the vicinity of the cleavage furrow.

Figure 3 shows the changes in the maximum \( E_{rms} \) and \( \nabla (E_{rms})^2 \) as a function of frequency, where \( \nabla (E_{rms})^2 \) was normalized by that of the mitotic model at a frequency of 20 kHz. The maximum \( E_{rms} \) peaked at 10–20 kHz in the mitotic model, which was more than six times higher than that in the spherical model. At this frequency, \( \nabla (E_{rms})^2 \) showed a maximum value.
Figure 2. Effect of frequency on distribution of effective electric field $E_{\text{rms}}$ (a) and squared electric field gradient $\nabla (E_{\text{rms}})^2$ (b). $E_{\text{rms}}$ was normalized by magnitude of external electric field $E_{\text{rms}}(\text{ext})$.

Figure 3. Maximum $E_{\text{rms}}$ (a) and $\nabla (E_{\text{rms}})^2$ (b) in cell as a function of frequency. $E_{\text{rms}}$ was normalized by magnitude of external electric field $E_{\text{rms}}(\text{ext})$, whereas $\nabla (E_{\text{rms}})^2$ was normalized by that of mitotic model at a frequency of 20 kHz.

By changing the cell size and the electrical properties with frequency, the effects on the distributions of $E_{\text{rms}}$ and $\nabla (E_{\text{rms}})^2$ were investigated. For example, the effect of the relative permittivity of the cell membrane is shown in figure 4. An optimum frequency was observed at which $E_{\text{rms}}$ and $\nabla (E_{\text{rms}})^2$ were extremely concentrated at the cell neck. The optimum frequency was shifted to a lower value as the relative permittivity increased. Figure 5 shows the changes in $E_{\text{rms}}$ and $\nabla (E_{\text{rms}})^2$ as a function of frequency, where the maximum $\nabla (E_{\text{rms}})^2$ was normalized by that for $\varepsilon_r = 5$ at 20 kHz. The optimum frequency at which $E_{\text{rms}}$ and $\nabla (E_{\text{rms}})^2$ showed a peak shifted to a lower value as the relative permittivity increased; however, the magnitude of these maxima did not change. Meanwhile, the conductivity of the cell membrane did not affect the
optimum frequency, as shown in figure 6. However, the maximum $E_{rms}$ and $\nabla (E_{rms})^2$ decreased with increasing conductivity, as shown in figure 7.

![Figure 4](image-url)

**Figure 4.** Effect of relative permittivity of cell membrane $\varepsilon_m$ on distribution of effective electric field $E_{rms}$ (a) and squared electric field gradient $\nabla (E_{rms})^2$ (b).

![Figure 5](image-url)

**Figure 5.** Effect of relative permittivity of cell membrane $\varepsilon_m$ on maximum $E_{rms}$ (a) and $\nabla (E_{rms})^2$ (b) in cell as a function of frequency.

![Figure 6](image-url)

**Figure 6.** Effect of electrical conductivity of cell membrane $\sigma_m$ on distribution of effective electric field $E_{rms}$ (a) and squared electric field gradient $\nabla (E_{rms})^2$ (b).
Figure 7. Effect of electrical conductivity of cell membrane $\sigma_m$ on maximum $E_{\text{rms}}$ (a) and $\nabla(E_{\text{rms}})^2$ (b) in cell as a function of frequency.

Similarly, figures 8–13 show the effects of cell diameter, cell membrane thickness, electrical conductivity, and relative permittivity of the intracellular fluid and extracellular medium on the maximum $E_{\text{rms}}$ and $\nabla(E_{\text{rms}})^2$. The cell diameter significantly affected $\nabla(E_{\text{rms}})^2$, which decreased as the diameter increased (figure 8). The optimum frequency shifted to a lower value with increasing diameter. Conversely, the optimum frequency shifted to a higher value as the cell membrane thickness increased (figure 9). The increase in the electrical conductivity of the intracellular fluid reduced $E_{\text{rms}}$ and $\nabla(E_{\text{rms}})^2$, as well as shifted the optimum frequency to a higher-frequency range (figure 10). The electrical conductivity of the extracellular medium showed a different effect from that of the intracellular fluid. The increase in extracellular conductivity increased $E_{\text{rms}}$ and $\nabla(E_{\text{rms}})^2$ slightly, but did not affect the optimum frequency (figure 12). Meanwhile, the relative permittivity of the intracellular fluid and extracellular medium did not affect $E_{\text{rms}}$, $\nabla(E_{\text{rms}})^2$, or the optimum frequency (figures 11 and 13).

Figure 8. Effect of cell diameter $d$ on maximum $E_{\text{rms}}$ (a) and $\nabla(E_{\text{rms}})^2$ (b) in cell as a function of frequency.
Figure 9. Effect of cell membrane thickness $\delta$ on maximum $E_{rms}$ (a) and $\nabla (E_{rms})^2$ (b) in cell as a function of frequency.

Figure 10. Effect of electrical conductivity of intracellular fluid $\sigma_i$ on maximum $E_{rms}$ (a) and $\nabla (E_{rms})^2$ (b) in cell as a function of frequency.

Figure 11. Effect of relative permittivity of intracellular fluid $\varepsilon_i$ on maximum $E_{rms}$ (a) and $\nabla (E_{rms})^2$ (b) in cell as a function of frequency.
Figure 12. Effect of electrical conductivity of extracellular medium $\sigma_e$ on maximum $E_{rms}$ (a) and $\nabla (E_{rms})^2$ (b) in cell as a function of frequency.

Figure 13. Effect of relative permittivity of extracellular medium $\varepsilon_e$ on maximum $E_{rms}$ (a) and $\nabla (E_{rms})^2$ (b) in cell as a function of frequency.

Table 2 summarizes the effects of cell size and electrical properties on the maximum $E_{rms}$, $\nabla (E_{rms})^2$, and optimum frequency that yielded these maxima. The maximum $E_{rms}$ and $\nabla (E_{rms})^2$ increased with the conductivity of the extracellular medium, whereas they decreased with the increase in the cell diameter and electrical conductivities of the cell membrane and intracellular fluid. The optimum frequency shifted to a lower value as the cell diameter and the permittivity of the cell membrane increased; conversely, it shifted to a higher frequency as the cell membrane thickness and the electrical conductivity of the intracellular fluid increased.

| Parameters | Range       | $E_{rms}$ | $\nabla (E_{rms})^2$ | $f_{opt}$ |
|------------|-------------|-----------|---------------------|-----------|
| $d$ (mm)   | 0.02 ~ 2    | Down      | Down                | Down      |
| $\delta$ (nm) | 5 ~ 500   | -         | -                   | Up        |
| $\sigma_l$ (S/m) | 0.1 ~ 3   | Down      | Down                | Up        |
| $\sigma_m$ (S/m) | $10^{-8}$ ~ $10^{-6}$ | Down      | Down                | -         |
| $\sigma_e$ (S/m) | 0.1 ~ 3   | Up        | Up                  | -         |
| $\varepsilon_i$ | 65 ~ 80   | -         | -                   | -         |
| $\varepsilon_m$ | 1 ~ 10    | -         | -                   | Down      |
| $\varepsilon_e$ | 65 ~ 80   | -         | -                   | -         |

The “-” sign denotes no change.
4. Discussion

To conduct an experiment using Xenopus eggs as a model cell for TTF treatment, the effects of the applied-voltage frequency, cell morphology, and electrical properties on the distribution of the intracellular electric field and dielectrophoretic force were investigated via numerical simulation using a finite element model. Owing to the insulating property of the cell membrane, low-frequency electric fields did not enter the cell. However, electric fields on the order of kilohertz to megahertz penetrated the cell and caused an electric field concentration at the neck of dividing cells. The maxima of the intracellular effective electric field \( E' \) and the squared electric field gradient \( \nabla (E')^2 \), which is proportional to the magnitude of the dielectrophoretic force, were affected by the cell diameter and the electrical conductivities of the intracellular fluid, cell membrane, and extracellular medium. The optimum frequency yielding these maxima was affected by the cell diameter, cell membrane thickness, intracellular conductivity, and extracellular permittivity.

A cell can be represented by an equivalent electrical circuit comprising resistors and capacitors, as shown in figure 14(a), where the intracellular and extracellular capacitances, \( C_i \) and \( C_e \), are negligible owing to their high electrical conductivities. The impedance of the cell membrane \( |Z_m| \) can be calculated as

\[
|Z_m| = \left( \frac{1}{R_m} \right)^2 + \left( 2\pi f_{ac} C_m \right)^2 \right]^{1/2}
\]

using the cell membrane resistance \( R_m \), capacitance \( C_m \), and applied-voltage frequency \( f_{ac} \). Because the first term of the denominator, \( 1/R_m \), is extremely small and hence negligible, the impedance of the cell membrane varies with \( f_{ac} \) and \( C_m \). Therefore, in a low-frequency electric field, the impedance becomes high and electrical flux lines pass through the outside, avoiding the cell. As the frequency increases, part of the electric field is admitted to the cell. Subsequently, the distorted electric field is concentrated in the neck of the mitotic cell. When the frequency increases further, the cell becomes completely transparent to the electric field. Hence, depending on the cell morphology and electrical properties, the most favorable frequency exists at which \( E_{rms} \) and \( \nabla (E_{rms})^2 \) in the cell are maximized.

![Figure 14](image_url)

**Figure 14.** Equivalent circuit model of a cell (a) and concentric sphere capacitor (b).

The distribution of the electric field and the optimum frequency, which were affected by the cell diameter and cell membrane thickness, can be explained by considering the cell as a concentric sphere capacitor as shown in figure 14(b). The cell membrane capacitance (spherical shell) \( C_m \) is expressed as

\[
C_m = \pi \varepsilon_0 \varepsilon_m \left( \frac{d^2}{\delta} + 2d \right)
\]

where \( \varepsilon_0 \) is the permittivity of vacuum, \( \varepsilon_m \) the relative permittivity of the cell membrane, \( d \) the cell diameter, and \( \delta \) the membrane thickness. Furthermore, the time constant of the cell membrane, \( \tau_m \), can be calculated from the following approximation [5]:

\[
\tau_m \approx \varepsilon_0 \varepsilon_m \left( \frac{\delta}{d} \frac{\sigma_i + \sigma_L}{2 \sigma_e + \sigma_i + \sigma_m} \right)^{-1}
\]
As the cell diameter $d$ increases, the membrane capacitance $C_m$ increases as shown in equation (4); subsequently, the impedance of the cell membrane $|Z_m|$ increases, based on equation (3), causing a small electric field to penetrate the cell. In addition, the optimum frequency shifts to a lower value because the time constant $\tau_m$ increases, requiring more time to charge the capacitor. These estimates are consistent with the results shown in figure 8. Conversely, the capacitance and impedance of the cell membrane decrease as the cell membrane thickness increases because it is equivalent to a relative decrease in the cell diameter. This results in an increased electric field in the cell and a shift in the optimum frequency to a higher band. In fact, $E_{rms}$ and $\nabla(E_{rms})^2$ increased slightly with the cell membrane thickness as shown in figure 9 although they were not included in table 2 owing to their insignificant effect.

As the electrical conductivity of the intracellular fluid increased, $E_{rms}$ and $\nabla(E_{rms})^2$ decreased (figure 10). This is attributable to the decrease in the potential difference caused by the small electrical resistance inside the cell. In addition, the optimum frequency shifted to a higher value because of the reduced time constant $\tau_m$. An increase in the conductivity of the cell membrane showed a similar effect, i.e., a decrease in $E_{rms}$ and $\nabla(E_{rms})^2$ (figure 7). This is because the electric field that penetrated the cell did not converge at the neck of the dividing cell but exited through the cell membrane. Conversely, an increase in the conductivity of the extracellular fluid would increase the current density outside the cell, resulting in an increase in the electric field inside the cell (figure 12). The relative permittivity of the intracellular fluid and extracellular medium did not affect $E_{rms}$, $\nabla(E_{rms})^2$, or the optimum frequency (figures 11 and 13), whereas an increase in that of the cell membrane shifted the optimum frequency to a lower value (figure 5). This might be due to an increase in the capacitance $C_m$ and time constant $\tau_m$ of the cell membrane.

In this study, the effects of cell morphology and electrical properties on the intracellular electric field, dielectrophoretic force, and the optimum frequency was revealed both quantitatively and qualitatively. However, they were the main effects of each parameter investigated with only one of the eight parameters varied and the remaining seven fixed. Therefore, the effect of simultaneously changing two or more parameters is unclear. To investigate the interaction of multiple parameters, the effects of multiple factors on the electric field must be analysed, for instance, using an experimental design method based on orthogonal tables.

Among the eight parameters, the cell diameter, cell membrane thickness, and electrical properties of the intracellular fluid and cell membrane were the most difficult to manipulate. However, this does not diminish the usefulness of the results of this study. For instance, cells exposed to TTF treatment showed a larger diameter than those without treatment [6]. Based on the analytical results that $E_{rms}$, $\nabla(E_{rms})^2$, and the optimum frequency decreased in the cell with a larger diameter, the outcome is predicted to decrease. To avoid a decrease in the therapeutic effect, it would be useful to increase the frequency or compensate for the decreased intracellular electric field by increasing the applied voltage. Another example is when the electrical properties of the intracellular fluid and cell membrane indicate significant variations in magnitude. The results of this study would be valuable for investigating these effects. Unlike the electrical properties of the intracellular fluid and cell membrane, those of the extracellular medium can be modified by administrating exogenous solutions. The analysis showed that TTF outcome would be enhanced by using a highly conductive solution as the extracellular medium.

5. Conclusions
The effects of applied-voltage frequency, cell morphology, and electrical properties on the distributions of intracellular electric field and dielectrophoretic force were investigated using a finite element model, where Xenopus eggs were used as a model cell because they can be used to predict the direction and timing of cell division in advance. Low-frequency electric fields did not penetrate the cell, whereas those on the order of kilohertz to megahertz did. The penetrated electric field in a certain frequency band caused a concentration in the neck of mitotic cells. The maximum electric field and squared electric field gradient, which was proportional to the magnitude of the dielectrophoretic force,
decreased as the cell diameter and the conductivities of the intracellular fluid and cell membrane increased; conversely, they increased with the extracellular conductivity. The optimum frequency yielding these maxima shifted to a lower value as the cell diameter and the relative permittivity of the cell membrane increased, whereas it shifted to higher frequencies as the cell membrane thickness and the conductivity of the intracellular fluid increased.

Acknowledgements
This study was supported by JSPS KAKENHI Grant Number 18H03520.

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