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RESEARCH ARTICLE

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Quantitative analyses of RBC movement in whole blood exposed to DC and ELF electric field

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Abstract

For the study of biological effects of direct current (DC) and extremely low frequency (ELF) electric fields, we have quantitatively analyzed red blood cell (RBC) movement in whole blood. Considering the inhomogeneous distribution of electric fields in vivo, five different electric field distributions were generated under a microscope. For theoretical analyses, we assumed electrophoresis and dielectrophoresis as basic motive forces and obtained the spatial distribution of blood cell velocity. The RBC velocity was measured using video image analysis. The spatial dependence of the velocity showed good agreement with that predicted by theoretical analysis. This result suggests the validity of the theoretical model based on electrophoresis and dielectrophoresis for the study of ELF electric field exposure to inhomogeneous animal and human bodies. Next, using the same measurement system, we attempted to find the electric field strength at which these effects occur. The threshold values were found to be 0.40 and 1.6 kV/m, respectively, for DC and AC electric field exposures. Furthermore, we investigated the reproducibility of the field effects in more realistic conditions of human exposure. The RBCs in microchannels were exposed to the electric field generated in capacitive coupling using electrodes separated by an air gap. Even in the new condition, similar effects were observed, which also verified the validity of the analysis described above. These results will provide useful information for the safety assessment of field exposure and for the future biomedical applications of electric fields to manipulate RBCs in vivo.

KEYWORDS

biological effects, dielectrophoresis, electrophoresis, ELF electric field, red blood cell, whole blood

1 | INTRODUCTION

We have been living in an electromagnetic environment with intensity that is unprecedented in human history. However, because this era has only begun, the long-term chronic effects of electromagnetic fields on human health and ecosystems remain unknown. Research investigating the biological effects of electromagnetic environments requires multifaceted evaluations from scientific, engineering, psychological, and statistical perspectives. Particularly, quantitative analysis must be conducted to verify the safety of human and ecosystems objectively. Quantitative evaluation necessitates identification of the mechanisms of targeted biological effects and clarification of the threshold for their occurrence.

Known biological effects of electromagnetic fields include thermal effects of high-frequency electromagnetic waves, such as microwaves, and body surface stimulation effects of low-frequency electric fields (National Research Council US, 1997; Reilly, 1992, 1998; Takebe et al., 1999). In the 1970s and 1980s, the civilian use of microwaves and high-voltage power networks spread widely. Since then, many studies of biological

effects have been conducted (Carstensen, 1987; National Research Council US, 1997; Reilly, 1992, 1998; Takebe et al., 1999; Wertheimer & Leeper, 1979; Wilson et al., 1990). As a result, international guidelines have been provided; safety standards have been established in various countries (ICNIRP, 2010; World Health Organization, 2007). Nevertheless, many issues remain unknown, such as working mechanisms. This study specifically examined extremely low frequency (ELF; herein, frequencies up to 300 Hz including direct current, DC) electric fields, many aspects of which remain unclear.

Extensive studies have been conducted to elucidate the biological effects of ELF electric fields (Carstensen, 1987; Odagiri et al., 1994; Shimizu & Shimizu, 1999; Takebe et al., 1999; Wertheimer & Leeper, 1979; Wilson et al., 1990; World Health Organization, 2007). These include many studies of the effects on blood in the body (Takebe et al., 1999; World Health Organization, 2007). Because of the large number of charged particles present in blood, physical and chemical effects might occur with their exposure to electric fields.

From physiochemical analyses of blood, blood cells are known to move through liquid by electrophoresis or dielectrophoresis when a voltage is applied to the blood (Cruz & Garcia-Diego, 1998; Hsu et al., 2003; Lu et al., 2003; Leonard & Minerick, 2011; Minerick et al., 2003; Su et al., 2012). In fact, the aggregation of red blood cells (RBCs) by electrophoresis (Baskurt et al., 2002), and the interaction of hemoglobin with proteins by electrophoresis (Su et al., 2010) have been reported. The use of dielectrophoresis for blood-plasma separation (Mahdi et al., 2015) and RBC analyses (Due et al., 2014; Edwin et al., 2022; Mahdi et al., 2015) have also been reported. However, almost all of these phenomena have been observed in diluted blood samples. Therefore, we have investigated blood cell movement when whole blood is exposed to an electric field (Kanemaki et al., 2022), bearing in mind electric field exposure effects on blood inside the body.

In our earlier work, we developed a system to measure blood cell movement under a microscope when whole blood is exposed to electric fields. From the preliminary analysis, we reported for the first time that exposure to an electric field clearly changes the migration velocity of blood cells in whole blood. Moreover, we showed that electrophoresis might be the main mechanism of this effect in the case of exposure to DC electric fields and dielectrophoresis in cases of AC field exposure. However, in the earlier study, the exposure field was uniform in the DC field, or the field gradient was uniform in the AC field. Furthermore, the electric field was exposed by application of a voltage with the electrodes in direct contact with the blood (Kanemaki et al., 2022). Therefore, for this study, we quantitatively analyzed the effects of electric field exposure under more realistic conditions, keeping in mind the exposure of a human body to electric fields in industrial and medical applications.

Highlights

- To evaluate the hematological effects of direct current (DC) and alternating current (AC) extremely low frequency (ELF) electric field exposure, we investigated the movement of red blood cells (RBCs) in whole blood. Video images of RBCs were captured under a microscope using specially designed electrode systems.
- We discovered that RBC movement is significantly influenced by ELF electric field exposure with much greater intensity than the internationally publicized guideline for human safety.
- In this study, we have conducted a quantitative analysis of RBC movement in whole blood, considering the inhomogeneous distribution of electric fields in vivo. The spatial dependence of RBC velocity exhibited good agreement with that predicted by the theoretical model based on electrophoresis and dielectrophoresis.
- The threshold values for the exposed electric field were found to be 0.40 and 1.6 kV/m for DC and AC field exposures, respectively.
- To investigate the effects of the electric field under more realistic conditions of human exposure, RBCs in microchannels were exposed to the electric field generated via capacitive coupling, using electrodes separated by an air gap. Even under these new conditions, we observed similar effects to those in the previous experiment, which verified the validity of the analysis described above.
- These results will provide valuable information for safety assessment of field exposure and for potential future biomedical applications of electric fields to manipulate RBCs in vivo.

2 | THEORETICAL ANALYSIS OF RBC MOVEMENT IN AN ELECTRIC FIELD

2.1 | Human body exposure to an electric field

Figure 1 presents a model of electric field exposure. Between two parallel plane electrodes is an object such as an experimental animal or a human body at the center of the bottom plane. The object is small compared with the field generating system. The object is exposed to an almost uniform electric field $E_0 \approx V/d$, where V and d are, respectively, the electric potential of an upper electrode plate and the distance between upper and lower electrodes.

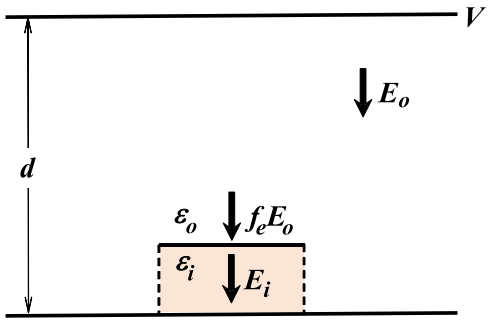

FIGURE 1 Theoretical model of an internal electric field during electric field exposure.

In the case of DC electric field exposure, the electric field inside the object body E_i can be approximated as

$$E_i = \frac{\varepsilon_o}{\varepsilon_i} f_e E_o, \tag{1}$$

where ε_i, ε_o, and f_e respectively denote the inside permittivity, the outside permittivity, and the factor of the field-enhancement caused by the protruding object.

In the case of AC electric field exposure, the internal electric field can be approximated as

$$E_i \approx \frac{\omega \varepsilon_o}{g_i} f_e E_o, \tag{2}$$

where ω and g_i respectively stand for the angular frequency of the applied electric field and the internal conductivity of the object. When the object is an experimental animal or a human body in the air, the internal conductivity is much greater than external permittivity. The internal electric field is greatly attenuated. However, the internal field becomes non-negligible when the external electric field E_0 is large or when the field enhancement factor f_e is large.

2.2 | Electric field effects on RBC movement

One biological effect of electric fields is their mechanical action on charged particles in the body. The body contains various charged particles. Because of their important role, we specifically examined RBCs in the blood as a representative example. The surface of RBC is known to be negatively charged under normal conditions (Jan & Shu, 1973). In other words, RBCs move under a force in the opposite direction to that of the electric field. The movement of RBCs in the body might be affected by exposure to an electric field from outside the body.

RBCs in blood exposed to an electric field are subjected to various physical forces. The main ones are electrophoresis force, dielectrophoresis force, electro-osmosis force, and gravity. Because blood vessels inside the human body run in all directions, the effects of gravity are canceled out almost completely. Moreover, the mobility of electroosmosis is much less than that of electrophoresis (Kaune & Forsythe, 1985). Therefore, for this study, we consider electrophoretic and dielectrophoretic forces. The final velocity $\vec{v}$ of a charged particle subjected to these forces in a liquid is given by the following equation using the respective mobility (Jan & Shu, 1973) as

$$\begin{aligned} \vec{v} &= \mu_{EP}\vec{E} + \mu_{DEP}\nabla E^2 \\ \mu_{EP} &= \frac{Q}{6\pi r\eta} \\ \mu_{DEP} &= -\frac{\pi r^2 f_{CM}\,\varepsilon_m}{3\eta}, \end{aligned} \tag{3}$$

where $\vec{E}$, μ_{EP}, μ_{DEP}, Q, r, η, f_{CM}, ε_m respectively represent the electric field at RBCs, electrophoretic mobility, dielectrophoretic mobility, particle surface charge, particle (equivalent) radius, medium viscosity, Clausius–Mossotti coefficient, and medium permittivity. Arrows on the variables denote vectors. The values of μ_{EP} and μ_{DEP} of RBCs suspended in phosphate-buffered saline (PBS) solution have been reported in the literature (Zhu & Chen, 2015).

Equation (3) holds irrespective of whether the electric field is DC or alternating current (AC). The direction of motion of the moving particles is the direction of the electric field vector in electrophoresis, and the direction of inclination of the spatial distribution of the squared electric field in dielectrophoresis. Because of the high conductivity in vivo, extremely large gradients in electric field are unlikely in a living body. Therefore, under general blood parameters ($Q = -3.19 \times 10^{-10}$ [C], $r = 2.70 \times 10^{-6}$ [m], $\eta = 1.58 \times 10^{-3}$ [Pa·s]) (Gudmundsson & Bjelle, 1993; Gutsul et al., 2012; Tokumasu et al., 2012; Zhbanov & Yang, 2015), the electrophoresis is overwhelmingly large. Therefore, electrophoresis is regarded as dominant under DC electric field exposure. By contrast, in the case of AC, the direction of the electric field vector reverses with time. If the particle movement is unable to follow the frequency change of the electric field, as in the case of RBCs in whole blood, then the electrophoretic action is canceled. These considerations suggest that electrophoresis is dominant when exposed to a DC electric field and that dielectrophoresis is dominant when exposed to an AC electric field.

2.3 | RBC movement in various field distributions

When a human body or an experimental animal is exposed to an electric field, the distribution of the electric field inside the body is not uniform: it varies both temporally and spatially (Chen et al., 1999). Therefore, we conducted a theoretical analysis of electrophoresis and dielectrophoresis in a spatially inhomogeneous electric field distribution. The outline of sample preparation in experiments and electrode configuration under analysis is depicted in Figures 2 and 3, respectively. Here, the electrodes are arranged in a curved shape with respect to the x-axis, bearing in mind the coordinate structure in the following experiments.

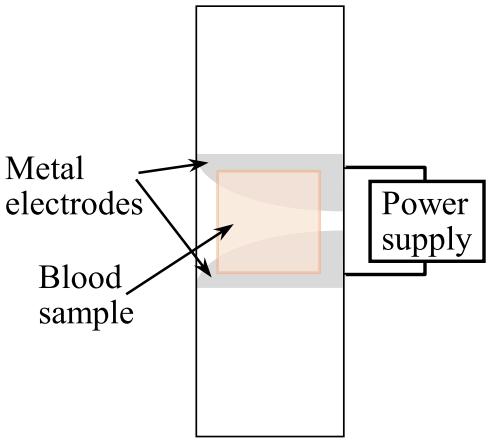

FIGURE 2 Schema showing sample preparation.

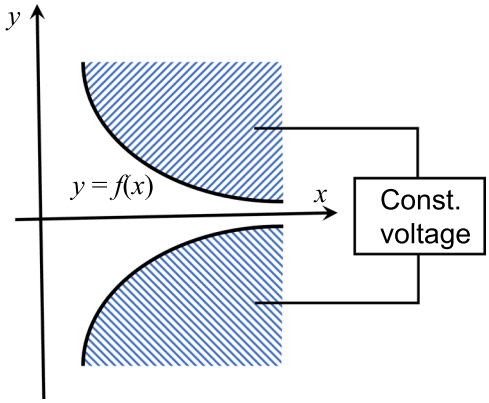

FIGURE 3 Structure of electrodes for theoretical analysis of various electric field distributions.

Based on the hypothesis presented in the preceding section, the migration velocity of RBCs is proportional to the electric field when exposed to a DC electric field. When exposed to an AC electric field, it is proportional to the slope of the squared electric field value. If the function of the upper and lower electrodes is $y = \pm f(x)$, then the electric field distribution along the x axis and the slope of the squared electric field value are given by the following equations.

$$\vec{E}(x) = C\frac{V}{2f(x)}\vec{i}_y, \tag{4}$$

$$\nabla|\vec{E}(\mathrm{x})|^2 = -C^2\frac{V^2}{2}f^{-3}(x)\frac{\partial f}{\partial x}\vec{i}_x. \tag{5}$$

In these equations, C, V, i_x, and i_y respectively signify the location-independent constant, the applied voltage, and unit vectors in the x and y directions.

As representative examples of various electric field distributions, electrodes with five different functions were considered. The functions used and their respective functional forms of Equations (4) and (5) are presented below as

$$case \quad 1.\, f(x) = a_1 x, \tag{6}$$

$$\vec{E}(x) = C\frac{V}{2a_1 x}\vec{i}_y, \tag{7}$$

$$\nabla|\vec{E}|^2 = -C^2\frac{V^2}{2a_1{}^2x^3}\vec{i}_x, \tag{8}$$

$$case\ 2.\, f(x) = a_2, \tag{9}$$

$$\vec{E}(x) = C\frac{V}{2a_2}\vec{i}_y, \tag{10}$$

$$\nabla|\vec{E}|^2 = 0, \tag{11}$$

$$case\ 3.\, f(x) = \frac{a_3}{\sqrt{x}}, \tag{12}$$

$$\vec{E}(x) = C\frac{V\sqrt{x}}{2a_3}\vec{i}_y, \tag{13}$$

$$\nabla|\vec{E}|^2 = C^2\frac{V^2}{4a_3{}^2}\vec{i}_x, \tag{14}$$

$$case\ 4.\, f(x) = \frac{a_4}{x}, \tag{15}$$

$$\vec{E}(x) = C\frac{Vx}{2a_4}\vec{i}_y, \tag{16}$$

$$\nabla|\vec{E}|^2 = C^2\frac{V^2x}{2a_4{}^2}\vec{i}_x, \tag{17}$$

$$case\ 5.\, f(x) = \frac{a_5}{x^2}, \tag{18}$$

$$\vec{E}(x) = C\frac{Vx^2}{2a_5}\vec{i}_y, \tag{19}$$

$$\nabla|\vec{E}|^2 = C^2\frac{V^2x^3}{a_5{}^2}\vec{i}_x. \tag{20}$$

In those case expressions, a_1–a_5 are independent constants with respect to positions x and y.

3 | EXPERIMENTAL ANALYSIS OF RBC MOVEMENT IN DIFFERENT FIELD DISTRIBUTIONS

3.1 | Measurement system

An overview of the measurement system is portrayed in Figure 4. It is a video-microscope system (Nikon Eclipse

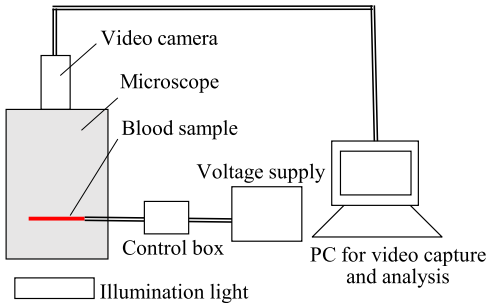

FIGURE 4 Measurement system of red blood cells movement in electric field exposure.

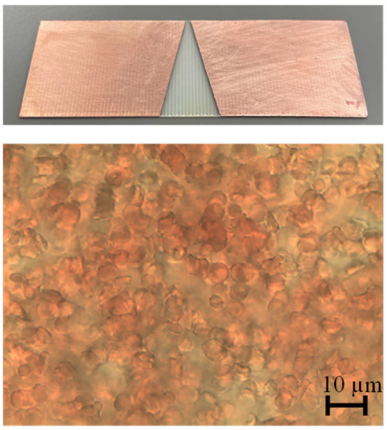

FIGURE 5 Appearance of manufactured electrodes, and example of an observed image.

80i; Nikon Corp.) with transmission-type illumination. For the experiment, a blood sample with electrodes described in Figure 2 was observed. Its video image was recorded using a video camera (Moticam 3.0 MP; Shimadzu Corp.). The electrodes were connected to a power supply (CVFT1-200H; Tokyo Seiden Co., Ltd.) through a voltage control box that can also change the DC voltage polarity easily.

A sample of whole blood was prepared by placing a drop of human blood between the electrodes. We covered it with a microscope cover glass to make a sample with constant 0.02 mm thickness. The blood sample was collected from the left-hand ring-finger tip by the subject's own self-puncture after obtaining the subject's informed consent. The subject was a 28-year-old healthy woman. No anticoagulant was used in the blood sample. This experiment was conducted at laboratory temperatures of 22–26°C. The sample temperature stability was confirmed as reported in an earlier report (Kanemaki et al., 2022).

As a fundamental condition in measurement, DC 7 V or AC 40 V at 50 Hz was applied to the electrodes of the blood sample. Microscopic images of RBCs were analyzed. MATLAB (2018) was used for video analysis. Blood cell velocity vectors were measured from frame-to-frame changes in blood cell positions. The video frame interval was set as 20 fps. After 10 RBCs within the observation field of view were selected arbitrarily, their individual motions were analyzed. Their average velocity was recorded for additional analyses.

Figure 5 presents the appearance of the representative electrodes and the RBC image. The electrodes used for the experiments were made by etching a 0.02-mm-thick copper foil on a translucent substrate similar to a slide glass for microscopy. In this way, constant thickness 0.02 mm of the blood sample was certified by covering these electrodes with a flat microscope cover glass. In all measurements reported in this paper, these copper foil electrodes were employed. A constant-voltage power souce was used, and the electric current flowing between the electrodes was several to several tens of μA in both DC and AC cases.

3.2 | Analysis of RBC movement

Considering the variation of the electric field distribution inside a living body, various electric field distributions were constructed under a microscope to verify whether the electrophoretic and dielectrophoretic hypotheses are widely applicable. According to Equations (6)–(20), the RBC velocity should show different x-position dependence depending on the electrode geometry. For example, the RBC velocity should be proportional to x^{-1} and x^2 in cases 1 and 5, respectively. We experimentally verified these hypotheses for various electric field distributions.

Using the measurement system described above, blood cell velocities were measured at several points on the x axis, that is, the axis of symmetry of the electrodes arranged in a line symmetrical position. To investigate the position dependence of the blood cell velocity on the x axis, we performed multidimensional curve approximations. Figure 6 shows examples of the measured position dependence of RBC velocity obtained with the electrodes in cases 1 and 5. As might be apparent in Figure 6a,b, respectively, the measured data show good agreement with the theoretically predicted –1 and 2 order curves. These results suggest the validity of the electrophoretic and dielectrophoretic hypotheses presented in the section "Electric Field Effects on RBC Movement" in Chapter II.

To clarify the validity of the hypothesis for different electric field distributions, we conducted the same analysis for all cases 1–5. After manufacturing electrodes with different curves, we measured the position dependence of the blood cell velocity on the x coordinate. For comparison among cases, we used the n-th order function $y = ax^n$ for curve approximation. The accuracy of the approximation was evaluated using the coefficient of determination of the approximation. The respective accuracies in the DC and AC field exposures are presented in Tables 1 and 2. Although a slight discrepancy was found in case 1 in Table 2, they show that the optimal order n of the approximation curve agrees well with each theoretically predicted order in Equations (6)–(20).

It is noteworthy that Equations (10), (11), and (14) predicted no x-position dependence of blood cell velocity. In the case of parallel electrodes of the case 2, the electric field is constant along the x-axis and independent of the x position. For the case 3 electrode, the slope of the field-squared value is constant and independent of the x position. Measurement results for these cases are shown in Figures 7 and 8. The results show good agreement with the theoretical predictions.

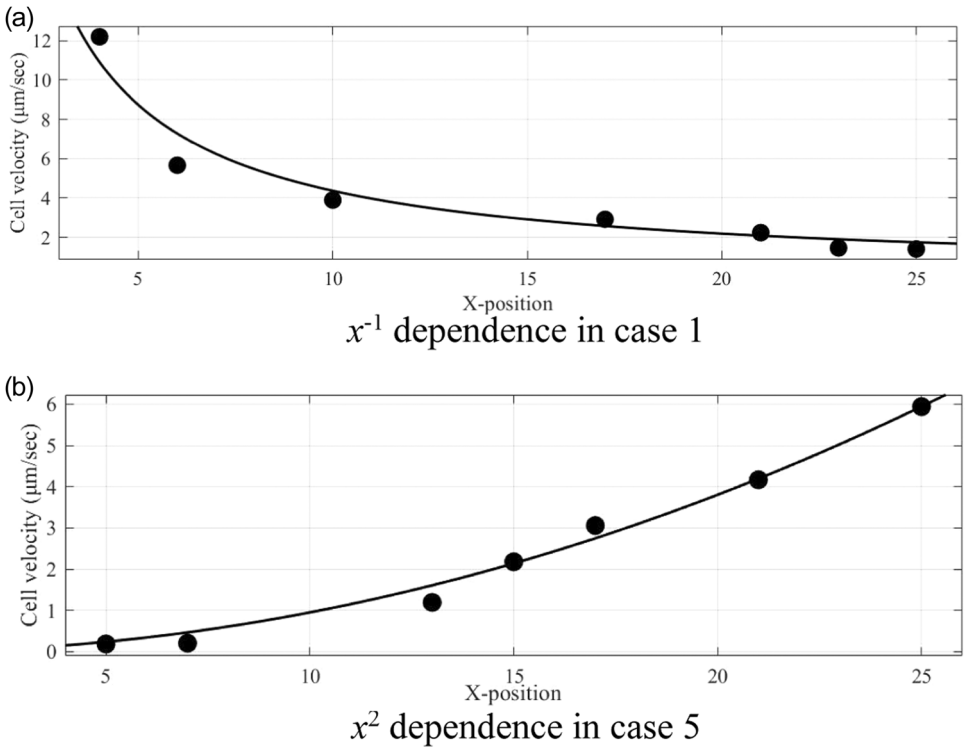

FIGURE 6 Dependence of red blood cells velocity on x-position: dots and curves indicate measured average velocities and regression lines. (a) X^{-1} dependence in case1 and (b) X^2 dependence in case 5.

TABLE 1 Analysis of determination coefficients for appropriate polynomial degree n to explain the x-position dependence of RBC velocity in DC field exposure: Dark boxes show the orders predicted in theoretical analysis in Section 2.3.

$y = ax^n$										
Case	**−4**	**−3**	**−2**	**−1**	**0**	**1/2**	**1**	**2**	**3**	**4**
1	0.539	0.665	0.839	**0.943**	—	−0.637	−0.990	−1.236	−1.306	−1.336
3	−8.724	−8.636	−8.249	−5.977	—	**0.955**	0.787	−0.324	−1.499	−2.498
4	−3.605	−3.502	−3.172	−2.065	—	0.698	**0.971**	0.849	0.492	0.143
5	−1.493	−1.486	−1.439	−1.102	—	0.506	0.803	**0.988**	0.922	0.776

Note: Bold characters show maximum values in the row.

TABLE 2 Analysis of determination coefficients for appropriate polynomial degree n to explain the x-position dependence of RBC velocity in AC field exposure: Dark boxes show the orders predicted in theoretical analysis in Section 2.3.

$y = ax^n$										
Case	**−4**	**−3**	**−2**	**−1**	**0**	**1/2**	**1**	**2**	**3**	**4**
1	**0.969**	0.961	0.909	0.686	—	−0.257	−0.363	−0.415	−0.425	−0.428
4	−3.460	−3.308	−2.895	−1.810	—	0.682	**0.962**	0.665	0.079	−0.384
5	−0.609	−0.608	−0.603	−0.522	—	0.334	0.584	0.851	**0.915**	0.883

Note: Bold characters show maximum values in the row.

Consequently, as shown in Tables 1 and 2, and in Figures 7 and 8, the measured blood cell velocities were all in good agreement with the x-position dependence predicted from Equations (6)–(20). These results suggest that electrophoresis in a DC electric field and dielectrophoresis in an AC electric field are the main mechanisms of the velocity change in field exposure, even when the electric field distribution varies inside the body.

4 | ANALYSIS OF VELOCITY CHANGE THRESHOLD

Studying the biological effects of electric fields requires clarification of the threshold of the electric field at which effects begin to occur. An earlier report (Kanemaki et al., 2022) described that the RBC velocity changes, and that changes occurred above 0.40 and 2.0 kV/m, respectively, for DC and AC electric fields.

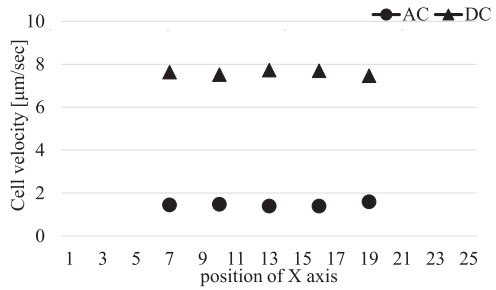

FIGURE 7 Dependence of red blood cell velocity on x-position in direct current and alternating current electric fields with parallel electrodes (case 2). AC, alternating current; DC, direct current.

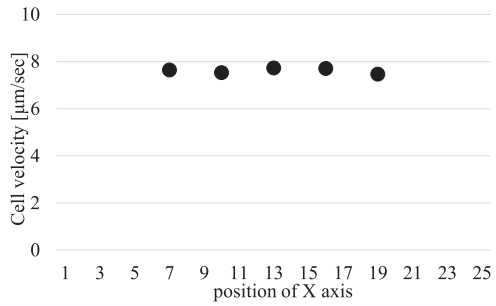

FIGURE 8 Dependence of red blood cell velocity on x-position in alternating current electric field with $x^{-1/2}$ electrodes (case 3).

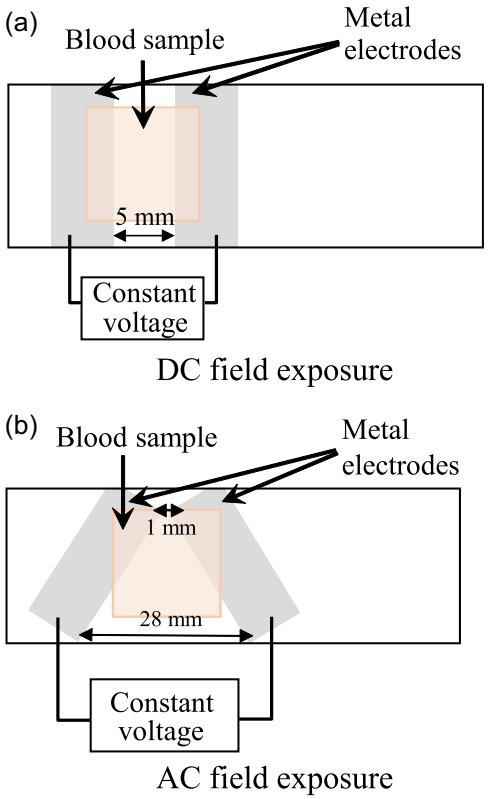

FIGURE 9 Electrode systems for threshold analysis. (a) DC field exposure and (a) DC field exposure. AC, alternating current; DC, direct current.

Nevertheless, the threshold values remain unknown. Therefore, for this study, we attempted to obtain a new threshold for RBC velocity change based on the natural movement of blood cells in the absence of an electric field.

The electrode configuration used for the experiment is shown in Figure 9. To facilitate estimation of the electric field values, parallel electrodes (case 2, Equation (9), Figure 9a) were used for the DC case and uniformly inclined electrodes (case 1, Equation (6), Figure 9b) for the AC case. Experiment conditions were the same as those described in the section *Measurement System*, except that the applied voltage was varied. Blood cell velocities were measured for two different ranges of electric fields to elucidate the wide-range characteristics and to obtain the threshold in detail.

The results for widely various DC and AC fields are shown respectively in Figure 10a,b. They are the average velocity of the arbitrary chosen 10 RBCs with error bars (mean ± half standard deviation, $N = 65$ points in time-series). Error bars show fluctuation of the RBC velocity during the 3 s at the end of each 5 s measurement period. In Figure 10a, there is a slight difference in RBC velocity when the applied voltage is increased or decreased. This difference is not consistent across repeated measurements. It is not regarded as a significant hysteresis.

Results obtained for a narrow range of DC and AC fields are shown respectively in Figure 11a,b. The abscissa is the voltage applied to the electrodes which is converted to electric field by dividing it with the electrode distance. To obtain the threshold, linear regression lines of the measured data were used. At each measured voltage, two regression lines were obtained using the data points less and more than the voltage of interest. In the voltage range with no RBC velocity change, the slope of the regression line was close to zero. Over the threshold voltage, the slope showed nonnegligible increase. The x-coordinate of the intersection of two regression lines which had the essentially zero slope and the significantly increased slope was determined as the threshold voltage. The voltage was converted into the threshold electric field. The thresholds for the onset of RBC velocity change were found to be 0.40 and 1.6 kV/m, respectively, for DC and AC electric field exposures.

5 | FIELD EXPOSURE IN A NARROW CHANNEL BY CAPACITIVE COUPLING

The study described above has shown that the RBC velocity in whole blood is apparently affected by electric field exposure. However, the exposure conditions in experiments were not exactly the same as practical conditions. The blood sample was confined in the space with 0.020 mm vertical thickness, which is of the same order as the blood capillary diameter. However, the sample was spread horizontally to approximately 10 mm size. In addition, electric fields were generated by application of voltage using a pair of metal electrodes that were in direct contact with the whole blood. In cases of practical exposure to electric

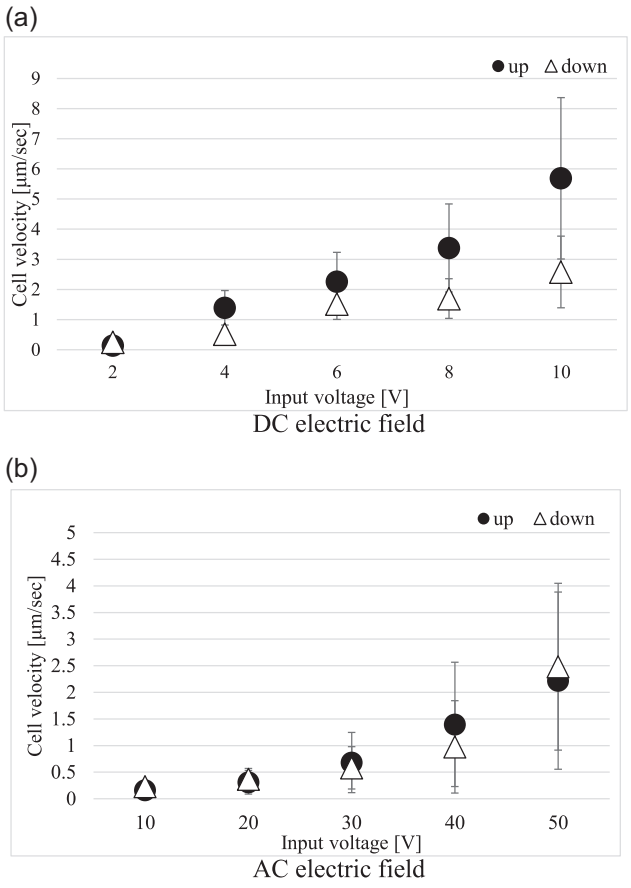

FIGURE 10 Dependence of red blood cell velocity on a wide-range of applied electric fields: Voltage was varied upward and downward. (a) DC electric field and (b) AC electric field. AC, alternating current; DC, direct current.

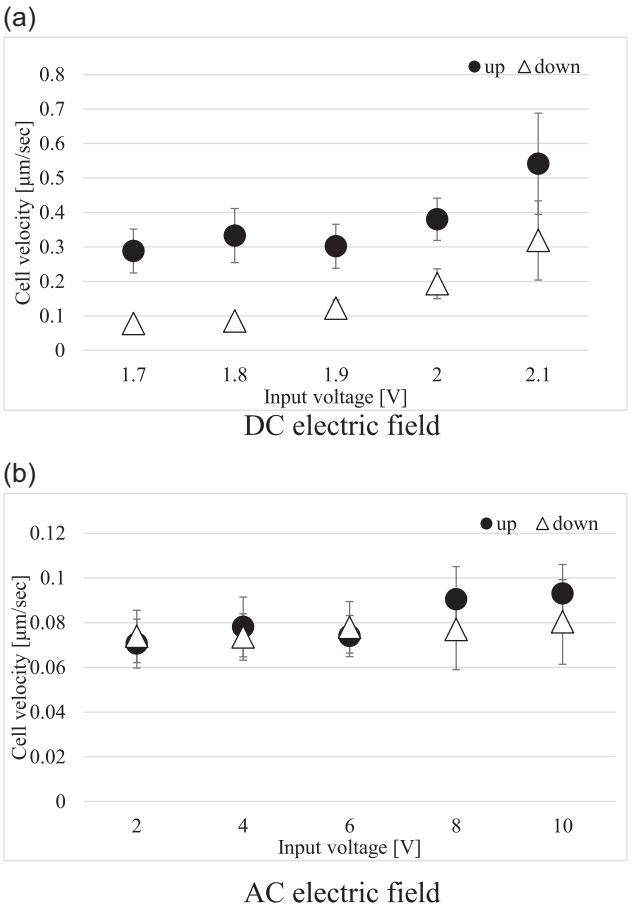

FIGURE 11 Dependence of red blood cell velocity around threshold of field effect: Voltage was varied upward and downward. (a) DC electric field and (b) AC electric field. AC, alternating current; DC, direct current.

fields under power lines or in electric field therapy equipment, capacitive coupling with an air layer or insulators between the electrode and a living body is more common. Therefore, we attempted to confirm observation of the same effects in the more realistic conditions, or in whole blood confined in a narrow channel when exposed to a capacitively coupled electric field generated through air gaps.

Figure 12 shows the outline of sample preparation. For this experiment, the blood was confined in a narrow channel with capillary-size diameter or approximately 0.020 mm. The pull-up method in thin-film etching was applied. To simulate the tissue around the capillary, we used a slime gel ($Na_2[B_4O_5(OH)_4]$ •$8H_2O$) as a substrate material. A 0.020-mm-diameter metal wire was placed on a slide glass and was covered by the slime gel. After the gel was solidified at room temperature, the wire was pulled up, leaving a narrow channel in the slime. To investigate the RBC movement in x and y directions, a cross-pattern-channel was made in the center area of the sample. The four ends of the channels were open to the air to certify free movement of RBCs in the channels. An air layer was secured between the metal electrodes and the slime substrate to ensure that the electrodes did not contact with the blood during the experiment.

The time course of the measurement is presented in Figure 13. The first 10 s period well before the field

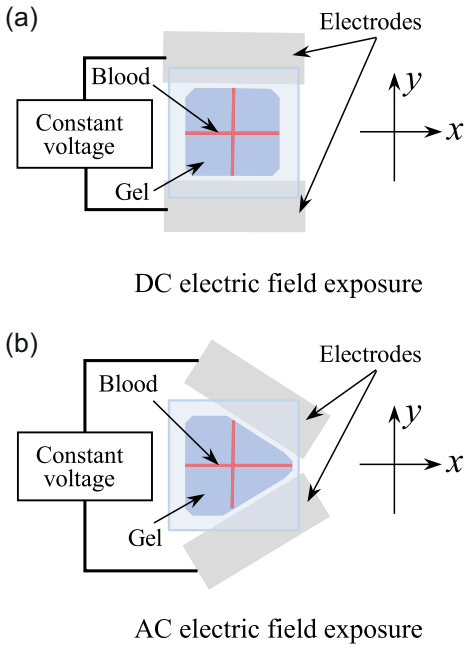

FIGURE 12 Sample preparation of blood in narrow channel exposed to capacitively coupled electric field. (a) DC electric field exposure and (b) AC electric field exposure. AC, alternating current; DC, direct current.

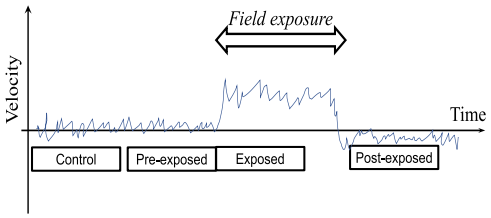

FIGURE 13 Time course of experiment for red blood cell velocity change in a capillary-size channel in field exposure with capacitive coupling.

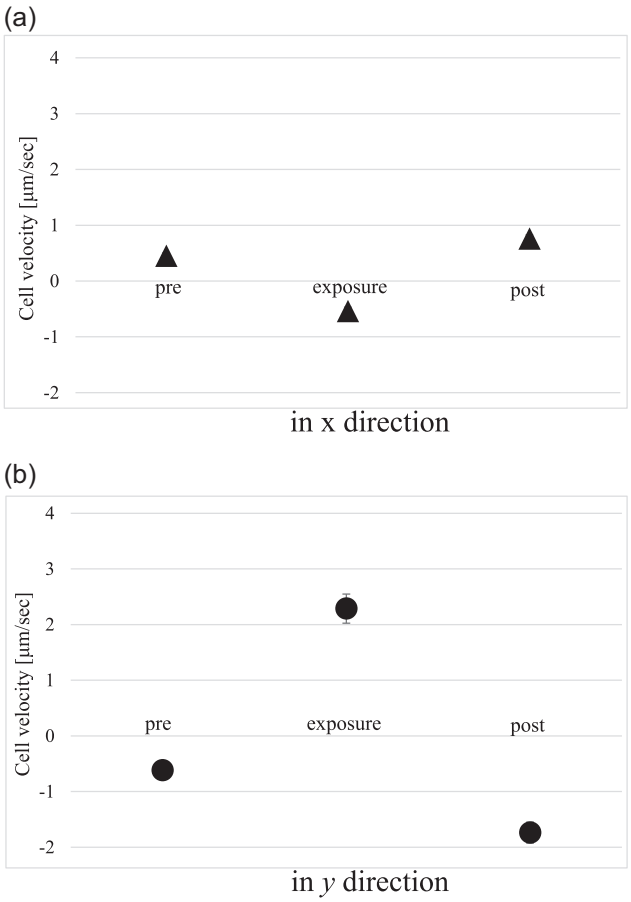

FIGURE 14 Effects of direct current electric field generated by capacitive coupling on red blood cell velocity in a capillary size channel.

exposure was designated as a control period during which the stability of measurement was confirmed. The 10 s periods before and after the start of the field exposure were designated respectively as pre-exposure and exposure periods. Another 10 s period after the end of the field exposure was designated as a postexposure period. During the exposure period, we applied voltages 500 and 1000 V in the DC and AC cases, respectively. In each period, the RBC velocity was evaluated at every second by tracing the position of arbitrarily chosen 10 RBCs in video images. The velocities averaged over the 10 RBCs are calculated and recorded as a time-series of each second.

Figures 14 and 15 present changes in blood cell velocity in the narrow channel in the field exposure made with the electrodes through the air gap. They are

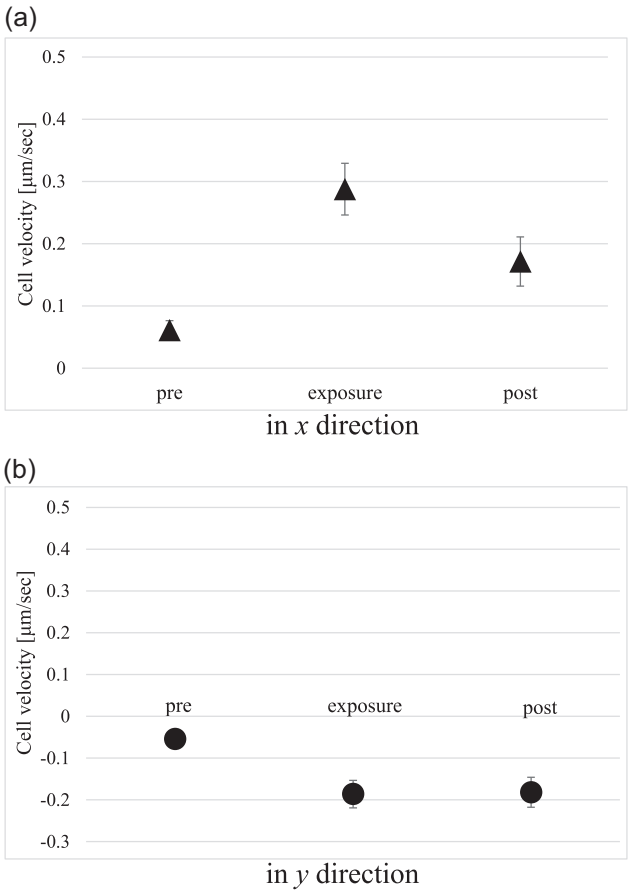

FIGURE 15 Effects of alternating current electric field generated by capacitive coupling on red blood cell velocity in a capillary size channel: (a) on x axis and (b) on y axis.

the average velocity of the arbitrary chosen 10 RBCs with error bars (mean ± half standard deviation, $N = 40$ points in time-series). Error bars show fluctuation of the RBC velocity during the 3 s at the end of each 10 s period specified in Figure 13. In the case of DC field exposure, blood cell velocity in the y direction increased significantly during the exposure, but almost no change was observed in the x direction. In the case of AC field exposure, we could not see as large a change as the DC field exposure. However, the result in Figure 15 showed the statistically significant difference in RBC velocity in x direction before and after the exposure. The difference was apparently larger in x direction than that in y direction. These results demonstrate that the RBC velocity is affected by electrophoresis and dielectrophoresis even in the capillary-size channel and during field exposure by capacitive coupling.

6 | CONCLUSIONS

Many studies have been conducted to elucidate the effects of electromagnetic fields on a human body. International guidelines for safety have been established. Nevertheless, some mechanisms of their effects remain unclear. Among these, we have particularly examined the effects of ELF electric fields, including those of DC field, on blood cell movement (Kanemaki

et al., 2022). For this study, we devised an experiment system for measuring and analyzing blood cell velocity in whole blood exposed to an electric field. This system revealed that electric fields affect the migration velocity of RBCs, even in undiluted whole blood. As described herein, the following points were clarified based on quantitative analysis.

(1) When blood is exposed to a uniform electric field, the main causes of blood cell movement are presumed to be electrophoresis and dielectrophoresis (Kanemaki et al., 2022). An animal body has an irregular external shape, and its internal electrical characteristics are not uniform. Therefore, even if the external electric field is spatially uniform, the electric field inside the body is non-uniform. For that reason, we investigated whether the principle of electrophoresis for DC field exposure and that of dielectrophoresis for AC field exposure can explain the blood cell movement, even in cases of a non-uniform electric field.

    Five different electrode shapes were used to construct inhomogeneous electric field distributions of various types. The RBC velocities in these electric fields were analyzed. First, the spatial dependence of RBC velocity was derived theoretically for each of five nonuniform field distributions. The RBC velocities measured using the developed system showed good agreement with the theoretical predictions. The analysis revealed that the RBC velocity in whole blood is affected even by a non-uniform electric field. It was also confirmed that the main mechanism of this effect is explainable as electrophoresis in DC field exposure and as dielectrophoresis in AC field exposure.

(2) The threshold for the above phenomena, i.e., the minimum electric field value at which the migration velocity of RBCs in whole blood begins to be affected, was investigated. Quantitative analysis clarified that the effect appears as small as 0.40and 1.6 kV/m respectively in DC and AC field exposures. The threshold value for the appearance of these effects is 10^3–10^5 times higher than the biological safety guideline values 0.02–0.8 V/m for basic restrictions for the public as stipulated in ICNIRP guidelines (ICNIRP, 2010).

    Compared to the guideline values for human safety, the results of this study are unlikely to raise new questions about the safety of long-term exposure and other issues related to exposure in general living spaces. Nevertheless, the threshold needs to be identified to prepare for future hazards. Electric fields can reach extreme values at some points depending on the shape of the object. Furthermore, occupational exposure to high electric field is also conceivable in factories using high-power electrical equipment and in medical institutions using high-voltage diagnostic and therapeutic instruments.

(3) In the previously reported experiments (Kanemaki et al., 2022), the blood sample was confined in a thin layer with 0.020 mm vertical thickness, but it was spread over an approximately 100 mm^2 horizontal area. To simulate the blood capillary condition better, we made a small channel of approx. 0.020 mm diameter. Then the blood sample was contained in the channel. In addition, to clarify the exposed electric field intensity, we have used electric fields generated with metal electrodes that were in direct contact with blood. However, in circumstances of practical field exposure such as under high-voltage power transmission lines or in electric field therapy equipment, electric fields are generally exposed to a human body by capacitive coupling through an air gap. Therefore, we created an air layer between an electrode and blood and investigated the movement of blood cells in this capacitively coupled electric field. The experimentally obtained results showed similar RBC velocity changes under practical field exposure conditions. These findings demonstrate that even a general capacitively coupled electric field can affect the RBC velocity in whole blood inside a blood capillary.

In conclusion, this study clarified the possibility, the principle, and the level of influence on the RBC velocity during electric field exposure. As described in Chapter 1, extensive research has been conducted on the electrophoresis of RBCs. Through these studies, it appears that changes in velocity are unlikely to impact the physiological state of RBCs themselves, but rather may affect their functions, such as the efficiency of oxygen exchange with surrounding cells in vivo. The physiological effects of this phenomena remain a subject for future investigation. In this study, we assumed an environment with very low blood flow velocity, such as in capillaries. If we seek to understand the effects on the blood flowing in arteries and veins, further experiments are imperative. Moreover, to assess the biological effects of field exposure, experiments involving living animals are ultimately necessary. For instance, observations of blood cell movement can be conducted using rabbit ears and rat mesentery, while the animals are kept alive. With the threshold in mind, these study results might open up possibilities for new biomedical applications such as local blood flow control using local inhomogeneous electric fields and their use for intracorporeal hemodialysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This research was conducted in strict adherence to ethical principles and guidelines. The study protocol

underwent a thorough review and received approval from the Ethics Committee of Hokkaido University of Science, ensuring that all experimental procedures were carried out responsibly and ethically.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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