Dielectric Measurement of *Euglena gracilis* as a Multi-parametric Approach for Non-invasive Biomonitoring of Aquatic Environment

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Abstract— *Dielectric spectroscopy was employed for monitoring biophysical parameters of Euglena gracilis in suspension to assess a possibility of using this method for a new biomonitoring system for detecting and identifying pollutants in aquatic environments. E. gracilis was subjected to different types of membrane-affecting toxic chemicals (1 mM chlorpromazine, 1 mM $HgCl_2$, or 1 mM Triton X-100), and dielectric measurement of the cell suspension was carried out over a wide frequency range between 5 kHz and 3 MHz. All of these chemicals at the designated concentrations induced similar changes in cell motility of Euglena cells; flagellar activity was inhibited and rounding-up movement of the cell body was induced. These chemicals also induced distinct changes in dielectric properties of the cell suspension, but the manner of changes in dielectric behavior was unique to individual chemical species, suggesting a possible use of this technique for quick identification of toxic materials in aquatic environments.*

Keywords— Biomonitoring, Dielectric spectroscopy, Impedance.

I. INTRODUCTION

Identification of toxic substances in various samples, such as drinking water and food specimens, is exceedingly important, but analytical methods by using chemical procedures require time-consuming and labor-intensive sample preparations [1]. Therefore, biomonitoring approaches have been developed to rapidly identify toxicity using living organisms such as microorganisms [2], animals [3, 4], unicellular algae [5], and protists [6] as biomonitors. Although biomonitoring allows evaluation of cumulative effects of various contaminants, it is not possible to identify and name the chemical substances contained in the polluted water. Tahedl and Häder [7, 8] reported an approach for monitoring water quality by using the motile unicellular flagellate *Euglena gracilis* as a monitoring organism. They developed an elaborate system to determine six different movement parameters including motility, swimming velocity, and gravitactic orientation, and showed that different chemicals affect different parameters [7]. *E. gracilis* is suitable as a biomoitor organism, since 1) methods for axenic mass cultivation has been established [9], 2) free-swimming motility ensures a long-lasting homogeneous cell suspension that is required for stable measurement, and 3) the cells have a uniform and symmetrical morphology that changes in response to various environmental factors [10].

Dielectric spectroscopy is a non-invasive technique by which multiple electrical and morphological parameters of the living cells in suspension can be obtained over a wide frequency range [11, 12]. In this paper, we have applied this method to *E. gracilis*, as an attempt to evaluate the possibility that different multiple parameters obtained by this technique might be useful to quickly unveil the chemical composition of unknown water samples.

II. MATERIAL AND METHOD

2.1 Cells

Euglena gracilis SM-ZK strain was obtained through the courtesy of Professor Y. Nakano of Osaka Prefecture University and cultured at 25°C for 7 days. SM-ZK is a streptomycin-bleached mutant strain derived from *Euglena gracilis* Z, which is permanently deprived of chloroplasts [13]. This mutant strain was used in the present study, since simpler intracellular organization may allow better theoretical consideration for the dielectric data analysis [14, 15, 16]. The culture medium consisted of 0.1% sodium acetate trihydrate (Nacalai Tesque, Japan), 0.1% polypeptone (Nihon Pharmaceutical, Japan), 0.2% tryptone (Nacalai Tesque, Japan), 0.2% yeast extract (Bacto, USA) and 0.001% calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, Nacalai Tesque, Japan). Cells were efficiently concentrated with two layers of membrane filters, one was an 11-μm nylon mesh filter and the other was a hydrophilic PTFE filter with 10 μm pores (Omnipore, Ireland) placed on the

bottom. Cells accumulated on top of the filters were collected and used for experiments after the cell density was adjusted to 5×10^5 cells/ml.

2.2 Chemical fixation and electron microscopic observation

After treated with chlorpromazine (1 mM), $HgCl_2$ (1 mM), or Triton X-100 (1 mM) for 5 min, *E. gracilis* cells were chemically fixed with glutaraldehyde and osmium tetroxide [17], dehydrated through an ethanol series, and embedded in Spurr's resin. Ultrathin sections were made using an ultramicrotome (Leica EM UC7), stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (Hitachi H-7100).

2.3 Dielectric measurement

The dielectric measurement system was composed of a measuring chamber and an impedance analyzer (Fig. 1). The measuring chamber was of a parallel-plate capacitor type, made from an acrylic glass tube (7.5 and 12.5 mm in inner and outer diameters, respectively), and a pair of Pt disks as parallel electrodes (diameter, 10 mm; thickness, 0.1 mm; cell constant, 5.1×10^{-14} F; placed 8.4 mm apart,) coated with Pt-black for reducing the electrode polarization [18,19,20]. The dielectric response was measured with an impedance analyzer (IM3570, HIOKI E. E. Co. Ltd, Japan, operated at 1 V) in the frequency range between 1 kHz and 5 MHz at 155 frequency points. Measurements were carried out at 25±1 °C, and a single series of measurements took about 50 sec. Data obtained between 5 kHz and 3 MHz were used for analysis. To avoid uneven distribution of the *Euglena* cells in suspension, that is occasionally observed as the result of bioconvection phenomenon, we designed a perfusion system with a peristaltic pump (AC-2120, ATTO, Japan), in which 3-ml cell suspension was circulated continuously through the measuring chamber at a rate of 4.2 ml/min.

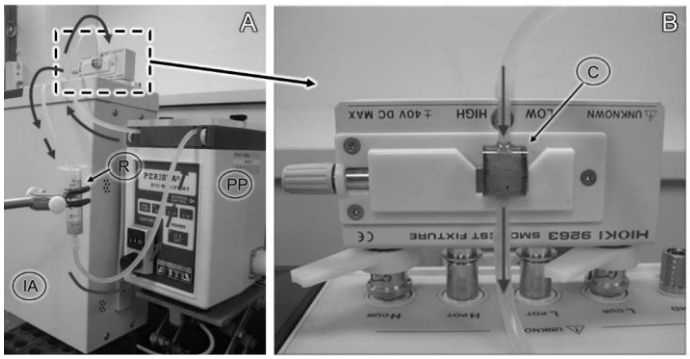

FIG. 1. A MICROGRAPH OF THE DIELECTRIC MEASUREMENT SYSTEM (A) AND AN ENLARGED PICTURE OF THE MEASURING CHAMBER ON A FIXTURE CONNECTED TO THE IMPEDANCE ANALYZER (B). THE SYSTEM WAS COMPOSED OF A PARALLEL-PLATE CAPACITOR TYPE MEASURING CHAMBER (C) CONNECTED TO AN IMPEDANCE ANALYZER (IA) AND A PERFUSION SYSTEM WITH A PERISTALTIC PUMP (PP). THE SAMPLE CELL SUSPENSION WAS CIRCULATED CONTINUOUSLY THROUGH THE MEASURING CHAMBER IN A DIRECTION SHOWN WITH ARROWS. A RESERVOIR (R) WAS PLACED IN THE FLOW PASSAGE, TO WHICH TEST CHEMICALS WERE INTRODUCED.

III. RESULTS

3.1 Effects of toxic chemicals on the dielectric behavior

Three different membrane-affecting chemicals were used to compare their effects on the dielectric behavior of *E. gracilis* cells in suspension. As shown in Table 1 and Fig. 2, all chemicals showed inhibitory effects on cell swimming, and rounding-up cell shape changes were induced. Each chemical, however, showed different effects on both relative permittivity and conductivity of the cell suspension as shown in Fig. 3. Chlorpromazine reduced the permittivity at around 100 kHz range (Fig. 3A). On the contrary, conductivity of the cell suspension was increased over the whole range of frequencies, especially in the lower frequencies (Fig. 3A'). $HgCl_2$ induced only a slight elevation in permittivity (Fig. 3B), while it strongly suppressed the conductivity of the cell suspension in the whole frequency range (Fig. 3B'). The effect of Triton X-100 was different from either that of chlorpromazine or $HgCl_2$. It reduced permittivity of the cell suspension as in the chlorpromazine-treated cells (Fig. 3C), but the conductivity decreased in the whole range, especially in higher frequencies (Fig. 3C'). The results were summarized in Table 1, showing that different chemicals provoked different effects on the dielectric behavior of cell suspension, even though morphological and motility changes were similar.

TABLE 1
EFFECTS OF TOXIC SUBSTANCES ON CELLULAR MORPHOLOGY, MOTILITY, AND DIELECTRIC PARAMETERS OF *EUGLENA GRACILIS* IN SUSPENSION.

	Chlorpromazine (1 mM)	$HgCl_2$ (1 mM)	Triton X-100 (1 mM)
Cell shape	partially rounded	rounded	partially rounded
Plasma membrane	intact	intact	intact
Mitochondria	swelling and fusion	intact	membrane disruption
Swimming	inhibited	inhibited	inhibited
$\varepsilon^{1)}$	decreased	slightly increased	decreased
$\kappa^{2)}$	increased	decreased	decreased

1) Relative permittivity of cell suspension. 2) Conductivity of cell suspension.

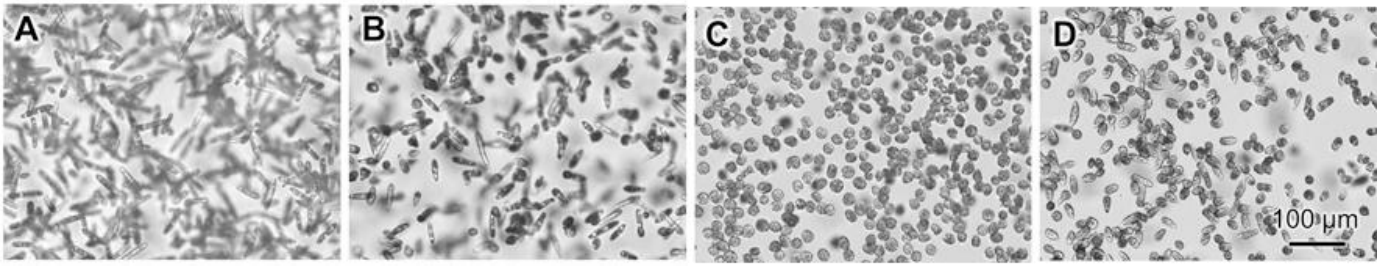

FIG. 2. LIGHT MICROGRAPHS OF *EUGLENA GRACILIS* SHOWING CONTROL CELLS (A), CELLS TREATED WITH 1 MM CHLORPROMAZINE (B), 1 MM $HgCl_2$ (C) , AND 1 MM TRITON X-100 (D). SWIMMING MOTIONS OF THE CELLS GRADUALLY CEASED BY THE ADDITIONS OF THESE CHEMICALS, AND THE CELLS BECAME ROUNDED-UP COMPLETELY BY $HgCl_2$, OR PARTIALLY ROUNDED BY EITHER CHLORPROMAZINE OR TRITON X-100. PICTURES IN B-D WERE TAKEN 5 MIN AFTER THE ADDITION OF THE CHEMICALS.

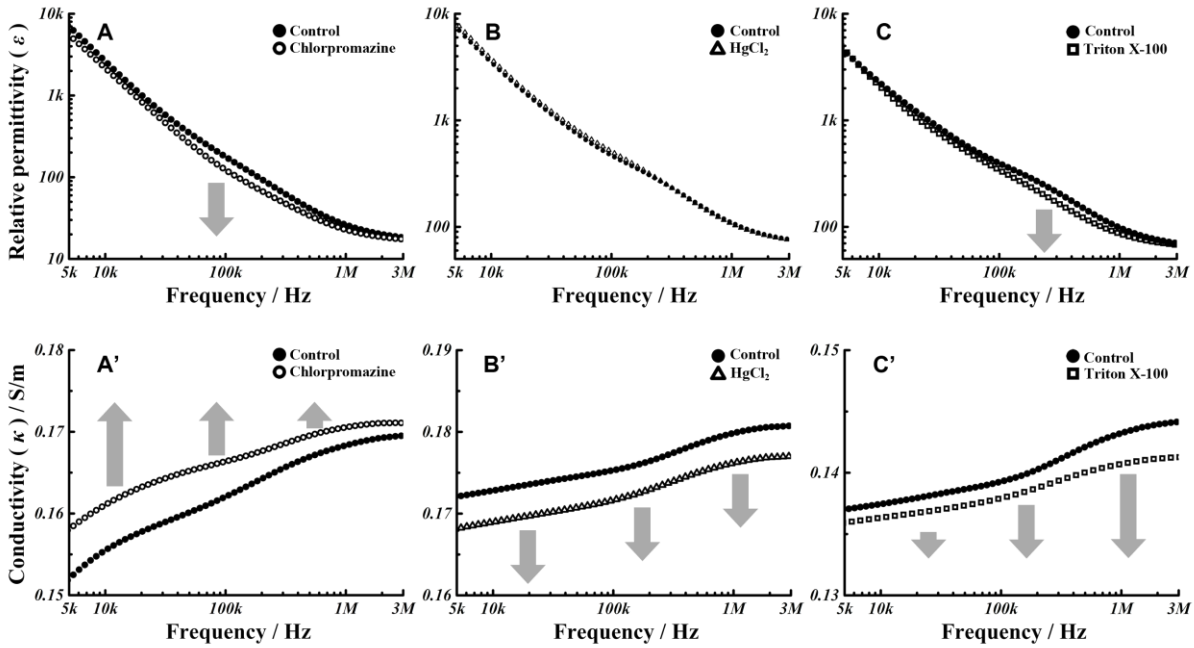

FIG. 3. FREQUENCY DEPENDENCE OF RELATIVE PERMITTIVITY *E* (A-C) AND CONDUCTIVITY *K* (A'-C') OF *E. GRACILIS* IN SUSPENSION. CONTROL CELLS (CLOSED SYMBOLS) AND THOSE MEASURED ABOUT 5 MIN AFTER THE ADDITION OF THE CHEMICALS (A/A'; CHLORPROMAZINE, B/B'; $HgCl_2$, C/C'; TRITON X-100) AT THE FINAL CONCENTRATION OF 1 MM (OPEN SYMBOLS) WERE SHOWN. ARROWS INDICATE THE CHANGING DEGREE AND DIRECTION INDUCED BY THE ADDITION OF THE CHEMICALS.

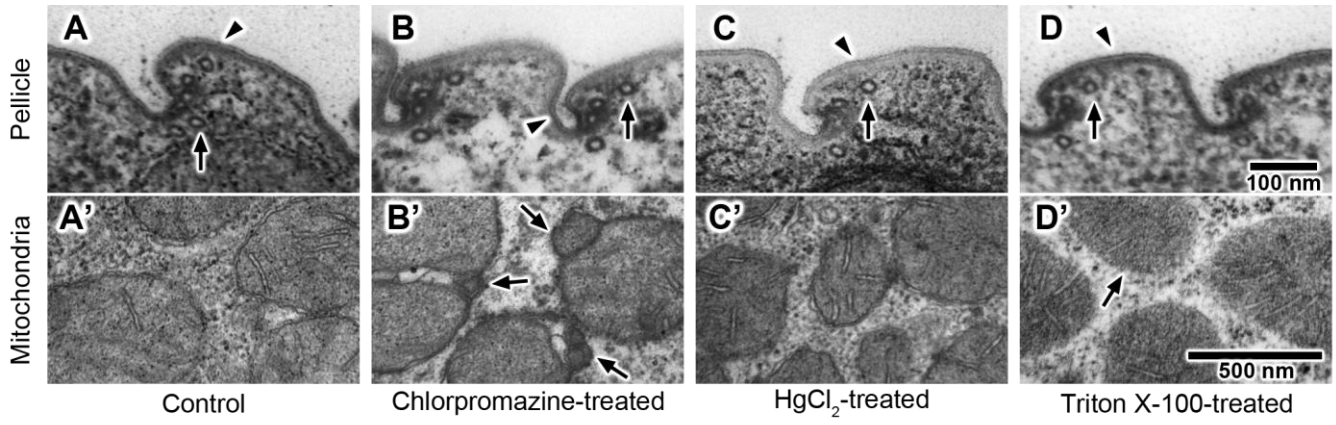

FIG. 4. ELECTRON MICROSCOPE IMAGES OF THE PELLICULAR STRUCTURES OF *E. GRACILIS*. A: CONTROL, B: CHLORPROMAZINE-TREATED, C: $HGCL_2$-TREATED, D: TRITON X-100-TREATED. IN ALL OF THE DRUG-TREATED SPECIMENS, NO DAMAGES WERE DETECTED IN THE PELLICULAR STRUCTURES (B-D). THE PLASMA MEMBRANES REMAINED INTACT (ARROWHEADS IN A-D), AND SUBPELLICULAR MICROTUBULES (ARROWS IN A-D) WERE ALSO UNAFFECTED. THE ULTRASTRUCTURE OF MITOCHONDRIA WAS ALTERED IN CHLORPROMAZINE-TREATED CELLS, WITH FORMATION OF SEPTA AND SMALL BLEBS (ARROWS IN B'). MITOCHONDRIA IN $HGCL_2$-TREATED CELLS WERE NOT ALTERED. IN TRITON X-100-TREATED CELLS, MITOCHONDRIAL MEMBRANES DISAPPEARED (ARROW IN D').

3.2 Electron microscopy observation

Transmission electron microscopy observation was carried out to investigate intracellular morphology of *E. gracilis* after treatment with the membrane-affecting chemicals. As compared with the control cells (Fig. 4A), no obvious damages were observed in the pellicular structures of the drug-treated cells, and the plasma membranes remained intact in all cases (Figs. 4B-D; arrowheads show the unit membrane structure of the plasma membrane). Large deformation of mitochondria was detected in the chlorpromazine-treated cells (Fig. 4B') with frequent fusion and formation of small blebs on the mitochondrial surface (arrows in Fig. 4B'). Intracellular organelles looked intact in $HgCl_2$-treated cells (Fig. 4C'). In the Triton X-100-treated cells, mitochondrial membranes were completely missing (Fig. 4D').

IV. DISCUSSION

Dielectric spectroscopy is a non-invasive technology that provides a rapid and useful way of biological research to analyze electrical properties of living cell suspensions [11, 12] and tissues [21, 22, 23], and also to monitor the growth of cultured cells [24]. In this study, we observed the dielectric behavior of *E. gracilis* in suspension in the range of frequency between 1 kHz and 5 MHz for detecting possible changes due to the presence of toxic chemical compounds in the surrounding medium. Characteristic changes in the dielectric behavior were induced by chlorpromazine, $HgCl_2$, and Triton X-100, which indicate that the toxic effects of different chemicals might have caused damages on different dielectric compartments of the cell.

Increase in conductivity by chlorpromazine may be explained by the raise in conductivity of the extracellular medium by a possible leaking of intracellular electrolytes toward outside. Chlorpromazine is a membrane-affecting positively-charged molecule that has an effect to enhance membrane permeability [25] and various electrolytes [26, 27]. It showed a deteriorating effect on the mitochondrial structure and increase in conductivity of the cell suspension of *E. gracilis*, which is probably ascribed to the enhanced ionic conduction of electrolytes across the damaged mitochondrial membranes.

The $HgCl_2$-treated cells showed no obvious damages on the cellular ultrastructure. However, it showed decrease in conductivity of the cell suspension as shown in Fig. 2B'. The reason for this phenomenon is not clear, and more detailed theoretical consideration is needed [14, 15]. The main cellular target of inorganic mercury is regarded as the plasma membrane, by influencing transport of water and electrolytes [28, 29]. The decrease in conductivity by the treatment with $HgCl_2$ may be the result of decrease in the medium conductivity, and an alternative explanation may be the possible increase in the volume fraction of the cells with concomitant decrease in the cytoplasmic conductivity. At this moment, it is difficult to distinguish these two possible mechanisms, and strong inhibitory effect of mercury to various cellular enzymes should also be considered [28].

Triton X-100 is a nonionic detergent that is probably the most commonly used permeabilization agent for biological researches. It has a strong effect on the living cell membranes by destructing the compactness and integrity of the lipid membranes [30]. The decrease in conductivity of the cell suspension as shown in Fig. 5C', especially notable in the high frequency range, may be explained by a decrease of the extracellular conductivity with possible concomitant decrease in the intracellular conductivity.

V. CONCLUSION

Biomonitoring of water quality is essential for the safe supply of drinking water, which offers many advantages over the classical physico-chemical methods of analyses. They usually involve simple observations, and requires only short time, small money and space. However, biomonitoring also has disadvantages. They are not able to provide an exact figure of water quality parameters, and cannot pinpoint the exact cause of water quality problems. It is principally because biomonitoring techniques usually employ only a single parameter (swimming behavior, electromyographic response, etc.), although chemical nature of toxicants is highly complicated. In the present paper, we found different dielectric responses that are characteristic to three different toxic chemicals, even though they showed similar morphological and behavioral responses to *E. gracilis*. If we can make good use of a set of multiple dielectric parameters extracted from the wide frequency-range data, it should provide us with a good starting point to identify individual toxicants contained in polluted water samples.

ACKNOWLEDGEMENTS

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