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A comparative study of cell death using electrical capacitance measurements and dielectrophoresis

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A B S T R A C T

The changes in the dielectric properties of cells that occur during their exposure to various lethal environmental stresses were measured using both dielectric spectroscopy and dielectrophoresis. It is shown that the dielectric properties of both dying and dead yeast cells were strongly dependent on the method used to induce cell death. Methods which directly affected the membrane permeability, and consequently the membrane conductivity and internal conductivity, resulted in large changes in the suspension capacitance and dielectrophoretic behaviour, whilst methods which affected the cell interior but had little effect on the cell membrane resulted in few or no changes in the dielectric properties of the cells. The findings indicate that, depending on the method by which cell death is induced, dielectric spectroscopy may not always be able to observe differences between viable and non-viable cells, and that dielectrophoresis will not always be able to separate viable from non-viable cells.

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1. Introduction

Cell death is accompanied by many changes in the cell [1–3]. Many of these changes can affect the electrical properties of the structures of which a biological cell is composed, which in turn lead to changes in the frequency-dependent dielectric properties of the cells [4–8]. A review of cell death, the changes that occur in the dielectric properties during cell death by different environmental stresses, and different methods to measure them, has been given elsewhere [1]. Of the techniques that are available, dielectric spectroscopy measurements on suspensions are somewhat less sensitive compared to electrokinetic techniques such as dielectrophoresis or electro-rotation [9]. However, the method is very simple, and – unlike electrokinetic measurements – gives an online and real-time signal which can be obtained directly in growth medium without risk of contamination. For electrokinetic experiments, in contrast, the cells usually have to be resuspended in iso-osmotic sugar solutions, and measurement of spectra can be time-consuming.

Dielectric spectroscopy, and in particular capacitance measurements, has found practical and commercial use in the on-line and real-time measurement of “viable” biomass [10–12]. However, to date there has been no systematic investigation of what is meant by “viable” biomass in dielectrics, even for the baker’s yeast Saccharomyces cerevisiae that is most commonly studied. Dielectrophoresis, the movement of particles in non-uniform electric fields, on the other hand has been used to separate “viable” from “non-viable” cells, including cells of baker’s yeast Saccharomyces cerevisiae that is most commonly studied. Dielectrophoresis, the movement of particles in non-uniform electric fields, on the other hand has been used to separate “viable” from “non-viable” cells, including cells of baker’s yeast Saccharomyces cerevisiae that is most commonly studied. Dielectrophoresis, the movement of particles in non-uniform electric fields, on the other hand has been used to separate “viable” from “non-viable” cells, including cells of baker’s yeast Saccharomyces cerevisiae that is most commonly studied. Dielectrophoresis, the movement of particles in non-uniform electric fields, on the other hand has been used to separate “viable” from “non-viable” cells, including cells of baker’s yeast Saccharomyces cerevisiae that is most commonly studied. Dielectrophoresis, the movement of particles in non-uniform electric fields, on the other hand has been used to separate “viable” from “non-viable” cells, including cells of baker’s yeast Saccharomyces cerevisiae that is most commonly studied. Dielectrophoresis, the movement of particles in non-uniform electric fields, on the other hand has been used to separate “viable” from “non-viable” cells, including cells of baker’s yeast Saccharomyces cerevisiae that is most commonly studied.

2. Materials and methods

2.1. Cells

Fresh baker’s yeast (S. cerevisiae) was purchased every week from St. Andrews Bakery in Manchester. The yeast was stored at 4 °C and used within 1 week. The number of live cells (as determined using methylene blue staining) was over 99% cells at the start of each experiment.

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2.2. Chemicals

The chemicals to which the cells were exposed in the experiments investigating the effect of toxic chemicals were chosen on the basis of differences in their action against cells. Iso-octanol was chosen because it is well known that long-chain aliphatic alcohols affect the dielectric properties of cells through a solvent-like effect [16–18]. Glutaraldehyde was chosen because of its ability to crosslink membrane components. Cycloheximide was chosen on the basis that it is an inhibitor of protein synthesis and its effect is mainly intracellular [19]. Finally, Virkon, a registered trademark of Dupont, was chosen because it has a double action: it oxidises the cell contents, and also has a detergent action. All chemicals were supplied by Sigma–Aldrich, and of the highest purity.

2.3. Capacitance measurements

An Aber Instruments 220 Biomass Monitor was used to measure the dielectric properties (capacitance and conductivity) of the suspension. For yeast, a capacitance signal of 1 pF corresponds to approximately $10^7$ cells ml$^{-1}$ (2.5 g fresh yeast l$^{-1}$). In the experiment investigating cell death by heat a 19 mm diameter annular probe was used; in all other experiments a 25 mm diameter four-pin probe was used. Both systems rely on a 4-electrode system to reduce electrode polarisation effects [20,21]. The annular probe is a new design which has a higher signal-to-noise ratio than the standard four-pin probe and is easier to handle in an industrial environment [22]. A magnetic stirrer was used to keep the cell suspension homogeneous. Data were obtained from the Biomass Monitor using a PC with Labview.

In the experiments in which cell death by heat was studied 100 g baker’s yeast was suspended in 100 ml of deionised water, and the volume was then adjusted to $6 	imes 10^9$ cells ml$^{-1}$ at 400 kHz) to make 120 ml of cell suspension. 300 ml 30 mM KCI solution (conductivity 5 mS cm$^{-1}$ at 400 kHz) was preheated to the desired temperature using a magnetic stirring heater, and 25 ml of prepared yeast suspension quickly added whilst continuously measuring the suspension capacitance. Capacitance measurements were performed at a single frequency of 400 kHz without filtering or automatic compensation for electrode polarisation. 400 kHz is on the low-frequency plateau, but high enough not to be significantly influenced by electrode polarisation.

In the experiments in which cell death by toxic agents was studied 75 g baker’s yeast was suspended in 100 ml of deionised water, and the volume was then adjusted to 200 ml. Following this, the conductivity of the suspension was adjusted to around 5 mS cm$^{-1}$ (at 400 kHz) by the addition of potassium chloride. Following this, the desired amount of the toxic agent was added to the suspension, and the dielectric properties of the yeast suspension were monitored in time. Frequency spectra in the range 100 kHz to 20 MHz were acquired for 60 s, averaged and stored in the computer. A typical spectrum, with 25 logarithmically spaced datapoints, took 11 s to acquire. When the experiment had finished the difference between the capacitance at 370 kHz and 10 MHz was determined as a function of time. Again, no filtering was used and no automatic compensation for electrode polarisation.

2.4. Dielectrophoretic measurements

To measure the dielectrophoretic properties of cells dying by heat exposure, 150 g baker’s yeast was suspended in water to make 200 ml of cell suspension. 300 ml of an approximately 30 mM aequous KCl solution (conductivity 5 mS cm$^{-1}$ at 400 kHz) was preheated to the desired temperature using a magnetic stirring heater, and the yeast suspension quickly added whilst continuously measuring the suspension capacitance. Samples were taken from the suspension and mixed with 1.5 ml of deionised water in an eppendorf. This was then centrifuged at 9000 × g for 5 min (Minispin Plus from Eppendorf). After centrifugation the supernatant was discarded and the cells were resuspended in fresh deionised water. This cycle was repeated 5 times to reduce the conductivity of the suspending medium to a value of 0.6 mS cm$^{-1}$, as determined using a Jenway 4010 conductivity meter.

To measure the dielectrophoretic properties of cells dying by exposure to toxic chemicals, samples were taken during the capacitance measurements described previously. The samples were prepared in the same way as described for the cells exposed to high temperatures.

Microelectrodes of the interdigitated alternately castellated design with characteristic size of 50 μm were fabricated using photolithography from indium tin oxide (ITO) coated microscope slides (Delta Technologies, USA), as described by Flores-Rodriguez and Markx [23]. A dilute sample of yeast cells suspended in deionised water was placed on the slide with microelectrodes and covered with a microscope cover slip. A Thurlby-Thandar TG120 function generator was used to apply signals of 0–20 V peak-to-peak ($V_{pp}$) within the frequency range 0.1–20 MHz to the electrodes. The dielectrophoretic behaviour of the cells was monitored at 200× magnification with a microscope (Nikon E800), fitted with a digital camera (Nikon, Coolpix 4500), and the frequency at which the dielectrophoretic behaviour turned from positive to negative determined.

3. Results and discussion

3.1. Capacitance measurements

3.1.1. Effect of thermal stress on the capacitance of suspensions of S. cerevisiae

In the first set of experiments, the effect of temperature on cell death was studied. Fig. 1 shows the change in the capacitance at 400 kHz as a function of time when yeast cells (S. cerevisiae) were subjected to different temperatures. At all temperatures (except 50 °C) the capacitance signal declined and then stabilised at around 6.2 pF cm$^{-1}$. At 60 °C a slow decline is seen at first, followed by a faster decline. A slow decline followed by a faster decline (not shown) in the capacitance was also observed at a temperature of 50 °C around 10 h after the start of the experiment; the capacitance again stabilised at a value of around 6.2 pF cm$^{-1}$. The increase in the decline in the capacitance in time is interesting, and indicates an acceleration in the physical decline of the integrity of the cells’ structures. A potential explanation of the observed behaviour is that at 50–60 °C the time-dependent denaturation of proteins leads to a progressively increasing destabilisation of the cell membrane.

The curves allow one to estimate death rates. The rate of cell death is often assumed to be proportional to the concentration of viable cells [24]. Making the additional assumptions that cell suspensions only consist of medium, dead and live cells, and that the contribution of dead and live cells to the total capacitance is proportional to their concentration, it is straightforward to show that the change in the capacitance during cell death can be described by an exponential decline from a high starting capacitance (at which all cells are alive) to a low plateau (at which all cells are dead). Exponential curves were fitted to the data, and an Arrhenius plot of the death rates is shown in Fig. 2. Arrhenius plots are useful for investigation of temperature effects on chemical reactions, but can also be used to effect to analyse the effect of temperature on more complex biological systems [24]. The data indicate that the mechanism by which the cells are disrupted changes with temperature.

![Fig. 1. Capacitance at 400 kHz of a Saccharomyces cerevisiae suspension as a function of time when subjected to heat at different temperatures.](image-url)
Fig. 2. Arrhenius plot of the death rates of baker’s yeast at different temperatures, as measured using dielectric spectroscopy.

However, the final capacitance values obtained were very similar, indicating that the dielectric properties of heat-killed cells are very similar, irrespective of the temperature at which they were killed.

Although no samples were taken here to determine the cell viability, in previous (unpublished) experiments samples were taken, and the cell viability determined using methylene blue. The experiments showed that the cell viability, as determined using methylene blue staining, dropped very quickly, much quicker than the suspension capacitance. These experiments confirmed previous findings [6] that capacitance measurements are relatively insensitive to changes in membrane permeability, and that very significant injury to the cell membrane is needed before they result in a drop in the capacitance.

3.1.2. Effects of chemical stress on the capacitance of suspensions of S. cerevisiae

In the next set of experiments the exposure of yeast cells to different chemicals was explored. Fig. 3 shows how the capacitance of a yeast suspension changes when subjected to different concentrations of iso-octanol. An initial rise in the capacitance can be seen, followed by a decline. Cells subjected to a lower concentration of iso-octanol (0.5%, 1% and 2%) start dying (lysing) later compared to cells subjected to higher concentrations (3% and 4%). A higher iso-octanol concentration decreases the time taken for cell lysis. The capacitance then stabilizes to a value higher than that of the medium, indicating that the dead cells have a residual capacitance. As the cell membrane ruptures, the cytoplasmic ions are also released into the suspending medium, giving a rise in the medium conductance. This kind of behaviour has previously been reported with n-octanol [6,17], and other solvents such as phenol, benzaldehyde, and others [18,19]. The initial increase has been attributed to the fact that the solvent partitions into the cell plasma membrane and increases its area [25], leading to an increase in \( r \) and/or \( C_m \), and hence \( \Delta C \) [6,17]. After a threshold level of partitioning into the membrane, cell lysis takes place, causing the capacitance to start decreasing [6,17]. However, recent results of modelling performed by Asami [26], investigating the effect of the presence pores on the dielectric properties of biological cells, point to an alternative explanation. He found that the introduction of one or several small pores in the cell membrane induced an additional \( \alpha \)-dispersion-like response at low frequencies. Pores of a similar nature may have been formed here during the early stages after the addition of the solvent. Asami’s modelling also showed that even despite extensive membrane loss a cell can still make a considerable contribution to the dielectric properties of a suspension.

It was observed that the capacitance in the case of 0.5% iso-octanol did not fall as much as at higher concentrations. To study this in more detail another set of experiments was conducted in which lower concentrations of iso-octanol were used. Fig. 4 shows the capacitance vs. time at different concentrations of iso-octanol (0.0% (blank), 0.2%, 0.4%, 0.6% and 0.8%). The figure shows that, when lower iso-octanol concentrations were used, the capacitance stabilised to different values. The actual value depended on the concentration used. This was different from the situation in which...
higher concentrations were used, as the capacitance then stabilised to a similar value. This suggests that at lower concentrations the cell membrane did not become completely ruptured.

To investigate whether rupture of the membrane plays a role in the observed effects of iso-octanol on the cell dielectric properties, in the next set of experiment cells were subjected to glutaraldehyde first and then to iso-octanol. Glutaraldehyde crosslinks proteins, and is used for fixing the cells before electron microscopy. It can therefore be expected that adding glutaraldehyde strengthens membranes.

Yeast was suspended in a KCl solution, and total of 1% (v/v) glutaraldehyde was added to the yeast cell suspension. A small rapid drop in the capacitance occurred, after which the capacitance became constant (see also later Fig. 6). When the capacitance had been stable for 15 min iso-octanol was added at different concentrations. Fig. 5 shows the observed changes in the capacitance in time.

The rise in the capacitance due to partitioning of iso-octanol into the membrane was observed to be quicker when the cells were pretreated with glutaraldehyde. After the initial rise the capacitance value at first got stabilised and then started falling. This fall in the capacitance is slower after pretreatment with glutaraldehyde than without pretreatment, and the capacitance value at which the capacitance finally stabilises appears to be higher. The observed effects may be explained by the fact that glutaraldehyde reacts with and crosslinks membrane proteins. The experiments confirm that membrane rupture plays a role in the observed changes in the dielectric properties of cells, as a crosslinked membrane will not be able to expand as much, and is stronger.

3.1.3. Comparison of different toxic agents

Different toxic agents have different action mechanisms, and can therefore be expected to have different effects on the dielectric properties of different cells. Already discussed were the effects of the solvent iso-octanol, which appears to make holes and solubilise the membrane, and glutaraldehyde, which crosslinks and stabilises it. To further investigate the effect of different toxic agents, the effects of cycloheximide and Virkon were studied. Cycloheximide inhibits protein synthesis in eukaryotes. Its action mechanism is therefore mainly internal. Virkon is a disinfectant which action is complex, but has at least two action mechanisms: it contains potassium persulphate which is involved in oxidising cell components such as proteins, nucleic acids and lipids, and also has a detergent action which solubilises the membrane and denatures proteins.

Fig. 6 shows the change in the capacitance that occurs in yeast suspensions when cells are killed with different methods. Addition of cycloheximide, at a high concentration of 500 mg l\(^{-1}\), had little or no effect on the dielectric properties of yeast cells. Virkon was added as a powder to a yeast cell suspension at different concentrations. Virkon contains a lot of salts, and a large change in the conductivity could be observed immediately after the addition of Virkon (data not included). Initially a small rise in the capacitance was observed after the addition of Virkon, after which the capacitance declined. The effects of the other agents have been discussed previously. The data clearly show that different methods of killing cells lead to different responses by the cells, and that the capacitance values at which the capacitance stabilises when cells are dead are different. This indicates that the dielectric properties of cells killed by different methods are different.

3.1.4. Investigation of the dielectrophoretic behaviour of cells killed by different environmental stresses

Capacitance measurements mainly give information on the properties of the cell membrane [1]. As shown in the appendix, measurement of the higher crossover frequency (>1 MHz) of yeast cells suspended in low conductivity media can be used to estimate the internal cell conductivity, which in turn is an indirect measure of the permeability of the cell membrane. To investigate this in more detail, yeast cells were exposed to high temperatures and various toxic agents. Samples were taken, and the crossover frequencies determined.

Fig. 7 shows the dielectrophoretic behaviour of cells treated with different concentrations of iso-octanol at different frequencies. It can clearly be seen that subjecting the cells to different concentrations of iso-octanol elicits different dielectrophoretic behaviours, becoming predominantly negative in the frequency range studied as the iso-octanol concentration is increased. Crossover frequencies of cells treated with different concentrations of chemicals are shown in Table 1. Also shown are estimates of the internal cell conductivities, based on the measured crossover frequencies. In a separate experiment with a self-built frequency generator which could generate signals in the frequency range 20–100 MHz it was shown that intact, live cells had a crossover frequency between 40 and 50 MHz, corresponding to an interior conductivity of 0.21 ± 0.03 S m\(^{-1}\). The data confirm our previous findings that cells killed with cycloheximide and glutaraldehyde stay mainly intact, with little change in the internal conductivity. The data for cells killed with heat confirms our previous finding that little difference exists in the dielectric properties between cells killed at different temperatures. Cells killed with iso-octanol have a lower
Fig. 7. Comparison of the dielectrophoretic movement of viable yeast cells and yeast cells subjected to different concentrations of iso-octanol at different frequencies.

internal conductivity than cells killed with heat; this ties in with our previous findings that the residual capacitance of cells killed with iso-octanol is slightly lower than that of cells killed with heat, indicating a lower membrane capacitance and hence higher membrane permeability. The capacitance data obtained with glutaraldehyde followed by iso-octanol indicate that the relation between capacitance, membrane permeability and residual conductivity is not a simple one. The cells that were first treated with glutaraldehyde and then with iso-octanol had a larger residual capacitance than cells that were not treated with glutaraldehyde before their exposure to

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Iso-octanol concentration (v/v)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>0.0%</td>
</tr>
<tr>
<td>2.0 MHz</td>
<td><img src="image1" alt="Image" /></td>
</tr>
<tr>
<td>3.0 MHz</td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>4.0 MHz</td>
<td><img src="image11" alt="Image" /></td>
</tr>
<tr>
<td>6.0 MHz</td>
<td><img src="image16" alt="Image" /></td>
</tr>
<tr>
<td>8.0 MHz</td>
<td><img src="image21" alt="Image" /></td>
</tr>
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</table>

Table 1
Crossover frequencies and cell interior conductivities of yeast cells exposed to different environmental stress conditions.

<table>
<thead>
<tr>
<th>Environmental stress condition</th>
<th>Crossover frequency (MHz)</th>
<th>Cell interior conductivities (S m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycloheximide (500 mg l⁻¹)</td>
<td>&gt;20</td>
<td>&gt;0.09</td>
</tr>
<tr>
<td>Glutaraldehyde (% v/v)</td>
<td>1</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Heat (temperature in °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>5 ± 1</td>
<td>0.0213 ± 0.0066</td>
</tr>
<tr>
<td>70</td>
<td>3 ± 1</td>
<td>0.0120 ± 0.0066</td>
</tr>
<tr>
<td>80</td>
<td>3 ± 1</td>
<td>0.0120 ± 0.0066</td>
</tr>
<tr>
<td>90</td>
<td>3 ± 1</td>
<td>0.0120 ± 0.0066</td>
</tr>
<tr>
<td>Iso-octanol (% v/v)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>&gt;20</td>
<td>&gt;0.09</td>
</tr>
<tr>
<td>0.4</td>
<td>4.5 ± 0.5</td>
<td>0.0190 ± 0.0033</td>
</tr>
<tr>
<td>0.5</td>
<td>3.5 ± 0.5</td>
<td>0.0143 ± 0.0033</td>
</tr>
<tr>
<td>0.6</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>0.8</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>1</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>2</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>3</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>4</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>0.2</td>
<td>2.5 ± 0.5</td>
<td>0.0097 ± 0.0033</td>
</tr>
<tr>
<td>0.4</td>
<td>1.95 ± 0.05</td>
<td>0.0071 ± 0.0003</td>
</tr>
<tr>
<td>0.6</td>
<td>1.8 ± 0.05</td>
<td>0.0064 ± 0.0003</td>
</tr>
<tr>
<td>0.8</td>
<td>1.65 ± 0.15</td>
<td>0.0057 ± 0.0010</td>
</tr>
</tbody>
</table>

Glutaraldehyde 0.5% (v/v) followed by iso-octanol at different concentrations (% v/v)
isooctanol. However, their internal conductivity is lower. The data indicate that either the membranes stay more intact, but are more permeable, or that the cells have become more permeable during their preparation for the dielectrophoresis experiments (which involved centrifugation as well as copious washing in deionised water).

4. Conclusions

The experiments show that the dielectric spectroscopy is a useful method for investigating cell death, but that the response obtained depends on the method by which cell death occurs. When a method is used to kill the cells which has an intracellular action mechanism (e.g. cycloheximide) or stabilises the cell membrane (e.g. glutaraldehyde) the change in the cells' dielectric properties is small, and the dielectric properties of dead cells are similar to that of live cells. When a method is used which involves the solubilisation of the membrane, such as the addition of (iso)octanol, the capacitance stabilises at a low value, indicating a large change in the cell dielectric properties. Heating affects the cell membrane indirectly and has an intermediate effect, whilst the effect of Virkon, who has both an oxidising and a detergent-like effect, is also intermediate.

The results obtained with dielectrophoresis support these findings. Methods of killing cells which result in large changes in the capacitance also cause large changes in the high-frequency dielectrophoretic behaviour. This is because the changes in the membrane permeability which cause the capacitance to become low also allow the ions to escape from the cell. The resulting low internal conductivity is the main cause of the large change in the high-frequency dielectrophoretic behaviour observed. Factors which do not affect the cell membrane do not change the internal conductivity, and therefore do not lead to changes in the high-frequency dielectrophoretic behaviour.

The results indicate that non-viable cells in a suspension make a significant contribution to the overall capacitance of the suspension. The extent of this contribution depends on the method by which the cell was killed, but in some extreme cases the dielectric properties of “non-viable cells” can be the same as those of “viable” cells. Thus, the statement that dielectric spectroscopy can be used to measure the level of “viable” biomass should be used with extreme caution, as it depends on the method by which the cells were killed. Although additional information can be obtained about cell properties using the whole frequency spectrum rather than measuring at one or two datapoints [27–29], even the most advanced multifrequency scanning dielectric spectroscopy and modelling techniques will not be able to determine the ratio of viable to non-viable cells in a suspension if there is little or no difference between the dielectric properties of viable cells and those of non-viable cells. Similarly, the ability of dielectrophoresis to separate “viable” from “non-viable” cells also depends on the method by which the cells were rendered non-viable. If the method by which the cell is rendered non-viable leads to an increase in the membrane conductivity and consequently a reduction in the internal conductivity, the cells can be separated. If not, non-viable cells are essentially non-distinguishable from viable cells, and their dielectrophoretic separation is not possible.

Acknowledgements

We wish to thank Aber Instruments for an Industrial Scholarship to P.M. Patel. We also wish to thank Prof Richard Snook, SCEAS, University of Manchester, for his help with project supervision.

Appendix A. Measurement of the internal conductivity of yeast cells using dielectrophoresis

Theory

Dielectrophoresis is the movement of particles in non-uniform electric fields. The dielectrophoretic force is the result of the interaction of the non-uniform electric field with the charges of the net dipole induced in the particle. If the particle is less polarisable than the medium, the dipole aligns against the field and the particle is repelled from regions of high electric field; if the particle is more polarisable than the medium the opposite happens, and the particle is attracted to the regions of high electric field strength. The force is dependent on the induced dipole, and is unaffected by the direction of the electric field, responding only to the field gradient. Since the alignment of the field is irrelevant, the dielectrophoretic force can be generated in by DC as well as AC electric fields.

The dielectrophoretic behaviour of biological cells is frequency dependent, and the same cell can show positive and negative dielectrophoresis in different frequency ranges. The crossover frequency is the frequency at which the dielectrophoretic behaviour changes from positive dielectrophoresis to negative dielectrophoresis. Dielectrophoretic spectra of cells without a cell wall (i.e. all animal cells) typically have two crossover frequencies. The lower crossover frequency has been used to obtain information about the properties of the cell membrane of animal cells [30]. The higher crossover frequency is dependent on the permittivity and conductivity of the cytoplasm. The relation between the higher crossover frequency and the dielectric properties of the cell can be described by the following equation [30]:

\[
f_{crossover} = \frac{1}{2\pi f_0} \sqrt{\frac{(\sigma_1 - \sigma_0)(\varepsilon_1 + 2\sigma_0)}{(\varepsilon_2 - \varepsilon_0)(\varepsilon_2 + \varepsilon_0)}} \quad (1)
\]

where, \(f_{crossover}\) is the crossover frequency; \(\varepsilon_0\) is the permittivity of vacuum; \(\sigma_1\) is the internal conductivity; \(\sigma_0\) is the external conductivity; \(\varepsilon_1\) is the internal permittivity; \(\varepsilon_0\) is the external permittivity.

This relation has previously been used to determine the internal conductivity of mammalian cells [31]. Yeast cells, however, have a cell wall. It can therefore not automatically be concluded that the same relation holds for the yeast cells.

To investigate this, the effects of cell interior conductivity, the cell membrane conductivity, cell wall conductivity and suspension medium conductivity on the dielectrophoretic behaviour of yeast cell were investigated by calculating the real part of the Clausius-Mossotti factor. The model, written in Matlab, was based on the multishell model as described by Huang et al. [32]; the values for the parameters used were also taken from the same paper. Unless stated otherwise, the medium conductivity was 0.6 \(\mu\text{s cm}^{-1}\). Results of the modelling are shown in Fig. 8.

Fig. 8A shows the effect of changing the interior conductivity. It can be seen that as the cell interior conductivity decreases, the crossover frequency also decreases. Cell membrane conductivity often increases as the cell loses viability. This is because cell death is often accompanied by disruption of the membrane. Fig. 8B shows how the cell membrane conductivity affects the Clausius-Mossotti factor. It can be observed that a change in the cell membrane conductivity does not affect the higher crossover frequency. Fig. 8C shows the effect of the cell wall conductivity on the Clausius-Mossotti factor. It can be seen that the cell wall conductivity has a minimal effect on the higher crossover frequency. The effect of the medium conductivity is shown in Fig. 8D. The higher crossover frequency can be seen to be little affected by the suspending medium conductivity until very high medium conductivity values (over 0.065 \(\text{S m}^{-1}\)) are reached.

The modelling indicates that for yeast cells the cell interior conductivity has a strong effect on the higher crossover frequency,
Fig. 8. Calculated real part of the Clausius-Mossotti factor of yeast cells as a function of frequency for different parameter values. The arrow indicates the direction in which a parameter was increased or decreased. (A) The cell interior conductivity was decreased from 0.2 to 0.01 S m\(^{-1}\) with a stepwise decrement of 0.01 S m\(^{-1}\). (B) The cell membrane conductivity was exponentially increased from 2.4 \(\times\) \(10^{-7}\) to 8.3 \(\times\) \(10^{-4}\) S m\(^{-1}\). (C) The cell wall conductivity was exponentially increased from 1.4 \(\times\) \(10^{-2}\) to 0.54 S m\(^{-1}\). (D) The suspending medium conductivity was exponentially increased from 6 \(\times\) \(10^{-5}\) to 0.2 S m\(^{-1}\).

and that other factors have relatively little effect. Eq. (1) indicates that, for low medium conductivities (0.5\(\sigma_c < \sigma_l\)), a linear correlation should exist between the crossover frequency and the cell interior conductivity. In order to explore whether this is also the case for yeast cells which have a wall, the high-frequency crossover frequency was calculated for yeast using the multishell model and plotted as a function of the cell interior conductivity. The results, shown in Fig. 9, show there is an excellent linear correlation between the cell interior conductivity and the crossover frequency for yeast cells under such conditions.

References


