Study on alteration in electrical double-layer structure in yeast cells by dielectrophoresis

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Systematically starting out with different electrical double layer configurations of yeast cells (*Saccharomyces cerevisiae*) in different phases of cell cycle, we determined excess permittivity (K_e) using cell balance technique. The variation in K_e of yeast cell as a function of cell fraction is determined at two frequencies 10 kHz and 1 MHz. Cytological and biochemical alterations would lead to changes in electrical double-layer structure in different phases of cell cycle. This aspect is detected from the knowledge of permittivity. K_e for single cell is 95 & 54, for cells with 1/3 bud is 388 & 162, for 2/3 bud cells is 247 & 63, and for double cell is 79 & 44, at 10 kHz and 1 MHz respectively. These results are in accordance with those obtained by microdielectrophoretic technique.

BIOLOGICAL cell dielectrophoresis is concerned with translational motion of cells in nonuniform electric fields (NUEF). Dielectrophoresis is useful as a tool for many practical applications like cell separation[1], cell fusion[2,3], cellular characterization[4], besides using it for determining cellular electrical properties[5–8]. Microdielectrophoretic (μDEP) technique has been used for detecting the electric fields arising due to cellular oscillations.

Cell balance technique in biological cell dielectrophoresis was used for the first time by Chen and Pohl[10]. Single cell dielectrophoresis[11] is a very useful tool to study the dielectric behaviour of intact individual cells. The dielectrophoretic force in ac fields is applied on yeast cells to help in characterizing their electrical polarization processes as a function of environment[12], age[13,14], physiological states[15], etc.

Electrical properties of cells are usually obtained from studies made on suspension by using appropriate mixture relationships[16]. Dielectrophoretic and cell rotation techniques[8] are straightforward and particularly useful to examine subtle changes in the cellular electrical make-up under different phases of cell cycle.

Earlier, using dielectrophoretic technique, Gopala Krishna *et al.*[17] studied dielectrophoretic collection rate and K_e of yeast cells from the same colony as a function of their relative sizes and some physical parameters like frequency, concentration of suspension, voltage, etc.[18]

An electrical double layer formed at the interface of a charged biological membrane and its extracellular ionic environment has been the subject of intense research since the appearance of Gouy-Chapmen–Debye–Huckel (GCDH) theory[19,20]. The concept of electric double layer has been employed in several ways to understand the field membrane coupling. Teorell[21] applied this to membrane process. However, additional surface properties were not considered.

As the cellular activity of a cell changes, the electrical composition of the electrical double layer changes which would influence its dielectric behaviour. Thus the main aim of this communication is to examine the variation of effective polarizability K_e as a function of cell fractions that are at different physiological states. The study has been confined to the following four important phases of cell cycle: (i) Single cell from which the cell cycle is assumed to start, (ii) Cell with small (1/3) bud in which mitotic activity is prominent, (iii) Cell with large (2/3) bud where mitotic activity is assumed to be almost completed and bud starts developing more, (iv) Fully grown bud (double cell), in which cell wall is growing between daughter and mother cells and cell is about to separate. These four phases of cell cycle are assumed to represent four different electrical double-layer configurations of yeast cell in the same ionic environment. We can say the cell cycle is completed by this stage. Two spot frequencies 10 kHz in α-region and 1 MHz in β-region are selected for the study.

An electrode chamber was constructed as described by Anwar Ali[22] by placing two thin platinum wires of diameter 26 μm with spacing 150 μm apart aligning them parallel on a glass slide to give rise to NUEF of cylindrical field geometry. The wires were covered with nonconducting material having a small hole to place the sample.

The yeast *Saccharomyces cereviseae* was cultured in YEPD medium (1% peptone, 2% glucose, 5% yeast extract, 2% agar) at 30°C. The active culture was collected from colonies and washed with double-distilled water whose conductivity is 5 $\mu\Omega\,cm^{-1}$ and a final suspension of yeast cells with conductivity around 20 $\mu\Omega\,cm^{-1}$ is made in double-distilled water. A drop of this sample was placed in electrode chamber and covered with a cover slip and placed on microscope stage. The microscope was tilted such that the axis of observation was horizontal and motion of the cells between the electrodes was vertical. Electric field was supplied using function generator (Agronic-72). Voltage was measured by ac millivoltmeter (Phillips CM-6012).

By applying electric field the cells were made to rest on the electrode. Specific cell was selected and keeping it under observation, voltage was reduced gradually. At certain voltage level, the cell detaches from the electrode and starts moving under the action of gravitational force. At this point the voltage was noted. It is known as release voltage. Each set of experiment was repeated 10 times for all cells belonging to the four stages of the cell cycle at 10 kHz and 1 MHz under

the same experimental conditions and mean deviation is shown in Figure 1.

The excess permittivity K_e is calculated using the expression given by Pohl[5].

$$K_e = \frac{(d_2 - d_1) g r^3 [\ln(r_1/r_2)]^2}{3\varepsilon_0 V^2} \quad (1)$$

where d_1 and d_2 are the densities of suspending medium and cells; r_1 and r_2 inner and outer radii of cylinder from intermediate radius; r, radius of platinum wire; V_1, release voltage measured from experiment; g, acceleration due to gravity; ε_0, permittivity of free space, a constant.

Using release voltage data from the experiment, K_e is computed by equation 1 for each cell at different growth phases of life cycle (Table 1). The reported n/p ratio, known as gathering factor obtained from microdielectrophoresis, is also shown in Table 1, where n is the number of particles (having high dielectric constant) associated with each cell compared to the number of free particles (p) immediately surrounding the cell. Percentage increase in K_e of other cell fractions with reference to single cell is also shown in Table 1. Figure 1 shows variation in K_e with cell fraction at 10 kHz and 1 MHz.

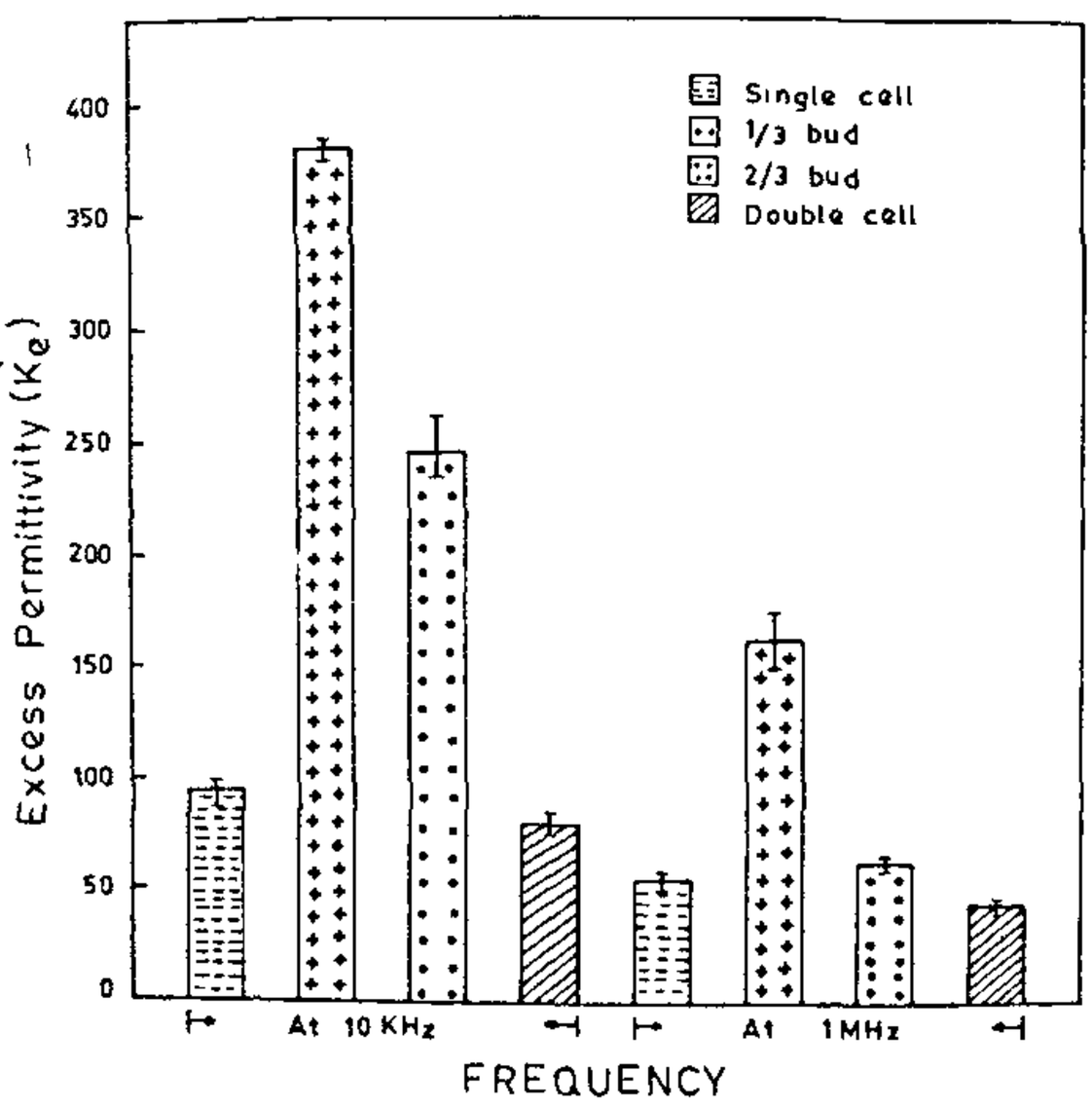

Figure 1. Variation in excess permittivity with cell fraction.

The prominent feature of Figure 1 is that K_e is maximum for 1/3 cell fraction at both 10 kHz and 1 MHz. It can be noted that K_e decreases with increase in frequency. The possible explanation is that, at higher frequencies cell membranes become progressively short-circuited as the resistance of the cell membrane becomes small, thereby allowing currents to flow in the cell interior. Thus, as frequency is increased, the effective permittivity of the suspension falls, whereas the conductivity increases. This is referred to as Maxwell–Wagner effect.

The dimensions of biological cells are in the range of classical colloids where surfaces play an important role in determining the properties of the system. When such surfaces are charged, the effect on the properties is more pronounced and can be calculated in terms of resulting electrical double layer[23]. The electrical double layer formed at the interface of a charged biological membrane and its extracellular ionic environment is different for different physiological states; as revealed by our dielectrophoretic experimental results (Table 1).

Single cell, cell with 1/3 bud, cell with 2/3 bud and double cell may be viewed as different electrical double-layer configurations. From this study it is obvious that even though the extracellular ionic environment is the same for all these configurations, the permittivity values are found to be different due to alterations in the electrical double-layer structure.

It has been postulated that electrical oscillation must accompany reproduction and, further, that without them reproduction cannot succeed[24]. This postulate has been experimentally verified by μDEP on yeast cells at different growth phases.

Cellular electric fields have been detected by microdielectrophoretic experiment on yeast cells[25] which reports that the electrical activity is found to have a maximum value during the mitotic phase (i.e. 1/3 bud), as shown in Table 1 where the n/p ratio is more

Table 1. Excess permittivity (K_e) of yeast at different growth phases

Cell fraction	Release voltage (mV)		K_e at		Variation in K_e (%)		n/p from μDEP
	10 kHz	1 MHz	10 kHz	1 MHz	10 kHz	1 MHz	
Single cell	210	290	95.2 + 5.9	54.2 + 3.45	—	—	2.3
1/3 bud	100	177	388.5 + 4.5	162.0 + 13.0	308	199	4.75
2/3 bud	135	268	247.0 + 15.3	63.0 + 3.2	160	16	2.75
Double cell	240	320	79.5 + 5.1	44.1 + 1.7	16	18	2.2

for this phase than for other phases. Our results on K_e as a function of cell fraction also indicate a maximum value for yeast cells at the mitotic phase of the cell cycle. This may be attributed to cytological and biochemical[26] evidences such as division of centriolar plaque, fusion of elements of endoplasmic reticulum and vacuoles, appearance of thread-like mitochondria and their introduction into bud and starting of nuclear division, local weakening of cell wall by secretion of suitable enzymes, rearrangement of glucan fibrils and starting of chitin synthesis. Dielectric behaviour at 2/3 bud stage is such that it is less dielectric than at 1/3 stage and its K_e value is higher than single cell value. As we examine the double cell we notice a further reduction in the dielectric behaviour of the cell which can be attributed to its size[27]. The variations exhibited by yeast cells at different growth phases are essentially owing to cytological and biochemical alterations and are in agreement with μDEP results. The biophysical experiment like cell balance technique throws light on the electrical character of the yeast cell at different growth phases from different points of view.

In conclusion, the alterations in electrical double-layer structure are important from biological and physiological points of view. But they have not been detected so far by electrophoretic experiments[23]. However, this dielectrophoretic study demonstrates that the variation in cytological and biochemical parameters influence the electrical double-layer structure of the membrane, which is reflected through its dielectric behaviour by dielectrophoresis. Thus the cell membrane can be regarded as a dynamic system whose electrical properties are demonstrated to be related to the physiological events of yeast cells.

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Chemical analysis of lead coins of the Ikshwaku period (3rd and 4th century AD)

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Fifty lead coins of the Ikshwaku period (3rd and 4th century AD) were chemically analysed both in respect of their major and minor constituents and the results interpreted in archaeo-metallurgical terms. Although, according to Puranas, seven kings have reigned in Andhradesa after Satavahana, coins belonging to only four Ikshwaku kings have been recovered from excavations so far. The chemical analysis does not show much difference in the coins of later Satavahana and Ikshwaku except for a few coins of Ikshwaku with higher silver content. Besides, evidences have been adduced to show that the coins were fabricated from the unrefined metal. Numismatic studies show that more than one mint were used in Ikshwaku period. Provenance of the ores used for minting coins have also been studied. No elaborate treatment for desilvering of crude lead seems to have been carried out for the lead coins.

THE Ikshwakus came to power during the first quarter of the 3rd century AD. Chantamula, the founder, established a mighty dynasty defeating the Satavahana at Vijayapuri and Dharanikota. The Ikshwakus of Vijayapuri ruled the Andhradesa during 3rd and 4th century AD. Many coins found at Nagarjunakonda, Tenali, Ongole, Nelakondapally, etc. of Andhra Pradesh provide the names of four known kings. Table 1 gives the genealogy of Ikshwakus and their legends. The Tenali hoard consists of the coins issued by all the four known kings and few coins from this hoard were chemically analysed.

Although Satavahana issued coins in many metals such as lead, copper, potin, silver, the Ikshwakus used