Glutaraldehyde enhanced dielectrophoretic yeast cell separation

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We introduce a method for improved dielectrophoretic (DEP) discrimination and separation of viable and nonviable yeast cells. Due to the higher cell wall permeability of nonviable yeast cells compared with their viable counterpart, the cross-linking agent glutaraldehyde (GLT) is shown to selectively cross-link nonviable cells to a much greater extent than viable yeast. The DEP crossover frequency (cof) of both viable and nonviable yeast cells was measured over a large range of buffer conductivities (22 $\mu\text{S}/\text{cm}$ –400 $\mu\text{S}/\text{cm}$) in order to study this effect. The results indicate that due to selective nonviable cell cross-linking, GLT modifies the DEP cof of nonviable cells, while viable cell cof remains relatively unaffected. To investigate this in more detail, a dual-shelled oblate spheroid model was evoked and fitted to the cof data to study cell electrical properties. GLT treatment is shown to minimize ion leakage out of the nonviable yeast cells by minimizing changes in cytoplasm conductivity over a large range of ionic concentrations. This effect is only observable in nonviable cells where GLT treatment serves to stabilize the cell cytoplasm conductivity over a large range of buffer conductivity and allow for much greater differences between viable and nonviable cell cof's. As such, by taking advantage of differences in cell wall permeability GLT magnifies the effect DEP has on the field induced separation of viable and nonviable yeasts. © 2009 American Institute of Physics. [doi:10.1063/1.3257857]

I. INTRODUCTION

Much work has been done to develop dielectrophoresis (DEP) for cellular separation and manipulation.^{1–5} As different types of cells and bacteria have distinctly different osmotic pressures, internal ionic strengths, sizes, shapes, action potentials, and membrane protein densities, there is much one can exploit to discriminate cell differences for effective separation. Dielectrophoretic cell separation takes advantage of the unique magnitude and/or direction of the field-induced DEP mobility that is hoped to be cell specific. Often differences between cell types, however, are subtle and various chemical or physical modifications are required to yield effective separation. Indeed, much work and creativity have been spent toward amplifying differences in cell types; virtually every degree of freedom available to the researcher has been exploited in some manner or another to achieve this. Engineering of electrolyte conductivity and permittivity has been a very successful cell isolation technique.^{6,7} For example, high permittivity buffers have been used for selective cell levitation and positioning, increasing the rate of cell concentration, and creating ideal dielectric conditions for DEP mobility measurements. Low conductivity solutions have been exploited to separate malaria-infected red blood cells from human blood.^{8,9} Others have chemically modified cell membrane electrical properties to amplify subtle differences among cell subpopulations.^{10–12} All of these techniques rely on the time consuming process of optimization,

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whereby the researcher must determine which differences are best exploited to achieve the desired separation scheme for a given suspension of cells. This is common for all cell separation techniques, but proves particularly difficult when the separation phenomenon of interest is DEP. DEP separation takes advantage of inherent or chemically modified differences in the electrical properties of the cell membrane, cytoplasm, and other organelles that become apparent when such cells are suspended in a specific electrolyte solution. Often, however, the buffer solution is nonideal and cellular constituents rapidly equalize with the surrounding buffer, either eliminating differences in DEP mobility or creating highly inconsistent time-dependent separations.⁹

In this work, we have developed an efficient method for determining and stabilizing optimum electrolyte parameters for DEP separation. We utilize a cell membrane fixation agent [glutaraldehyde (GLT)] to rapidly cross-link aminated membrane proteins of viable and nonviable yeast cells to seal cell ion channels and prevent cell ion leakage in low conductivity buffer solutions. As such, ion channel activity ceases, and cytoplasmic dependency on buffer conductivity (a common problem in DEP separation) is minimized. Owing to these effects, a simple buffer conductivity study determines conditions for optimal cell separation. More specifically, due to ion-channel deactivation, a cell's inner ionic strength becomes stabilized in low conductivity buffer, enabling improved DEP yeast cell separations. We hope to demonstrate the potential role GLT treatment could have in future DEP cell separation applications.

II. DIELECTROPHORESIS

As described in Ref. 13 the classical theory models the DEP force F_{DEP} induced by an ac electric field E of frequency ω on a homogeneous spherical particle of radius a as¹³

$$F_{\text{DEP}} = 2\pi a^3 \epsilon_m \text{Re}(f_{\text{CM}}) \nabla |E|^2, \quad (1)$$

where ϵ_m is the real part of the permittivity of the suspending medium, and ϵ_p^* and ϵ_m^* are the complex permittivities of the medium and the particle, respectively, each described by $\epsilon^* = \epsilon + \sigma/(i\omega)$. For a homogeneous lossy dielectric sphere, the factor $f_{\text{CM}} = (\epsilon_p^* - \epsilon_m^*)/(\epsilon_p^* + 2\epsilon_m^*)$ is the frequency-dependent Clausius–Mossotti (CM) factor. This equation reveals that permittivity dominates particle polarization at high frequency, while the conductivity σ becomes the governing factor at low frequency. A positive real part of the CM factor indicates that the DEP force pushes a polarized particle toward a local electric field maximum, which is known as positive dielectrophoresis (pDEP), while a negative CM factor pushes the particle away from the field maximum and into regions of weak electric field, known as negative dielectrophoresis (nDEP). The frequency at which the CM factor equals zero is known as the crossover frequency (cof),¹⁴

$$\omega_c = \frac{1}{2\pi} \sqrt{\frac{(\sigma_m - \sigma_p)(\sigma_p + 2\sigma_m)}{(\epsilon_p - \epsilon_m)(\epsilon_p + 2\epsilon_m)}}. \quad (2)$$

As conductive polarization involves ion migration, it is a relatively slow phenomenon. On the other hand, dielectric polarization involves relatively fast charge segregation at the molecular level. Consequently, conductive polarization is observed at low frequencies while dielectric polarization dominates at high frequencies. The cof separates these two distinct polarization mechanisms. For particles with conductivity higher and permittivity lower than that of the medium, pDEP is expected below the cof and nDEP above the cof according to this simple model. Since most cells and bacteria have lower permittivities than the surrounding electrolyte buffer, lowering buffer conductivity below that of the cell interior should produce a single cof. Yeast cells, however, are nonhomogeneous nonspherical particles, and due to the existence of a cell wall, cell membrane, and cytoplasm, the classical homogeneous model must be modified to account for each of these domains.

A yeast cell is approximated as a dual-shelled oblate spheroid of 7 μm in diameter, with a 5.8 μm domain of cytoplasm bound by a membrane of ~ 8 nm, shelled by a cell wall of ~ 200 nm for a total of three interfaces,¹ as shown in Fig. 1. A Maxwell–Wagner relaxation process is introduced for each interface that separates the three different dielectric layers: an inner

FIG. 1. Dual-shell oblate spheroid model for a yeast cell, where r and c represent the spheroid primary and secondary axes, respectively.

cytoplasm-membrane, membrane-cell wall, and an outer electrolyte-cell wall. The thin cell wall-membrane region behaves as a capacitor, which when fully charged prevents field penetration into the cell cytoplasm at frequencies below both the cell wall and membrane inverse RC charging time scale ω_o . This capacitive charging behavior limits low frequency polarization to the cell wall and outer cell membrane interfaces.

An analysis of the three polarizable domains (cell wall, cell membrane, cytoplasm) is required to better understand the DEP behavior of viable and nonviable yeast cells. Based on reported values for both viable and nonviable yeast cells, the cell membrane is considered to be less conductive than the cell cytoplasm and cell wall.² The higher conductivity of the cell wall will therefore dominate the direction of the electric field induced polarization at low frequencies (typically 100–500 kHz). In the case of viable cells, where the cell wall conductivity is less than that of the medium, low frequency field polarization less than the cell wall ω_o will result in an observed nDEP behavior, as the cell wall is less polarizable than the media. At intermediate frequencies greater than ω_o , the electric field penetration into the cell interior and the resulting polarization is dominated by differences in the cytoplasm (higher) and media conductivity, giving rise to pDEP. At high frequencies, or frequencies larger than the cytoplasm inverse charging time scale, ranging between 1 and 4 MHz, polarization is governed by differences in media (higher) and cytoplasm permittivity, resulting in nDEP. A dual-shelled yeast cell therefore exhibits two cof: one at low frequency (cof_1) governed by the relaxation time of the media/cell wall, and another at a higher frequency (cof_2) dependent on the relaxation time of the media/cytoplasm. As such, for relatively low conductive (less than cell wall) and comparatively high permittivity (greater than cytoplasm) buffers, one has the advantage of an extra cof when considering conditions for cell separation.

For nonviable cells with a more conductive cell wall, both low and intermediate frequency regimes will result in observed pDEP behavior. As the cytoplasm relative permittivity is still less than the media, applied ac fields at high frequency result in nDEP behavior. Hence nonviable yeast cells with conductive cell walls will only exhibit one cof as determined by the inverse RC charging of the cytoplasm.

As the CM factor used in the DEP force expression is only valid for homogeneous spherical

particles, it is necessary to modify this factor to take into account both the cell wall and cell membrane of the nonspherical yeast cell. In this case, both the cell wall and membrane are modeled as thin shells surrounding an oblate spheroidal homogeneous cytoplasm core, each domain having a specific complex permittivity. An effective complex permittivity of this three-domain system is derived; first the cytoplasm and surrounding membrane complex are replaced with a single effective complex term,¹⁴

$$\epsilon'_{\text{cyto-mem}} = \epsilon_{\text{mem}}^* \frac{\epsilon_{\text{mem}}^* + (\epsilon_{\text{cyto}}^* - \epsilon_{\text{mem}}^*)[A_{ip} + v(1 - A_{op})]}{\epsilon_{\text{mem}}^* + (\epsilon_{\text{cyto}}^* - \epsilon_{\text{mem}}^*)[A_{ip} - vA_{op}]}, \quad (3)$$

where ϵ_{mem}^* and ϵ_{cyto}^* are the complex permittivities of the cell membrane and cytoplasm, respectively. Repeating the analysis, an additional complex effective term is derived to include the complex permittivity of the cell wall (ϵ_{wall}^*),

$$\epsilon'_{\text{eff}} = \epsilon_m^* \frac{\epsilon_{\text{wall}}^* + (\epsilon_{\text{cyto-mem}}^* - \epsilon_{\text{wall}}^*)[A_{ip} + v(1 - A_{op})]}{\epsilon_{\text{wall}}^* + (\epsilon_{\text{cyto-mem}}^* - \epsilon_{\text{wall}}^*)[A_{ip} - vA_{op}]}. \quad (4)$$

The parameter A_{op} describes the geometry-specific degree to which the exterior of the cell, extending from the outer membrane edge to infinity, depolarizes along the principal axis. For each of the two shells, A_{ip} describes the depolarization from just inside the membrane or cell wall to infinity, where $v = r^2 c / [(r+d)^2(c+d)]$ is the volume ratio of the cell exterior to interior, r is the cell radius (along the primary axis), c is the cell half-length (secondary axis), and d is the membrane thickness. In the case of a yeast cell, the cell membrane and cell wall are approximately 8 and 200 nm, respectively,¹ and at least an order of magnitude less than r and c ; thus one can make the approximation $A_{op} = A_{ip}$. A useful expansion of this approximation can be expressed in terms of the scaling factor $\gamma = c/r$, as shown in Eq. (5),¹³

$$A_{op} = A_{ip} = \frac{[1 + \frac{3}{5}(1 - \gamma^{-2}) + \frac{3}{7}(1 - \gamma^{-2})^2 + \dots]}{3\gamma^{-2}}. \quad (5)$$

For a sphere, $\gamma = 1$, and thus $A_{op} = A_{ip} = 1/3$, which forces this model to converge to the well-known CM factor for a dual-shelled sphere. The yeast cells in this work have a measured scaling factor of approximately $c/r \sim 2.8 \mu\text{m}/3.5 \mu\text{m} \sim 0.8$ giving a depolarization factor $A_{op} = A_{ip} \sim 0.170$. For the case of a dual-shelled oblate spheroid with major axis aligned parallel with the applied field, the CM factor can then be shown to be¹³

$$f_{\text{CM}} = \frac{1}{3} \epsilon_m \frac{\epsilon'_{\text{eff}} - \epsilon_m^*}{(\epsilon'_{\text{eff}} - \epsilon_m^*)A_{op} + \epsilon_m^*}. \quad (6)$$

Using reported electrical properties for viable and heat treated nonviable yeast cells² suspended in 1M aminohexanoic acid (AHA) solution ($\sigma = 22 \mu\text{S}/\text{cm}$), the CM factor is shown plotted in Fig. 2. To complicate matters further, the conductivity of a yeast cell wall is dependent on the media conductivity.² For common viable bakers yeast, *Saccharomyces cerevisiae*, suspended in an electrolyte solution of conductivity less than 30 mS/m, the conductivity and relative permittivity of a viable yeast cell wall, membrane, and cytoplasm are approximately 100 $\mu\text{S}/\text{cm}$ and 50, 0.25 $\mu\text{S}/\text{m}$ and 6, and 200 mS/m and 60, respectively.²

Nonviable cells are typically created by subjecting viable cells to heat treatment at 90 °C for 20 min. This has been shown to permeabilize the cell wall, increasing the conductivity to a reported range between 200 and 300 $\mu\text{S}/\text{cm}$ and decreasing cytoplasm conductivity to a range between 100 and 800 mS/cm.³ Such a large range can be attributed to the variable “leakiness” of nonviable cells. This can be problematic when determining buffer conditions for optimal DEP separation, as the ionic strength of each sample can vary widely. In this work, we combine low conductivity/high permittivity zwitterion buffers with membrane-sealing GLT, and subsequent frequency changes to separate viable yeast cells from nonviable. In the past we have demonstrated the effect that GLT has on the electrical properties of bovine red blood cells and its use as a

FIG. 2. $\text{Re}[k(\omega)]$ calculated from the dual-shell oblate spheroid model plotted against frequency for viable and nonviable yeast cells.

facilitator to cell separation.⁶ As GLT reacts with aminated proteins on the surface and within the cell membrane, an intact yeast cell wall should prevent GLT infusion through the cell wall and subsequent membrane cross-linking. In contrast, nonviable cells with permeable cell walls should in theory be more susceptible to GLT cross-linking effects. We capitalize on this concept with yeast cells to demonstrate improved stability and DEP separation.

III. MATERIALS AND METHODS

A. Yeast cell preparations

5 g dry weight of commercial grade bakers yeast (*Saccharomyces cerevisiae*) was dissolved in 50 ml of culture medium, pH 5, consisting of 5 g l⁻¹ yeast extract, 5 g l⁻¹ bacterial peptone, and 50 g l⁻¹ sucrose. The yeast was grown at 37 °C for 36 h and four 1 ml samples were harvested, each washed four times in 280 mM mannitol. Two samples were rendered nonviable by heating at 90 °C. Nonviable cell samples were washed as before and all four samples stained with 80 μl of methylene blue (Sigma-Aldrich), as described in other studies.^{2,3} Cells were washed three times in 280 mM mannitol, cross-linked in 150 μl of 25% w/v GLT (Sigma-Aldrich), and agitated for 30 minutes, whereby the reaction was immediately quenched by rinsing cells three times in 280 mM mannitol.

B. Media preparation

Buffer solution was composed of 6-aminohexanoic acid obtained from Sigma. A 1M AHA solution was prepared and treated with 100 g/l of Dowex MR-3 resin (Sigma-Aldrich) to remove trace salts. As such, buffer conductivity was reduced from 150 to 22 μS/cm. Aliquots of de-ionized buffer were conductivity adjusted using small amounts of NaCl to attain ten buffers of varying conductivity: 25, 41, 51, 61, 73, 82, 150, 200, 300, and 400 μS/cm. Cross-linked cell

FIG. 3. The fabricated quadrupole electrode array when activated can produce (a) pDEP or (b) nDEP.

samples were washed in 280 mM mannitol, aliquot into four sets of ten 200 μl volumes: viable and nonviable uncross-linked and viable and nonviable cross-linked, each suspended in 1 ml of conductivity adjusted buffers.

C. Dielectrophoretic measurements

DEP cof experiments were performed utilizing a quadrupole electrode array. These electrodes were fabricated by patterning dual titanium-gold layers onto precleaned $50 \times 75 \text{ mm}^2$ glass slides. Slides were patterned with the image reversal photoresist Shipley AZ-5214 to define the electrode patterns, onto which 50 Å of titanium and 2500 Å of gold were evaporated. The resist was dissolved and metal was lifted-off from the slides using acetone. The arrays were designed as four triangular posts with an inner square side length of 25 μm and an electrode width of 60 μm .

The fabricated array, as shown in Fig. 3, was attached to a function generator set to 10 V_{pp} (Agilent, model 33220A) via copper tape and wire leads such that alternating electrodes were deemed active. This created an electric field minimum in the center region of the four electrodes, while the electrode edges were regions of absolute field maxima. The array was covered with a plastic coverslip and injected with yeast cell suspension. The frequency was slowly increased from 1 kHz to 20 MHz in order to determine yeast cell cof values for each conductivity buffer. The suspension cof was determined when the cells migrated from the electrode edges [Fig. 3(a)] to the electrode center [Fig. 3(b)].

D. Microchip fabrication

Following DEP characterization, viable and nonviable yeast cell separation was performed on a polydimethylsiloxane (PDMS)-glass microfluidic chip. Electrodes were patterned onto pre-cleaned glass slides as before. The microchannel geometry was fabricated using common soft-lithography techniques. Briefly, thick photoresist (SU-50, Microchem) was spun and patterned on a clean silicon wafer to create the microchannel mold. PDMS (Sylgard 184, Dow Corning) elastomeric base was mixed with curing agent in a 10:1 ratio, poured onto the channel mold, degassed under vacuum for 30 min, and cured at 65 °C for 1 h. The cured PDMS was removed from the mold and fluid access ports were punctured into the channel entrance and exit using a 75 mm biopsy punch (Ted Pella). The cured PDMS was cleaned in acetone, isopropanol, and methanol for 1 min each and dried in a gentle stream of nitrogen. The PDMS was aligned to the electrode patterned glass slide and held 1 mm off the glass surface using an in-house constructed micromanipulator. The micromanipulator/microchip was placed in an oxygen plasma chamber and oxidized at 1450 W for 1 min. The device was removed from the chamber and the PDMS/glass surface was immediately brought into contact and held firmly for 1 min. This resulted in complete sealing of the electrode-channel aligned device. Polyethylene (PE-20) tubing was connected to the microchip entrance ports and yeast cell suspension was injected at a controlled flow rate of 3.5 $\mu\text{l}/\text{min}$ via

FIG. 4. Effects of cross-linking on the high and low cofs on viable yeast cells as a function of media conductivity for viable yeast cells. (a) Low frequency cof. (b) High frequency cof. GLT has a minimal effect on viable yeast cell electrical properties. (c) Low frequency cof. (d) High frequency cof. Unlike viable cells, GLT has a large effect on nonviable yeast cell electrical properties.

syringe pump (Harvard Apparatus, PHD 22/2000). Electrical contact to the electrodes was achieved, as described earlier, using copper tape and wire leads connected to a function generator.

IV. RESULTS AND DISCUSSION

A. DEP yeast cell characterization

GLT reacts with the aminated surface membrane and *trans*-membrane proteins, forming a cross-linked network.⁶ Since the yeast cell wall has minimal protein content, reactivity with GLT is expected to be minimal. During heat treatment to produce nonviable cells, however, the cell wall becomes permeabilized, resulting in an increase in cell wall conductivity.¹⁻⁵ This increase in the cell wall permeability could possibly improve GLT penetration and reactivity with the underlying cell membrane proteins. GLT cross-linking effects on viable and nonviable yeast cells are analyzed below.

DEP cof data of cross-linked and uncross-linked viable cells are shown in Figs. 4(a) and 4(b). As the yeast cell has three interfaces of polarization, the effective polarizability of this interfacial network is a complicated function of the electrical properties of the media, cell wall, cell membrane, and cytoplasm. When this network is effectively more polarizable than the media, the cell exhibits two cofs. When media conductivity exceeds this effective value, the yeast cell wall/membrane becomes less polarizable than the media and cells exhibit only one high frequency cof related to the polarizability of the cell cytoplasm.

For media of conductivity less than 61 $\mu\text{S/cm}$, noncross-linked viable yeast cells only produced one cof, while above this conductivity range they produced two. In contrast, cross-linked viable yeast cells exhibited negligible differences in measured cofs with only a minor conductivity increase to 73 $\mu\text{S/cm}$, which suggest that small degrees of cell wall permeabilization or mem-

FIG. 5. [(a) and (b)] Calculated effects of GLT on viable yeast cells. (a) Calculated cell wall conductivity. The observed peak relates to optimal buffer conditions where the cell wall ion channels are most open. (b) Cytoplasm conductivity. The slight difference over the active ion channel range is most likely due to cytoplasm leakage into the media. [(c) and (d)] Calculated effects of cross-linking on nonviable yeast cells. (c) Cell wall conductivity. (d) Cytoplasm conductivity.

brane cross-linking took place with GLT treatment. The high frequency cof_2 measurements, cof_2 , are essentially identical datasets for both cross-linked and noncross-linked viable cells. This suggests that GLT is unable to penetrate the cell wall and therefore has minimal modification of the electrical properties of viable yeast cells.

Unlike viable cells, GLT had a significant effect on nonviable cell cof . As shown in Fig. 5(a), cof_1 for uncross-linked nonviable cells largely differs from the cross-linked variety for conductivities greater than 200 $\mu\text{S/cm}$. This suggests a large increase in cell wall conductivity with heat treatment. Also, GLT cross-linking of nonviable cells resulted in no measurable cof_1 over the entire media conductivity range, indicating that the cell wall/membrane had a greater effective conductivity than the media and that of cross-linked viable cells. One possible explanation is the effect of GLT on the cell membrane. Previously, GLT has been shown to increase membrane conductivity of bovine red blood cells.⁶ Heat treated permeable cells appear to allow GLT to penetrate the cell wall, cross-link the cell membrane proteins, and increase the cell membrane conductivity.

Unlike cof_1 , which characterizes the conductivity of the cell wall/membrane, cof_2 describes cell cytoplasm conductivity. As shown in Fig. 5(b), nonviable cell cof_2 was also modified when cells were subjected to GLT. Nonviable cells were observed to have a slight increase in cof_2 in the low media conductivity range, followed by a relatively constant cof_2 value at intermediate values, and finally disappearing at media conductivity values greater than 200 $\mu\text{S/cm}$. The stability of the measured cof_2 suggests minimal cytoplasm conductivity dependence with media conductivity, perhaps due to the observed GLT membrane cross-linking effect. This concept is analyzed and discussed in more detail below.

To better understand how GLT affects yeast cell parameters as a function of media conduc-

tivity, cof data were analyzed and fit to a dual-shell oblate spheroid model. Due to the large difference between yeast cell membrane conductivity ($0.25 \mu\text{S/m}$) and cell wall conductivity (200 mS/m), we assume that field induced polarization is dominated by the much larger cell wall conductivity. Also, relative permittivity values of the cell wall (50), cell membrane (6), cell cytoplasm (60), and cell membrane conductivity (200 mS/m) were assumed fixed, while cell wall and cell cytoplasm conductivity were adjusted to fit the measured experimental cof data. This follows the assumption that the observed cell behavior is largely dominated by changes in interface and cytoplasm conductivity, which is consistent with previous experimental observations.⁶ As such, the dual cof measurements were used to determine the effect GLT had on both cell wall and cell cytoplasm conductivity.

Cell wall conductivity was analyzed for viable and cross-linked viable yeast cells. Results shown in Fig. 5(a) indicate that for both GLT-treated and untreated samples, viable cell wall conductivities were strongly dependent on media conductivity. This was observed as an increase in cell wall conductivity to a peak value at low media conductivities, followed by a monotonic decline to approximately 100 nS/cm at high conductivities. It follows that at low media conductivities, the cell protects itself against loss of electrolytes by closing ion channels, whereas at high conductivities the cell prevents ion invasion by again closing these channels. In a more physiological friendly surrounding, the cell opens ion channels, suggesting that enhanced membrane ion exchange is possible at these intermediate conductivities.

From the data, GLT appears to have affected cell wall conductivity to a small degree, which likely reduces the cell's ability to exclude the passage of small ions. This suggests that GLT-treated cells have a higher degree of ion transport over the physiological buffer conductivity range. Overall, however, because GLT has a minimum effect on viable cells there appears to be virtually no effect on their corresponding cof .

Calculated cytoplasm conductivity for viable and cross-linked viable cells is shown in Fig. 5(b). While both samples show negligible differences at low and high media conductivity, intermediate physiological conductivities produce cytoplasmic conductivity differences. Over a conductivity range at $50\text{--}200 \mu\text{S/cm}$, cross-linked viable yeast cells with high cell wall conductivity had lower cytoplasm conductivity values, while cytoplasm conductivity of untreated viable cells with lower cell wall conductivity was approximately 30% higher. It follows that GLT treatment increases cell wall permeability and leads to enhanced ion leakage and lower cytoplasm conductivity. Again, however, the resulting effect on DEP cof appears to be minimal.

Nonviable cells were also analyzed in similar fashion. As shown in Fig. 5(c), the cell wall conductivity of GLT treated nonviable cells was very different from untreated cells over the tested media conductivity range. Untreated cells exhibit a rise-fall trend over the conductivity range, peaking at approximately $150 \mu\text{S/cm}$. This trend again suggests that even nonviable cells are able to regulate cell wall conductivity in an attempt to limit ion transport. GLT treated cells, however, appear to lack this ion transport limiting feature. As shown in the figure, cross-linked cell wall conductivity increased linearly with increasing media conductivity. At media conductivities greater than $200 \mu\text{S/cm}$, the effective conductivity of the cell wall/membrane network exceeded the media conductivity; no cof was observed, a degree of freedom was lost, and cell wall conductivity could no longer be determined. As shown in Fig. 5(d), cytoplasm conductivity of untreated nonviable cells exhibited a similar exponential increase with media conductivity. However, cof_2 measurements indicate that cytoplasm conductivity of GLT treated cells actually decreased slightly with media conductivity. This is counterintuitive as treated cells appeared to lose their ability to regulate ion transport, as suggested by the observed linear increase in cell wall conductivity. One possible explanation is that increased cell permeability resulting from heat treatment allowed GLT to penetrate the cell wall, contact the underlying yeast cell membrane, cross-link cell wall and membrane proteins, and seal the membrane ion channels within the highly cross-linked cell.

B. Optimized cell separation

The above observation is important in DEP cell separation applications, as it is quite common for large variations in cell DEP mobility to occur due to time-evolved ion leakage, such as in the

FIG. 6. Comparison of Δcof for [(a) and (b)] uncross-linked and [(c) and (d)] cross-linked cells. (a) Low frequency crossover over media conductivity range for viable and nonviable cells. A small gap between measured crossover (possible separation) exists between 150 and 200 $\mu\text{S/cm}$. (b) Differences in cof observed for the high frequency crossover. Separation is possible over larger conductivity range (>150 $\mu\text{S/cm}$). (c) The observed low frequency crossover difference is much larger when the cells are cross-linked. (d) High frequency crossover data: cells have a large range of media conductivity where cell separation is possible.

DEP separation of malaria.¹⁰ By definition, DEP separation is possible when two or more species of cells and/or particles have differences in measured cof , defined as Δcof . With increasing Δcof , the separation becomes more efficient, as this will guarantee large differences in DEP mobility between each separating species. Therefore, when determining optimum separation conditions, the objective is to find media conductivity and frequency conditions which maximize DEP mobility differences. As shown in Figs. 6(a) and 6(b), a Δcof exists between viable and nonviable cells, and separation is possible. Due to active cell wall ion channel regulation, however, Δcof is very small over the observed physiological ranges where the cell is most “leaky.” This could pose a problem during separation as ion channel leakage could lead to cytoplasm conductivity changes during processing, and perhaps push one type of cell into the cof window of another type of cell, reducing separation efficiency. Based on the data, one could theoretically raise media conductivity and separate at higher frequencies. High frequency high conductivity DEP separation, however, is notoriously slow and can lead to unwanted electrochemical reactions.¹⁴

In this work, we enhance separation efficiency by utilizing what was observed during GLT treatment. As shown in Figs. 6(c) and 6(d), GLT cross-linking significantly increases both Δcof and the conductivity range for possible separation. Moreover, based on cytoplasm conductivity measurements, nonviable cells are less leaky and cytoplasm conductivity is more likely to remain stable during separation. Based on cof measurements, optimum separation conditions, where cells exhibited sharp separation with the fastest DEP response, were determined to exist at media conductivities between 70 and 150 $\mu\text{S/cm}$.

The DEP separation between untreated and GLT cross-linked cells was tested and compared using the quadrupole array. Two 1:1 viable/nonviable sample mixtures ($\sim 10^4$ cells/ml) were

FIG. 7. Effects of GLT on viable (transparent) and nonviable (blue) DEP separation in cells are suspended in media conductivity of $100 \mu\text{S}/\text{cm}$ with a field frequency of 2.5 MHz. (a) Untreated 1:1 cell mixture indicates poor separation. (b) GLT treated cell mixture has improved DEP separation over the untreated sample.

prepared—one reacted with GLT and the other untreated—and placed on the quadrupole array. Each suspension had an identical media conductivity of $100 \mu\text{S}/\text{cm}$. The device was activated, viable and nonviable cells maneuvered to their appropriate field trapping regions, and resulting separation of each was compared. As shown in Figs. 7(a) and 7(b), the resulting DEP separation of the untreated cell suspension at low frequency [Fig. 7(a)] is partially unsuccessful, as it is rather obvious that both live and dead cells appear to be dispersed in both the high and low field regions. In contrast, however, the GLT treated cells, as shown in Fig. 7(b), exhibit a much sharper degree of separation. While there are still a small number of nonviable cells collected within the high field regions, this number is substantially reduced.

V. CONCLUSIONS

Cell separation via DEP is a well-developed area of study. In this work we introduced GLT treatment as a means to increase the DEP mobility differences between viable and nonviable yeasts to improve DEP induced cell separation. GLT was shown to stabilize nonviable yeast cell cytoplasm conductivity, sealing ions within the cell and preventing leakage into the buffer solution. Viable yeast cells, however, remain unaffected by GLT treatment. Hence, GLT cell treatment can be used as an effective discrimination technique for improved DEP yeast cell separation.

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