HIGH-THROUGHPUT CELL AND PARTICLE CHARACTERIZATION USING ISO-DIELECTRIC SEPARATION

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ABSTRACT

We present a new method for characterizing the electrical properties of cells and particles across a range of both frequency and medium conductivity. Using a recently developed separation method - iso-dielectric separation (IDS) - we characterize cells and particles spanning three orders of magnitude in volume and conductivity and explore the influence of medium conductivity on interfacial electrical properties.

KEYWORDS: Electrical properties, cell characterization, dielectrophoresis

INTRODUCTION

The electrical properties of cells and particles offer insight into their composition and structure as well as provide an intrinsic handle upon which separations can be based. Over the past several decades, dielectrophoresis (DEP) [1], electrorotation [2] and impedance spectroscopy [3] have been used to characterize the electrical properties of cells. Not surprisingly, these techniques – in particular, DEP - have also proven effective for cell sorting [1]. One significant barrier in developing effective electrical sorts of cells, however, is our relatively poor understanding of cells' electrical properties and how they vary under different environmental conditions. Better understanding of how phenotype and genotype manifest themselves through the electrical properties of a cell is crucial for developing new screens.

THEORY

We recently developed a separation method, called iso-dielectric separation (IDS), that specifically sorts

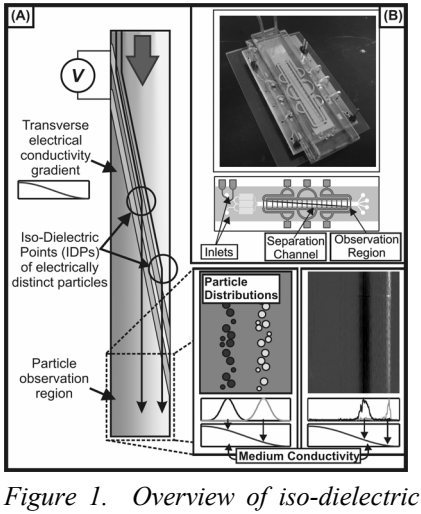

Figure 1. Overview of iso-dielectric separation. (A) Concept for sorting and characterizing particles. In an electrical conductivity (σ_m) gradient, different particles will have different dielectrophoretic equilibrium positions. By varying the operating conditions and observing the particle distributions across the conductivity gradient that results, we are able to determine the electrical properties of the particles. (B) Photograph and layout of the IDS device.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

cells based upon their electrical properties in a *continuous, size-independent* manner (Figure 1) [4]. In IDS, cells are dielectrophoretically positioned at the place in an electrical conductivity gradient where their polarizability vanishes: their iso-dielectric point (IDP). By observing distributions of particles as they flow through the device, we determine the conductivities at which they localize for a particular frequency. By combining these frequency-conductivity mappings with models for force balance throughout the device [4] and for the electrical properties of cells, we are able to measure the electrical properties of different layers within a cell or particle.

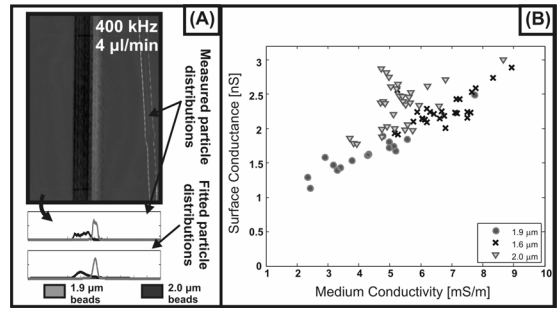

Figure 2. Characterizing polystyrene beads. (A) Observed distributions of modified and unmodified beads with different radii and the fits to these distributions (lower traces below the fluorescent images), from which we obtain the beads' surface conductances. (B) Surface conductance values for three types of beads (corresponding to those presented in (A) as well as 1.6 µm COOH-modified beads) as a function of medium conductivity.

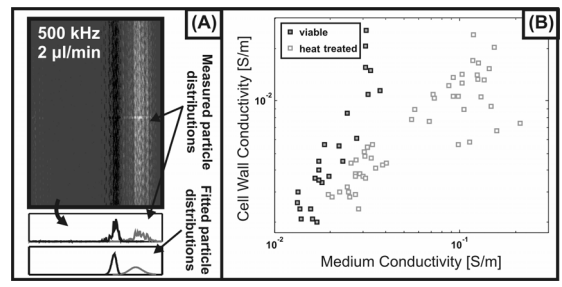

Figure 3. Characterizing the dependence of the yeast cell wall conductivity on the conductivity of the external medium. (A) Distributions of viable (dark) and heat treated (light) cells. Below the fluorescent images are the measured and fitted distributions used to determine the electrical properties of the cell layers. (B) Cell wall conductivities plotted against medium conductivity, suggesting an increase in porosity or decrease in wall thickness of a factor of ~4 upon heat treatment inferred from the slope of the two curves.

EXPERIMENTAL

Because IDS allows us to monitor the electrical responses of large numbers of cells in a medium with spatially varying conductivity, we can use it to determine the dependence of electrical properties on the conductivity of the surrounding medium. This allows us to simultaneously determine the frequency and conductivity dependence of a particle's electrical properties by varying the conditions of the separation (Figure 1). This gives experimental access to properties of cells and particles that is not afforded by traditional measurements, which are carried out in a homogeneous medium.

RESULTS AND DISCUSSION

We have applied IDS to measure the electrical properties of polystyrene microspheres (Figure 2), the budding yeast *S. cerevisiae* (Figure 3), and mouse pro B cells (Figure 4), representing three orders of magnitude in particle volume (~1-1000 μm^3) and conductivity (~0.001–1 S/m). From a fundamental biophysics standpoint, we have found that the surface conductance of the microspheres and the conductivity of the yeast cell wall both increase with the medium conductivity, as observed by others [5], while the specific capacitance and cytoplasmic conductivity of the mammalian cells appear to be independent of the medium conductivity under the conditions we use. Importantly, this suggests that the cells are able to maintain ion homeostasis over the course of the measurement, offering insight into the unperturbed properties of the cells.

CONCLUSIONS

The ability we demonstrate here to accurately characterize the properties of cells across both frequency and conductivity and, as they are being separated, relate these properties to phenotypic differences (e.g. changes in the porosity and thickness of the yeast cell wall) could enable high-throughput screens of environmental stress response across genetic libraries.

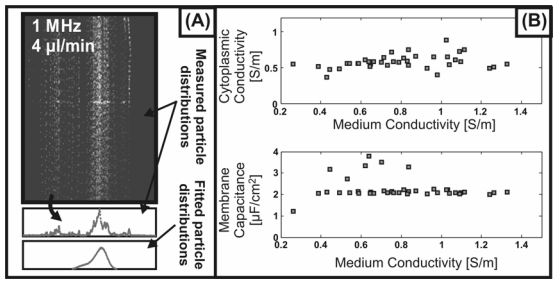

Figure 4. Measuring the cytoplasmic conductivity and membrane capacitance of mouse pro B cells using the cells' growth media as the high conductivity solution. (A) Distributions of cells with fits to determine the membrane capacitance and cytoplasmic conductivity. (B) Values for the cytoplasmic conductivity and membrane capacitance at different medium conductivities.

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