**Electrically Addressable Vesicles: Tools for Dielectrophoresis Metrology**

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Dielectrophoresis (DEP) has emerged as an important tool for the manipulation of bioparticles ranging from the submicron to the tens of microns in size. Here we show the use of phospholipid vesicle electroformation techniques to develop a new class of test particles with specifically engineered electrical properties to enable identifiable dielectrophoretic responses in microfabricated systems. These electrically addressable vesicles (EAVs) enable the creation of electrically distinct populations of test particles for DEP. EAVs offer control of both their inner aqueous core and outer membrane properties; by encapsulating solutions of different electrolyte strength inside the vesicle and by incorporating functionalized phospholipids containing poly(ethylene glycol) (PEG) brushes attached to their hydrophilic headgroup in the vesicle membrane, we demonstrate control of the vesicles’ electrical polarizabilities. This combined with the ability to encode information about the properties of the vesicle in its fluorescence signature forms the first steps toward the development of EAV populations as metrology tools for any DEP-based microsystem.

**Introduction**

Giant unilamellar vesicles (GUVs) have garnered considerable interest as both model cellular compartments and model membranes. GUVs have been used as model systems for studying a range of cellular phenomena and machinery ranging from cellular compartmentalization, actin polymerization, gene expression, and membrane fusion. GUVs serve as good model systems due to their large size (1–100 μm) and ease of visualization with conventional light microscopy. They also offer exquisite control over both their inner aqueous core and outer membrane properties. Various techniques have been explored for encapsulating particles and molecules ranging from mammalian cells to large DNA strands within the aqueous core of vesicles. Additionally, standard formation techniques for GUVs allow for the creation of complex mixtures of lipids and small molecules within the membrane. We have leveraged this inherent flexibility in the preparation of GUVs to generate vesicles with distinct electrical properties which have identifiable dielectrophoretic responses. By modulating these electrical properties, we have demonstrated the concept of electrically distinct vesicle populations to serve as test particles for dielectrophoresis (DEP)-based microsystems. We term these new test particles as electrically vesicles (EAVs).

Although much recent work on GUVs has focused on the dynamics and structure of the vesicles themselves, the potential of engineering the electrical properties of vesicles has been largely unexplored. The ability to control the composition of the aqueous core and membrane, in addition to enabling the study of membrane biophysics, also allows for the creation of vesicles spanning a wide range of electrical and mechanical properties. Accordingly, any system intended to manipulate cells or particles on the basis of their electromechanical properties can be characterized more thoroughly by using test particles specifically designed to encompass the entire range of these properties.

**DEP-based microsystems** are widely used for patterning, or sorting cells. DEP, the force on a polarizable object in a spatially nonuniform electric field, depends on the electric field and the electrical properties of the particle (assumed to be spherical) relative to the surrounding medium. The dipole contribution to the DEP force is given by the equation

\[ \mathbf{F}_{\text{DEP}} = 4\pi \varepsilon_0 a^3 \text{Re}[\mathbf{K}(\omega)] \cdot \mathbf{E} \times \nabla \mathbf{E} \]

(1)

Here, \( \varepsilon_0 \) denotes the permittivity of the medium, \( a \) denotes the particle radius, \( \mathbf{E} \) denotes the electric field, and \( \mathbf{K}(\omega) = (\sigma_p - \sigma_m)/(\sigma_p + 2\sigma_m) \) is the Clausius–Mossotti function, a dimensionless factor describing the frequency-dependent electrical properties of the particle (\( \sigma_p = \sigma + i\omega\epsilon_p \) and medium (\( \sigma_m = \sigma_m + i\omega\epsilon_m \)). Since the induced dipole moment of a particle is proportional to \( \text{Re}[\mathbf{K}] \), knowing the Clausius–Mossotti function for a specific set of particles is critical to determining the magnitude and sign

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of the DEP forces throughout a system. A prerequisite for knowing the Clausius–Mossotti function is to know the particles’ structure, electrical conductivity, and electrical permittivity.

Current test particles for DEP consist primarily of polystyrene microspheres, which are commercially available in several sizes and with various surface functionalizations (for example, carboxyl groups). While functionalized microspheres (FMs) can be further engineered with phospholipids in an effort to mimic biological membranes, they poorly mimic the electrical properties of live cells. This is largely attributable to the disparity between the bulk conductivity and permittivity of polystyrene and that of a cell’s cytoplasm; polystyrene lends the particles a negative polarizability at high frequencies (≈1 MHz) in nearly any aqueous solution. Furthermore, because the electrical properties of FMs are controlled primarily through the charge density presented at the surface of the particle, tailoring beads to exhibit a specific conductivity is generally not straightforward and may require potentially laborious sequences of reactions to achieve the desired electrical response. To circumvent some of these difficulties, it is possible to take the notion of surface modification to its extreme by coating otherwise electrically insulating particles with a conductive metal layer. These metal-coated particles then present an alternative to the low conductivity and permittivity of polystyrene relative to water. Although such particles have been successfully employed in testing systems where positive dielectrophoresis (pDEP) is essential, they are not as widely used as FMs. This may be attributable to the fact that they exhibit nearly uniform positive polarizability regardless of the frequency of the applied field or the conductivity of the surrounding medium. In addition to FMs, multilayered lipid-based particles such as multilamellar and oligolamellar vesicles have been used in electrorotation experiments to verify dielectric multishelled models for cells. While such multishelled particles can serve as surrogates for cells (particularly bacterial and yeast cells which possess a multilayered cell wall), they can be challenging to reliably replicate and are therefore not well-suited for building electrically distinct populations as test particles for DEP-based systems.

For many applications, a particle whose dielectrophoretic response is sensitive to its environment (both electric field frequency and medium electrical properties) is desirable. EAVs offer an alternative in which both the bulk properties of the particle (that is, the aqueous core) as well as its membrane may be tailored to exhibit a particular DEP spectrum. EAVs enable the generation of test particles over a large range of conductivities (varying over ~100× of physiologically relevant conductivities) which cannot be achieved easily with commercially available FMs. The additional ability to label EAVs with membrane-bound or internalized aqueous fluorescent markers allows information regarding the vesicle’s properties to be encoded so that vesicles can be visually identified. Taken together, the degrees of freedom afforded by EAVs with respect to their electrical polarizability and fluorescent signatures make these particles compelling model systems for characterizing DEP-based microsystems.

**Results**

**Creating EAVs.** We have generated EAVs with customizable electrical properties through the process of electroformation. Figure 1A shows a schematic of EAVs in which the composition of both the aqueous core and phospholipid membrane may be tuned independently (or in concert) to confer distinct electrical

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Figure 1. Electrically addressable vesicles (EAVs). (A) Schematic (not to scale) of an EAV where we have independent control over the properties of the vesicle membrane and aqueous core. Control of vesicle membrane and aqueous membrane properties results in distinct electrical polarizabilities and consequently identifiable dielectrophoretic responses. Modulating the membrane capacitance with PEG brushes (schematic, center panel) results in shifting of the DEP spectra toward lower frequencies (plot, upper panel). Modulating the aqueous core conductivity by altering the ionic strength of the encapsulated solution results in lowering the peak polarizability of the respective DEP spectra (plot, low panel). (B) Demonstration of control of membrane and aqueous core properties. Fluorescence microscopy image of a representative EAV with lissamine-rhodamine-labeled membrane. The inset shows merged phase and fluorescence microscopy images with encapsulation of fluorescein salt (green) in the aqueous core of EAVs. (C) Merged fluorescence image of a population of differentially labeled EAVs: lissamine-rhodamine (red, lipid), carboxyfluorescein (green, lipid), and dansyl PE (blue, lipid). Scale bars: 50 µm.


properties to a vesicle population. The electrical properties of the aqueous core are readily tuned in the electroformation process, by controlling the conductivity of the electroformation buffer and thereby conferring a specific internal conductivity, $\sigma_c$, to the formed vesicles. Therefore, by leveraging the electroformation process, it is straightforward to vary the value of $\sigma_c$ over a broad range. Specifically, we have successfully formed vesicles in solutions ranging in conductivity from $10^{-4}$ S/m (deionized water) to $\sim 1.5$ S/m ($1 \times$ phosphate buffered saline, ionic strength $\sim 150$ mM), though we have observed that the size and yield of electroformed vesicles diminishes at higher conductivities (typically above $\sim 0.1$ S/m). Without significantly altering the conductivity or size distribution, we have also encapsulated fluorescent dyes, including fluorescein (Figure 1B, inset) and rhodamine. The encapsulation of fluorescent dyes facilitates the tagging of vesicles of specific conductivity with an identifiable fluorescence spectrum.

An additional degree of freedom in creating vesicles with distinct dielectric spectra is conferred by the phospholipid membrane. Long chain polymers (such as PEG) can be conjugated to the membrane bilayer to change its dielectric properties. Poly(ethylene glycol) (PEG) brushes serve to modulate the effective membrane thickness and consequently the membrane capacitance, conferring unique electrical properties to a vesicle population. Similar to the aqueous core, the vesicle membrane is also capable of supporting fluorescent labels. Figure 1B depicts a representative vesicle formed with a fluorescein-labeled phospholipid, and Figure 1C shows a merged fluorescence image of sample EAV populations and indicates the ability to optically distinguish between differentially labeled EAVs. The ability to label vesicles not only allows us to encode information about the properties of the EAVs in their fluorescence signature but also facilitates the tracking and visualization of EAVs in Microsystems.

**Biasing EAV Size.** As is evident from Figure 1B, electroformation techniques yield EAVs with a wide range of sizes. To narrow the size distribution of EAVs and tailor them to the DEP system being characterized, we performed filtrations using syringe-attached inline glass frit filters (of 5 and 10 µm pore sizes). Figure 2A shows quantitative results for sizes of EAVs filtered through 5 and 10 µm pores (where sizes were measured using light microscopy). Filtration through a 5 µm pore allows for narrowing the mean EAV size from 4.6 to 1.8 µm, whereas filtration through a 10 µm pore tunes the EAV size from 4.6 to 3.4 µm. The fluorescence images in Figure 2B show qualitative agreement with these results. The top panel shows unfiltered EAVs (labeled with lissamine-rhodamine for fluorescence visualization), and the middle panel shows a fluorescence image after filtration through a 5 µm pore where few large (>10 µm) vesicles are visible, indicating that they were blocked or ruptured in the glass frit filter. The bottom panel shows a representative fluorescence image after filtration through a 10 µm pore where several large vesicles are still visible. These results indicate that filtration can bias the EAV size distribution and thereby create vesicle populations that are amenable for use in Microsystems.

In the following sections, we describe the ability to use polydisperse EAV populations for use as test particles for DEP.

**EAVs as Test Particles.** The structure of electroformed GUVs, comprising an internal aqueous core surrounded by a lipid bilayer membrane, lends these particles a dielectric spectrum which is qualitatively (and potentially quantitatively) similar to that of live cells. This spectrum is characterized by both high- and low-frequency dispersions and, in the case of EAVs, may be controlled through the conductivity of the aqueous core, $\sigma_c$. By varying $\sigma_c$, it is possible to create test particles with specifically engineered spectra; this is in contrast to many widely available alternative test particles, including both metallic and polystyrene beads, which tend to have uniformly negative and positive dielectrophoretic spectra, respectively. Accordingly, it is possible to create EAVs for which the transition from negative to positive polarizability occurs at a controlled frequency (Figure 3A).

In addition to offering spectra distinct from commercially available microspheres, EAVs offer the ability to create suspensions of particles that are both visually and electrically distinct by varying the membrane-bound fluorophore and the conductivity of the internal solution. Figure 3A shows an experiment in which EAVs labeled with rhodamine (red) and carboxyfluorescein (green) and encapsulating different conductivities (0.1 and 80 mS/m, respectively, with a medium conductivity of 40 mS/m) respond differently to the application of an electric field at a frequency of 1 MHz. In this part of the spectrum, vesicles with internal conductivities in excess of the medium conductivity ($\sigma > \sigma_m$) exhibit positive polarizability and are attracted to the electrode edge, whereas vesicles with less conductive cores are negatively polarizable and are repelled to the regions above and between the electrodes. Figure 3B presents a related demonstration, where we used red fluorescent polystyrene microspheres to demonstrate the more complex electrical properties that EAVs can present. While the microspheres exhibit a flat dielectric spectrum over a broad range of frequencies, the EAVs undergo a dispersion at $\sim 200$ kHz, going from negative polarizability at 50 kHz to positive polarizability at 500 kHz. Figure 3C illustrates in greater detail the dielectrophoretic spectrum of an EAV suspension comprising a range of sizes. At 50 kHz, these vesicles have a strong negative polarizability, which weakens as the frequency is increased to 100 kHz. By 500 kHz, nearly all vesicles exhibit positive polarizability, a condition that persists up to about 2 MHz, where the higher of the two crossover frequencies is observed. These dielectrophoretic spectra demonstrate the ability of EAVs to encompass a more complex set of electrical properties than other commonly used test particles for DEP.

**Determining Membrane Characteristics from Crossover Frequency.** To characterize the electrical properties of individual EAVs and to compare these properties to those predicted by established theories for single-shelled spherical particles, we
The form of eq 2 comes from the standard formulation for a sphere bounded by a thin membrane. Here, we have combined the membrane properties into a single capacitance-per-unit-area \( \varepsilon_{\text{mem}} \equiv \varepsilon_{\text{mem}} \). We neglect the effects of membrane conductance \( g_m \) on the assumption that it has little effect on the membrane’s electrical properties. We have neglected any contribution arising from the vesicles’ surface conductance; for a vesicle with a surface conductivity of -0.1 nS (typical for a charged bilayer membrane) and a radius of -1 \( \mu \)m, the surface conductance will contribute -0.2 mS/m to the particle’s overall conductivity, roughly -1% of the typical conductivities used in our experiments. To simplify eq 2, we begin by addressing only the low-frequency behavior of the polarizability. This allows us to focus more directly on the contribution of the membrane to the vesicles’ dielectrophoretic response. In this frequency regime, eq 2 simplifies to

\[
K(\omega) = \frac{-\sigma_m \sigma_c + i\omega \varepsilon_c (\sigma_m - \sigma_c) - (\sigma_m \varepsilon_m + \sigma_m \varepsilon_c)}{2 \sigma_m \sigma_c + i\omega \varepsilon_c (\sigma_m + 2 \sigma_c) + 2 (\sigma_m \varepsilon_m + \sigma_m \varepsilon_c)}
\]

where \( \sigma \) represents the radius of the vesicle and \( i = (-1)^{1/2} \). This expression is valid provided the frequency is significantly lower (that is, by about one decade) than the inverse of the charge relaxation times for both the vesicle interior and exterior \( (\omega \ll \sigma_c / \varepsilon_c \text{ and } \omega \ll \sigma_m / \varepsilon_m, \text{ respectively}) \). Using eq 3 and setting \( \text{Re} \{K(\omega_0)\} = 0 \), we find an expression for \( \omega_0 \), the lower of the two possible crossover frequencies permitted by eq 2:

\[
\omega_0^2 = \frac{2 (\sigma_m \sigma_c)^2}{\varepsilon_c \varepsilon_m (\sigma_m - \sigma_c) (\sigma_c + 2 \sigma_m) + 2 (\sigma_m \varepsilon_m + \sigma_m \varepsilon_c)}
\]

To better understand the properties of the EAV membrane, it is convenient to rearrange eq 4 so as to relate the crossover frequency, which we observe directly, to \( \delta \), the membrane thickness:

\[
\delta = 2 \varepsilon_c \varepsilon_m \left( \frac{\sigma_m}{\sigma_c} - 1 \right) \left[ \frac{\sigma_m (\sigma_c + 2 \sigma_m)}{\varepsilon_c (\sigma_m + \sigma_m \varepsilon_c)} \right]^{1/2} - 1
\]

Measuring crossover frequency across different core and medium conductivities \( (\sigma_c \text{ and } \sigma_m) \) and across different batches of electroformed EAVs yields an effective membrane thickness of 9.3 ± 4.3 nm. The sensitivity of \( \delta \) to \( \omega_0 \) for typical parameters is such that a 10% change in crossover frequency produces a change of ~8% in predicted membrane thickness. Our measurements are in reasonable agreement with the expectation for the thickness of a lipid bilayer and support the validity of this simple model for vesicle polarizability. Specifically, we find that extracted
values of $\delta$ are fairly independent of the vesicle radius (correlation coefficient: $-0.0026$ across 80 individual vesicles) as well as the crossover frequency (correlation coefficient: 0.18). While low coefficients of correlation are not sufficient to prove independence, they do suggest that our measurements are consistent with the functional dependencies of $\delta$ on these parameters given by eq 5. Additionally, we find that the specific fluorophore incorporated in the membrane does not lead to a change in electrical properties, with carboxyfluorescein-labeled and rhodamine-labeled vesicles exhibiting no observable difference in crossover frequency (not shown).

**Modulating Membrane Capacitance.** In addition to the ability to control the electrical properties of the EAVs through their aqueous core, we also demonstrate the ability to modify the vesicles' dielectric spectra through the structure and composition of their membrane (Figure 4A). Since the thickness and dielectric constant of the lipid membrane are fairly constant over a wide range of lipids, we decided to alter the membrane properties by incorporating into the membrane lipids conjugated with polymers, which would increase the effective membrane thickness while also altering its effective dielectric constant. We incorporated 5 mol % PEG-modified fluorescently tagged lipid into our membranes to verify this hypothesis. The presence of a fluorescence signal in the membrane (Figure 4A, inset) indicates that the PEG-modified lipid indeed organizes into the membrane of the vesicle. Additionally, we counterstained PEGylated EAVs with lissamine-rhodamine-labeled fluorescence to confirm that the incorporation of PEG is comparable across vesicles of different sizes. Specifically, we used image processing techniques to quantify the average fluorescence in the membrane for vesicles ranging in size over an order of magnitude. We found that the average fluorescence intensity over this size range is within 5%, indicating that the PEG-lipid concentration across vesicles is uniform (data not shown). As shown in Figure 4B, these membrane-modified EAVs displayed different electrical properties than unmodified EAVs. Using the same method for measuring the sizes and crossover frequencies of individual EAVs and interpreting the results in the context of a single-shell spherical model (eq 5), we are able to discern changes in the low-frequency dielectrophoretic response of the EAVs. While the unmodified EAVs exhibit crossover frequencies consistent with the previously discussed single-shell model, PEG-functionalized EAVs deviate from this pattern suggesting that a more complex model is needed to accurately describe their electrical properties, such as a multishell model incorporating separate lipid and PEG layers. As mentioned previously, when fit to the single-shell model, we extract a membrane thickness for the unmodified vesicles that is independent of particle radius and consistent in value ($\sim5-10$ nm) with expectations for a phospholipid bilayer. In contrast, extracted membrane thicknesses for PEG-functionalized vesicles increase with vesicle radius. We are able to reliably detect the increase in crossover frequency of PEG-functionalized EAVs over their nonfunctionalized counterparts associated with this change; however, the increasing extracted membrane thickness suggests that the single-shell model of eq 5 with the membrane thickness $\delta$ independent of all other parameters is no longer sufficient to describe the membrane capacitance of PEG-functionalized EAVs. This may be attributable to differences in PEG conformations within membranes of different curvature or to the effects of PEG on membrane morphology. Additionally, we electroformed PEG-functionalized EAVs with low concentrations (0.1 mol %) of PEG. Crossover frequency measurements were made with these EAVs, and the results were compared to those predicted from a single-shell model. Effective membrane
thicknesses extracted from these comparisons yielded 8.4 ± 2.3 nm, which is of the same order as those of EAVs without incorporation of PEG. This suggests that a mole percentage of at least a few percent PEG may be necessary to obtain particles with a well-organized PEG-brush and consequently a significantly altered membrane capacitance.

To determine the predicted PEG-functionalized EAV membrane thickness we considered the physicochemical properties of PEG polymers that would govern their morphology and organization within the lipid bilayer membrane. The physicochemical aspects of lipid membranes with grafted polymers have been the subject of considerable past study. Based on the concentrations and molecular weight of PEG-lipid used in our experiments, we expect that the PEG organization structure is above the “mushroom-to-brush” structural transition point. Hence, in our case, the PEG-lipids assume a stretched, brushlike configuration in which the PEG chains extend out from the membrane surface. As previously determined, the transition between the mushroom and brush regimes occurs at the concentration of PEG-lipid for which the surface-associated polymer chains first begin to overlap. This condition is fulfilled at mole fractions of polymer lipid given by $\chi_{\text{PEG}} = (A_{f}/\pi a_{m}^{2}) - n_{p}/a_{m}$, where $A_{f}$ is the membrane surface area per lipid molecule, $a_{m}$ is the size of the monomer unit, and $n_{p}$ is the degree of polymerization. Based on these parameters (which are extensively explored by Marsh et al.), the mushroom-to-brush transitions for PEG lipids of a molecular weight of 2000 (as in our case) occurs at mole percentages of 0.5% above (the mushroom configuration is typically only relevant for low molecular weight lipids with short chain lengths). Further, the anticipated membrane thickness can be derived from the minimization of the free energy of the membrane and is determined as previously described by $L \approx n_{\text{PEG}}^{5/3}(X_{\text{PEG}}/A_{1})^{1/3}$. This effective PEG brush thickness $(L)$ is determined from the above equation as 5.02 nm, using $n_{p} = 45$ (from Marsh et al.), $a_{m} = 0.39$ nm (determined from the monomer volume in aqueous solution), $X_{\text{PEG}} = 0.1$ (from Marsh et al.), and $A_{1} = 0.65$ nm$^{2}$ (from Marsh). This is in good agreement with X-ray diffraction measurements of the thickness of comparable PEGylated distearoyl-phosphatidylcholine (DSPC) lipid bilayer measurements which report a thickness of 5.6 nm.

As the PEG brush extends outward from both the inner and outer leaflets of the membrane, it is incorporated twice (∼10 nm total brush thickness) along with the estimation of the effective membrane thickness of an unmodified EAV (as ∼5 nm) yielding ∼15 nm total estimated membrane thickness. Further, this anticipated membrane thickness fits well with our measurements of effective membrane thickness of PEGylated EAVs of ∼20 nm (for smaller vesicle sizes, as seen in Figure 4B). The discrepancies of larger effective membrane thickness for larger PEGylated EAVs (as seen in Figure 4B) could arise from steric interactions of PEG polymers in the brush regime. Such interactions have been known to exert lateral pressures to expand the lipid membrane and have been experimentally verified.

Discussion

Our results demonstrate the feasibility of creating populations of particles with distinct and controllable optical and electrical properties. These results should open new avenues in both the characterization of dielectrophoretic traps and in the electrical manipulation of vesicles. Previously, DEP traps have been primarily characterized with polystyrene microspheres, which prove to be constraining in their electrical properties. Additional functionalizations and surface chemistry have been performed, but they can involve considerable complexity, which makes the route to multiplexing more difficult. Conductive test particles such as metal or metal-coated microspheres are challenging to fabricate and are commercially available in a very limited size range. Thus, most systems that leverage these highly conductive particles use pDEP and are limited to nanometer-scale particles, for example, for self-assembly. Hence, EAVs fill a much needed void in the application space in metrology tools for characterizing DEP traps.

We have shown that EAVs have unique advantages over conventional test particles. However, they do have some limitations that impact the design of electrically distinct populations, specifically the size polydispersity that results from electroformation. Nonetheless, electroformation has been the predominant technique for the generation of vesicles even though several alternate techniques for formation of vesicles have emerged. These techniques range from lipid film patterning to pulsatile jet flow and microfluidic sheathing flows. To the best of our knowledge, none of these techniques are capable of producing vesicles of very large size (>100 μm), which would preclude their use in DEP-based Microsystems for manipulating cell-sized bioparticles in the 1–50 μm size range. The generation of monodisperse vesicles in the size range of typical cells continues to be a major research challenge. To circumvent this challenge, we describe techniques for the use of EAVs that are applicable even in the case of polydisperse vesicle populations.

Since electroformation is the predominant method of vesicle formation, it is important to understand how to design desired electrical properties in vesicle populations in the presence of size polydispersity. Size impacts electrical properties by affecting the membrane capacitance of the resulting particle; specifically, larger vesicles exhibit larger membrane capacitances and thus their low frequency dispersion occurs at lower frequencies than that of smaller vesicles. Equation 3 shows that the crossover frequency may vary strongly with variations in the vesicle radius, $a$, with $\omega_{0} \propto a^{-1}$ for larger vesicles (typically corresponding to $a \sim 1–10$ μm). Thus, to design a population with a known dielectric response, one approach is to operate at the plateaus of the dielectric spectra rather than near the crossover frequency, since the plateaus are less affected by size (Figure 3C).

Alternatively, operating at high frequency allows the electrical properties of the vesicle to be dominated by the vesicle’s internal conductivity and permittivity, which are very well controlled. For intermediate to high frequencies, the crossover frequency is determined approximately from

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For sufficiently large vesicles (for which $ac_m \gg \epsilon_m$, $\epsilon_c$), eq 6 becomes independent of the vesicle size, reducing to $\omega_0^2 = (\epsilon_c - \epsilon_m)(a + 2\epsilon_m)(\epsilon_m - \epsilon_c)^{-1}(\epsilon_c + 2\epsilon_m)^{-1}$.

The creation of two distinct EAV populations is most easily achieved by selecting the internal conductivity of one vesicle to be below that of the surrounding medium so that its polarizability is uniformly negative at all frequencies, while the other vesicle population, with an internal conductivity higher than that of the surrounding medium, will exhibit polarization that will be positive over some (programmable) frequency range. The difference in the internal conductivity of the vesicle and that of its surrounding solution can result in an osmotic gradient. A vesicle is in osmotic equilibrium when the osmotic pressure drop arising from the solute concentration difference is balanced by the Laplace pressure arising from the tension of the membrane. This leads to the equilibrium condition $RT(c_i - c_o) = -2\gamma/a$, where $R$ is the ideal gas constant, $T$ is the ambient temperature, $c_i$ and $c_o$ are the internal and external osmolarities, respectively, $\gamma$ is the membrane tension, and $a$ is the vesicle radius. In our experiments, the difference in osmolarity is on the order of 0.5 mM (1× PBS diluted by a factor of ~300x), with the higher osmolarity typically inside the vesicle, driving the flux of water into the vesicle and causing it to swell. For the membrane tension, we assume that the membrane tension $\gamma = \kappa\langle AA/A \rangle$ is the product of the elastic area expansion modulus ($\kappa$) and the fractional change in membrane area. For a 20 µm diameter vesicle with $k \sim 360$ dyn/cm, the fractional change in area is under 3.5%, corresponding to a 5% increase in vesicle volume that would create a corresponding decrease in the internal conductivity, in cases where the internal and external conductivities differ by ~2x, this does not significantly influence the vesicles’ dielectrophoretic response. Accordingly, in interpreting our experiments, we have neglected this effect. Since vesicles typically cannot withstand fractional area changes of ~10% (a volume change of ~15%), vesicles will typically rupture before the flux of water entering the vesicle has a substantial effect on the internal conductivity. Furthermore, osmotic stress can be mitigated altogether through the addition of nonionic solutes (for example, sucrose or glucose) to the internal or external phases.

Besides limitations on the osmolarity difference between internal and external solutions that the vesicle membrane is able to support are considerations regarding the electrical permittivity of the internal and external solutions that can be used. Specifically, the use of solvents significantly less polar than water can fundamentally interfere with the stability of the vesicle membrane. Accordingly, the degree of control one may exercise over the core and medium permittivities of the EAVs, $\epsilon_c$, and $\epsilon_m$, respectively, is much more limited than that possible for their corresponding electrical conductivities.

In the case where more than two vesicle populations with distinct spectra are desired, the choice of the internal conductivity is constrained by the range of crossover frequencies, $\Delta \omega_{0\sigma}$, exhibited across each subpopulation. Specifically, one wishes to pick internal conductivities that result in EAVs without overlapping dielectrophoretic responses over as large a range of frequencies as possible. For simplicity, we express the internal conductivity as a multiple of the medium conductivity ($\sigma_i = k\sigma_m$, with $k > 1$). To estimate the implications of this requirement, one can approximate the variation in $\omega_0$ associated with variations in both vesicle size and internal conductivity. Equating these variations and assuming $\epsilon_i \approx \epsilon_m$ gives

$$\frac{d\omega_0}{\omega_0} \sim \frac{[ac_m(\epsilon_i - \epsilon_m) - (\epsilon_i + 2\epsilon_m)]}{[ac_m(\epsilon_m - \epsilon_c) + \epsilon_m\epsilon_c][ac_m(\epsilon_i + 2\epsilon_m) + 2\epsilon_m\epsilon_c]}$$

(6)

By narrowing the size distribution of the EAVs, and choosing the internal conductivity as prescribed approximately by eq 7, it is possible to create suspensions comprising EAVs undergoing dispersions in distinct ranges of frequency. Particularly useful values of $k$ are those for which the crossover frequency is more sensitive to variations in internal conductivity than to variations in size (see the Supporting Information). For example, a vesicle with $a = 2$ µm, $\epsilon_m = 0.002$ F/m², and $\epsilon_o = 80\epsilon_0$, will be more sensitive to changes in internal conductivity when the internal conductivity is greater than that of the surrounding medium by a factor of ~1.8 or less, with the caveat that if $k$ is too close to unity the crossover frequency will no longer exist ($k \sim 1.45$ for these parameters). Alternatively, as described in eq 5, operating at high frequencies allows one to form multiple electrically distinct vesicles largely independent of the size distribution of the population. Accordingly, even in the presence of considerable polydispersity, it is possible to create populations of particles undergoing transitions from positive to negative polarizability over narrow frequency ranges.

Further control is made possible by modulating the vesicle’s membrane capacitance at the same time as its interior conductivity, enabling the creation of EAV populations in which each type of vesicle has a frequency range over which it is the most polarizable member of the population. Increasing the membrane capacitance shifts the spectrum to lower frequencies, whereas decreasing the internal conductivity reduces the peak polarizability of the spectra. A population of EAVs for which $\epsilon_m$ and $\sigma_i$ are controlled to vary inversely produces a set of spectra in which each member of the population exhibits the maximum polarizability within the population over a particular range of frequencies.

The modification of giant vesicle membranes with polymers has been the subject of considerable past study, specifically in the application of studying the interface of polymers on membrane curvature. Additionally, polymers have been encapsulated within the aqueous phase of giant vesicles and used to study compartmentalization in aqueous two-phase systems. Thus, polymers such as PEG have served roles in the modification of both the internal aqueous phase properties and external membrane properties. By decorating vesicles with PEG molecules, we have further extended the use of polymer functionalizations to affect changes in the electrical properties of vesicles. This ability to modulate the specific capacitance of membranes has implications from the study of fundamental properties of membranes (and the organization of polymers within membranes) and the use of polymer-functionalized vesicles as electrically distinguishable surrogate cells for DEP-based manipulation and separation.

Besides the sign of a particle’s polarization in an electric field, a second means of addressing particles is applicable to the manipulation of their polarizability. If we define the dielectrophoretic polarizability

$$\epsilon_{polarizability} = \kappa/\epsilon_m$$

velocity of a particle as the dielectrophoretic force divided by the Stokes drag coefficient, we have that $U_{\text{DEP}} \propto a^2 \text{Re} \{ K(\omega) \}$. The dependence of $\text{Re} \{ K \}$ on vesicle size at relatively low frequencies can be determined from eq 3, with smaller particles typically exhibiting more negative polarizabilities. It is thus possible to minimize the sensitivity of the dielectrophoretic velocity on particle size by choosing a frequency such that $\partial U_{\text{DEP}}/ \partial a \approx 0$ over the targeted size range. For general sizes and conductivities of interest ($a \sim 1$–10 μm and $\sigma_c > \sigma_m$), optimal insensitivity to size is obtained by selecting the frequency according to

$$\omega \approx \frac{2\sigma_m \sigma_c}{(\sigma_c + 2\sigma_m) \left[ \left(1 + \frac{1}{2} \frac{\sigma_c + 2\sigma_m}{\sigma_c - \sigma_m} \right)^{1/2} - 1 \right]^{1/2} \frac{1}{\sigma_m}}$$

Although this does not remove the dependence of $U_{\text{DEP}}$ on size for an arbitrarily large range of sizes, it does improve it significantly. For example, using core and medium conductivities typical for our experiments and operating at a frequency of ~350 kHz, eq 8 predicts that vesicles in a size range from 3 to 7 μm will exhibit dielectrophoretic velocities within 60% of each other, compared to a greater than 5-fold difference for particles for which $\text{Re} \{ K \}$ is independent of particle size.

In all, the ability to generate populations of vesicles with distinct polarizabilities opens new avenues for the study of electric field interactions with phospholipid vesicles. Previous work has focused on using electric fields to apply forces to vesicles to study deformations of phospholipid membranes, complex mechanisms of membrane fusion, and alterations in vesicle morphology. Thus, considerable effort has focused on using electric fields to study vesicles and membranes. Our work opens an avenue of investigation in the use of vesicles to study electric field phenomena, providing the ability to characterize systems that use electric field based manipulation techniques (such as DEP). Vesicles can now find valuable applications as surrogates for living cells in the characterization of microscale cell manipulation devices.

Conclusions

We have demonstrated the ability to generate EAVs as test particles for DEP-based microsystems. These vesicles can be specifically engineered to allow for their dielectrophoretic manipulation in microsystems. We have demonstrated the ability to create electrically distinct particles using crossover frequency measurements with a canonical DEP device. Further, we have shown that it is possible to alter the effective membrane thickness of EAV membranes using PEG-conjugated lipids. This shows that vesicles allow considerable control over their chemical composition (and therefore electrical properties) and lend themselves well to the generation of electrically distinct populations of test particles.

We believe these proof-of-principle studies demonstrate that the electrical properties of vesicles can be specifically engineered to allow their dielectrophoretic manipulation. Moreover, the generation of EAVs form the first steps toward the development of vesicles as metrology tools for DEP-based microsystems.

Materials and Methods

EAV Electroformation. EAVs were prepared using a previously described electroformation technique. Briefly, electroformation was performed in a chamber consisting of two 50 × 75 mm² indium tin oxide (ITO) slides (SPI Supplies) separated by a 1 mm thick silicone gasket (Press-to-seal gaskets, Invitrogen). ITO slides were cleaned in 1% Micro-90 solution, followed by ultrasonication 2× in acetone and rinsing by ultrasonication 2× in isopropyl alcohol, and subsequently dried with a nitrogen stream. 1-Stearoyl-2-oleoyl-glycero-3-phosphocholine (SOPC, Avanti Polar Lipids) stock solutions at 10 mg/mL concentration were first diluted to 1 mg/mL concentration (in chloroform) and subsequently pipetted on the bottom slide of the electroformation chamber. The lipid solution was then allowed to dry for approximately 1 h in a vacuum desiccator. The internal aqueous solution was pipetted in the gasket reservoirs and then capped with the top ITO slide (with the conducting surfaces facing each other) and clamped with binder clips. A sinusoidal waveform (2 V p-p, 10 Hz) was applied for 2 h. For the preparation of fluorescently labeled EAVs, 1 mol % lissamine-rhodamine phosphadylethanolamine, fluorescein phosphadylethanolamine, or dansyl phosphadylethanolamine (respectively, Lissamine rhodamine PE, Fluorescein PE, and Dansyl PE, Avanti Polar Lipids) was added to the 1 mg/mL SOPC solution prior to vacuum drying. For the generation of PEG-conjugated EAVs, 5 mol % 1,2-distearoyl-glycero-3-phosphoethanolamine-N-polyethylene-glycol-2000-carboxy-fluorescein (DSPE PEG2 CF, Avanti Polar Lipids) was added to the 1 mg/mL SOPC solution prior to vacuum drying. For the generation of counterstained PEG-conjugated EAVs, 5 mol % 1,2-distearoyl-glycero-3-phosphoethanolamine-N-polyethylene-glycol-2000-carboxy-fluorescein and 1 mol % lissamine-rhodamine phosphatidylethanolamine was added to 1 mg/mL 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC, Avanti Polar Lipids).

Light Microscopy. EAVs were imaged immediately after electroformation on an inverted microscope (Axiovert 200, Carl Zeiss Microimaging) using phase microscopy. Once vesicle formation was confirmed, vesicle suspensions were aspirated from the gaskets and resuspended in iso-osmolar glucose solutions (50 mM). The difference in density allowed for the vesicles to settle to the bottom of the chamber and allowed for ease of imaging on an inverted microscope using both phase and fluorescence microscopy. All phase images were captured at 8-bit resolution using a cooled camera (SPOT RT, Diagnostic Instruments). Fluorescence illumination was provided by an XCite 100 (EXFO Life Sciences and Industrial Division) illumination source. EGFP (Set 38, Carl Zeiss MicroImaging), Texas Red, and DAPI filter sets (31000 and 31002, Chroma Technology) were used for imaging green fluorescent, red fluorescent, and blue fluorescent vesicles, respectively. Fluorescence images were captured at 12-bit resolution using cooled cameras on both inverted and upright microscopes (SPOT RT, Diagnostic Instruments and Imaging). A schematic depicting this image processing technique has been included in the Supporting Information (Figure S1).

Interdigitated Electrode Array Design and Fabrication. Interdigitated electrode (IDE) arrays were fabricated using a standard gold lift-off process on Pyrex substrates that has been previously described. Briefly, 6 in. Pyrex substrates were cleaned in a piranha solution and subsequently rinsed. Image reversal resist (AZ5214, Clariant) was spun on the wafers and the wafers were prebaked at 90 °C for 30 min. Subsequently, wafers were exposed at 10 mJ/cm² through a custom-designed chrome photolithography mask (Filineline Imaging) for 5 s and then postbaked for 30 min at 120 °C. Wafers were then flood exposed for 10 s and developed for ~1 min. A total of 100 Å of titanium and 1000 Å of gold were then deposited using an electron-beam deposition system. The resist was subsequently removed by immersion in an acetone bath overnight. The wafers were then diced with a diamond saw to yield individual chips. 

![Image](http://example.com/image.png)

**Interdigitated Electrode Array Packaging.** For our IDE devices, we packaged individual chips by mounting them on glass slides using double-sided tape. We then affixed the slides to a standard upright microscopy stage insert. We used laser-cut PDMS gaskets (250 µm thick, Bisco Silicones Inc.) around the active chip areas to form flow chambers. We then filled the chambers with vesicle suspensions and capped them with coverslips. We made electrical connections to the on-chip electrodes using alligator clips and delivered signals using an arbitrary waveform generator (Agilent 33250A).

**Crossover Frequency Measurements.** We measured the crossover frequencies of vesicles and fluorescent polystyrene beads (F8834, Invitrogen) using an interdigitated electrode array in which the pitch and spacing of the electrodes are both 50 µm. By diluting the EAV solution (with some volume fraction of EAVs) with deionized water at a ratio of 2:1, we set the conductivity of the external medium to approximately half that of the vesicle interior. We pipetted ~10 µL of the diluted EAV suspension into a laser-cut silicone gasket placed over the electrode array and sealed the chamber with a glass coverslip. The device was placed under a fluorescence microscope (Zeiss Axiolmager, Carl Zeiss MicroImaging) and imaged using a 20× (0.5 NA) objective. Individual EAVs were selected so as to broadly sample the total distribution of sizes, down to approximately 1 µm. The electrodes were then activated at 3–5 Vp-p and frequencies ranging from ~50 kHz to 1 MHz while we observed the behavior of the vesicle. We narrowed the range of excitation frequencies until the vesicle exhibited minimal response to the application of the electric field. We supplemented this determination of the crossover frequency with an image of the vesicle, from which we determined its radius.

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**Supporting Information Available:** Plot showing the EAV core conductivity sensitivity as a function of changes in EAV radius and image processing of PEGylated EAVs to confirm uniform PEG-lipid incorporation in EAVs. This material is available free of charge via the Internet at http://pubs.acs.org.

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