VESICLE LIBRARIES – TOOLS FOR DIELECTROPHORESIS METROLOGY

Salil P. Desai, Michael D. Vahey and Joel Voldman
Massachusetts Institute of Technology, USA

ABSTRACT

We demonstrate the use of phospholipid vesicle electroformation techniques [1] to develop a new class of metrology tools for systems leveraging dielectrophoresis (DEP) to manipulate, pattern, and sort micron-scale particles. These cell-sized phospholipid vesicles, commonly called giant unilamellar vesicles or GUVs, possess specifically engineered electrical properties and thus exhibit identifiable dielectrophoretic responses in microfabricated systems.

Keywords: Vesicles, dielectrophoresis, libraries

1. INTRODUCTION

Dielectrophoresis (DEP) has emerged as an important tool for the manipulation of bioparticles ranging from submicron to the tens of microns in size. Despite the wide biological applicability of microfabricated systems for DEP, the particles available for their characterization serve as poor models for living cells. For example, polystyrene microspheres commercially available in several sizes and surface functionalizations (e.g. carboxyl groups) are the most widely used test particles for characterizing DEP systems. While functionalized microspheres (FMs) can be further engineered with phospholipids in an effort to mimic biological membranes [2] their density, rigidity, conductivity and permittivity are generally quite different from those of biological cells.

As an alternative to FMs, we explore the use of phospholipid vesicles as test particles for DEP. The close parallels between the physical structure of these synthetic particles and live cells make them ideally suited as substitutes for studying the electromechanical response of cells to external electric fields. Importantly, these cell-sized GUVs are straightforward to synthesize and can be customized to exhibit a range of fluorescence and electrical properties by modulating the composition of their aqueous interior and lipid bilayer membrane.

2. THEORY

Electroformation of GUVs has been studied extensively for over 20 years. Notably, because this technique offers flexibility in the choice and composition of both polar and non-polar phases, it is possible to simultaneously control the fluorescence and electrical properties of both the aqueous core and membrane of the vesicles. For example, electroformation enables the encapsulation of electrolytes spanning several orders of magnitude of physiologically relevant electrical conductivities. The impermeability of lipid bilayers to charged species allows these vesicles to retain their electrical properties when resuspended in osmotically balanced solutions of differing conductivity. Incorporating water-soluble fluorescent molecules inside the vesicles visually encodes their electrical conductivity, making the creation and use of large libraries tractable. Finally, conjugating polymeric molecules to the phospholipids comprising the membrane and adding

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hydrophobic fluorescent markers to the lipid film prior to electroformation lends control over the specific capacitance of the membrane and offers an additional means of visually encoding information (Figure 1).

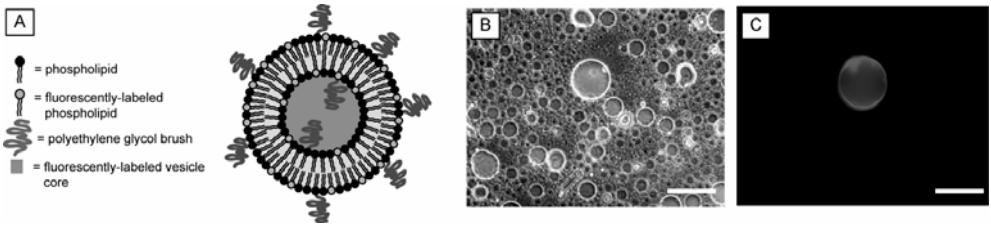

Figure 1: Vesicle libraries (A) Schematic (not drawn to scale) of the concept of a vesicle library where we have independent control over the properties of the vesicle membrane and aqueous core. Fluorescence microscopy images of electroformed vesicles containing fluorescent dyes in the aqueous core (B) and phospholipid membrane (C). Scale bar 50 μm.

3. EXPERIMENTAL

We characterize the electrical properties of the GUVs by performing cross-over frequency (COF) measurements on the vesicles using interdigitated electrode (IDE) arrays. By determing the frequency and medium conductivity at which the DEP force vanishes, we compare the properties of our vesicles with the predictions of widely used models for the electrical properties of layered particles. We perform these measurements on vesicles with different sizes, internal conductivities, and membrane composition to infer from the existing models the effective thickness of the lipid membrane.

4. RESULTS AND DISCUSSION

To demonstrate the capabilities of vesicle-based metrology we construct vesicles encapsulating different conductivity solutions. Figure 2 shows electroformed vesicles labeled with red and green fluorescent dyes randomly oriented on an IDE array. Application of a 1 MHz waveform results in the more polarizable green vesicles undergoing positive DEP and the less polarizable red vesicles undergoing negative DEP.

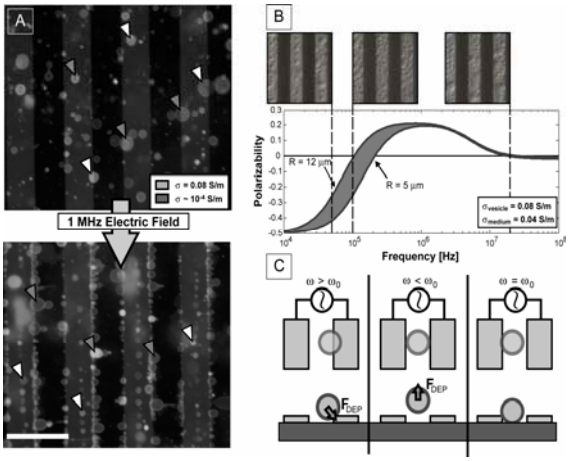

Figure 2: Vesicle measurements. (A) Fluorescence microscopy images of vesicles on IDEs before and after applying a 1 MHz electric field, showing a distinct DEP response of the red (white arrowhead) and green (grey arrowhead) vesicles. (B), (C), We sweep the frequency, ω, observing a single vesicle as it is pulled to and pushed from the electrode edge (C). We narrow the range of frequencies swept until we have converged on the cross-over frequency, ω_0, where the vesicle no longer responds to the electric field. Size and internal conductivity of the vesicle are recorded to generate the spectrum shown in (B). Scale bar 50μm.

Electroformed vesicles with functionalized phospholipids containing PEG brushes attached to their hydrophilic head group along with a fluorescent marker allowed us to test our ability to modulate membrane capacitance (Figure 3). Fluorescence imaging of the electroformed vesicles confirmed that the PEG-functionalized lipids had organized in the vesicle membrane. Interestingly, while COF measurements of non-PEG-functionalized lipid vesicles suggested a membrane thickness of ~5 nm, in agreement with the expected size of a unilamellar lipid bilayer (Figure 3B, black), PEG-coated vesicles exhibited substantially lower effective membrane capacitance, consistent with increased membrane thickness.

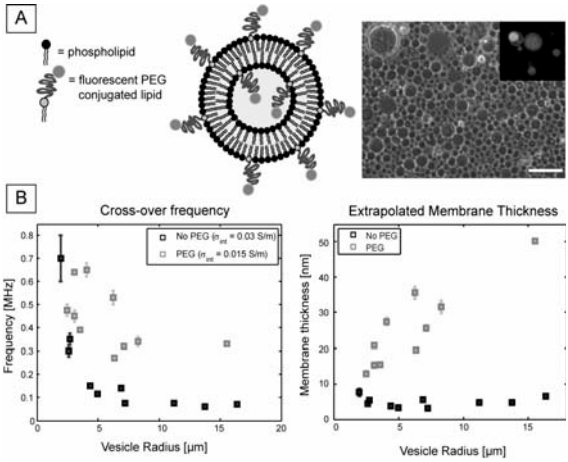

Figure 3: Modulating membrane thickness. (A) Schematic of PEG-modified vesicle (not drawn to scale) and phase microscopy images of electro-formed PEG-vesicles (inset shows fluorescence image of vesicles, confirming PEG-modification). Scale bar 50 µm. (B) Plot of cross-over frequency vs vesicle radius and plot of estimated effective membrane thickness. Vesicles formed in the absence of PEG show a uniform membrane thickness of ~5 nm, as expected for a single lipid bilayer, PEG-coated vesicles exhibit an increased effective membrane thickness that varies with vesicle radius.

5. CONCLUSIONS

We have demonstrated the feasibility of using GUVs as metrology tools for DEP by modulating the conductivity of the aqueous core, the capacitance of the membrane, and the fluorescent signature of both polar and non-polar phases. This, together with post filtration and electrical characterization of the vesicles, allows the creation of combinatorial vesicle libraries and provides a valuable tool in the design and characterization of DEP-based biological microsystems.

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