Dielectrophoretic Capture of Viral Particles from Media of Physiological Ionic Strength

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Abstract

Non-uniform AC fields (dielectrophoresis), created by a set of microelectrodes, are examined in terms of their capabilities to collect viral particles from dilute suspensions of physiological ionic strength. This study forms the basis for the development of a sensitive, miniaturized device that provides rapid, in situ sampling and detection of potentially infectious biological agents.

Introduction

The growing need for methods that permit rapid detection of infectious biological agents, such as viruses, within small sample volumes and without need of amplification of the agent, can be met by a set of promising approaches that involve the selective capture of viral particles from a sample onto a surface, and subsequent detection via optical transduction. While optical transduction is now possible by a number of methods, ranging from conventional fluorescent antibody labeling[1] and mass-sensitive detection[2,3] to emerging label-free methods such as liquid crystals[4,5], the still unresolved difficulty with these surface-based approaches is the slow diffusive transport ("random walk") of the (sub-micron-sized) viral particles from dilute suspension to the capture surface.

One possible approach that overcomes the aforementioned difficulty is based on the forced transport of viral particles to the desired surface by dielectrophoresis (DEP), i.e., the vectorial transport of particles by non-uniform electric fields[6]. Because transport by DEP depends on the particle volume ($\sim r^3$), dielectrophoretic capture of viral particles requires electric fields of high V^{-1} and divergence (see below). Such non-uniform electric fields can be created without large applied voltages by using microelectrodes separated by only a few micrometers.

Manipulation of viruses inside spatially non-uniform, time-dependent (AC) electric fields using microelectrodes has been reported previously[7-12]. In all these past reports, collection of (usually fluorescently labeled) virus particles took place inside "model" suspensions of very low ionic strength (typical range: 1-100 mSm^{-1}) and/or high particle concentrations (e.g., $>10^{10}$ particles/ml). The aforementioned experimental conditions allow for convenient demonstration of the capabilities of dielectrophoresis, namely, manipulation and observation of virus collection patterns, trapping, differential separation of virus populations, as

well as characterization of their dielectric and biophysical properties. What has not yet been determined, however, is whether (i) dielectrophoresis-based, *in situ* collection of viruses from suspensions of physiological ionic strength (e.g., ≥880 mS m^{-1}) is possible, and (ii) dielectrophoresis can lead to detectable signals of surface-bound virus particles at low virus concentrations (e.g., <10^6 particles/ml). Answers to these questions will permit assessment of the potential utilization of dielectrophoresis in portable microscale analytical systems for rapid detection of virus particles from dilute samples (it is known, for example, that during the acute phase of many viral diseases, viruses are shed in biological fluids at concentrations typically ranging from 10^4-10^8 pfu/ml).

The present article describes a study that aimed to address the aforementioned questions. A series of experiments was performed, in which theoretically predicted values of critical experimental variables (voltage, AC frequency, electrode design) were tested in terms of their capability to cause sizable dielectrophoretic effects on viral particles inside high conductivity media and within relatively short time scales. The effect of virus concentration on sampling intensity is also examined.

Theory

Dielectrophoresis of particles is an electrokinetic phenomenon that is brought about by induced polarization effects inside spatially non-uniform electric fields. Because of the electric field divergence, the induced particle polarization gives rise to a dipole moment and, hence, to a non-zero net force (termed "dielectrophoretic force", F_{DEP}) on the particle. The magnitude of F_{DEP} acting on a polarizable spherical particle (e.g., a virus) inside an AC electric field can be calculated from the following equation[6]:

$$< \vec{F}_{DEP} >= 2\pi r_p^3 \varepsilon_M \mathrm{Re}[K_e^*] \vec{\nabla} \vec{E}_{rms}^2 \quad (1)$$

where $< \vec{F}_{DEP} >$ is the time-averaged force, r_p is the radius of the polarizable particle, ε_M is the real part of the dielectric permittivity of the suspending medium, $\vec{E}_{rms}$ is the root-mean-squared value of the electric field intensity, and $\mathrm{Re}[K_e^*]$ is the real part of the effective polarizability (also known as Clausius - Mossotti factor):

$$K_e^* = \frac{\varepsilon_p^* - \varepsilon_M^*}{\varepsilon_p^* + 2\varepsilon_M^*} \quad (2)$$

where ε_p^* (= $\varepsilon_p + \sigma_p/j\omega$), ε_M^* (= $\varepsilon_M + \sigma_M/j\omega$) are the complex permittivities and σ_p, σ_M are the conductivities of particle and suspension medium, respectively, ω is the angular frequency (=$2\pi f$) of the alternating electric field, and $j=\sqrt{-1}$.

The numerical sign of F_{DEP} depends on the value of the effective polarizability ($-0.5 \le \mathrm{Re}[K_e^*] \le 1.0$), which is a function of the dielectric properties of particles and suspension medium. Positive values of effective polarizability give rise to "positive" dielectrophoresis, which leads to the transportation of particles to regions of maximum divergence of the electric field (typically the edges of the microelectrodes). The opposite phenomenon ($\mathrm{Re}[K_e^*]<0$) is termed "negative" dielectrophoresis and directs particles to regions corresponding to minima in the divergence of the electric field (e.g., the center of the microelectrode array, or away from the electrode plane). In the present case, i.e., virions suspended in a fluid of physiological

ionic strength, $\mathrm{Re}[K_e^*]$ assumes negative values throughout the range of AC frequencies. This type of dielectrophoretic behavior stems from the fact that the medium conductivity (ionic strength) is higher than that of the particle interior ($\sigma_M > \sigma_P$). The latter relation is embedded in Eqn. (2) and has been confirmed by numerous studies on viruses, bacteria, and biological cells [13, 14].

Materials and Methods

Microelectrodes. Non-uniform AC electric fields were generated by sets of gold microelectrodes fabricated on the surfaces of oxidized (SiO_2 thickness: 500 nm) silicon substrates. The overall dimensions of each microelectrode chip were 1.5x1.5 cm. The chips were fabricated by using photolithography and metal evaporation (gold deposition). The adhesion of the gold electrodes (thickness ~100 nm) onto the substrate was enhanced by the deposition of a thin layer (20 nm) of titanium between the gold and silicon oxide. The gap between opposing electrodes was ℓ=2 μm. Prior to each experiment, the microelectrodes were coated with poly-L-lysine, which served to create a positively charged surface for the electrostatic capture of the virus particles directed to the surface by dielectrophoretic forces. Power to the microelectrodes was supplied by a 20 mW power amplifier (Mini-Circuits, Model: ZHL-32A), connected in series to a signal generator (BK Precision, Model: 2005A). The microelectrodes were connected to the source in an alternating fashion (180^o phase difference between adjacent electrodes) and the value (peak-to-peak) of the applied voltage (V= 8 Volts) was monitored by an oscilloscope (Leader, Model: LBO-520).

Virus and suspending media. Vesicular stomatitis virus-Indiana (VSV) was obtained from ATCC (Chantilly,VA) and was prepared using procedures published previously[4]. These procedures led to stock suspensions of VSV of concentration 10^8 infectious particles per milliliter (denoted pfu/ml, plaque forming units per milliliter). Suspensions of VSV were diluted from freshly thawed aliquots of VSV stock in TSE (10 mM Tris-Cl, 0.1 M NaCl and 1 mM EDTA) at pH=8.0. A range of samples at different virus concentrations was prepared through dilutions of the stock suspension with TSE buffer. The conductivity of the stock suspension (880 mS m^{-1}) was measured at room temperature with a conductivity meter.

Sample handling and imaging. All experiments were carried out at room temperature (21 ^{o}C) under biosafety level 2 conditions. All work involving suspended virus was conducted in a Class II biosafety cabinet. The viral suspensions were used immediately after dilution. The experiments were performed on a custom-designed stage that supported the microelectrodes and provided connections to the electrical source. Suspensions of VSV (8 μl) were dispensed directly onto the microelectrodes using a micropipette.

Observations of the DEP-facilitated virus collection patterns on the microelectrodes were made under a fluorescent microscope coupled to a CCD camera. First, the samples were incubated with dilute solution of rabbit anti-VSV IgG (affinity purified, Sigma, St. Louis, MO) for 2 hrs, and then rinsed with TSE buffer and incubated in solutions of bovine serum albumin (IgG-free, 1 mg/ml, Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hr. This incubation step was necessary to prevent non-specific adsorption of the fluorescently-labeled antibody (next step), especially to areas of electrode damage. The samples were then rinsed with TSE buffer and incubated with drops of fluorescently-labeled goat anti-rabbit IgG

(Cy3-conjugated IgG, 30-40 nM (Sigma, St. Louis, MO) for 1 hr. All incubation steps took place at room temperature in a water-saturated environment.

Results and Discussion

From Eqn. (1) it becomes apparent that the generation of a strong force on the virus requires high electric field intensity and divergence (non-uniformity). Both these conditions can be easily satisfied with the use of microelectrodes separated by a distance of, e.g., ℓ=2 μm across. When a voltage of 8 V (peak-to-peak value) is applied across these microelectrodes, the resulting maximum electric field strength is 5×10^6 Vm^{-1}. Calculations reported elsewhere [15,16], indicate a maximum dielectrophoretic force on the virus in the order of 10^{-15} N. Assuming steady state dielectrophoresis of such viral particle (r_P=100 nm) inside the non-uniform electric field, F_{DEP} can be equated to the Stokes force. The apparent transport of a virus can then occur at a linear velocity, u, of:

$$u = \frac{F_{DEP}}{6\pi\eta r_p} \cong 1\,\mu ms^{-1} \tag{3}$$

which is, theoretically, capable of collecting particles onto the electrode plane from a radius of, e.g., 100 μm away within a couple of minutes. This time scale is much smaller compared to virus transport by gravity or Brownian motion alone. For the latter case, combining the Einstein and Stokes-Einstein equations, an order of magnitude calculation suggests that the corresponding time for a particle of same dimensions to traverse a distance of x=100 μm is:

$$t = \frac{3\pi r_p \eta}{kT} x^2 \cong 2000\,s \tag{4}$$

In reality, the magnitude of F_{DEP} decays approximately with the third power of the distance from the electrode plane. The collection, however, is significantly enhanced by secondary phenomena, such as fluid streaming[17, 18], which ensure convective transport of virus to the electrode plane. This principle has recently been demonstrated with the collection of submicron-sized spores[19]. One should also notice in Eqn (1) the direct dependence of F_{DEP} on the square of the applied voltage across the electrodes, which provides an additional means of leveraging the collection rate.

For viral particles undergoing negative dielectrophoresis, their attraction and capture on the chip surface requires the existence of a region of electric field minimum on the electrode plane. This becomes possible with the use of quadrupolar microelectrodes, a form of which was used in the present experiments[20]. Simulations of the spatial electric field variation (∇E^2_{rms}) generated on the electrode plane by such microelectrodes are shown in Figure 1. It can be seen that electric field strength is maximum at the edges of the electrodes, which form the areas of attraction for particles subject to positive dielectrophoresis. Away from the electrodes the field becomes progressively weaker, forming a local minimum at the center of the electrode arrangement. The latter represents the expected collection area of particles driven by negative dielectrophoresis[21]. These simulation results represent very closely the electrodes and operating conditions (namely, applied voltage) used in our experiments. The electric field profile of Figure 1 is independent of the applied AC frequency and, for continuous and dielectrically homogeneous suspension media, it can also be assumed invariable throughout the experiments.

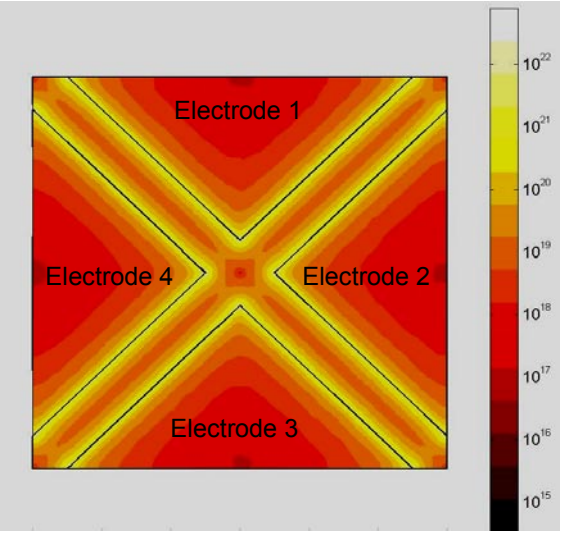

Figure 1: Computer simulated profile of the electric field divergence (∇E^2) at x_o=100 nm, created by a set of quadrupolar electrodes. Only the tips of the four electrodes are shown (electrode separation, ℓ=2 μm across; V_{rms}=5.6V). The scale bar indicates local ∇E^2-values (in V^2m^{-3}).

The hypothesis that detectable virus collection can be achieved within short time spans was put to test with a series of experiments. Specifically, the efficiency of the dielectrophoretic collection for a period of t=2 min was evaluated with virus suspensions in a range of concentrations from 10^7-10^4 pfu/mL and ionic strength of 880 mSm^{-1} (physiological buffer). The microelectrodes were energized by a sinusoidal signal with amplitude (peak-to-peak) V=8 Volts and frequency *f*=1 MHz.

The results are presented in a qualitative manner in Figure 2 as fluorescent images of immuno-stained virus collected on the electrode surface. The experimental variable in Figure 2 is the virus concentration in physiological buffer (TSE). A fluorescent image of a control sample (no electric field exposure) is also included (Fig. 2d) for comparison. At a first glance, the experimental results are overall in a very good agreement with the theoretically predicted response. According to Figures 1, virus suspended in TSE should exhibit negative dielectrophoresis and collect, primarily, in the center of microelectrodes (local electric field intensity minimum) and, to a lesser degree, midway in the gap between adjacent electrodes. As can be seen in Figure 2, this is unambiguously the collection pattern that was consistently observed. As expected, the fluorescent intensity of the images is related to the virus concentration in the sample. At concentration 10^5 pfu/mL (Fig. 2c) only virus aggregates can be seen collected on the electrodes and, finally, at even lower concentrations (10^4 pfu/mL) no collection is observed (results not shown). The weak signal emitted from the electrode edges is attributed to non-specific adsorption of fluorescent antibody.

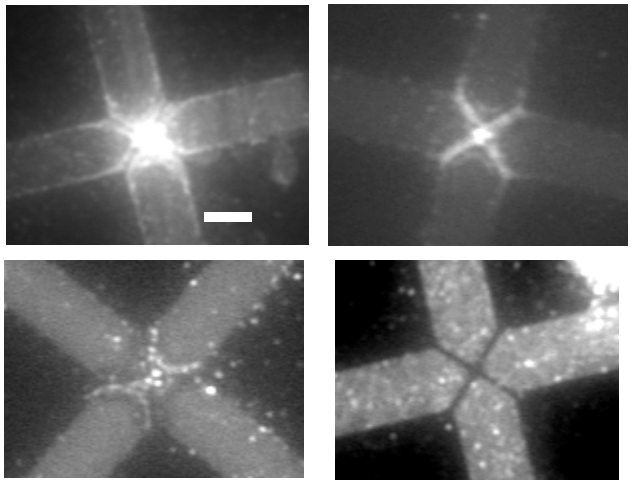

Figure 2: Fluorescence images showing dielectrophoretic sampling of virus from suspensions at various concentrations in TSE buffer (sM=880 mSm-1). The accumulation of virus between the electrodes is consistent with negative delectrophoresis. Concentrations (pfu/ml): (a) 10^7, (b) 10^6, (c) 10^5, and (d) 10^7 (Control sample, no electric field). Experimental conditions: *f*=1 MHz, V_{ptp}=8V, t_{DEP}=2 min.

Conclusions

The principal conclusion of the study reported here is that dielectrophoresis can significantly accelerate the transport of viral particles from suspensions with physiologically

relevant ionic strengths. Dielectrophoretic transport led to the detection of virions captured on surfaces at titers (concentration) of virus as low as 10^5 pfu/mL inside physiological buffer (TSE), whereas passive diffusion did not lead to detectable levels of captured virus when using titers of VSV as high as 10^8 pfu/ml. Theoretically predicted dielectrophoretic collection patterns were found to be in qualitative agreement with experiment as a function of virus titer.

During the acute phase of many viral diseases, virus is shed in biological fluids at concentrations typically ranging from 10^4-10^8 pfu/ml. The results presented in this study are encouraging, particularly because the experiments took place under conditions (e.g., electrode design and dimensions, applied voltage, AC frequency, virus detection method) that have not yet been optimized. This leads us to anticipate that detection of virus particles at substantially lower titers could be possible by using dielectrophoresis.

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