HIGH-THROUGHPUT ELECTRODELESS DIELECTROPHORESIS OF VIRUSES IN POLYMERIC MICRODEVICES

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Abstract

The electrodeless dielectrophoretic trapping and concentration of viruses was demonstrated. Dielectrophoresis is the motion of matter caused by polarization effects in a nonuniform electric field. Experiments were performed on a glass chip with insulating posts in order to study the dielectrophoretic behavior of the viruses and extend the application of insulative (electrodeless) Dielectrophoresis (iDEP) to polymeric microdevices. The ultimate goal of his research project is to create a high-throughput EDEP system using polymers as substrate. Only two electrodes were present in the system. In the presence of an applied DC electric field the viruses exhibited streaming and trapping dielectrophoresis.

Keywords: electrodeless dielectrophoresis, virus, high-throughput

1. Introduction

Water contamination has recently become a prominent health safety issue [1]. There has been a growing interest in developing techniques for the monitoring, detection, and removal of low levels of microorganisms from the nation's water supply. Dielectrophoresis (DEP) is a transport mechanism with great potential for selective concentration to support water monitoring of biological pathogens.

Dielectrophoresis is the motion of polarizable and conductive particles under the influence of a nonuniform electric field that induces dipole moments and electric current in the particles. The electrostatic potential energy of such particles decreases with increasing electric field so particles experience a dielectrophoretic force toward regions of high field intensity [2-3]. The immersion fluid, experiencing a similar force. tends to buoy the particles away from the high-field region. The net dielectrophoretic transport is toward or away from high-field regions depending on the relative strength of the forces. DEP is a non-destructive characterization and separation method and therefore has enormous potential for applications involving microorganisms. Recently, there has been significant activity on utilizing DEP for the isolation of microbes and bio-particles [4-10].

The present work is part of a comprehensive research project for dielectrophoretic concentration of microorganisms in water. This project involves the application of insulative (electrodeless) Dielectrophoresis (iDEP) for manipulating and trapping of viruses in water. This is the first reported account of iDEP trapping of viruses. Previous studies of dielectrophoretic virus trapping have employed arrays of

7th International Conference on Miniaturized Chemical and Biochemical Analysis Systems October 5–9, 2003, Squaw Valley, California USA microelectrodes as the trapping media [4-6]. Major disadvantages of using arrays of electrodes are charging effects on the electrodes surface, electrochemical reactions, and the complexity of the fabrication methods. In addition, devices based on arrays of microelectrodes can not be scaled up due to limitations of the fabrication techniques. The ultimate goal of this project is to develop a high-throughput iDEP system using polymers as the substrates. Polymeric materials were selected since the malleability of plastics facilitates the scale-up of DEP devices for high throughput operations. Polymeric structures can be utilized as the substrate and insulating-post structure for iDEP. Figure 1 illustrates an array of microbumps that was replicated using a Ni micro-screen under load and in a heated Carver press. These microbumps are 100- μ m in diameter and 40- μ m in height. Several polymeric materials were tested and two materials have been identified (Zeonor and polyethylene) with excellent characteristics for the fabrication of iDEP devices. These materials will be used in the current investigation to build a high-throughput device for the iDEP trapping of viruses.

Our objective is to create a thin-film structure with an array of microbumps. These microbumps will work as insulating posts to concentrate the applied electric field. Once the thin-film has been fabricated, it will be rolled up using a supporting material in order to create a high-throughput and large-volume system for water analysis. In this paper we present the preliminary results of this effort. iDEP trapping of viruses was performed by using a glass chip. The results obtained will be the guidelines for developing a high-throughput system made of polymeric material for iDEP analysis of water.

2. Theory

The dielectrophoretic force acting on a particle depends on the volume and shape of the particle. Several viruses have ellipsoidal shapes. For an ellipsoid, whose mayor axis is parallel to the electric field, the DEP force can be written as [4]:

$$F_{DEP} = \frac{2\pi abc}{3} \varepsilon_m \operatorname{Re}\left\{\frac{\sigma_p^* - \sigma_m^*}{\sigma_m^*}\right\} \nabla E^2$$
(1)

where: σ_p^* and σ_m^* are the complex conductivities of the particle and the medium respectively, ∇E^2 defines the local field strength, *a*, *b* and *c* are the dimensions of the ellipsoid (Figure 2).

Here, iDEP is produced by applying an electric field across an array of insulating posts. Electric field lines between the two electrodes of the systems have to travel between the insulating post structures, creating zones of higher and lower field intensity. Figure 3 shows a cartoon of electric field lines being squeezed between the insulating posts.

From Equation (1) it can be seen that the dielectrophoretic force acting on a particle can be positive or negative. If the permittivity of the particle is higher than that of the medium, the particle will exhibit positive dielectrophoretic behavior and it will be trapped in the regions with higher field intensity, i.e., the narrow spaces

between the insulating posts (Figure 3). Particles that exhibit negative dielectrophoretic behavior will trap in regions with lower field intensity.



Figure 1. Images of microbump array. Optical image at 8x magnification of an array of polymeric features produced from a Ni microscreen that have been replicated in Zeonor® film. Films are tilted at 45°. Features are approximately



Figure 2. Diagram of an ellipsoid with volume = $4/3 \pi abc$



Figure 3. Representation of electric fields lines being concentrated in the narrow spaces between the circular insulating posts, creating zones of higher field intensity.

3. Experimental

Glass chips: Glass structures were fabricated using standard photolithography techniques. The microfluidic chip contains 12 subcircuits. Each subcircuit contains a total of 6 separate patterned microchannels located between two liquid reservoirs. The chip contains a total of 16 liquid reservoirs (Figure 4). The depth and length of the microchannel is 10 µm and 1.25 cm respectively.

Apparatus: Experiments were conducted on an inverted fluorescence microscope (Olympus, Napa, CA). A flow manifold. made in-house was utilized. The DC electric fields were applied by using a high-voltage power supply (Stanford Research Systems, PS350, Palo Alto, CA). The data was collected in the form of videos using a Sony digital camera (Sony, San Dicgo, CA.

Virus labeling: T4 virus (a DNA, nonenveloped, bacteriophage virus; head size 65 x 80 nm, tail size

120 x 20 nm) was labeled by using the green fluorescent dye SYTO[®] 9 (Molecular Probes, Eugene, OR). Live T4 virus were obtained suspended

> in BU buffer (50 mM Na₂HPO₄•7H₂O, 22 mM KH₂PO₄, 70 mM NaCl) at a concentration 1×10^{12} virus/mL. The virus concentration was adjusted to 1×10^{10} virus/mL by using diluted (1%) BU buffer. 3 µL of dye Syto 9 ® was added per mL of diluted virus culture. The dye was incubated for 15 minutes and the background dye was eliminated bv centrifugation at 14,000 rpm for 30 minutes.

4. Results and Discussion

Two forms of dielectrophoresis of T4 virus were observed when an electric field of 40 V/mm is applied (Figure 5). Both dielectrophoretic phenomena have the potential to be used for concentration and manipulation of T4 viruses. Streaming dielectrophoresis of T4 virus is observed since the flow of viruses is aligned with the electric field. Streaming DEP can be used for continuous concentration of particles. In addition, positive dielectrophoretic trapping is observed at the corners of the posts, where the electric field is most concentrated. Trapping DEP can be used for batch concentration of particles.

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5. Conclusions

Streaming and trapping iDEP of viruses was demonstrated on glass chips. Insulating posts made of glass were utilized in order to produce a nonuniform electric field. These preliminary results shown that iDEP has tremendous applications for the trapping of viruses in water. The data obtained from these results will be used to develop a high-throughput system, using a polymeric substrate, for the iDEP trapping of viruses in water.

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Figure 5. Image of EDEP of T4 Virus. 10 X magnification. T4 viruses are in white. A field of 500 V/cm, which is surprisingly low, was applied. The background electrolyte was diluted BU buffer. Uniform square array of square posts arranged on 200-µm centers with 160-µm sides.

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