Micron Particle Segregation in Lower Electric Field Regions in Very Dilute Neutrally Buoyant Suspensions

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ABSTRACT

When a dilute suspension of neutrally buoyant poly alpha olefin particles and corn oil is exposed to a highly non-uniform AC electric field generated by a spatially periodical electric electrode array, particles are first transported by dielectrophoresis force to the lower electric field region. However, after these particles enter the lower electric field region, they don't form a staple particle strip, instead, are segregated to form island-like structures that it is experimentally testified are suspended somewhere above each ground electrode. Given a suspension of 0.1% (v/v) and an applied voltage of 5kV/100Hz, the DEP induced particle transport time is 10 minutes, the particle segregation time is 30 minutes, and so a driving force for the particle segregation, if any, is estimated as 1% of the DEP force. Two applications are proposed of this finding that are to monitor a surviving rate of cells after electroporation, and sort out dead cells from human being breast cancer cells before perfusion respectively.

Key Words: dielectrophoresis, segregation, electroporation, cell surviving rate, island-like structure

1 INTRODUCTION

Techniques of dielectrophoresis (DEP) based separation and manipulation still have further application potentials in biotechnology. Two previous papers of ours reported DEP-induced transport of particles in dilute suspensions in non-uniform electric fields [1][2]. The single particle model works well because the interactions among particles are so weak that they are negligible. This paper will report an unexpected finding of island-like structure formation of poly alpha olefin particles in corn oil in the lower electric field region.

2 EXPERIMENTAL

2.1 Setup and Suspension

As shown in Figure 1A, the DEP chamber consists of a transparent cover with one side ITO coated and grounded, a Teflon gasket as its four-side wall, and an insulating bottom plate with an electrode array. Its cavity is 150x70x3(mm). Eight pairs of 1.6x1.6mm square brass bars act as the ground and high voltage electrodes that are inlaid into the insulating plate in parallel with a uniform gap of 2 mm. The top surface of each electrode is flattened and polished with fine sand paper and metal polishing agents. The 3mm thick and 5mm wide close gasket of Teflon constitutes its four-side wall. The chamber cover has two layers of a clear

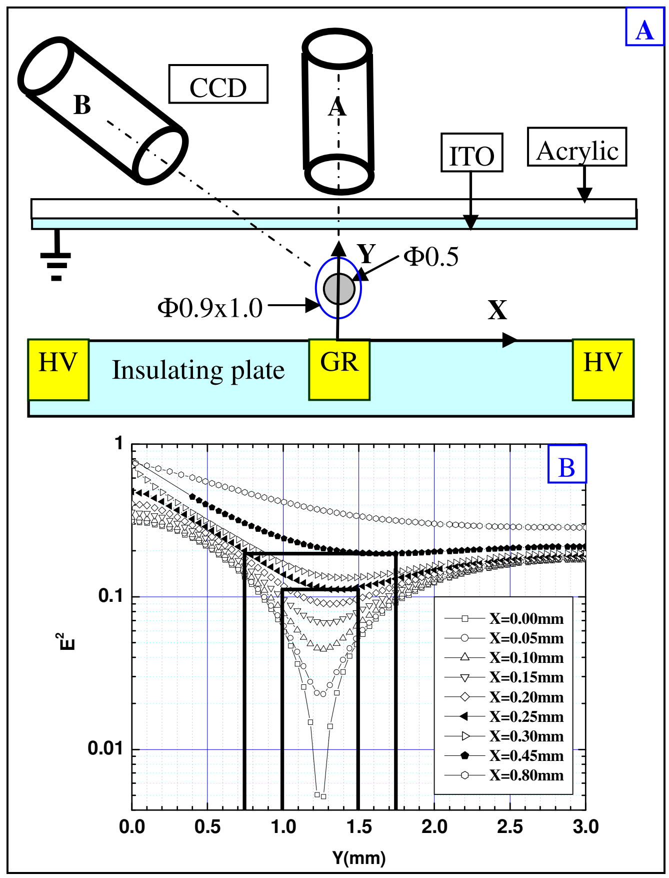

Figure 1 A: The cross section of the DEP chamber with a spatially periodical electrode array. The unit cell area is 3.0x3.6mm. HV and GR denote electrically energized electrodes and ground electrodes respectively. B: The spatial distribution of electric field. The lowest electric field is located at (0, 1.245mm). X-axis and Y-axis are shown in the Figure 1A and Z-axis is along the electrode direction. No drawings are scaled.

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acrylic plate and ITO-coated glass. The ITO-coated glass and all the ground electrodes are electrically connected together and grounded. The electrode array is energized by a high voltage AC power supply. Mono-sized poly alpha olefin particles of diameter of 86.7µm are used for experimental studies. The particles have the exactly same density of 0.92g/cm^3 as corn oil only at 19°C. The dielectric mismatch factor（β）between particles and corn oil was measured with 4-volt excitation voltage as -0.143 in the frequency rang from 50 to 10000Hz. The viscosity of corn oil (η) is 0.060 Pas at 19°C. A CCD camera was used to record particle distribution with time under an AC electric field from the direction of A or B shown in the Figure 1A.

2.2 Experimental Results

Experiments of particle transport, aggregation and segregation induced by an AC field were carried out with the DEP chamber and the suspension described above. A typical set of experimental photos is shown in Figure 2 for a suspension of 0.1% (v/v) under 5kV/100Hz. The dynamic responses of the particles to the high gradient ac electric field have two stages. First, the particles are transported to the lower electric field region because the dielectric mismatch factor of the suspension is negative, -0.143. Particle dynamics in this stage is reflected in the images of A to D in Figure 2. The single particle model works well for this stage. In the second stage when the particles reach the lower electric field region, they don't form a uniform staple column, instead, are further segregated to form island-like structures, as shown in the images of E to H.

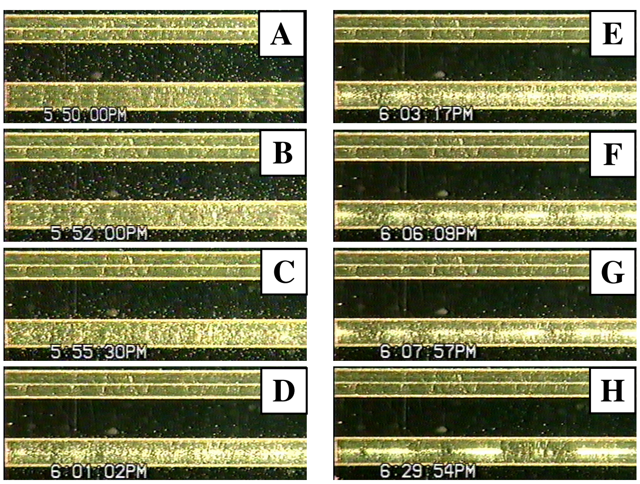

Figure 2: Particle transport, aggrergation and segregation under 5kV/100Hz in the DEP chamber. The suspension consists of poly alpha olefin particles and corn oil at 0.1% (v/v). All the images were taken when the camera was in Position A in Figure 1A. The voltage was applied to the suspension from 5:50:00pm. Time on each image was real time. Images of A to D show the particle transport to the lower field region and images of E to H show the particle aggregation and segregation in the lower field region.

For a better understanding of two dynamics processes and their relations, we numerically simulated the final particle distribution after the suspension was exposed to the electric field for a long enough time [3]. In this simulation, we did not consider the particle aggregation and segregation although we used a continuous model that is very close to the single particle model for a dilute suspension. We first calculated the spatial distribution of particle concentration with time under an externally applied electric field. When the time was approaching to infinite, we got the final distribution of particle concentration. The distribution of mean particle concentration was defined as $<\Phi(X)>=\frac{1}{3}\int_0^3\Phi(X,Y,t)dY$ and $<\Phi(Y)>=\frac{1}{3.6}\int_0^{3.6}\Phi(X,Y,t)dX$. $<\Phi(X)>$ against X and $<\Phi(Y)>$ against Y are shown in Figure 3, data for the suspension of 0.1% initial particle concentration under 5kV/100Hz are expressed by the solid squares. All the particles are finally collected within a circle with a diameter of 0.5mm and its center is at (0, 1.245mm) in Figure 1A. For the suspension of 1% initial concentration under 5kV/100Hz, the distribution3 of mean concentration against X and Y are shown in Figure 3, expressed by the empty circles. The particles are finally collected in an ellipse with long and short axes of 1.00 mm and 0.90mm and its center is at (0, 1.245mm) also, as shown in the Figure 1A.

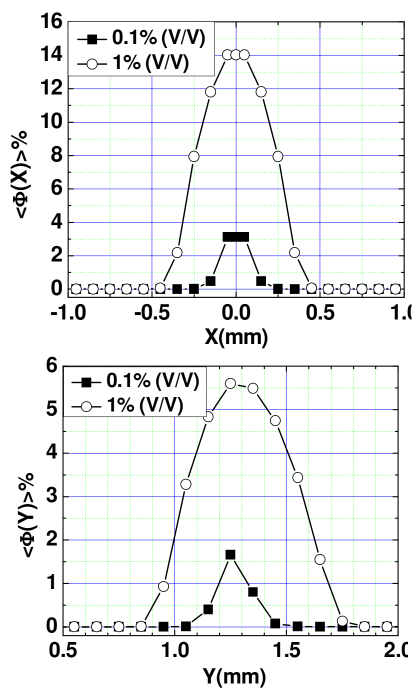

Figure 3: Mean particle concentration $<\Phi(X)>$ versus X and $<\Phi(Y)>$ versus Y calculated based on a continuous model when the exposure time t was long enough [3]. The excitation voltage of 5kV/100Hz and β = - 0.15 were used in the above calculations. The solid squares refer to the 0.1% initial concentration suspension and the empty circles refer to 1%. X- and Y-axes are shown in Figure 1A.

We set the CCD camera in Position B in Figure 1A so as to determine locations of island-like structures relative to the ground electrode. Figure 4 shows that these island-like structures are suspended somewhere above the ground electrode, but don't touch the ground electrode surface. The experiment shows that the island-like structures are in the lower electric field region shown in Figure 1B, which is in qualitative agreement with the calculations.

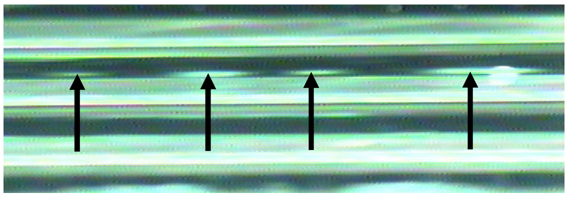

Figure 4: Island-like structures are located somewhere above the ground electrode, as each arrow points. The continuous brighter stripes are the electrode surfaces. This image was taken when the camera was in Position B in Figure 1A. This experiment shows that the island-like structures are in the lowest electric field region.

The particle instability always exists while formation of island-like structures is conditional in the suspension. Effects of initial concentrations of suspensions on the particle segregation are scrutinized with experiments. It was experimentally verified that it depends on the initial concentration whether island-like structures are formed in a suspension or not. The experiments were performed with the suspensions of 0.1% to 1.126% initial concentrations. A critical initial concentration for the island-like structure formation is experimentally determined as 1%. When the initial concentration of suspensions is less than 1%, particles are completely segregated into the island-like structures and there are pure corn oil regions between the island-like structures of particles, as shown in the image H in Figure 2. When an initial concentration is higher than 1%, no island-like structures occur in such a suspension, instead, particles form staple strips in each lower field region. The below experiment confirms existence of the critical initial concentration of 1%. Shown in Figure 5A is a result for the suspension of 1.126% initial concentration at the 611^{th} second after the suspension was exposed to a voltage of 5kV/100Hz. No island-like structures appear and only uniform straight stripes of particles are formed as shown in Figure 5A. The width of particle stripes is 0.618 of the electrode width. Being contrary to what is shown in Figure 5A, Figure 5B shows that particle instability does exist in the same experiment when some amount of particles was removed from one end of the ground electrode with help of the edge effect of the electrode array in the chamber. Clear wavy structures are added on each particle stripe above each ground electrode. The convincing evidence of particle instability is that particle stripes were not uniformly reduced from the central part to the end of the ground electrode; instead these stripes have some sawtooth waveforms on them. The thickest of stripes was measured as 0.561 of the ground electrode width which is less than the limit value of 0.9/1.6=0.5625.

It should be pointed out that all the experiments except for this one in Figure 5 were carried out with a close gasket and that the boundary condition of zero particle flux is valid.

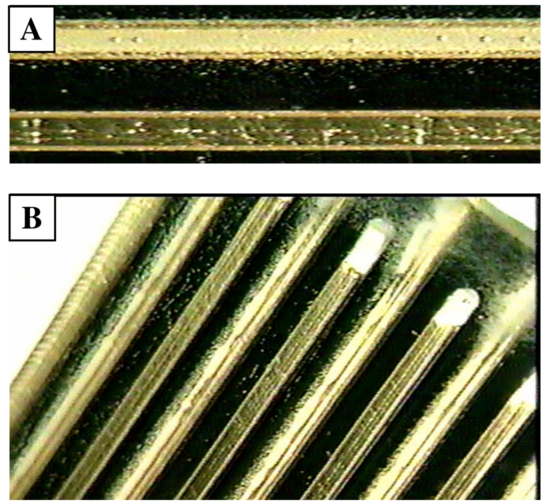

Figure 5 A: Image A shows that uniform stripes of particles were formed in the suspension of the initial concentration of 1.126% (v/v) at the 611^{th} second after 5kV/100Hz was applied to the suspension. The width of the particle stripes is 0.618 of the electrode width. B: Wavy structures are developed on each particle stripe after some amount of particles was removed from one end of the ground electrode with help of the edge effect of the electrode array. The widest of particle stripes is 0.561 of the electrode width. Image B was taken at the 70th minute after 5kV/100Hz was applied to the suspension.

3 MECHANISMS RESPONSIBLE FOR ISLAND-LIKE STRUCTURE FORMATION

What force drives the particle segregation in the lower field region? Why are the island-like structures formed in dilute suspensions of less than 1% initial concentration? How does transverse (that means the DEP force is normal to the electrode direction) DEP force influence island-like structure formation or particle instability in suspensions of different initial concentrations?

Let's look at the distribution of E^2 shown in Figure 1B again. There are deep valleys in the curve families of E^2 versus (X, Y) and their centers are located at (Xmm, 1.245mm). The valleys rapidly become shallow with increase in X and disappears at X = 0.45mm. It is found that the valleys of E^2 area should be an ellipse with long and short axes being 1.00mm and 0.90 mm, respectively and the center is at (0, 1.245mm). It will shed light on the formation of island-like structure or development of particle instability to compare the distribution of electric field with the final particle concentration distributions of the 0.1% and 1% initial particle concentration suspensions under the same excitation voltage of 5kV/100Hz.

For the suspension of the initial concentration of 0.1% under 5kV/100Hz, particles are always distributed within the deep valley no matter before or after the particles are segregated. As shown in Figure 2, although island-like structures are formed, the particles in the island-like structures are still in the deep valley and so these particles only see the very weak transverse DEP force. However, according to the estimation from Figure 3, when the initial particle concentration of a suspension is increased to 1%, the distribution of particles reaches the rim of the valley. This means that, if the initial particle concentration is further increased, particles not only take up the valley but outside it also. As shown in Figure 1B, there exists a very huge difference of the square of electric field intensity (E^2) between inside and outside the valley. Particles outside the valley experience the much stronger transverse DEP force and will be forced to enter the valley as many as possible. Even though there is the particle instability along the electrode direction in the suspension of initial particle concentration of 1% or higher, no island-like structures are formed or even the particle instability cannot be developed in such a suspension because the transverse DEP force in the transit region from inside the valley to outside the valley is strong enough to suppress the particle instability completely. As a result, there appear no island-like structures or development of the particle instability in such a suspension and the particles only form uniform straight stripes, which have been testified by the experiments. As shown in Figure 5A, for the suspension of the initial concentration of 1.126%, the stripe width is 0.618 of the electrode width that is larger than the limit value of 0.9mm/1.6mm = 0.5625, and so the particles take up both inside and outside the valley and no island-like structures or particle instability was developed. However, as shown in Figure 5B, the particle stripes become thinner by removing some particles with help of the edge effects of the electric field, the particle instability or pre-island-like structure appears because the widest of stripes is 0.560 of the electrode width, slightly less than the limit value of 0.563. Also, as shown in Figure 2, the width of particle stripes in the images of E to H is always less than the limit of 0.563 shown in Figure 3 and so particle instability and island-like structure are always developed to be observable.

Furthermore, it is necessary to estimate the relative strength of a driving force responsible for the particle segregation to the transverse DEP force. A possible driving force can be estimated as $F_{DRIVE} = 6\pi\eta aV = 6\pi\eta aL/\tau_{seg}$, where τ_{seg} is the segregation time, a the particle radius and L a characteristic distance for particles to move during the segregation. The transverse DEP force can be written as $< F_{TDEP} >= 2\pi\varepsilon_0\varepsilon_l\beta a^3 < \nabla E^2 >$, where $< F_{TDEP} >$ is to take average over the involved region and ε_l. is the dielectric constant of corn oil. Therefore, the ratio is given as $\frac{< F_{seg} >}{< F_{TDEP} >} = \frac{3\eta L}{\varepsilon_0\varepsilon_l\beta\tau_{seg}a^2 < \nabla E^2 >} \approx 0.01$. It demonstrates that, compared with the average transverse DEP force, a possible driving force responsible for the particle segregation is so weak! Fortunately, because there is the lowest electric field region where the transverse DEP force has the least effect on particle instability or island-like structure development, we are able to observe such a tantalizing phenomenon due to so weak a driving force!

4 CONCLUSIONS AND FURTHER WORK

The particle instability is recognized in the neutrally buoyant suspensions of poly alpha olefin particles and corn oil, and island-like structures are observed in the suspensions of less than 1% initial concentration when the suspension was exposed to an AC gradient electric field that is generated by a spatially periodical electrode array. It is testified that the island-like structures are located in the lowest electric field region above each ground electrode. In short, experiments demonstrate that there is particle instability in the suspension; when the initial concentration is less than 1%, the particle instability is developed to form island-like structures; when the initial concentration is higher than 1%, the particle instability is suppressed by the strong transverse DEP force and particles form uniform straight stripes above each ground electrode.

So far, we have not had a clear physics picture about how the particle instability in the suspension is initialized. We are going to perform 3-D MD simulations so as to crack the nut. In the mean time, we proposed two applications of this finding. One application is to monitor a surviving rate of cells in a real time domain after electroporation which is one crucial parameter to electroporation techniques like target drug delivery and cancer target therapies. The other application is to sort out dead cells from human being breast cancer cells before perfusion.

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