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Dielectrophoretic Isolation and Detection of cfc-DNA Nanoparticulate Biomarkers and Virus from Blood

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Abstract

Dielectrophoretic (DEP) microarray devices allow important cellular nanoparticulate biomarkers and virus to be rapidly isolated, concentrated and detected directly from clinical and biological samples. A variety of sub-micron nanoparticulate entities including cell free circulating (cfc) DNA, mitochondria and virus can be isolated into DEP high-field areas on microelectrodes, while blood cells and other micron-size entities become isolated into DEP low-field areas between the microelectrodes. The nanoparticulate entities are held in the DEP high-field areas while cells are washed away along with proteins and other small molecules which are not affected by the DEP electric fields. DEP carried out on 20 μ L of whole blood obtained from Chronic Lymphocytic Leukemia (CLL) patients showed a considerable amount of SYBR Green stained DNA fluorescent material concentrated in the DEP high-field regions. Whole blood obtained from healthy individuals showed little or no fluorescent DNA materials in the DEP high-field regions. Fluorescent T7 bacteriophage virus could be isolated directly from blood samples, and fluorescently stained mitochondria could be isolated from biological buffer samples. Using newer DEP microarray devices, high molecular weight (hmw) DNA could be isolated from serum and detected at levels as low as 8–16 ng/mL.

Keywords

DNA; cfc-DNA; CLL; nanoparticulates; mitochondria; virus; biomarkers; dielectrophoresis; high conductance buffers; blood

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COMPETING INTEREST STATEMENT

We declare no financial conflict of interest with any manufacturer of products listed in the report, regarding the theoretical work presented in Figure 1, the DEP results for CLL blood samples (Figure 2), the DEP results for T7 bacteriophage (Figure 3) and the DEP results for mitochondria (Figure 4). The DEP work on HMW-DNA was done using microarrays from Biological Dynamics. This company was recently started by Dr. Rajaram Krishnan to develop new DEP technology. Dr. Raj Krishnan presently serves as the CEO for Biological Dynamics and Dr. Michael J. Heller serves as a consultant.

1 INTRODUCTION

The rapid isolation and detection of nanoparticulate biomarkers such as cell free circulating (cfc) DNA directly from blood and other clinical samples is a major challenge for many molecular diagnostic applications. CFC-DNA is now considered an important biomarker for early detection of cancer [1–4], residual disease [5,6] and for monitoring chemotherapy [7]. Cancer related cfc-DNA biomarkers are often found in the blood along with smaller apoptotic DNA fragments. CFC-DNA from cancer can occur in the blood at levels of more than 1000 ng/mL, while normal apoptotic DNA levels in blood are around 30 ng/mL [8–11]. In addition to cfc-DNA, challenges also exist for the rapid detection of many other important nanoparticulate biomarkers including exosomes/cfc-RNA [12, 13], mitochondria [14], viral pathogens [15] and drug delivery nanoparticles [16–19].

At this time, the sample preparation processes for isolating cfc-DNA and other nanoparticulate entities directly from blood and other biological samples are relatively complex, time consuming and expensive. These procedures can involve centrifugation, filtration, washing and extraction of the DNA by phenol/chloroform methods, ion exchange binding or other laborious procedures [4, 8–10]. Frequently the cfc-DNA is run on PAGE gels to determine its size and concentration and then genotyped using PCR techniques [9, 20]. Other disadvantages include the extended amount of time between blood drawing, cell separation, DNA extraction and final DNA analysis. Delay in processing blood to plasma/serum causes release of DNA molecules by normal cells, and the processing itself leads to shearing degradation of DNA into smaller fragments [8, 9]. Additionally, sample processing is inefficient and DNA can be lost in the procedure, especially when present at very low concentrations [11]. Thus, for research and clinical diagnostic applications it is important to develop a rapid, sensitive and inexpensive method for the isolation and detection of cfc-DNA and other nanoparticulate biomarkers directly from whole blood.

AC dielectrophoresis (DEP) has been known to provide effective separations of cells, nanoparticles, and biomolecules [21–27]. However, until recently DEP techniques had remained impractical for general use with high conductance solutions (~10 mS/cm) and with complex biological samples such as whole blood [24–27]. As examples, DEP work on separating bacteria from blood [23, 26], separations of cells [27–29], virus [30], polystyrene nanoparticles [31–35], DNA [36–38] and proteins [39–40] all required sample dilution and low-conductance conditions (below 1 mS/cm). While some progress has been made on carrying out DEP under high conductance conditions, this work has been generally limited to separations of cells and micron-size entities by negative DEP forces using hybrid DEP devices [28, 41–45]. Recently we have developed DEP microarray devices and methods that allow nanoscale entities including high molecular weight (hmw) DNA and nanoparticles to be separated and detected from high conductance buffer solutions [46–48], as well as from whole blood samples [19]. Such DEP microarrays could be operated at up to 20 volts peak-to-peak (pk-pk) at 10 kHz in buffers and biological samples with conductances of more than 10 mS/cm. Under these DEP conditions, hmw-DNA and nanoparticle entities which are more polarizable than the surrounding media experience positive DEP (p-DEP) and are concentrated into the DEP high-field regions over the microelectrodes, while micron-size or larger particles (blood cells) which are less polarizable experience negative DEP (n-DEP)

and are concentrated into the DEP low-field regions between the microelectrodes. The polarizability of these entities depends on the solution in which they are suspended as well as the frequency of the applied non-uniform electric, a relationship which is described by the Clausius-Mossotti Factor (CMF). At a given frequency, the sign of the CMF (positive or negative) indicates whether an entity will experience p-DEP or n-DEP. Any transition between a positive and negative CMF is referred to as a crossover frequency. Choosing to apply a non-uniform electric field with a frequency at which one entity has a positive CMF and another has a negative CMF allows the two entities to be separated with DEP [25]. After the DEP separation is completed, a simple fluidic wash removes the larger particles (cells) in the low-field regions, while the nanoparticles remain concentrated in the high-field regions. Generally, proteins and lower molecular weight biomolecules are not affected by the DEP fields and are also removed by the washing procedure. This study now demonstrates the rapid DEP isolation and fluorescent detection of cfc-DNA from whole blood samples obtained from Chronic Lymphocytic Leukemia (CLL) patients, T7 bacteriophage virus from blood samples and mitochondria from biological buffer solutions. This study also demonstrates the isolation and detection of low levels of hmw-DNA from serum samples using new DEP microarray devices.

2 DEP THEORY

Electrokinetic methods such as DC electrophoresis and dielectrophoresis have proven very useful in manipulating biological samples [21–27]. While DC electrophoresis induces movement of an entity based on its net charge and the interaction of that charge with a DC electric field, dielectrophoresis uses an electric field which must be applied asymmetrically by controlling the geometry of the electrodes or the shape of the fluid chamber [49, 50]. When an entity experiences an asymmetric electric field, a dipole forms and is acted upon asymmetrically. The net force experienced due to this electric field is called the dielectrophoretic force and is described by the equation:

$$F_{DEP} = 2\pi r^3 \epsilon_m \text{Re}\{K(\omega)\} \nabla E^2$$

Where r is the effective radius of the entity, ϵ_m is the permittivity of the medium, E is the applied electric field, and $K(\omega)$ is the Clausius-Mossotti Factor (CMF), defined below for spherical entities, which describes the relationship between the entity and the fluid medium in which it is suspended. The complex dielectric constant ϵ^* includes the bulk permittivity ϵ_r , ϵ_0 , the conductivity σ , and frequency ω .

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}$$

$$\epsilon^* = \epsilon_r \epsilon_0 + \frac{\sigma}{j\omega}$$

If the real part of the CMF is positive, this indicates that the interaction of the induced dipole and the applied non-uniform electric field will result in a force towards the areas of increasing electric field strength (positive DEP). If the real part of the CMF is negative, the entity will experience a force directed away from areas of high field strength (negative DEP).

With regard to nanoparticulate entities such as hmw-DNA, mitochondria and virus, the simplified model above for determining the direction and magnitude of DEP force is useful for understanding how the force originates, but does not adequately predict the behavior of particles at low frequencies or when the electric double layer is large, as it is when considering nanoparticles and DNA [51–53]. It has been known for some time that many biological entities experience positive DEP at low frequencies despite the fact that simplifying their multilayer components to an effective bulk conductivity and permittivity predicts the opposite [54,55]. To predict the DEP response of particles at low frequency with electric double layers of arbitrary size, a promising method is to numerically solve the Poisson–Nernst–Planck equation [56]. While such analysis is enlightening, it is not absolutely necessary for appreciating the practical clinical significance of the experimental observations below.

3 EXPERIMENTAL SECTION

3.1 DEP Device and Methods

The devices used to create the asymmetric DEP electric field were the Nanochip 100 site microelectronic arrays (Nanogen) which have 100 individually-addressable platinum microelectrodes, each 80 μm in diameter with a spacing of 200 μm between electrode centers. These microarrays are coated with a 10 μm thick polyacrylamide hydrogel layer. The microarray is contained within a fluidic chamber with a glass cover and has a volume of 20 μL [19, 46–48]. These 100 site microelectrode arrays were used to carry out the CLL, T7 bacteriophage and mitochondria DEP experiments. New prototype DEP microarray devices designed by Biological Dynamics containing 1000 platinum microelectrodes (80 μm diameter) were used to carry out DEP isolation of hmw-DNA from human serum experiments. These microarrays are coated with a 10 μm layer of a polyHEMA hydrogel and have an 80 μL sample volume. In studies with these new DEP devices all 1000 microelectrodes on the microarray were activated.

3.2 Experimental Setup and Imaging

The microelectrode arrays were connected to a bank of three-position switches allowing each electrode to be disconnected or connected to the positive or negative terminal of an Agilent 33120A Arbitrary Function Generator. A subset of nine electrodes in a three by three group was used for each experiment. Electrodes were connected in a “checkerboard” pattern as seen in Figure 1A and 1B, which results in the DEP force vector field shown in Figure 1C which was simulated using COMSOL Multiphysics software (v4.0a). A sinusoidal waveform was used at a frequency of 10 kHz at 20 V peak-to-peak (pk-pk) amplitude. The system was visualized using an Olympus BX41 upright epifluorescent microscope with filters for FITC (520 nm) and Texas Red (650 nm). Both bright field and

fluorescent images were captured using an Olympus 16-bit (per channel) 5-megapixel RGB Bayer filter CCD camera.

3.3 Whole Blood Samples from CLL Patients

Whole blood samples were collected from CLL patients and healthy volunteers in accordance with the policies of the Institutional Review Board of the University of California, San Diego under IRB protocol #070643. The evacuated blood collection tubes contained lithium heparin (Becton Dickinson). SYBR Green I Dye (Invitrogen) was diluted from 10,000× stock to a concentration of 100× in 1× TBE (Fisher Scientific). 10 µL of the 100× SYBR Green I solution was added to 190 µL of blood from each CLL patient or from samples obtained from healthy individuals, for a final dye concentration of 5×. The sample was then allowed to incubate at room temperature for 15 minutes before 20 µL were loaded onto the microarray. The DEP field was applied at 10 kHz and 20 V_{pk-pk} for 15 minutes. The microarray was washed three times with 0.5× PBS and then fluorescent images were taken.

3.4 Virus Preparation

T7 Bacteriophage were grown to express mCherry fluorescent protein using Novagen's T7Select® Phage Display System. The titer of the isolated virus was 10¹⁰ mL⁻¹, determined by plating dilutions of phage and counting the resulting plaques. 10 µL of the isolated virus solution was added to 190 µL of settled blood and gently mixed before being added to the DEP microelectrode array. The function generator was set to output a 10 kHz sinusoid at 14 V_{pk-pk}. The DEP field was applied for 20 minutes, the microarray was then washed three times with 0.5× PBS and then fluorescent images were taken.

3.5 Mitochondria Preparation

Mitochondria from Jurkat cells were first stained using the MitoTracker Red CMXRos (catalog number M-7512) from Invitrogen and then isolated using a Mitochondria Isolation Kit from Sigma (catalog number MITOISO2). Lyophilized dye was dissolved in dimethylsulfoxide (DMSO) to a concentration of 1 mM. Suspension cells were centrifuged (1000 rpm for 10 minutes) and resuspended in 37 °C RPMI 1640 (catalog number: 11875093 from Invitrogen) containing 10% FBS and 500 nM of MitoTracker Red. The cells were then incubated for 45 minutes at 37 °C with 5% CO₂ before pelleting for mitochondria isolation. After the cells were stained, they were centrifuged at 1000×g for 5 minutes (All centrifugation steps were performed at 4 °C). After another wash with PBS and centrifugation, the cell pellet was resuspended in 0.75 mL Lysis Buffer and incubated on ice. After the 5-minute incubation, 1.5 mL of Extraction Buffer was added and then centrifuged at 1000×g for 10 minutes. The supernatant was transferred to a fresh tube and centrifuged at 3500×g for 10 minutes. This supernatant was removed and the remaining pellet was resuspended in 200 µL storage buffer. The field applied was a 10 kHz sinusoid at 50 V_{pk-pk} for 30 minutes.

3.6 Buffers, Blood Samples and Conductivity Measurements

Tris-Borate-EDTA (1× TBE) buffer solution was obtained from Fisher Scientific. Dulbecco's Phosphate Buffer Saline (1× PBS) solution was obtained from Invitrogen (Carlsbad, CA) and diluted to 0.5×. Blood for the controls and disrupted blood experiments was collected from a human adult male volunteer in accordance with the policies of the Institutional Review Board of the University of California, San Diego under IRB protocol #070643. The collection tubes contained lithium heparin (Becton Dickinson). For "settled blood" experiments, the heparinized blood was allowed to settle for 20–30 minutes and 200–400 µL of supernatant was removed. This supernatant is primarily plasma, but still contains some red and white blood cells. To disrupt settled blood, about 10 mg of porous glass microbeads were added to 100 µL of settled blood, which was then agitated by vortexing for 1 minute. This procedure causes disruption of many of the cells, releasing high molecular weight DNA (nuclei, nucleosomes, DNA nanoparticulates) from white blood cells. The DEP field was applied to the settled blood samples at 10 kHz and 20 V_{pk-pk} for 15 minutes. Conductivity measurements were made with a Horiba B-173 Compact Conductivity Meter. Conductivities were measured to be 7.4 mS/cm for whole blood, 11.5 mS/cm for settled blood, and 1.46 mS/cm for mitochondria storage buffer.

4 RESULTS AND DISCUSSION

Our earlier work has demonstrated the ability to use DEP devices and techniques to separate and detect both hmw-DNA and nanoparticles from high conductance buffers [46–48]. More recently, the rapid isolation and detection of hmw-DNA and nanoparticles directly from whole blood samples has been demonstrated at clinically relevant levels [19, 57, 58]. This new study further demonstrates the potential clinical relevance of this DEP technology by now showing: (1) the rapid isolation and detection of SYBR Green stained DNA materials from 20 µL whole blood samples from Chronic Lymphocytic Leukemia (CLL) patients; (2) the rapid isolation and detection T7 (*mCherry*) bacteriophage from whole human blood; (3) the rapid isolation and detection of human mitochondria from biological storage buffer; and (4) the use of new DEP microarray devices for the rapid isolation and detection of low levels of hmw-DNA (8–16 ng/mL) in serum samples.

4.1 DEP Separation and Detection of CFC-DNA from CLL Patient Blood Samples

Chronic Lymphocytic Leukemia (CLL) has been considered a homogeneous disease of immature, immune-incompetent, minimally self-renewing B cells, which accumulate constantly because of a faulty apoptotic mechanism. CLL is now viewed as originating from antigen-stimulated mature B lymphocytes, which either avoid death through the intercession of external signals or die by apoptosis, only to be replenished by proliferating precursor cells [59]. With regard to CLL disease diagnostics, the Immunoglobulin V_H (IGHV) somatic mutation status has been shown to be of value in predicting outcomes for CLL patients [60–62]. To obtain this information, a typical protocol involves the isolation of peripheral blood mononuclear cells (PBMCs) from a CLL patient, extraction of DNA, PCR amplification of the IGHV region, sizing on an agarose gel, excision and extraction of the amplified band, and sequencing of the resulting DNA. The objective of this study is to determine if DEP can be used to rapidly isolate CLL related cfc-DNA directly from a small blood sample. For

these studies, fresh blood samples from CLL patients were obtained from the UCSD Moores Cancer Center (Dr. Thomas Kipps Lab). Samples were usually run within two to four hours of the blood draw. Figure 2 shows results for DEP isolation and fluorescent detection of cfc-DNA from: (A), (B), (C) normal blood samples; (D), (E), (F), (G), (H) five different CLL patient blood samples; and (I) a normal “disrupted” settled blood sample. Normal blood samples, such as shown in Figure 2A–2C, generally do not show any significant collection of SYBR Green stained fluorescent DNA in the DEP high-field areas. In contrast, all the CLL patient samples (Figures 2D–2H) show significant amounts of SYBR Green stained DNA in the DEP high-field areas on the nine activated microelectrodes. The normal “disrupted” settled blood sample (Figure 2I) also shows significant collection of SYBR Green stained fluorescent DNA in the DEP high-field areas of the nine activated microelectrodes. Since the “disrupted” buffy coat blood represents a good model for hmw/cfc-DNA [19] there is high confidence that the fluorescent stained materials in the three CLL samples is cfc-DNA. Qualitatively, it is estimated from our earlier DEP work [19] and from results shown in Figure 5, that these particular CLL samples contain more than 50 ng/mL of cfc-DNA.

4.2 DEP Isolation and Detection of T7 (*mCherry*) Bacteriophage from Blood

All our previous DEP work has involved the isolation and detection of either hmw-DNA or fluorescent polystyrene nanoparticles from blood and buffer solutions. In order to further investigate the different nanoparticulate entities that can be separated from blood, a fluorescent T7 (*mCherry*) bacteriophage was selected as good (safe) model for virus detection. The T7 bacteriophage is capable of infecting many types of bacteria, including most strains of *Escherichia coli*. The virus has complex structural symmetry, with a spherical protein capsid with an inner diameter of 55 nm and a tail 19 nm in diameter and 28.5 nm long attached to the capsid. The T7 bacteriophage DNA is enclosed within the capsid structure. In this study, DEP isolation and detection was carried out on a 20 μ L sample of blood containing fluorescent T7 (*mCherry*) Bacteriophage at a concentration of about 5×10^8 virus/mL. After 20 minutes of applying the AC field at 10 kHz and 14 V_{pk-pk} and three washes with 0.5 $\times$ PBS buffer, the fluorescent image in Figure 3B shows considerable concentration of the T7 (*mCherry*) Bacteriophage in the DEP high-field areas relative to the (no virus) negative control in Figure 3A. While this is only a qualitative example demonstrating the DEP separation of virus directly from blood, the actual number of virus seen on one microelectrode structure is estimated to be about 5×10^5 .

4.3 DEP Isolation and Detection of Mitochondria from Biological Buffer

Again, all our previous DEP work has been focused on the isolation and detection of hmw-DNA and fluorescent polystyrene nanoparticles from blood and buffer solutions. This study now involves the isolation and detection of human (Jurkat Cell) mitochondria from a biological buffer with a conductance of ~ 1.5 mS/cm. These particular mitochondria are approximately 500–600 nm in size with an outer membrane coat. For this study a 20 μ L sample of MitoTracker Red fluorescent stained mitochondria in storage buffer was applied to the DEP microarray device. DEP was carried out at 10 kHz and 50 V_{pk-pk} for 30 minutes. Figure 4B shows intense red fluorescence from the MitoTracker Red fluorescent stained mitochondria which have become highly concentrated in the DEP high-field areas. This

study was meant to be a qualitative example of using DEP to isolate and detect another important cellular nanoparticulate (organelle) which has potential to be a clinical diagnostic biomarker.

4.4 Isolation and Detection of HMW-DNA from Serum Using New DEP Microarray Devices

New prototype DEP microarray devices were used to carry out the isolation and detection of hmw-DNA in serum. In these experiments, DEP was carried out on eight samples of a dilution series where the concentration of hmw-DNA in serum ranged from 0 ng/mL to 500 ng/mL. The AC field was applied to each sample at 10 kHz and 7 V_{pk-pk} for 15 minutes. Figure 5A shows that detectable fluorescence was observed in the DEP high-field areas on the microelectrodes for all the hmw-DNA containing samples, including the very low concentrations of 8 ng/mL and 16 ng/mL. Figure 5B shows an enlarged image of the 0 ng/mL negative control, and Figure 5C shows the enlarged image for the 250 ng/mL sample. Due to incomplete removal of the silicon dioxide layer, occasionally an electrode will not collect DNA. The ability to detect these low levels of hmw-DNA in human serum (conductance of ~10–11 mS/cm) will be important for future diagnostic applications. For example, cancer related cfc-DNA occurs in the blood at levels from 0 to over 1000 ng/mL, with an average value of about 180 ng/mL [2, 4, 8, 9]. Thus, the ability to detect levels at the 10 ng/mL means the technology might be used for early cancer diagnostics.

5 CONCLUDING REMARKS

The ability to rapidly isolate and detect cancer and other disease related cfc-DNA, cellular nanoparticulates (exosomes, mitochondria, etc.) and virus directly in blood, plasma, serum and biological buffers will be important for many future clinical diagnostic applications. This study demonstrates the potential clinical relevance of this DEP technology by showing the rapid isolation and detection of SYBR Green stained cfc-DNA from 20 µL whole blood samples from Chronic Lymphocytic Leukemia (CLL) patients. The isolation and detection of T7 (*mCherry*) bacteriophage from whole human blood is an important result that demonstrates that the DEP technology has potential for clinical applications related to virus and other pathogen detection. With regard to other important cellular nanoparticulate (organelle) biomarkers, the rapid isolation and detection of human mitochondria from biological storage buffer shows potential for related clinical and research applications. Finally, the results from the new DEP microarray devices demonstrating the rapid detection of low levels of hmw-DNA in serum samples show promise for early cancer diagnostics. Overall the results of this study support the enormous potential of DEP as a “seamless sample-to-answer” technique for the rapid detection of cfc-DNA and nanoparticulate biomarkers directly from blood and other complex biological samples.

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Figure 1.

DEP Force Vector Diagram. (A) Nine of the 80 μm diameter circular microelectrodes in a 3 $\times$ 3 section of the 100 site microarray used to carry out DEP experiments. (B) The checkerboard pattern used to create the DEP AC field asymmetry, which results in DEP high-field regions on the microelectrodes (red dashed circles) and DEP low-field regions between the microelectrodes (blue dashed circles). (C) The resultant DEP force vector field pattern as modeled with COMSOL Multiphysics (CMF=1, particle diameter=100 nm).

Figure 2.

DEP Isolation and Fluorescent Detection of CFC-DNA in Blood Samples from CLL Patients. About 20 μ L of whole blood was applied to the DEP microarray device. An AC field was then applied at 10 kHz and 20 V_{pk-pk} to the nine microelectrodes in columns 2, 3, and 4 (yellow dotted area) for 15 minutes. No voltage was applied to the three microelectrodes in column 1 (left side), which serve as a negative control. The DEP microarray was washed three times with 0.5 $\times$ PBS after which epifluorescent microscope imaging detection was carried out. Figures 2 (A), (B), and (C) show the results for blood samples from three normal (non-CLL) individuals, Figures 2 (D), (E), (F), (G), and (H) show the results for five different CLL patient bloodsamples, and (I) shows the result for a normal “disrupted” buffy coat blood sample. The normal blood samples (A), (B), and (C) show no significant collection of SYBR Green stained fluorescent DNA in the DEP high-field areas. All five CLL patient samples (D), (E), (F), (G) and (H) show relatively significant amounts of SYBR Green stained DNA in the DEP high-field areas on the nine activated microelectrodes. The normal “disrupted” buffy coat sample (I) also shows significant collection of SYBR Green stained fluorescent DNA in the DEP high-field areas of the nine activated microelectrodes. It should be pointed out that the fluorescent signal

often appears significantly higher for the middle microelectrodes of columns 2 and 4, and the upper and lower microelectrodes of column 3, due to greater electric field gradient at these locations. In all cases, the three un-activated microelectrodes in column 1 show no fluorescence.

Figure 3.

DEP Separation of T7 (*mCherry*) Bacteriophage in Blood. 20 μ L samples of whole blood without T7 (*mCherry*) Bacteriophage (A), and with T7 (*mCherry*) Bacteriophage (B) were applied to the DEP microarray devices. An AC field was then applied to each sample at 10 kHz and 14 V_{pk-pk} to the nine microelectrodes in columns 2, 3, and 4 (yellow dotted area) for 20 minutes. No voltage was applied to the three microelectrodes in column 1 (left side), which serve as a negative control. The DEP microarrays were washed three times with 0.5 $\times$ PBS after which epifluorescent microscope imaging detection was carried out. Figure 3B shows intense red fluorescence from the concentrated T7 (*mCherry*) Bacteriophage in the DEP high-field areas (yellow arrows), relative to the image of the blood sample without T7 (*mCherry*) Bacteriophage (Figure 3A).

Figure 4.

DEP of Fluorescent Stained Mitochondria. A 20 μ L sample MitoTracker Red fluorescent stained mitochondria in storage buffer was applied to the DEP microarray device. An AC field was then applied to each sample at 10 kHz and 50 V_{pk-pk} to the nine microelectrodes in columns 2, 3, and 4 (yellow dotted area) for 30 minutes. No voltage was applied to the three microelectrodes in column 1 (left side), which serve as a negative control. The DEP microarrays were washed three times with 0.5 $\times$ PBS after which epifluorescent microscope imaging detection was carried out. Figure 4A shows the microarray before the DEP field was applied, and Figure 4B shows the microarray after the DEP field was applied and washed with 0.5 $\times$ PBS buffer. Figure 4B now shows intense red fluorescence from the MitoTracker Red fluorescent stained mitochondria concentrated in the DEP high-field areas (yellow arrows).

Figure 5.

Detection of hmw-DNA in Serum Using New DEP Microarray Devices. New prototype DEP microarray devices were used to carry out the isolation and detection of hmw-DNA in serum. In these experiments, DEP was carried out on eight samples of a dilution series where the concentration of hmw-DNA in serum ranged from 0 ng/mL to 500 ng/mL. An AC field was then applied to each sample at 10 kHz and 7 V_{pk-pk} for 15 minutes. The DEP microarrays were washed three times with 0.5× PBS after which epifluorescent microscope imaging detection was carried out. Figure 5A shows the results for the series where detectable fluorescence is observed in the DEP high-field areas on the microelectrodes for all hmw-DNA containing samples including the lowest concentrations of 8 ng/mL and 16 ng/mL. Figure 5B shows an enlarged image of the 0 ng/mL negative control and Figure 5C shows the enlarged image for the 250 ng/mL sample.