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# New High Conductance DEP Devices for Detection of Cancer Related DNA Nanoparticulate Biomarkers and Nanoparticles in Whole Blood

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## ABSTRACT

The ability to rapidly detect cell free circulating (cfc) DNA nanoparticulate biomarkers, drug delivery nanoparticles and virus directly from blood is a major challenge for nanomedicine. Previously, we have developed high conductance dielectrophoretic (HC-DEP) microarray devices which allowed cfc-DNA, cellular nanoparticles (mitochondria, etc.), nanoparticles and virus to be rapidly isolated from undiluted whole blood. Now we have developed novel capillary tube and pipette tip DEP devices which can be used to isolate cfc-DNA directly from a few microliters of whole blood. An AC voltage from 50-150 volts is applied at frequencies of 5kHz-10kHz which causes cfc-DNA to become concentrated on the tip of the device. The material captured on the tip can then be transferred to a collection tube for further analysis (PCR, genotyping, sequencing). The new HC-DEP devices are being used to collect cfc-DNA from Chronic Lymphocytic Leukemia (CLL) whole blood patient samples. Blood sample to PCR is achieved in less than twenty minutes. HC-DEP sets the stage for new "seamless" sample to answer diagnostic systems which will allow a variety of important cancer and other disease biomarkers to be rapidly isolated and analyzed directly from blood and other clinical samples.

***Keywords***: Dielectrophoresis (DEP), DNA, cfc-DNA, nanoparticles, blood, biomarkers, cancer diagnostics

## INTRODUCTION

While the potential medical applications of nanotechnology are rapidly growing, a number of issues still need to be resolved before nanomedicine moves from the lab to the bedside. Two very important challenges in nanomedicine will be the detection of early disease nanoparticulate biomarkers, and the monitoring of drug delivery nanoparticles. The ability to rapidly detect low levels of cell free circulating (cfc) DNA, RNA and other nanoparticulate biomarkers directly in blood would represent a major advance for early cancer detection and screening, residual disease detection and chemotherapy monitoring. Cfc-DNA and cfc-RNA are potentially important biomarkers for early cancer detection [1]. These biomarkers are high molecular weight (hmw) DNA/RNA clusters that are released into the blood stream by tumor cell necrosis [2]. Unfortunately, it remains a challenge to isolate and detect hmw-DNA and other early disease biomarkers *directly* in complex samples like blood [3]. Often, the detection of early disease biomarkers is a statistical problem requiring that a relatively large sample (1-5ml) be processed. Even though highly sensitive detection technologies (PCR, FACS) are available for subsequent analysis [4], the sample preparation process adds considerable time, labor and expense to the diagnostic assay. Furthermore, sample preparation (centrifugation, filtration, etc.) can also cause considerable degradation and loss of hmw-DNA. Additionally, with the enormous amount of activity now being directed at new drug delivery nanoparticle therapeutics, it will also be important to develop rapid, sensitive and inexpensive monitoring techniques for this nanomedicine application. Thus, there is a critical need for novel robust technology, which will allow a variety of important nanoscale entities to be manipulated, isolated and rapidly detected directly from whole blood and other biological samples. DEP is a separation technique which uses AC electric fields to manipulate cells and nanoparticles. While high resolution separation of cells, bacteria, virus, and DNA has been carried out by DEP, serious performance limitations have prevented the technology from being used for practical applications. In particular, DEP's limitation to low ionic strength (conductance) solutions requires that blood be processed and diluted 10-100 fold before separation [5]. Previously, using microarray devices we were able to demonstrate high conductance (HC) DEP that allowed both hmw-DNA nanoparticulates and nanoparticles to be manipulated, isolated and detected under high ionic strength conditions [6-8]. We were also able to show the rapid isolation and detection of hmw-DNA and nanoparticles in undiluted whole blood using the high conductance DEP microarray devices. We now show that high molecular weight (hmw) DNA and cfc-DNA can be isolated directly from small amounts of cancer patient whole blood samples using novel capillary tube and pipette tip DEP devices. HC-DEP sets the stage for new "seamless" sample to answer diagnostic systems which will allow a variety of important nanoscopic biomarkers and drug delivery nanoparticles to be rapidly isolated and analyzed from clinically relevant amounts of complex un-diluted biological samples.

## RESULTS AND DISCUSSION

Figure 1A–1C shows the basic scheme for the DEP separation of hmw-DNA in whole blood using a microarray device. Figure 1A shows the DEP microarray device with whole blood (red and white cells) containing hmw-DNA clusters (green dots). Figure 1B shows the DEP separation of hmw-DNA clusters into the high-field regions (represented as domes) where they are held firmly on the microelectrodes, and blood cells moving into the low-field regions between the microelectrodes where they are held less firmly. A fluidic wash now easily removes the blood cells while the hmw-DNA remains in the high field regions (Figure 1C). The hmw DNA can then be detected and further analyzed by PCR. The actual experimental results showing the isolation and detection of OliGreen fluorescent stained 45kb hmw-DNA in whole blood (0.7 S/m) is shown in Figure 2A-2D. Figure 2A shows twelve 80 um microelectrodes (on the microarray) clearly visible before the blood sample is applied. The set of nine microelectrodes in dotted square area will be activated, while the three microelectrodes on the left side remain un-activated. About 20µL of whole blood containing 260ng/mL of hmw-DNA (40-45kb DNA clusters stained with OliGreen fluorescent dye) were now added. After the blood/hmw-DNA sample is added, the microelectrodes are no longer visible because of the high cell density (Figure 2B). The hmw-DNA is then separated from the blood cells by application of a DEP field at 10,000 Hz (20 volts pk-pk) for 15 minutes. The microarray was then washed three times with 0.5x PBS to remove the blood cells. The OliGreen stained fluorescent hmw-DNA can clearly be seen concentrated around the microelectrodes (Figure 2C). The three un-activated control microelectrodes in column 1 show no fluorescence. Finally, MATLAB was used to produce 3D fluorescent intensity images for better quantitative analysis of the hmw-DNA concentrated on the microelectrodes (Figure 2D). The next DEP experiment demonstrates the separation and detection of 40nm red fluorescent nanoparticles in undiluted whole blood (0.52 S/m). These experiments were carried out at 10,000Hz and 20 volts pk-pk. In addition to HC-DEP microarray devices, we now have developed unique and simple capillary tube and pipette tip devices which can carry out HC-DEP. Figure 3 shows a basic DEP pipette tip device, which represents a general type of high voltage AC/DC device which can be constructed from simple components. The pipette tip component has a pore/hole structure, a hydrogel filling in the lower section, a buffer reservoir above the hydrogel and an electrode. The lower sample chamber provides a reservoir for the sample and has an circular electrode. The figure shows the DEP separation of nanoparticles from blood cells (red), where the smaller green fluorescent 40nm nanoparticles (cfc-DNA) are concentrated around the DEP high field region, which generally occurs at the edge of the pipette tip hole structure; and micron-size blood cells are concentrated in the low field region radiating out to the platinum ring electrode (in

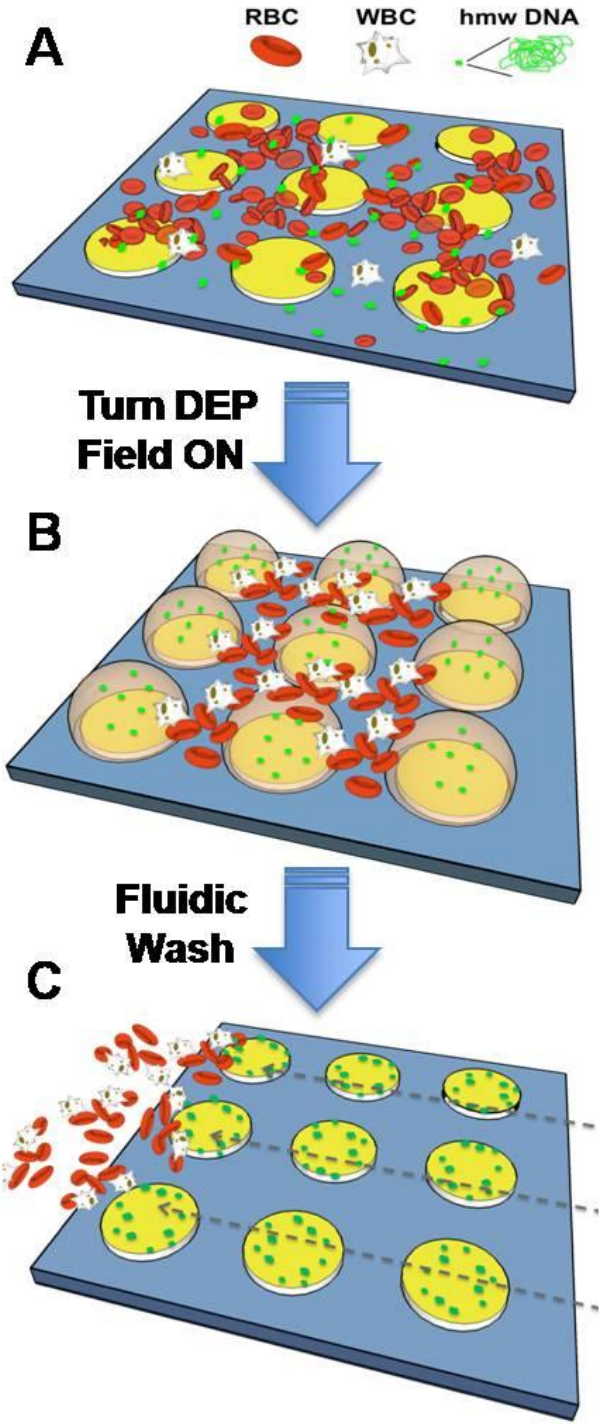


**Figure 1 - The DEP separation of hmw-DNA clusters in whole blood. (A) Shows the microarray with blood cells and hmw-DNA. (B) Shows the array after the DEP field is applied, with hmw-DNA clusters (green dots) in the high field regions and the red and white blood cells in the low field regions between the microelectrodes. (C) Shows fluidic wash removing the blood cells, with hmw-DNA clusters remaining in the high field regions.**

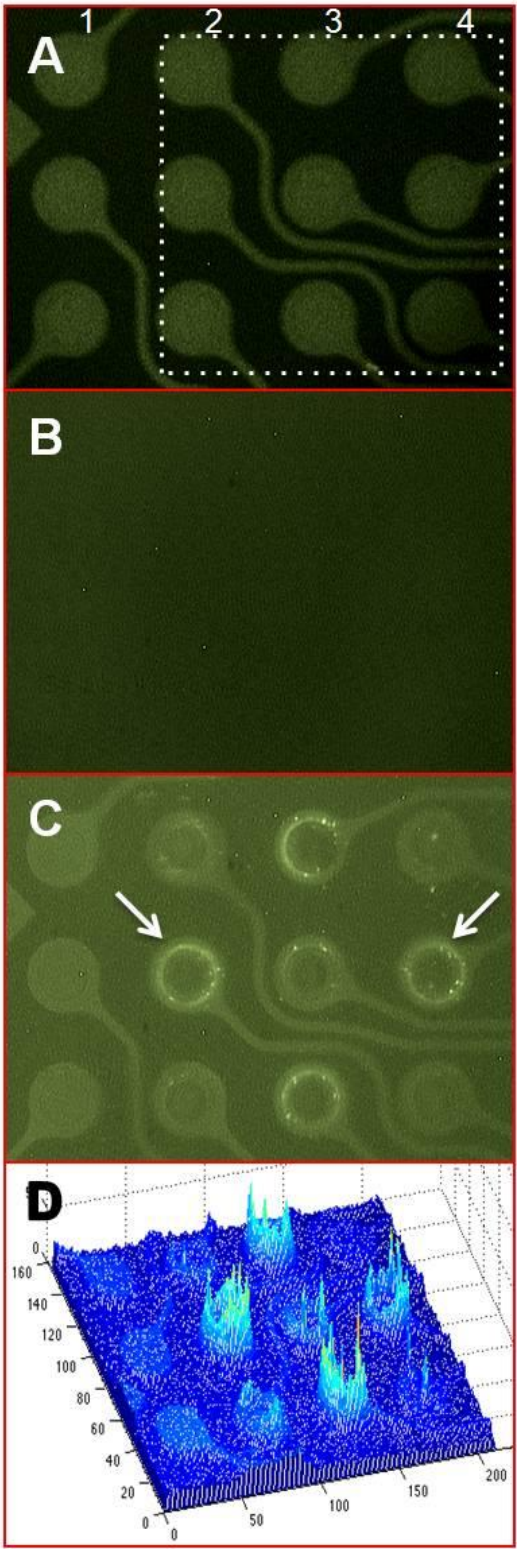


**Figure 2 - Experimental results for separation of OliGreen fluorescent stained hmw-DNA in whole blood. (A) Shows twelve microelectrodes (80um diameter) with the three microelectrodes in column 1 being un-activated controls. (B) Shows the microarray after the whole blood sample was added. (C) Shows green fluorescence from the hmw-DNA concentrated around the microelectrodes, after DEP field was applied for 15 minutes and the cartridge was washed 3 times with 0.5x PBS. (D) Shows the relative 3D fluorescent intensities on all nine of the microelectrodes (produced using MATLAB).**

the sample chamber). The DEP high field region occurs around the edges of the pipette tip hole structure because this is where the DEP field between the sample separation chamber and inner area (inside the pipette) is most constricted.

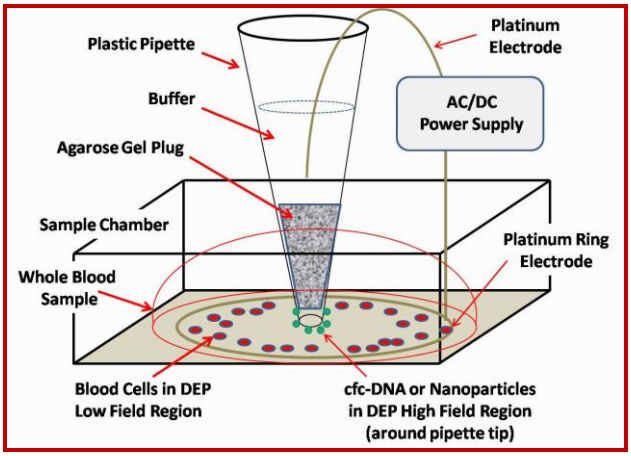


**Figure 3 – Shows a DEP pipette tip device and sample chamber. The pipette tip component has a pore/hole structure, a hydrogel filling in the lower section, a buffer reservoir above the hydrogel and an electrode. The lower sample chamber provides a reservoir for the sample and has an electrode. The figure shows the DEP separation of nanoparticles from blood cells (red), where the smaller green fluorescent 40nm nanoparticles (cfc-DNA) are concentrated around the DEP high field region, which generally occurs at the edge of the pipette tip hole structure; and micron-size blood cells are concentrated in the low field region radiating out to the platinum ring electrode (in the sample chamber). The DEP high field region occurs around the edges of the pipette tip hole structure because this is where the DEP field between the sample separation chamber and inner area (inside the pipette) is most constricted.**

Overall, in these presented and other related DEP experiments hmw-DNA could be detected at a level of <260ng/ml, and 40nm fluorescent nanoparticles at <9.5 x $10^9$ particles/ml. These detection levels are well within the range for viable clinical diagnostics and drug nanoparticle monitoring. More recently, using the pipette tip devices we have been able to detect cfc-DNA stained materials directly in blood from patients with Chronic Lymphocytic Leukemia (CLL). (CLL samples obtained from Dr. Thomas Kipps at the UCSD Moores Canter Center). We have shown

that this technique can be used to isolate cfc-DNA and other nanoparticulates from Chronic Lymphocytic Leukemia (CLL) patient blood samples. At DEP frequencies of 5-10 kHz SYBR Green fluorescent-stained cfc-DNA separates from the blood and becomes highly concentrated at specific DEP high field regions over the microelectrodes, while blood cells move to the DEP low field regions. The blood cells can then be removed by a simple fluidic wash and the highly concentrated fluorescent stained cfc-DNA detected. Early PCR results show that we can amplify the genes for the VH families. This also verifies that the nanoparticulates collected contain cfc-DNA.

## CONCLUSIONS

DEP allows us to overcome the sample preparation challenges that have limited the clinical relevance of cfc-DNA as a diagnostic biomarker.

- **DEP is an *enabling* technology**
- **Observed visible quantities of SYBR Green I stained material in stage I and stage II CLL patients**
- **Isolated cfc-DNA for PCR identification of $IgV_H$ family**
- **New pipette tip devices enhance recovery for downstream processing (PCR)**
- **Carried out PCR analysis of cfc-DNA isolated from CLL patients.**

## FUTURE WORK

- **Continue cfc-DNA /RNA isolation from CLL, pancreatic and ovarian cancer samples**
- **ID and verify nature of cfc-DNA/RNA from CLL and other blood samples**
- **In-situ RT-PCR and fluorescent antibody (seamless sample to answer)**
- **Infectious disease (bacteria/virus) applications, MI and cardiac disease applications**

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