Detection of Cancer Related DNA Nanoparticulate Biomarkers and Nanoparticles in Whole Blood

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ABSTRACT

The ability to rapidly isolate and detect DNA nanoparticulate biomarkers (cell free circulating DNA) and drug delivery nanoparticles directly in blood is a major challenge for early disease detection and nanomedicine. We now show that a microarray dielectrophoretic (DEP) device can be used to rapidly isolate and detect high molecular weight (hmw) DNA nanoparticulates and nanoparticles directly from whole blood. At DEP frequencies of 5kHz-10kHz both fluorescent-stained hmw-DNA and 40nm fluorescent nanoparticles separate from the blood and become 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 while the hmw-DNA and nanoparticles remain highly concentrated. The hmw-DNA could be detected at a level of <260ng/ml, and the nanoparticles at $<9.5 \times 10^9$ particles/ml, detection levels that are well within the range for viable clinical diagnostics and drug nanoparticle monitoring. DNA stained materials could also be detected directly in blood from patients with Chronic Lymphocytic Leukemia (CLL).

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]. Recently, we have developed a high conductance (HC) DEP method that allows both hmw-DNA nanoparticulates and nanoparticles to be manipulated, isolated and detected under high ionic strength conditions [6-8]. 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. We now report on a dielectrophoretic (DEP) approach that allows rapid isolation and detection of hmw-DNA and nanoparticles in undiluted whole blood.

RESULTS AND DISCUSSION

Figure 1A–1C shows the basic scheme for the DEP separation of hmw-DNA in whole blood. 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. 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.

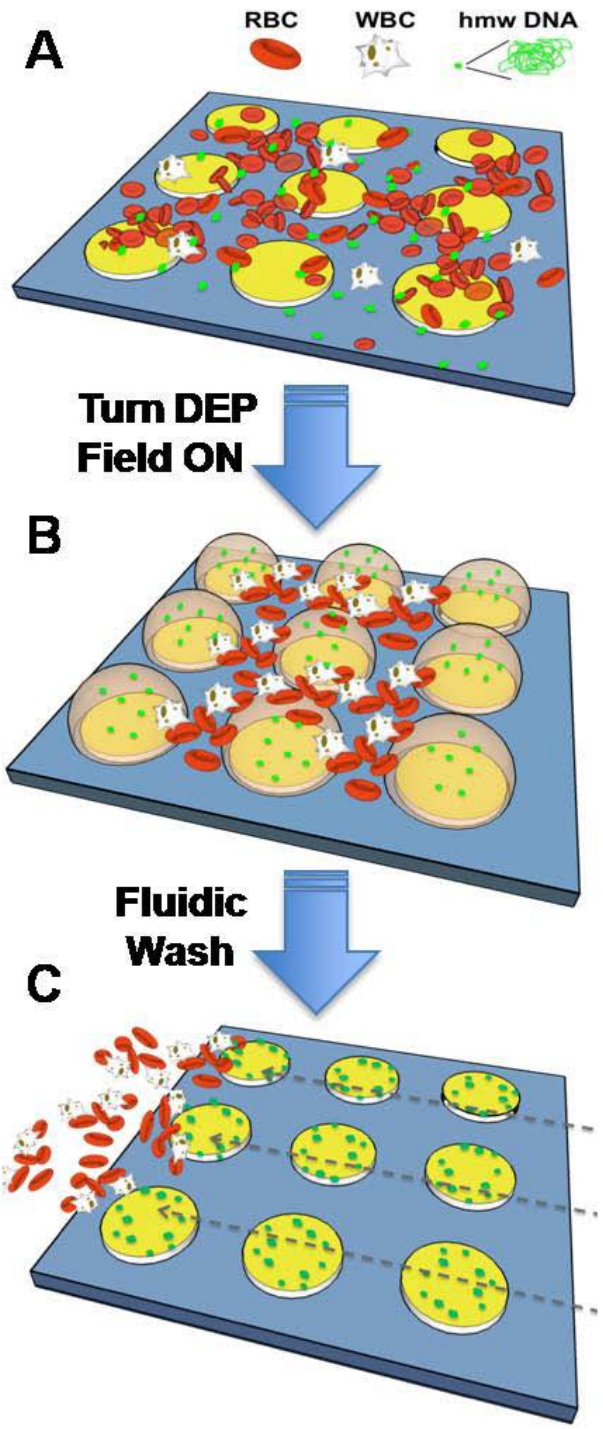

Figure 1 - The DEP separation of hmw-DNA clusters in whole blood. (A) Shows the microarray with blood cells and hmw-DNA. (B) Shows the array after the DEP field is applied, with hmw-DNA clusters (green dots) in the high field regions and the red and white blood cells in the low field regions between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

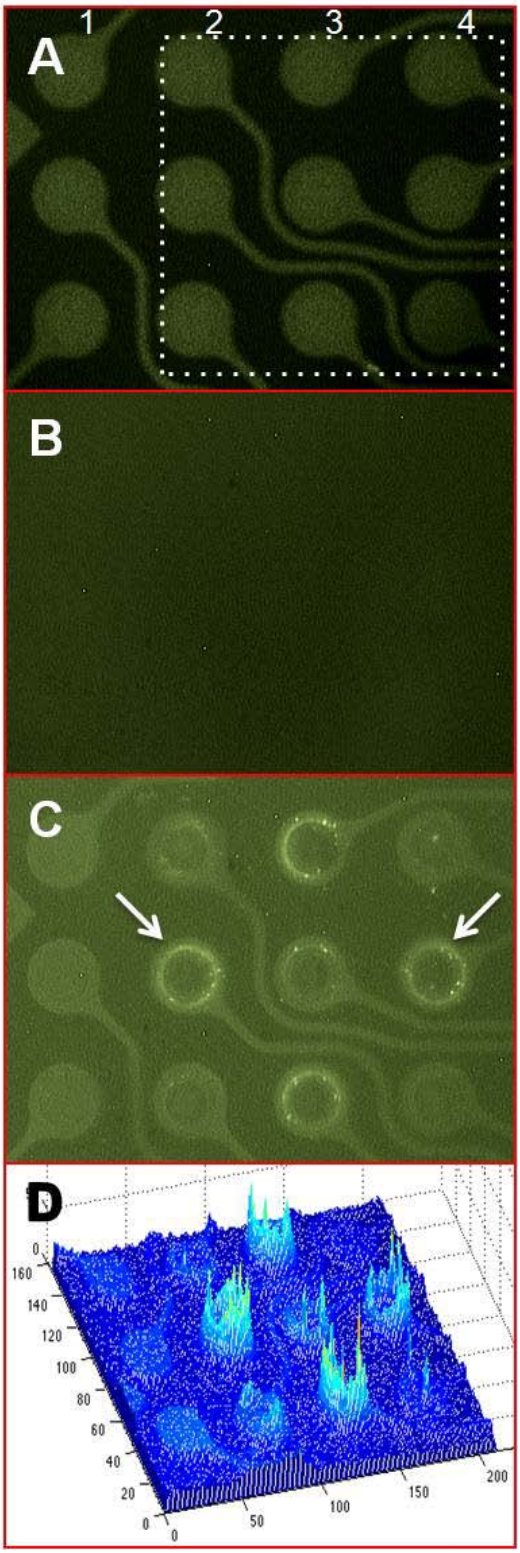

Figure 2 - Experimental results for separation of OliGreen fluorescent stained hmw-DNA in whole blood. (A) Shows twelve microelectrodes (80um diameter) with

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).

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. Figures 3a and 3b show the microarray in green light and red fluorescence before the whole blood sample was added. Figure 3c and 3d show images of the microarray in green light and red fluorescence after 20µL of whole blood containing 300ng/µL of 40nm red fluorescent nanoparticles was added, but before the DEP field has been applied. The microelectrodes are no longer visible in the green light image because of the high blood cell density, but a red fluorescent background from the nanoparticles can now be seen in the fluorescent image (Figure 3d). The DEP field was applied to the nine microelectrodes for 11 minutes. For whole blood, the movement of cells into the low field regions and concentration of fluorescent nanoparticles into the high field regions was only partially detectable while the blood remained on the microarray. After the blood was removed, the green light image in Figure 3e shows the microelectrodes and the red fluorescent image in Figure 3f shows the fluorescent nanoparticles concentrated over the microelectrodes (columns *2, 3, and 4*). No red fluorescence is seen over the three microelectrodes in column *1*, which were not activated. The microarray was then washed five times with 0.5x PBS, and the final images show no cells remaining (Figure 3g), and some red fluorescence from the nanoparticles still remaining on the microelectrodes (Figure 3h). Figure 3i is a fluorescent intensity image after the first 0.5x PBS wash showing the relative levels of fluorescence on all nine microelectrodes (produced using MATLAB).

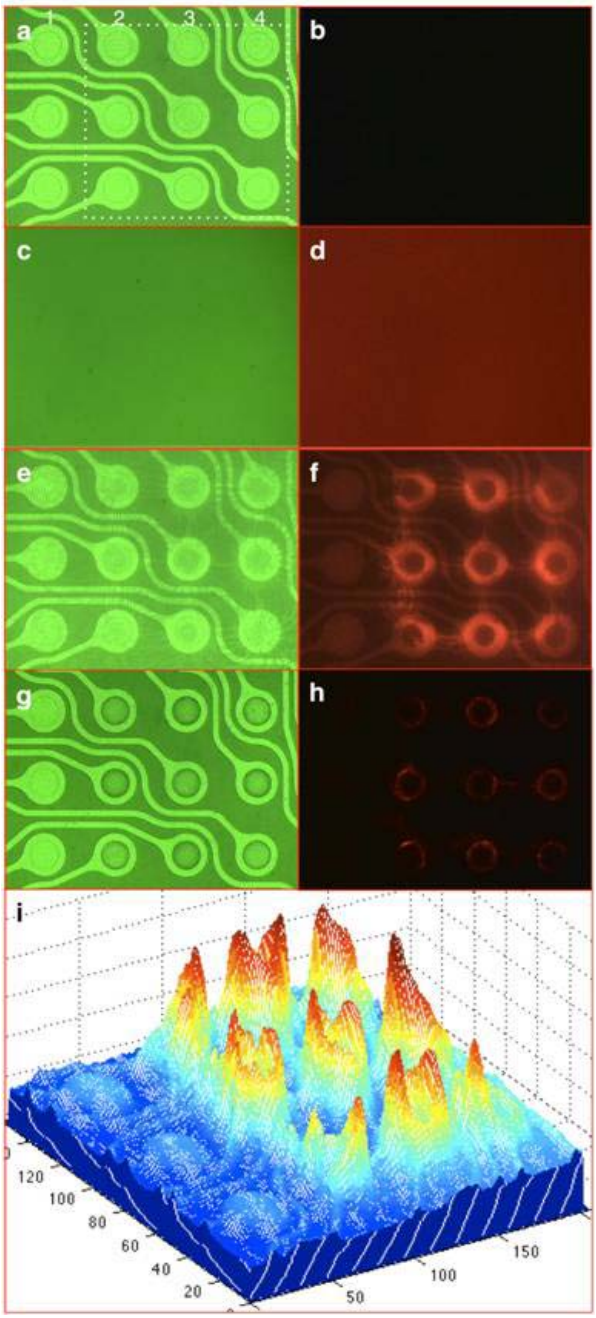

Figure 3 - Separation and detection of 40nm red fluorescent nanoparticles in whole blood. (a, b) Show the microarray in green light and red fluorescence before the whole blood sample was added. (c, d) Show the microarray in green light and red fluorescence after 20µL of whole blood containing 300ng/µL of 40nm red fluorescent nanoparticles were added, but no DEP field has been applied. (e, f) Show the microarray after the

DEP field was applied at 10,000 Hz and 20 volts pk-pk for 11 minutes to a set of nine microelectrodes (columns 2, 3, and 4), while the three electrodes in the column 1 remained unactivated, and then the blood was removed. The green light image shows the underlying microelectrodes with some blood cells scattered across the surface, and the red fluorescent image shows the red fluorescent nanoparticles concentrated over the microelectrode structures (columns 2, 3, and 4). (g, h) Show the microarray after being washed five times with 0.5x PBS, the final (green light) image shows no cells remaining on the microarray, and the fluorescent image shows red fluorescence from nanoparticles still remaining on the microelectrodes. (i) A 3D fluorescent intensity image after the first 0.5x buffer wash showing the relative levels of fluorescence on all nine microelectrodes (produced using MATLAB).

CONCLUSIONS

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, 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)**

Some important DEP device performance observations were made while carrying out separations under high conductance conditions. These included the formation of bubbles on some of the microelectrodes and darkening of the microelectrodes. As was discussed in our previous work, heat and competition from DC electrochemistry may be the reason for bubbles and the microelectrode darkening [6-8]. The fact that concentration of nanoparticles still takes place may be due to the arrangement of the circular microelectrodes and/or the thin porous hydrogel layer covering the microelectrodes. The thin hydrogel layer provides some level of protection from electrochemical effects including H_2 and O_2 bubbles, and extremes of pH, which occurs under high conductance conditions. Future efforts on this project are being directed at better understanding the electrokinetic mechanisms, and on developing a new generation of robust high performance DEP devices. Overall, we believe the present work has set the stage for a new generation of viable "seamless" sample to answer diagnostic systems; i.e., a process where a complex sample is run through the device, and the specific analytes are rapidly concentrated onto microscopic locations and subsequently detected. Such devices will allow highly complex clinical and other biological samples such as blood, plasma and serum to be rapidly and directly analyzed for rare cells, DNA biomarkers and drug delivery nanoparticles.

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