Rapid detection of cancer-DNA biomarkers and nanoparticles

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A new, seamless, sample-to-answer technology (high-conductance dielectrophoresis) enables rapid analysis of high-molecular-weight DNA clusters and drug-delivery nanoparticles based directly on whole blood.

The ability to rapidly isolate cancer-related DNA biomarkers and drug-delivery nanoparticles directly from whole blood is a major challenge for early disease detection and nanomedicine. Using a microarray dielectrophoretic (DEP) device (in which a force is exerted on dielectric particles subjected to a nonuniform electric field) and new DEP parameters, we have demonstrated that we can rapidly isolate and directly detect high-molecular-weight (hmw) DNA and nanoparticles from whole blood. DEP electric fields cause these components to separate from blood and become highly concentrated at specific DEP high-field regions (over the micro-electrodes), while blood cells move to the low-field regions. The blood cells can then be removed using a simple fluidic wash, while the hmw-DNA and nanoparticles remain highly concentrated.

Cell-free-circulating (cfc) DNA is an important biomarker for early cancer detection, consisting of hmw-DNA clusters that are released into the blood stream by premature death (necrosis) of tumor cells. Unfortunately, isolating and detecting this cancer-cell-derived DNA directly from complex samples (like blood) remains a challenge. In addition, with the significant level 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, a novel, robust technology is critically needed that will allow manipulation, isolation, and rapid direct detection of a variety of important nanoscale entities from whole blood and other biological samples.

DEP is a potentially useful separation technique that uses AC electric fields to manipulate cells and nanoparticles. Although high-resolution separation of cells, bacteria, viruses, and DNA has been carried out successfully using DEP, serious performance limitations have prevented development of practical applications. In particular, DEP’s limitation to low-ionic-strength (conductance) solutions requires that blood be processed and diluted 50 to 100 times before separation. We recently developed a high-conductance (HC) DEP method that allows manipulation, isolation, and detection of both hmw-DNA nanoparticulates and nanoparticles under high-ionic-strength conditions.1–3

Figure 1. Detection of high-molecular-weight (hmw) DNA and fluorescent nanoparticles from whole blood and a chronic lymphocytic leukemia (CLL) blood sample. (A) Normal blood sample after dielectrophoresis (DEP) and washing. No fluorescence is observed on any of the microelectrodes. (B) Blood sample spiked with SYBR Green-stained hmw-DNA. (SYBR Green, a registered trademark of Synergy Brands Inc., is a cyanine dye used as a nucleic-acid stain.) After DEP and washing, green fluorescence is observed on the nine activated microelectrodes. (C) Blood from a CLL patient sample to which SYBR Green was added but no DNA. Following DEP and washing, green fluorescence is observed on the microelectrodes, indicating that hmw-DNA was present in the CLL blood. (D) Blood spiked with 40nm red fluorescent nanoparticles. Upon completion of DEP and washing, intense red fluorescence is observed on the nine activated microelectrodes.

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Figure 1 shows recent results demonstrating isolation and detection of hmw-DNA and fluorescent nanoparticles directly from blood, as well as isolation of cfc-DNA from a chronic lymphocytic leukemia (CLL) blood sample. In these experiments, we added approximately 20μl of blood to the DEP microarray (micro-electrode diameters: ~80μm). We applied the DEP field for 12 to 15 minutes at 10,000Hz/20V (peak to peak) to a set of nine micro-electrodes—see the white dotted square in Figure 1(A)—while the three electrodes on the side remained unactivated. The blood cells move away from the micro-electrodes while any SYBR® Green-stained DNA or red fluorescent nanoparticles begin to concentrate on the electrodes. The microarray was washed with 0.5× phosphate-buffered saline to remove the blood cells, and then examined by red or green fluorescence. Figure 1(A) shows a normal blood sample after DEP and washing. No fluorescence is observed on any of the micro-electrodes. Figure 1(B) shows a blood sample that was spiked with SYBR Green-stained hmw-DNA. Following DEP and washing, green fluorescence is observed on the nine activated micro-electrodes. Figure 1(C) shows blood from a CLL patient sample to which SYBR Green was added but no DNA. After DEP and washing, green fluorescence is observed on the micro-electrodes, indicating that hmw-DNA was present in the CLL blood. Finally, Figure 1(D) shows blood that was spiked with 40nm red fluorescent nanoparticles. Upon completion of DEP and washing, intense red fluorescence is observed on the nine activated micro-electrodes.

The HC-DEP method could enable new, seamless sample-to-answer diagnostic systems that allow rapid isolation and analysis of a variety of important nanoscopic biomarkers and drug-delivery nanoparticles from blood and other clinically relevant biological samples. We recently formed a new company, Biological Dynamics, to pursue development and commercialization of this technology for early cancer detection and chemotherapy/residual disease monitoring.

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References