Dielectrophoretic (DEP) Isolation and Detection of Cancer Related Cell Free DNA, RNA and Exosome Biomarkers from Blood and Plasma (Abstract # 600)

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ABSTRACT

The analysis of cell free (cf) DNA and cf-RNA nanoparticulate biomarkers continues to become more widely used for cancer diagnostics and management. We have now demonstrated a dielectrophoretic (DEP) based approach that allows cf-DNA biomarkers from chronic lymphocytic leukemia (CLL) patients to be isolated in about 10 minutes directly from a small volume (25ul) of patient whole blood and plasma. Cf-DNA isolated from fifteen CLL patient blood samples and eleven CLL patient plasma samples were PCR amplified to identify the VHL genotype and then sequenced. The DNA sequencing results for cf-DNA isolated by DEP were compared to two gold standard methods for CLL analysis. In further work, cf-RNA for ROR1 has also been isolated from CLL samples, and RNA containing exosomes excreted by glioblastoma cells have been isolated by DEP.

Keywords: DEP, Cancer, Cell Free DNA, Cell Free RNA, Biomarkers

1. Introduction

Cell-free (cf) DNA and cf-RNA are important biomarkers for early detection of cancer, residual disease, monitoring chemotherapy and other aspects of cancer management. The isolation of cancer-related cf-DNA from plasma may allow "liquid biopsies" to replace more invasive tissue biopsies for detecting and analyzing cancer mutations. However, the present methods for isolating cf-DNA from plasma are complex, time-consuming and relatively expensive processes that rule out use for point-of-care (POC) diagnostic applications. Other limitations of conventional sample preparation processes include: requirement of at least one or more milliliters of plasma; processing of blood to plasma; a large number of manipulations, which increases the chance for technician errors; decrease of recovery efficiency with decrease in sample size and concentration; degradation of cf-DNA by mechanical sheering during the processing steps; and limiting PCR analysis to shorter target DNA sequences due to the degradation of cf-DNA. Finally, other potentially important cancer-related biomarkers such as cf-RNA, exosomes and microvesicles also require relatively long and involved processes for their isolation from plasma. With regard to hematological cancers such as chronic lymphocytic leukemia (CLL) and lymphomas, DNA for PCR and sequencing can be obtained from transformed cells, as well as from cf-DNA isolated from plasma. In the case of CLL, B cells from patients can be segregated into one of at least two major subsets on the basis of whether or not the immunoglobulin (IG) variable region has somatic mutations. Patients with CLL cells that express unmutated IG heavy chain variable region genes (IGHV genes) tend to have an aggressive clinical course relative to that of patients who have CLL cells that express IGHV with somatic mutations. For CLL diagnostics and management, genomic DNA is isolated from the peripheral blood mononuclear cells (PBMCs). The PBMCs are usually purified from the CLL patient blood samples by density centrifugation using Ficoll-Hypaque 1077. This is a long and labor-intensive process, which adds considerable cost to patient management and precludes any POC applications. To assess the unique patient-specific IGHV expressed by the CLL B cells, PCR and DNA sequencing are performed on the isolated genomic DNA to determine the mutation status for the expressed IGHV gene.

Electrokinetic technologies, like AC dielectrophoresis (DEP) have long been known to provide effective separation of cells, nanoparticles, DNA and other biomolecules. However, until recently, DEP techniques remained impractical for use with high-conductance solutions (5-15 mS/cm), as well as with whole blood, plasma and serum. In earlier work, sample dilution to low-conductance conditions (<1 mS/cm) was required before effective DEP

separations could be carried out. While some progress was made using DEP under high-conductance conditions, these efforts have been limited to separations of cells and micron-sized entities by negative DEP forces using hybrid electrokinetic devices. Such devices still could not be used with whole blood samples, and, more importantly, they did not provide efficient isolation of DNA from the sample. More recently, we have been able to develop electrokinetic techniques that allow nanoscale entities, including high molecular weight (hmw) DNA and nanoparticles, to be isolated from high-conductance (>10 mS/cm) buffer solutions and whole blood samples. We were also able demonstrate isolation of virus from blood and fluorescent detection of cell free (cf) DNA from CLL patient blood samples. Most recent, we were able demonstrate PCR and Sanger DNA sequencing results for cf-DNA biomarkers isolated by DEP using only 25 ul samples of unprocessed CLL patient blood . The PCR and Sanger sequencing results for the DEP process were equivalent to results obtained using conventional sample preparation of cf-DNA from 1 ml of CLL patient plasma, and to the "gold standard" DNA sequencing results obtained using an established method for isolating DNA from the leukemic cells of CLL patients that requires 15-20 ml of blood. In addition to comparing the DEP isolation of cf-DNA from CLL patient blood with CLL patient plasma, we are also showing some initial results for the DEP isolation RNA containing exosomes from Glioblastoma cell cultures.

2. Materials and Methods

New dielectrophoretic (DEP) microarray devices specifically designed for the isolation of cf-DNA and cf-RNA from high conductance buffer, blood and plasma samples were obtained from Biological Dynamics. DEP experiments were carried out by adding about 25-50 ul of blood/plasma sample to the DEP microarray device. The DEP field was applied at 10 kHz and 20 Vp-p for 5-10 minutes. The microarray was then washed with 0.5x PBS with the DEP field on and examined by epifluorescent microscopy. Cf-DNA was then removed from device and PCR amplified using VHL patient specific primers.

3. Results and Discussion

Figure 1 below shows the fluorescent image results for cf-DNA isolated by DEP from one normal blood sample (Normal-1e B) and five representative CLL blood samples (CLL-1e B, CLL-2e B, CLL-3e B, CLL4e B, CLL-5e B) on the left side, compared with one normal plasma sample (Normal-1e P) and five representative CLL plasma samples (CLL-1e P, CLL-2e P, CLL-3e P, CLL4e P, CLL-5e P) on the right side The 3D fluorescence intensity images created by MATLAB, which provide better visualization of the relative amounts of cf-DNA are also shown. Overall, the fluorescent DNA levels were higher in most of the CLL patient samples when compared to the fluorescent DNA levels obtained from the normal blood and plasma samples. Using the electrokinetic DEP microarray device, cancer related cf- DNA from only 25 ul of unprocessed patient blood or plasma could be detected within five minutes. Elution of the cf-DNA from the DEP device for quantification and PCR analysis required less than ten minutes. The PCR and DNA sequencing results for cf-DNA isolated from 15 CLL patient blood samples by the DEP process were exactly comparable to results obtained using conventional methods, which take several hours to complete, are more laborious (many steps) and required much larger plasma/blood samples i.e., one ml plasma for Qiagen cf-DNA extraction method, and 15-20ml blood for isolation of genomic DNA from CLL patient leukemic B cells. The results for eight of eleven CLL plasma samples compared well with the gold standards.

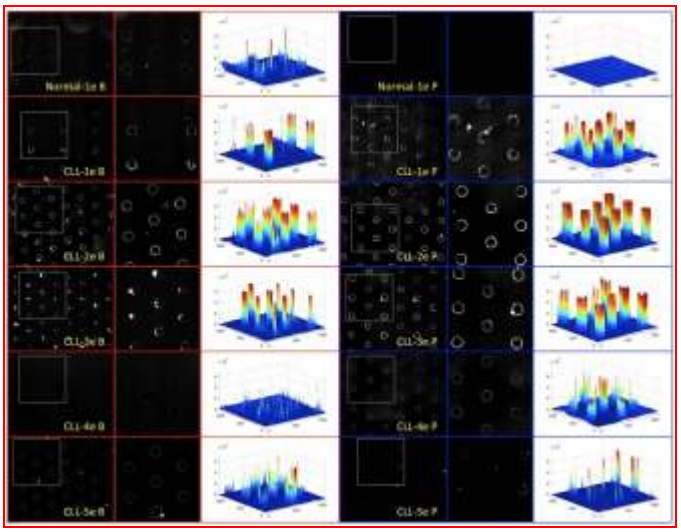

Figure 1 *- Fluorescence Detection and analysis of cf-DNA from one normal blood sample and five CLL patient blood samples (left side), compared with one normal plasma sample and five CLL patient plasma samples.*

In further work, DEP was used to isolate cf-RNA containing ROR1 mRNA from CLL patient blood

samples (Figure 2), and RNA containing exosomes excreted by glioblastoma cells (Figure 3).

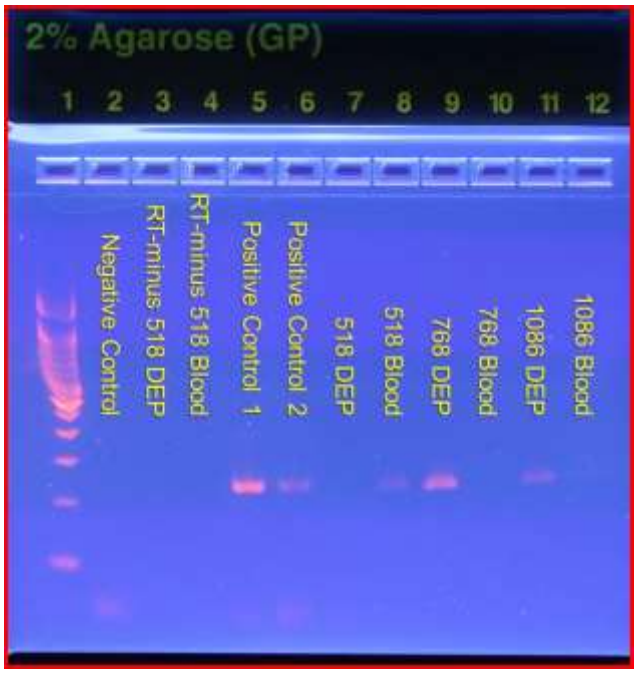

Figure 2 – *PCR results showing cf-RNA isolated from CLL patient blood contains ROR1 mRNA.*

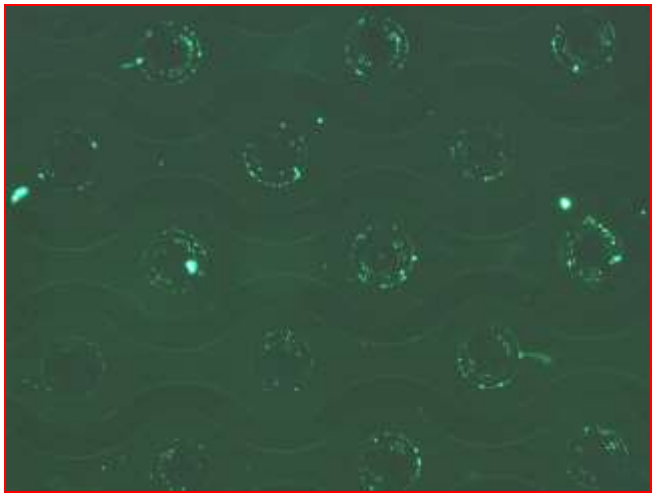

Figure 3 *–DEP isolation of extracellular cf-RNA containing exosome from glioblastoma brain cell culture.*

4. Conclusions

Generally, cf-DNA and cf-RNA for "liquid biopsies" is isolated form plasma using relatively long and involved processes which are not suitable for point of care (POC) diagnostics. New DEP devices are being used to collect cf-DNA and cf-RNA directly from Chronic Lymphocytic Leukemia (CLL), including PCR identification of ROR1 mRNA from cf-RNA. Blood sample to PCR is now achieved in less than ten minutes. Further work has now also demonstrated that DEP can be used to isolate RNA containing exosomes released by glioblastoma cells. Thus, the new DEP technology and devices set the stage for "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 whole blood and other clinical samples.

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