Low-cost and Rapid micro-RNA Assay for Identification of Pancreactic Cancer

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***Abstract*— Electromagnetic based micro-RNA sensor was developed and applied to analyze pancreatic cancer biomarkers in plasma samples. Results confirmed the previous finding of differential expression levels of micro-RNA between healthy and pancreatic cancer patients, but in a one-step, rapid, and higher specificity manner. Keywords—microRNA, Dielectrophoresis, biosensing, fluorescence enhancement**

I. Introduction

Pancreatic cancer (PC) is the fourth leading cause of cancer deaths in the United States (US). In the last decade, the prevalence of PC has steadily increased, making PC a major health concern in the US [1, 2]. The high lethality rate of pancreatic cancer is due to lack of specific, cost-effective screening tests, which continues to hinder the ability to reliably detect PC at early stages when disease treatment is most effective. The ability to detect early could have significant personal and public health impacts by enabling early treatment, which could increase 5-year survival rates by more than 10-fold (from 6% to 60-100%). There current diagnostic options such as CT, MRI and endoscopic ultrasound do not provide sufficient resolution, sensitivity, and applicability to be useful screening tools at early stage. Furthermore, these methods are highly invasive, emit harmful ionization radiation and expensive and therefore not recommended for routine screening process.

Carbohydrate antigen 19-9 (CA19-9) is the only PC biomarker for screening pancreatic cancer after surgery and sometimes used as early stage screening marker although the result is unreliable. Other biomarkers, including carcino-embryonic antigen (CEA) and genetic markers, such as K-RAS and p53, maybe used as secondary markers but also not recommended to be used as screening markers. Clinicians rely on CA 19-9 as a prognostic tool for managing patients with late stage disease, monitor reoccurrence, and determine operability, but CA 19-9 has inadequate specificity and unreliable sensitivity for screening applications [1-2].

One potential avenue for screening is microRNA (miRNA). These short (19-23 nt long) non-coding RNAs post-transcriptionally regulate gene expression to modulate a host of biological processes. For example, through binding of a miRNA to a complementary sequence in the 3′-UTR of a target mRNA, miRNA could regulate the gene expression. Recent studies, however, demonstrated that miRNA could be used as sensitive biomarker for early detection of cancer, including PC.

Despite great progress in miRNA biomarker research, these molecules have not yet been translated to screening or clinical diagnosis of cancers, including PC due to technical challenges, such as limitations of various detection technologies, inconsistent results between studies. In the circulation, miRNAs are protected from degradation, and therefore are present at sufficient levels [typically at $\leqslant$ picomolar (pM)] for quantitative analyses. However, the detection of a minute portion (<1% by weight) of target miRNA from a large background of other mRNAs, nucleic acids, and cellular debris presents a major technical challenge. In addition, the contamination of clinical samples during a long pre-processing time (>4 h) may lead to hemolysis, which further complicatex the detection process. This issue is critical because, among tumor-associated circulating miRNAs reported in the literature, 58% are highly expressed in blood cells, and hemolysis alters circulating miRNA levels by about 50-fold. Therefore, hemolysis could lead to inaccurate diagnosis and increased background, further complicating miRNA detection.

In addition, current miRNA detection methods do not provide absolute molarity of target miRNAs (e.g.: RT-qPCR, LAMP, microarray), inefficient for short miRNAs (e.g: NGS, RT-qPCR), longer pre-processing time (e.g: microarray, electrochemical, hybridization methods), insufficient sensitivity (RT-qPCR, microarray), low dynamic range (plasmonic, electrochemical) and expensive (all). One way to minimize hemolysis is to introduce a rapid miRNA analysis, preferably in the clinics and hospitals.

II. Experiments

To address this critical issue, we developed a disposable device for sensitive, low-cost and speedy miRNA detection in plasma samples. To detect miRNA in clinical samples, first we isolated the miRNAs using commercially available miRNA isolation kit (QIAGEN miRNeasy Serum/Plasma Advanced kit, Cat No: 217204) , then we selectively hybridized the target miRNA with its complementary DNA tagged with a fluorophore and produce miRNA-DNA duplex molecules. The sample is then pipetted to the device for quantification. An electric potential of 10 Vpp with known frequency was applied to the

interdigitated electrodes on the device. Fig. 1 shows the miRNA sensors and electrodes.

MiRNA-DNA duplexes have electric polarizability that is identical to double-stranded nucleic-acid molecules of the same length; other molecules in the sample have the polarizability of single-stranded nucleic acid molecules.

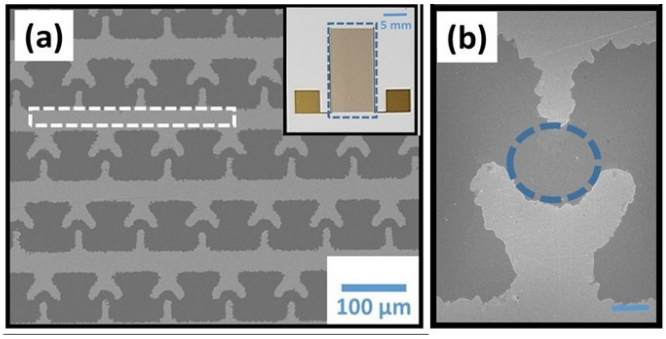

Fig 1: MiRNA sensing device. (a) Scanning electron microscope (SEM) image of the interdigitated electrodes. Inset show a picture of the sensor. The sample is pipetted on the area highlighted in broken blue rectangle. Rectangle with white broken line show an area where free complementary DNA molecules are concentrated. (b) SEM image of the electrode area (circle drawn with broken blue color) where conjugated miRNA-DNA molecules are concentrated.

At 1 MHz, miRNA-DNA molecules have large positive polarizability, while other molecules have negative polarizability that produce a larger dielectrophoretic force on miRNA-DNA molecules. We used the dielectrophoretic force (DEP; magnitude is proportional to polarization), thermophoresis and electrothermal flow to selectively enrich miRNA-DNA molecules in plasmonic nanostructures called hotspots [3]. Hotspots are located in the periphery of interdigitated gold micro-electrodes. Other molecules are repelled from the hotspots and concentrated in other areas of the device [1].

Critically, miRNA-DNA duplex molecules produce weak fluorescence because each duplex molecule has one fluorophore molecule. Significant enhancement of fluorescence is therefore required to detect miRNA-DNA duplex molecules with high sensitivity and selectivity. Traditional fluorescence enhancement methods produce a few-thousand-fold enhancement, limiting the detection to the nM range—insufficient for screening. To address this issue, we have recently developed a novel approach in which we employed externally applied electric fields for fluorescence enhancement. The electric field-enhanced fluorescence utilizes multiple nanoscale metal-fluorophore interaction mechanisms to eliminate the fluorophore quenching and promotes fluorescence enhancement to produce million- to billion-fold fluorescence enhancement, which could increase the detection limit to a several-attomolar level [1].

III. Results

To demonstrate the effectiveness of the miRNA sensor in clinical applications, we have used the sensor to evaluate the miRNA levels of PC patients and healthy individuals. PC patients were identified previously by real-time Taqman RT-PCR. Subjects were recruited and samples were collected at the Valley Hospital, New Jersey, NJ. An IRB approval was taken before the subject recruitment and sample collection

The samples were kept frozen and shipped to North Dakota State University to analyze. We used the miRNA sensor to quantify miRNA levels of 3 targets (*miR-642b*, *miR-885* and *miR-let-7i*) that are related to PC; these miRNA targets were proposed as a diagnostic panel with cohorts of patients and controls and found to yield high sensitivity (91%) and specificity (91%) with an area under the curve of 0.97 ($P < 0.001$).

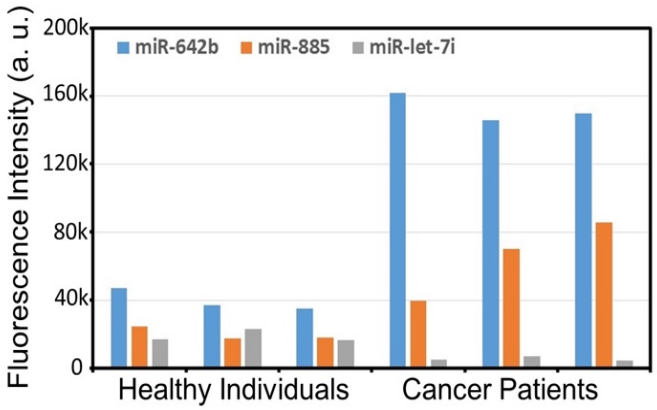

Fig. 2: Fluorescence intensities produced by miR-642b, miR-885 and miR-let-7i in the samples of healthy and PC patients.

Compared to the FDA approved antigen biomarker, CA19-9 at 73%, the three-miRNA panel has higher sensitivity although CA19-9 has higher specificity of 100% [2]. Fig. 2 demonstrate the variation of biomarker levels detected by our miRNA sensor in 3 healthy individuals and 3 PC patients. These samples were also analyzed by qRT-PCR and found that both qRT-PCR and miRNA sensor produced similar patterns for all 6 samples.

Results show that miR-642b and miR-885 were over-expressed in PC patients by over 3-fold. miR-7i expression was suppressed in PC patients by at least 4-fold. Although we have tested smaller number of samples (total of 6), this result show that effectiveness of the miRNA biomarkers and the sensing device toward identifying the PC patients. The ability of the biomarkers or sensor to identify early state of the PC patients is still unknown. However, Chang's group have demonstrated that miR-642b, miR-885 and miR-22 combinedly could identify early-stage PC patients with 91% sensitivity and 91% specificity [2]. We plane to extend our work toward applying this miRNA biomarker sensing towards early-stage PC.

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