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# Risk stratification of heart failure from one drop of blood using hand-held biosensor for BNP detection

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ABSTRACT

Continued risk assessment by evaluating cardiac biomarkers in healthy and unhealthy individuals can lower the mortality rate of cardiovascular diseases (CVDs). In this research, we have developed a hand-held biosensor system to rapidly screen for brain natriuretic peptide (BNP) from a single drop of whole blood. The sensor methodology is based on extended gate design of electrical double layer (EDL) field effect transistor (FET), that can directly detect BNP in whole blood, without extensive sample pre-treatments, thereby eliminating the limitations of charge screening in high ionic strength solutions. A simple sensor array chip is fabricated to integrate with the MOSFET sensor system. Sensing characteristics are elucidated using purified BNP samples in 1 × PBS (with 4% BSA), spiked BNP samples in whole blood and clinical whole blood samples. The blood cells can be gravitationally separated without the use of any external actuation. The sensor exhibits very high sensitivity over wide dynamic range of detection. The sensing characteristics are not adversely affected by the presence of background proteins or blood cells, even without gravitational blood cell separation. Thus, the biosensor system can allow users to perform rapid whole blood diagnostics with minimal user protocols, in 5 min. The features of high sensitivity, cost-effectiveness and convenience of usage empower this technology to revolutionize the mobile diagnostics and healthcare industry.

## 1. Introduction

Molecular biomarkers that provide physio-pathological information play an important role in the diagnosis and prognosis of diseases such as cardiovascular diseases (CVDs) and cancers (Mayeux, 2004; Liu et al., 2014). Timely diagnosis aids in the prevention of CVDs, which are the leading causes of death worldwide. Continuous monitoring of molecular biomarkers provides additional risk stratification for CVDs, beyond the traditional biomarkers (Gilstrap and Wang, 2012; Yin et al., 2014). High rates of morbidity and mortality could be prevented through regular assessment and management of cardiovascular risk, by providing cost-effective and convenient means of health monitoring systems available to all. Congestive heart failure (CHF) is essentially the inability of the heart to pump blood to all parts of the body, due to weakened heart muscles or defects (Taylor, 1996). Since heart failure can be presented with a variety of symptoms, physicians face challenges in definitive diagnosis and correct prognosis. Protein based biomarkers of heart failure such as brain natriuretic peptide (BNP) and N-terminal pro brain natriuretic peptide (NT-proBNP) are identified as the standard biomarkers for diagnosis and prognosis of heart failure (Lin et al., 2014; Natriuretic Peptides Studies, 2016). Clinical determination of the natriuretic peptides from blood plasma can be performed using conventional spectroscopic techniques such as radioimmunoassay and chemiluminescence (Cowie et al., 2003; Nishikimi et al., 2013). More recently surface plasmon resonance (SPR) based immunoassays and electrochemical enzyme immunoassays have also been developed to quantify BNP levels (Matsuura et al., 2005; Jang et al., 2014). However, these assays are quite complex, performed using bulky and costly equipment, often requiring trained laboratory staff to perform the assay.

Miniaturized electronic sensors present an opportunity to perform high sensitivity immunoassays at considerably low cost. The ease of integration of these miniaturized biosensors with the measurement system facilitates compact and portable sensor units that can be used for point-of-care assays. In this genre, field effect transistor (FET) based

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biosensors are highly desirable owing to its characteristic features such as high sensitivity, low cost, easy signal read-out and fast response times. However, FET based sensors suffer from charge screening effect in high ionic strength solutions such as blood due to the extremely short Debye length (Stern et al., 2007; Zheng and Lieber, 2011). As a result, extensive sample pre-treatment methods are adopted to facilitate biomolecule detection, such as dilution and desalting (Zheng et al., 2005; Zheng and Lieber, 2011; Stern et al., 2010). This severely impacts the reliability and practicality of FET based assays in clinical applications especially if a rapid, inexpensive in-vitro diagnostic platform is desired.

In the present research, we have devised and characterized a hand-held biosensor system for the determination of BNP levels from a single drop of whole blood. The sensing methodology is based on extended gate electrical double layer (EDL) FET biosensor that can directly detect the target analyte in high ionic strength solution such as whole blood, offering bio sensing beyond the Debye length. EDL FET biosensors have demonstrated high sensitivity and selectivity in biomolecule detection (Chu et al., 2017). In the extended gate design, device to device variations are considerably reduced allowing easy sensor calibration for variety of clinical applications. Also, FET is protected from harsh chemical environments of the physiological fluids, providing enhanced stability and robustness. The use of single FET for multiple electrical measurements significantly lowers the assay cost. In here, we demonstrate the detection of BNP in 1 × PBS with 4% BSA and clinical whole blood samples obtained from different patients. The sensor exhibits very high sensitivity, selectivity and wide dynamic range of detection. Simple gravitational separation of blood cells from blood plasma is performed, without any external actuation to eliminate any interference of cell components of whole blood. A hand-held biosensor measurement unit is developed to integrate the sensor chip and the electrical test results are displayed in the LCD display. We demonstrate that our unique sensing methodology can facilitate rapid screening of protein biomarkers from a single drop of whole blood (< 10 μL) in 5 min, and the simplistic assay can be performed by consumers with minimal protocols, as per their convenience and requirements.

## 2. Experimental

### 2.1. Fabrication of sensor array chip

A simple sensor array chip is fabricated on an epoxy substrate, containing multiple gold electrode pairs. Epoxy substrate is fabricated by pouring thermo-curable epoxy resin in PDMS mold and curing at temperatures of 125 and 165 °C for 1 and 1.5 h respectively. Post curing, electron beam (e-beam) evaporator technique is used to deposit gold electrode pairs (Ti (200 Å) and Au (2000 Å)) on the epoxy substrate. Typically, an eight-electrode pair array is fabricated. The two electrodes forming the gold electrode pair are separated by a gap of 185 μm. The whole sensor array chip is passivated using photoresist and openings of 3600 $\mu m^2$ (600 μm x 600 μm) are made on each of the electrodes. When test sample is dropped on the sensor, the solution connects the two openings on the gold electrodes. Gate bias voltage is applied to one electrode and the other electrode is connected to the gate terminal of the MOSFET.

### 2.2. Sensor surface functionalization

Monoclonal antibody (mAb) is used to capture BNP samples from the test solution. BNP antibody is purchased from Hytest Inc (19c7). A mild reducing agent such as 2-mercaptoethylamine (2-MEA) is used to cleave the disulfide hinge region of IgG molecule, yielding free thiols to bind with gold. mAb and 2-MEA are mixed together in the molar ratio 10,000:1. The mixture is then incubated in reaction tube at 37 °C for 15 mins and on the sensor surface for 1.5 h in room temperature. The sensor is allowed to remain in 4 °C for 12 h following which the unbound antibodies are thoroughly washed away in 1 × PBS.

### 2.3. Purified BNP samples

Purified BNP purchased from Abcam, is diluted to target concentrations in 1 × PBS (pH 7.4, 150 mM NaCl, 10 mM $PO_4^{3-}$) containing 4% Bovine Albumin Serum (BSA). The target concentrations of purified BNP samples tested are 0, 100, 200, 500 and 1000 pg/mL. 4% BSA is added to the reaction buffer solution to simulate the real conditions of human blood serum.

### 2.4. Clinical BNP samples

The clinical whole blood samples are obtained from different patients admitted to the hospital as per the IRB No. 16MMHIS112. The whole blood samples are collected from different individuals at different times, in a vacutainer treated with anti-coagulant. The whole blood sample obtained from healthy individual with undetected BNP is also treated with anti-coagulant, as per the IRB no. 10610HE074.

### 2.5. Sensor regeneration

After each sample measurement, the sensor surface is thoroughly washed with 3 mL gentle protein elution buffer (Pierce gentle ag/ab elution buffer, pH 6.6) and 1 × PBS, alternatively for one hour, to remove target proteins and non-specifically bound proteins. Thus, the sensor surface is regenerated, and electrical baseline is restored.

### 2.6. Sensor measurement

A hand-held biosensor system is used to evaluate the sensor characteristics. The system houses a microcontroller unit, signal acquisition, LCD display, and read out circuitry. N-Channel Depletion-Mode DMOS FET (LND150) is used to carry out all electrical measurements and is connected to the hand-held device as shown in Fig. 1. The hand-held device is able to measure 8 sensors on one chip, which is inserted into a PCI socket in the hand-held device. The circuit in the device provides pulse Vd and Vg to each sensor electrode, by using switch component to lead the bias given to each sensor electrode. The device can be connected to laptop with USB wire or Bluetooth. A rechargeable Li-battery is embedded in the device. FET biasing, signal acquisition and display of measurement results are controlled with user interface software, which facilitates two distinct modes of operation: single and burst. In single mode, one measurement data is recorded and in burst mode, multiple data are recorded. Throughout this study, burst mode of operation has been used. A short duration gate pulse with a width of 100 μs and amplitude of 1 V is applied as the gate bias to one of the electrodes. A steady DC bias of 2 V is applied as the drain-source voltage during sensor operation. The detachable sensor chip containing gold electrode pairs is connected to hand-held system as shown in Fig. 1. More details about the test parameters, current gain calculations and typical drain current response are shown in Supplementary information.

## 3. Results and discussion

The schematic representation of the extended gate design of EDL gated FET biosensor is shown in Fig. 1(a). The real image of the sensor mounted on the handheld biosensor system is shown in Fig. 1(b). The sensor structure is uniquely different from traditional FET based biosensors. In the extended gate design, the gate metal is connected to a pair of gold electrodes which are separated by a short gap. The gold electrode pair simulate a two-plate capacitor, with the test solution (containing the target protein) as the dielectric. One of the gold electrodes of the pair is functionalized with the receptor and the other is connected to the FET gate metal. The test solution is dropped on the gold electrode pair, forming a dielectric between the two gold surfaces. In this way, the FET does not come in contact with the bio sample