A HANDHELD MAGNETIC SENSING PLATFORM FOR ANTIGEN AND NUCLEIC ACID DETECTION

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ABSTRACT

The core requirements for point-of-care (POC) diagnostics necessitate low-cost, high multiplexing, portability, easily integrated sample preparation, and quick measurement time [1, 2]. Frequency-shift based magnetic sensing is a measurement technique utilizing a complementary metal-oxide-semiconductor (CMOS) integrated-circuit (IC) chip for magnetic label detection. Using this technology, we have developed a complete handheld, low-power, low-cost, disposable cartridge-based diagnostic device (Fig. 1a,b) with two fully implemented assays for antigens and nucleic acids. We have demonstrated reliable measurements down to 100 pM for a 31 base-pair oligomer and 1 pM for the protein interferon-γ (IFN- γ).

KEYWORDS: Nucleic Acid, Antigen, Biosensor, Magnetic

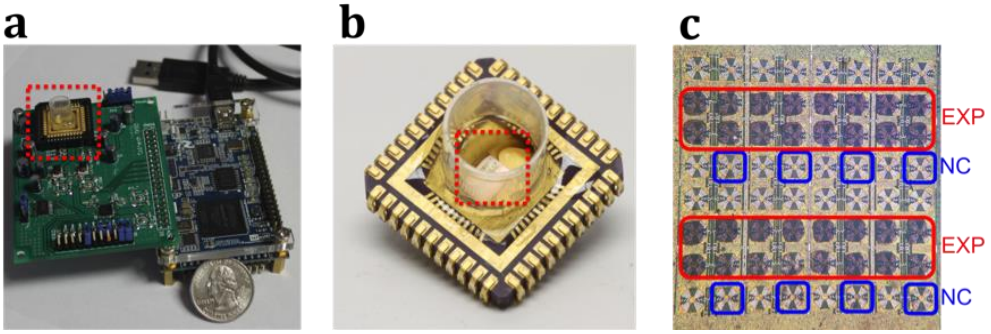

Figure 1: (a) Handheld Diagnostic Device. The device consists of a plug-in cartridge, circuit board, and single USB data interface cable for communication and power. (b) Disposable Cartridge. The disposable cartridge consists of an electrically connected integrated circuit chip inside a polypropylene well. (c) Chip Surface after Immunoassay. The chip inside the cartridge has 48 sensor sites and 16 reference sensors. Sensor sites designated by EXP and NC were used for printing the capture probe or negative control, respectively. Some sensing sites were left blank to examine background binding. In the DNA and antigen assays, the presence of biological targets leads to the accumulation of magnetic beads over the sensor.

INTRODUCTION

Point-of-care (POC) diagnostics is a growing segment of the healthcare industry [3]. Although many types of sensors have been proposed with high sensitivities, few have demonstrated the key features that will allow a device to reach widespread use: low-cost, multiplexing, portability, easily integrated sample preparation, and quick measurement time [1, 2]. In this manuscript, we present a complete handheld diagnostic device (Fig. 1a) with two fully implemented assays for antigens and nucleic acids (Fig 2a,b). It is based on the novel "magnetic freezing" scheme that removes the need for baseline measurements, allows for effective multiplexing, and eliminates the long warm-up and calibration phases. We have devised a disposable, low-cost single chamber cartridge-based approach, which simplifies the sample handling significantly and allows for multi-analyte detection without microfluidics (Fig. 1b). We eliminate expensive and bulky infrastructure that limits other sensing technologies such as microfluidic pumps and optical elements. We have developed all the necessary surface chemistry to functionalize the surface of a standard complementary metal oxide semiconductor (CMOS) chip, further reducing the cost and complexity of this handheld diagnostic device without the use of expensive post-processing steps. Standard CMOS IC chips have the advantage of decades of manufacturing development for superior reliability, scalability, and low-cost. The electrical details of the CMOS sensor can be found in [4]. We utilize these enhancements to the previous work for the room temperature, amplification-free detection of a 31 base-pair DNA oligomer and the interferon-γ (IFN- γ), a protein relevant for tuberculosis diagnostics. We have demonstrated reliable measurements down to 100pM for the DNA assay (Fig. 3a) and 1pM for the protein (Fig. 3b).

THEORY

The diagnostic system consists of a USB-interfaced reader and a disposable open-well cartridge. At the heart of the cartridge is a CMOS IC chip containing an array of sensing inductors. Each of the sensing inductors relies on detecting the shift in the natural frequency of a free-running oscillator based on an electromagnetic resonator in the presence of external magnetic material. The resonator is often implemented on a silicon chip using an inductor, L, and a capacitor, C, forming an LC resonator. When a magnetic bead is in the vicinity of the inductor, it increases its inductance, which in turn lowers the resonance frequency. This leads to a small, yet discernible drop in the oscillation frequency that is

registered by electronically counting the number of cycles over one or multiple time intervals. The reader is powered through the USB interface and consumes less than 1 W of power.

To eliminate the warm-up requirement, reduce oscillator frequency drift noise, enable multiplexed measurements, and eliminate baseline measurement before or during biological assay, a new technique called magnetic freezing is implemented. This is accomplished by first measuring the sensor at the end of the assay using the previously described frequency shift technique, as seen in Fig. 3. In an unfrozen state, the beads are able to "track" the magnetization induced by the inductor. Next, an external magnet is used to saturate the magnetization of the bead. This "holds" the magnetization to a point of saturation. The magnetic field of the inductor cannot modulate the magnetization vector of the beads due to the magnetic saturation of the beads. Thus, the beads have no significant effect on the inductance. A small (9.5mm × 9.5mm ×9.5mm) neodymium magnet with a surface field of 0.5T is used to saturate magnetization. This prevents the beads from increasing inductance and lowering the resonant frequency of the electrical oscillator.

EXPERIMENTAL

Both the DNA and antigen sandwich assays (Fig. 2) consisted of four major steps; surface functionalization, probe printing, processing, and measurement. The standard CMOS IC chip was functionalized with poly-L-lysine for the DNA assay and epoxysilane for the antigen assay. Next, the capture strand or antibody was printed onto the chip surface using a contact pin microarray printer. After probe printing, a polypropylene housing was attached to the chip to form an open-well cartridge. The cartridge was stored in a dessicator until required for measurement. Measurement is taken by rinsing the cartridge with an associated buffer, introducing the target sample with negative control, bead incubation, then a final wash. The bead solution consisted of 10 µl of 10 mg/ml Dynabeads MyOne Streptavidin C1 (Invitrogen, Carlsbad, CA).

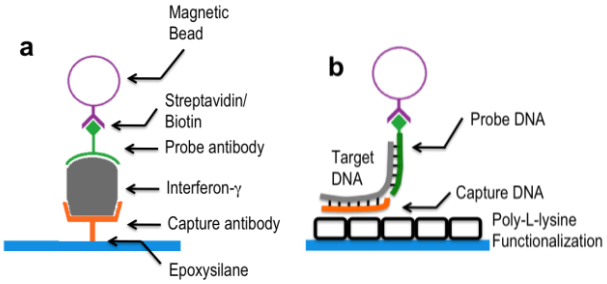

Figure 2: (a) Sandwich Assay for IFN-γ. Surface functionalization consists of an Epoxysilane group to covalently attach the capture antibody. The probe antibody binds to a separate epitope of IFN- γ. The magnetic bead attaches to the probe antibody through a streptavidin/biotin attachment. (b) Sandwich Assay for DNA oligo. A capture DNA strand complementary to a portion of the oligo strand is attaches to the PLL surface through electrostatic adsorption. The probe DNA strand is complementary to a second portion of the target strand and attaches to the bead through a streptavidin/biotin attachment.

For the DNA assay, we employed a sandwich assay consisting of a capture (5'-TTT TTC TGG TTG GGT TGA TTG GAT TTA GCT TGG C-3'), target (5'-ATC CAA TCA ACC CAA CAA TAT TGA TAA GGA T-3'), and a biotinylated probe strand (5'-Biotin-ATC CTT ATC AAT ATT-3') for indirectly labeled detection. A non-complementary NC capture strand (5'-ATG CGA AAC GAT CCT CAT CCT GTC TCT TGA-3') is also printed to test cross binding. The target, capture, and NC strands were incubated for 30 min at room temperature in a hybridization buffer (1X PBS, 1mg/mL BSA, 5nM EDTA, 0.1% Tween-20, 0.1mg/mL salmon sperm DNA).

For the antigen assay, IFN-γ and Granulocyte-macrophage colony-stimulating factor (GM-CSF) ELISA MAX Standard kits were obtained from Biolegend (San Diego, CA). IFN-γ capture antibodies were used for the primary assay. GM-CSF capture antibodies were used as negative control. A total of 100 µl of protein solution was diluted in 1% BSA and added to the well and incubated for 2 hours at room temperature with shaking at 125rpm.

At the conclusion of the assay, the cartridge was inserted into the reader and measured for a total of 4 min for 48 sensing sites. Midway through the measurement, a permanent magnet was attached to the underside of the reader to initiate magnetic freezing.

RESULTS AND DISCUSSION

Areas with the printed capture strand result in beads binding to the sensor surface, as seen in Fig. 1c. Reference sensors and sensors printed with the NC capture strand exhibit some fluctuations in frequency shift measurements due to variations in background bead binding to the chip. However, the NC sensors did not appear to have a different level of binding than the background binding level, no discernable cross binding of the sandwich assay. Sensor-to-sensor variations were caused by non-uniform capture strand printing, due to slight misalignments in printing (Fig. 1c).

Quantifiable target DNA concentrations could be detected over two orders of magnitude (Fig. 3a). The limit of detection for the sensor was 100 pM, where the frequency shift was more than two times the background level. The sensor reached a saturation level at 10 nM. Although this sensitivity is less than traditional amplification-based technologies, this

device did not depend on the stringent heating/cooling cycles nor the bulky optical elements of amplification technologies. This allows for a simpler, more reliable detection system.

Compared with the DNA assay, the immunoassay had a higher level of background binding and variability. The results of the immunoassay are shown in Fig. 3b. The minimum detectable concentration of protein was 1 pM. The sensor saturated at approximately 30 pM. Two additional concentrations were measured outside of the quantifiable range of the ELISA kit: 60 pM and 150 pM. It is important to note that even though a single antigen was detected using a standard sandwich ELISA kit, the IC surface chemistry and detection system are compatible with any other sandwich immunoassay.

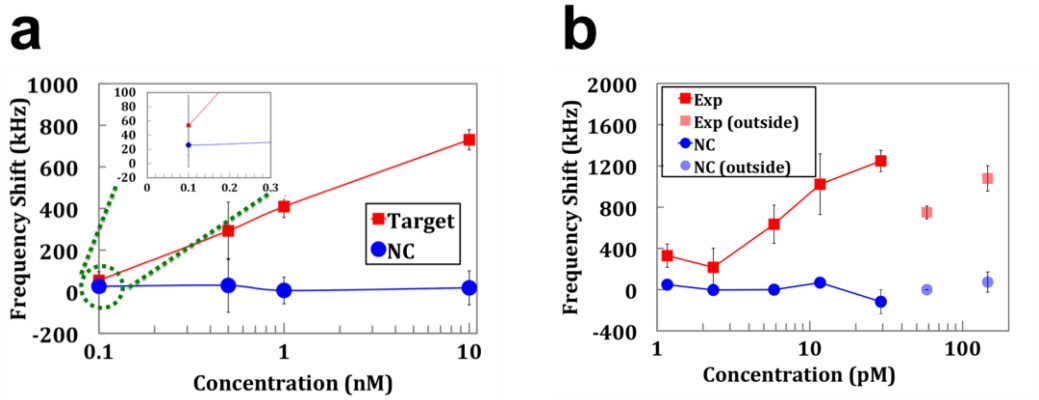

Figure 3: (a) DNA Quantification. In the DNA assay, target concentrations can be detected from 100 pM to 10 nM. At the limit of detection, 100pM, the frequency shift from the target sensor is over two times the shift from a non-complementary NC sensor. (b) Immunoassay Quantification. The limit of detection for IFN-γ was approximately 1pM. The sensor surface was saturated with beads at 30 pM. The negative control (NC) consisted of sensors sites printed with an antibody specific to Granulocyte-macrophage colony-stimulating factor (GM-CSF). Two additional concentrations were tested at 60pM and 150pM. However, these concentrations were outside the quantifiable range of the ELISA kit and therefore not as reliable.

CONCLUSION

An important trend in biotechnology is the emergence of nucleic acid testing (NAT) [5]. Although NAT testing has demonstrated significant progress in diagnostics, a more diverse variety of biomarkers can be detected with traditional immunoassay. Therefore, a diagnostic compatible with both nucleic acid and antigen detection is of significant utility. Our handheld diagnostic device consists of two major components: a small, disposable cartridge (Fig. 1b) and the electronic reader (Fig. 1a). The disposable cartridge consists of 48 sensing sites in a single well design, obviating complicated microfluidics. The sensing system has many desirable features: ability to multiplex, portable, low power, low-cost, and quick measurement time. A reliable protocol was developed to not only adapt our frequency shift CMOS IC, but any future standard silicon IC for either a DNA assay or immunoassay. The immunoassay is capable of incorporating the hundreds of available two-site antibody/antigen kits.

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