



A novel approach based on magneto-electric torque sensor for non-contact biomarkers detection

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ABSTRACT

A rapid system for the sensitive non-contact detection of proteins using actuated magnetic particle labels, which are measured with a magneto-electric torque (MET) sensor, is described. The system contains MET sensor with the cantilever of magnet and the “test strip” of magnetic beads has been fabricated. The performance of MET sensor is demonstrated using magnetic beads and its detection limit was found to be 0.05 mg/mL. Furthermore, the biomarkers of α -fetoprotein is captured by immobilized anti-AFP antibodies on the surface of the “test strip” and detected using magnetic beads labeled with anti-AFP antibodies. The detection limit was found to be 100 ng/mL. The low sample volume, high analytical performance and high detecting speed coupled with the inexpensive, mass-producible, hand-held MET biosensor make the system especially suitable for sensitive testing outside of laboratory environments.

1. Introduction

Cancer is a major cause of death worldwide [1,2]. World Health Organization reported that more than 30% of these death could be avoided if the cases of cancer resulting in death can be detected and treated at an early stage [3]. Biomarkers, being characteristic for identifying certain diseases or state of a particular disease, are vital for detecting patients with complex diseases such as cancers in molecular diagnosis [4]. Therefore, the detection of cancer biomarkers at the early stage has attracted considerable attention in the field of clinical diagnosis [5,6].

To provide a rapid and highly specific means of identifying and quantifying biomarker, immunoassay-based techniques employ a label as a biochemical probe for detection. Conventional labeled assays, including enzyme-linked immunoassay (ELISA) [7], radioimmunoassay (RIA) [8,9], time resolved fluoroimmunoassay [10], chemiluminescence immunoassay [11,12], electrochemical luminescence (ECL) [13,14], electrochemistry immunoassay (EL) [15,16] and so on [17], are highly sensitive in detection, but suffer from certain limitation. For example, radioactive labels have a tedious waste disposal process. Optical and electrochemical detection is difficult to miniaturize because

it requires the use of large equipment [18]. Thus, there is a need for investigation into alternative methods that are highly sensitive, conducive to miniaturization and suitable for mass production [19].

The magnetic biosensor which consists of magnetic beads (MBs) and magnetic sensor is well suited to the requirements of medical diagnostics because they are highly sensitive, easily scalable, and use little power [20,21]. In comparison with other detection methods, such as those based on fluorescent markers or electrochemical measurements, MBs that are coupled with magnetic sensors can detect biomolecules at very low sample concentrations and have an extensive linear dynamic range [22]. Additionally, the absence of other magnetic compounds provides an advantage in minimizing the noise and possible interference with the measurements. MBs, which can bind specifically to biomolecules for the detection of target diseases, are currently used in diagnostic procedures [23]. The magnetic sensor can infer the presence of MBs by detecting fringe magnetic fields of the MBs under the external magnet. The strength of the magnetic fringe fields is proportional to the numbers of the MBs, which indicates the concentration of the specific target diseases [24,25].

Magnetic sensors includes giant-magneto-resistance (GMR), Hall resistance, magneto impedance (MI) sensor, magnetoelectric torque

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(MET) sensor, etc [26–28]. Compared to other sensors, the MET sensor has many advantages such as easy preparation and low cost, which is implemented in single magnetostrictive and piezoelectric materials and requires no specialty complex, inexpensive processing. And the MET sensor has high sensitivity and stable performance which can reach several hundred V/cm·Oe at the low frequency and as high as several thousand V/cm·Oe at the resonance [29,30]. Additionally, the conventional biosensors are contact detection, which the labeled assays should be taken place on the sensor directly. Thus the biosensors can be only used one time, which yield the high cost for detection. Nevertheless, the MET sensor can realize the non-contact detection, which magnetic labeled assays can be completed on the “test strip”, and detected by the MET sensor.

This report presents a novel non-contact detection technique for α -fetoprotein (AFP), biomarker of liver cancer, a detection limit in the 100 ng/mL range is obtained. To obtain the rapid detection and mass production, the silicon substrate was used for the “test strip”. The magnetic beads were labeled on the “test strip”, and then the “test strip” was tested by MET sensor directly. Massive samples can be labeled on the “test strips”, and rapid detection of the “test strips” can be realized by the MET sensor. In contrast to commonly proposed immune-sensors, this non-contact detection technique makes the magnetic sensor use repeatedly. This report establishes the feasibility of using MET-based detection of magnetic labels towards an inexpensive, mass-producible, hand-held sensor for biomolecules.

2. Materials and methods

2.1. MET sensor

The MET sensor composed of piezoelectric layer, elastic layer and magnet (NdFeB) is shown in Fig. 1a. The elastic layer is Cu metal ($100 \times 10 \times 1$ mm in size), used as beam. The top layer is piezoelectric PVDF material ($50 \times 10 \times 0.6$ mm in size). One end of the sensor is clamped and the other end is free with the NdFeB magnet ($2 \text{ mm} \times \varnothing 8 \text{ mm}$ in size) loaded on it. The MET device was located in the Helmholtz coil which was used to supply an AC magnetic field $H_{ac} = 0.5$ Oe. We measured the ME voltage output by an SR7270 DSP lock-in amplifier.

2.2. Conjugation of antibodies to magnetic beads

Firstly, a 5 μ L drop of the MBs (Dynal Biotech ASA) was transferred

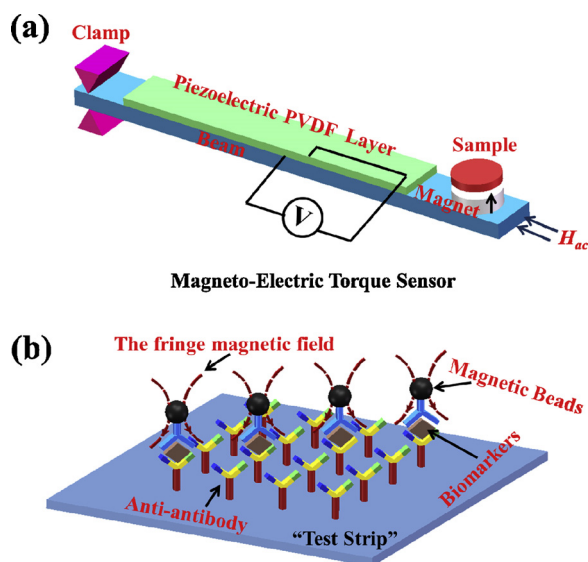


Fig. 1. The detection principle of the biomarkers based on MET sensor.

into a 1.5 mL eppendorf tube, the MBs were concentrated using a magnet for 2 min. Secondly, the MBs were washed twice with 100 mL buffer solution and resuspended in a 100 μ L the second monoclonal anti-AFP (1 mg/mL). Thirdly, the antibodies were captured onto the beads during 12 h at room temperature under continuous stirring (600 rpm). Subsequently, the second antibody AFP-modified MBs were washed twice with 100 mL of PBS buffer solution (pH 7.4). Thereafter, the unreacted groups of the MBs were blocked by bovine serum albumin (BSA). At last, the antibody AFP-modified MBs were kept at 4°C for further use.

2.3. Magnetic immunoassay

The procedure for preparing magnetic biomarkers is shown in Fig. 1b. The 5×5 mm silicon wafer was used as the “test strip”. An 80 nm Au layer was sputtered on the “test strip” for immobilization the first anti-antibody. Before functionalization with secondary antibodies, the Au layer was washed with ethanol and acetone, dried in a N_2 stream. The first antibody of AFP was immobilized on the Au surface via chemical self-assembled monolayers conjugation of 16-mercaptohexadecanoic acid [31,32]. Subsequently, the “test strip” was incubated in 100 ng/mL antigen AFP (100 μ L) at 37°C for 30 min. Finally the “test strip” was incubated in 0.25 mg/mL antibody functionalized MBs (100 μ L) at 37°C for 30 min after washing by the buffer (0.02 wt.% Tween 20 in PBS). Unbound beads were removed by performing a magnetic wash away from the sensor surface for 2 min.

3. Results and discussion

3.1. MET sensor

The working principle for the MET sensor is firstly demonstrated. When the applied AC magnetic field H_{ac} interacts with the magnetic moment of the magnet inducing a magnetic torque, the torque bends the layers. According to the direct piezoelectric effect, the piezoelectric layer induces ME voltage. The MBs labeled on the “test strip” bring weak stray magnetic field when external field is applied, which interacts with the magnet, thus induce the change of ME voltage. Therefore, the concentration of biomarkers can be detected through the detection of the concentration of MBs. To obtain the high sensitivity of MET sensor, the optimization of parameters is essential. First, the frequency dependence of the MET sensor is investigated. The ME voltage was measured from 4 to 20 Hz with AC magnetic field of 0.2 Oe applied by a Helmholtz coil. The ME voltage was measured. Fig. 2a shows a typical frequency dependence of the ME voltage for the MET sensor. When the frequency is smaller than 10 Hz, the ME voltage is nearly unchanged with a small value of approximately 3 mV. However, the ME voltage reaches the highest value of about 600 mV at the resonant frequency of approximately 10.58 Hz, which is about 200 times of the ME voltage at low frequency. Therefore, the magnetic field dependence is investigated at the resonant frequency of 10.58 Hz. The induced ME voltage as a function of magnetic field was illustrated in Fig. 2b. The ME voltage is increasing with the increase of magnetic field showing a good linearity. The smallest magnetic field can be detected in the range of nT.

3.2. Detection of MBs

MBs are polymer beads with an even dispersion of iron oxide (γ -Fe₂O₃) nanoparticles. They have a highly uniform spherical shape with a diameter of 2.8 μ m and a susceptibility of 0.04 [33]. The fringe magnetic field of single MBs is about tens of nT at the distance of 1 mm when the MBs are in the state of saturation magnetization by simulation. Therefore, the MET sensor can satisfy the detection sensitivity of MBs. First, the hysteresis loop of MBs was measured by the Vibrating Sample Magnetometer (VSM) systems. Fig. 3 shows the hysteresis loop of the MBs at room temperature in the range of 1 T. The magnetic

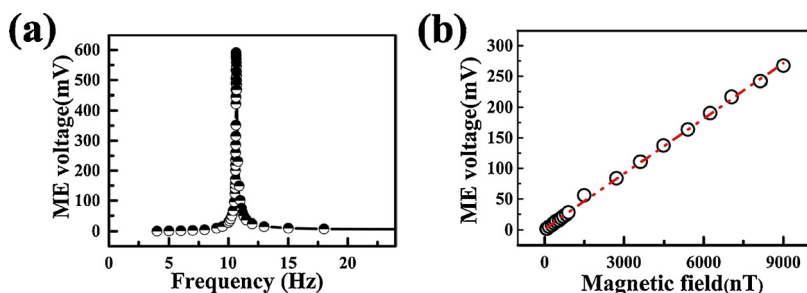


Fig. 2. The property of MET sensor. (a) Frequency dependence of ME voltage (b) Induced ME voltage as a function of magnetic field at 10.58 Hz.

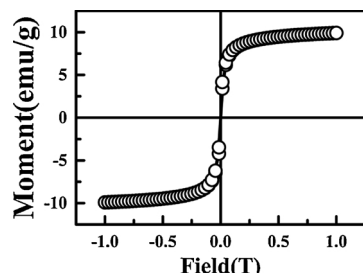


Fig. 3. The hysteresis loop of the m MBs at room temperature.

hysteresis loop of the particles shows a good superparamagnetic property with a saturated magnetization about 10 emu/g. This means the MBs will occur the fringe magnetic field when the extra magnetic field is applied and disappear when the applied magnetic field is removed, which guarantee the repeatability of the detection.

As the fringe magnetic field generated by the MBs is determined by the concentration of MBs, it is important to measure the concentration dependence of ME voltage. 5 μ L MBs with different concentrations from 0.05 to 15 mg/mL were dropped the “test strip”. Then the “test strip” with MBs was approached to the MET sensor (the distance was fixed to be about 1 mm) and magnetized by the NdFeB magnet of 0.1 T. Fig. 4a

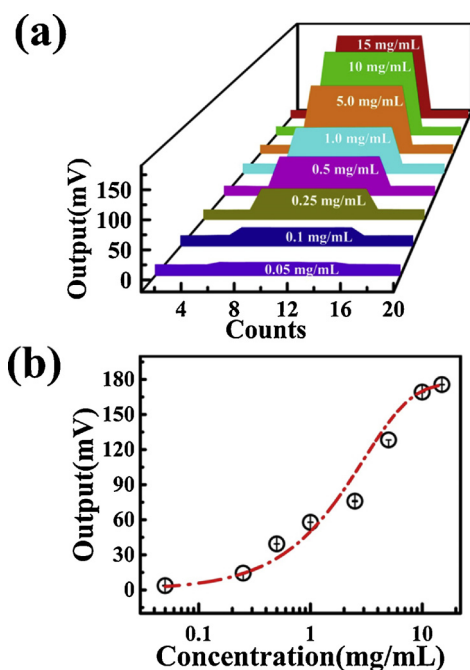


Fig. 4. Real time monitoring of different concentrations of magnetic beads using MET sensor. (a) The relationship between the concentration of MBs and the output voltage of the MET sensor. (b) The curve between the concentration of MBs and the output voltage of the MET sensor.

shows real time monitoring of the ME voltage for different concentration of MBs at resonant frequency of 10.58 Hz with AC magnetic field of 2×10^{-5} T (0.2 Oe) applied by a Helmholtz coil. No change of ME voltage was observed at the initial state, which suggests the stability of the MET sensor and provides a base signal. When the MBs are approached, the ME voltage suddenly changes and stabilizes at a high value. With the increase of the concentration of MBs, the stable ME voltage increases, as one can be seen clearly from Fig. 4a and b shows the concentration dependence of ME voltage. The ME voltage increases with increasing the concentration and the detection limit is about 0.05 mg/mL. The error bars provided in the figure suggests that sensor has shown only 0.4% maximum error, which demonstrates a high accuracy. The result is similar to those that have been well documented by the contact mode [34].

3.3. The reproducibility of MET sensors

The reproducibility of MET sensor was also investigated. Five sensors with the same size were prepared. The ME voltage was measured from 4 to 20 Hz with AC magnetic field of 0.2 Oe applied by a Helmholtz coil. The frequency dependence of the ME voltage for the five MET sensors was also measured. Fig. 5a shows a frequency dependence of the ME voltage for the MET sensors. Sensors have shown resonant frequencies i.e. 10.51, 10.54, 10.56, 10.59, 10.60 Hz respectively, which are almost same. Resonant frequencies of all sensors remain same because all the sensors are made up of same material and structure having constant properties. Minor difference occurred in output of sensors only due to human error. The ME voltage for different concentration of MBs at resonant frequency was also measured by the five MET sensors with AC magnetic field of 2×10^{-5} T (0.2 Oe) applied by a Helmholtz coil. Stable ME voltage increases with the increase of the concentration of MBs as it is obvious from Fig. 5b. Small variation ($< 1\%$) in output of five sensors, demonstrates that all the sensors exhibit outstanding reproducibility.

3.4. Detection of biomarkers

The number of detected MBs can indicate the concentration of the antigen AFP. First, the antigen AFP should be labeled by MBs through magnetic immunoassay. After anti-AFP was covalently coupled to the “test strip”, the remaining bonding sites were blocked with BSA. The blocking step was important to prevent nonspecific absorption of antigen AFP onto the “test strip”. Following this step, the “test strip” was soaked in antigen AFP and secondary antibody functionalized MBs solution. Once secondary antibody functionalized MBs recognized any of the antigen AFP on the surface, they would be immobilized tightly. During the repetitious washing procedures, any nonspecifically adsorbed antibody functionalized MBs could be removed. The recognition processes were proved by scanning electron microscope (SEM), which can provide information concerning the appearance, the homogeneity and the structure of the adsorbed material. Fig. 6a shows a typical SEM topographic image of the gold deposited on the “test strip”. The gold film is uniform. When antibody AFP was immobilized on surface, the

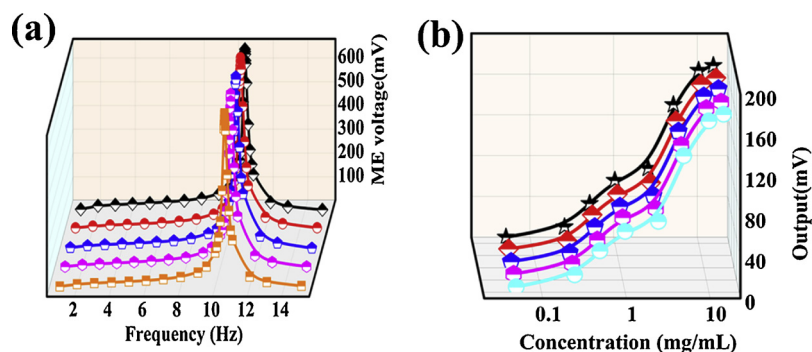


Fig. 5. The reproducibility of MET sensors. (a) A frequency dependence of the ME voltage of five MET sensors. (b) The relationship between the concentration of MBs and the output voltage of the five MET sensors.

topography strikingly changes, as shown in Fig. 6b. Many small spheroidal spots appear around the gold surface. After 100 ng/mL antigen AFP and secondary antibody functionalized MBs were recognized, the surface morphology changes again (Fig. 6c). Lots of bigger and brighter spheres adsorb on the surface. Their average diameter is about 2.8 μm from Fig. 6d, consistent with the size of secondary antibody functionalized MBs. Appearance of the spheres indicated the possibility to form antibody AFP / antigen AFP / secondary antibody AFP functionalized MBs complexes. Finally, the “test strip” was placed near the MET sensor (the distance was about 1 mm). Fig. 7 shows the real time monitoring of the ME voltage when the “test strip” of 100 ng/mL AFP is approaching. It can be seen that there is about zero output when the “test strip” of no AFP is approaching. Moreover, the ME voltage jumps to about 2.5 mV when the “test strip” of 100 ng/mL AFP is approaching, and drops to about 0 mV with the removal of the “test strip” of 100 ng/mL AFP. The results demonstrate the MET sensor provides a novel

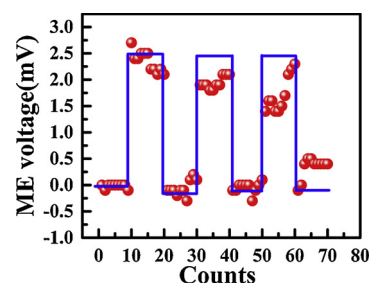


Fig. 7. Real time monitoring of AFP with magnetic beads using MET sensor.

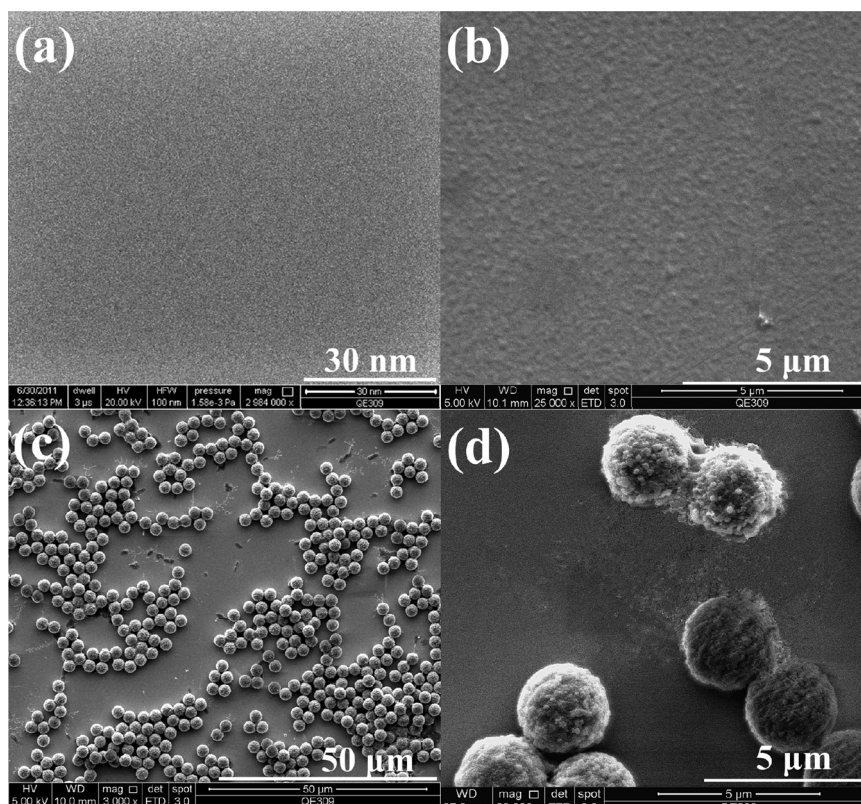


Fig. 6. SEM image of magnetic immunoassay.

(a) The gold film deposited on the “test strip”, (b) The antibody AFP immobilization on gold surface, (c) (d) Secondary antibody functionalized MBs immobilized onto antigen surface.

approach for rapid point-of-care diagnostics of cancers.

4. Conclusions

In summary, the MET sensor with high sensitivity was fabricated by magnetostrictive and piezoelectric materials, which can detect the weak magnetic field in the range of nT. Furthermore, the different concentrations from 0.05 to 15 mg/mL can be detected through non-contact mode. The detection limit is about 0.05 mg/mL. Finally the sensor was able to detect the 100 ng/mL of AFP through non-contact mode. The advantages of this sensor over other similar sensors are the faster results, reusability and outstanding reproducibility. All in all, the MET biosensor can be used for rapid point-of-care diagnostics in food safety and biomedical applications.

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