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Planar Hall magnetoresistive aptasensor for thrombin detection

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ABSTRACT

The use of aptamer-based assays is an emerging and attractive approach in disease research and clinical diagnostics. A sensitive aptamer-based sandwich-type sensor is presented to detect human thrombin using a planar Hall magnetoresistive (PHR) sensor in cooperation with superparamagnetic labels. A PHR sensor has the great advantages of a high signal-to-noise ratio, a small offset voltage and linear response in the low-field region, allowing it to act as a high-resolution biosensor. In the system presented here, the sensor has an active area of 50 μm × 50 μm with a 10-nm gold layer deposited onto the sensor surface prior to the binding of thiolated DNA primary aptamer. A polydimethylsiloxane well of 600-μm radius and 1-mm height was prepared around the sensor surface to maintain the same specific area and volume for each sensor. The sensor response was traced in real time upon the addition of streptavidin-functionalized magnetic labels on the sensor. A linear response to the thrombin concentration in the range of 86 pM–8.6 μM and a lower detection limit down to 86 pM was achieved by the proposed present method with a sample volume consumption of 2 μl. The proposed aptasensor has a strong potential for application in clinical diagnosis.

1. Introduction

The detection of protein that binds with sequence-specific DNA (Aptamer) plays a significant role in disease research and clinical diagnosis, due to the long term stability of the probe DNA. In molecular biology, thrombin is one of the important proteins because, through its concentration, thrombin performs a crucial role in physiological and pathological conditions (Zhang and Sun, 2011) particular, thrombin is one of the target biomarkers for cardiovascular disease, being essential to several coagulant effects as well as acting as a useful tumor marker for the diagnosis of pulmonary metastasis (Edwards et al., 2010, Nierodzik and Karpatkin, 2006, Yoon et al., 2013). Therefore, the development of the devices for the monitoring and detection of thrombin with high sensitivity and selectivity is of enormous importance in medical applications.

DNA aptamers are single-stranded (ss) DNA, which bind to their target molecules with high affinity, specificity and stability that act as model diagnostic in vitro reagents and are potential replacements for antibody biomarkers in the use of nanobiotechnology in biomolecular sensors (Cho et al., 2008, Qiu et al., 2010, Zhang and Sun, 2011, Shimada et al., 2012, Xu et al., 2006). DNA aptamers exhibit several superior properties compared to antibodies, such as long-term and thermal stability, inexpensive production, consistent chemical synthesis, labeling and selective changes in sequences (Cho et al., 2008, Edwards et al., 2010, Yang et al., 2009). As a result, the development of aptamer-based detection methods has received great interest for use in detection of thrombin. Hence, various types of DNA-aptamer based techniques have been used, including fluorescence, surface plasma resonance, quartz crystal microbalance and electrochemistry, for thrombin detection (Lu et al., 2009, Bai et al., 2013; Chen et al., 2010, Fan et al., 2012).

Magnetic labels coupled with magnetoresistive (MR) sensors can detect very low bio-molecules concentrations and have an extensive linear dynamic range compared with the above mentioned detection methods (Oh et al., 2013). In addition, other compounds present in the investigated samples do not exhibit any magnetic behavior, consequently minimizing the noise and possible interference of foreign substances in the MR signal measurements (Larsson et al., 1999). Hence, the development of MR sensors is significantly increasing towards bio-applications in both industrial and basic academic research as well as for point-of-care diagnostics. Moreover, MR sensors are low-power consumuption devices that are highly sensitive, easily scalable, inexpensive and portable (Oh et al., 2013). Several types of MR sensors, such as

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semiconductor Hall sensor (Besse et al., 2002), anisotropic magnetoresistive (AMR) (Miller et al., 2002), giant magnetoresistive (GMR) (Baselt et al., 1998, Ferreira et al., 2003, Mak et al., 2010), tunneling magnetoresistive (TMR) (Albisetti et al., 2013) and giant magneto-impedance (GMI) (Kurlyandskaya and Levit, 2005; Yang et al., 2010) sensors, have been reported in the field of bio-sensing applications. Although PHR sensors have great advantages of high signal-to-noise ratio, small offset voltage at zero field and very linear response in the low field region (Sinha et al., 2013, Hung et al., 2010, Sinha et al., 2012), biosensing application of PHR sensors for molecular diagnostics has not been reported yet.

In this study, we report the development of a sensitive aptamer-based PHR sensor for human α-thrombin detection. The sensing mechanism is based on the sandwich-type aptamer assay. The sensor surface was modified with thiol-labeled primary aptamer, and then thrombin was applied to the aptamer immobilized surface. After that, biotin-labeled secondary aptamer was used to bind with thrombin. Finally, streptavidin-coated magnetic nanoparticles were used to measure the MR signal. The MR signal was measured by the changes in the thrombin concentration which results in the changes in the amount of biotin-labeled secondary aptamer for the binding of streptavidin-coated magnetic nanoparticles in the assay.

2. Materials and methods

2.1. Materials and chemicals

The sputtering targets of $Ni_{80}Fe_{20}$, Ta, $Ir_{25}Mn_{75}$, Cu, Au and SiO_2 with a purity of 99.99% were procured from Kojundo Chemical Laboratory Co. Ltd., Japan, and used to fabricate the PHR sensor. The SiO_2 substrates were purchased from Wafermart, Korea. The Photoresist (PR) AZ 5214E and Developer AZ 500MIF were purchased from AZ Electronic Materials USA Corp. The epoxy (PT-135K) was obtained from Poly-tech Co. Ltd. Korea. Streptavidin functionalized magnetic nanoparticles embedded in starch with an average size of 100 nm were procured from Chemicell GmbH, Germany. Phosphate buffered saline (PBS, pH 7.4) was purchased from Bioneer Corporation, Korea. Tris-EDTA buffer (pH 8.0) and human α-thrombin were obtained from Sigma-Aldrich. All of the oligonucleotides (aptamers) were purchased from GeneChem Inc, Korea with a thiol modified 15–mer primary aptamer with a poly tail of thymine and a biotinylated 15-mer secondary aptamer. The base sequences used in this study are as follows:

1. Thiolated primary aptamer: 5′-SH– $(CH_2)_6$-TTTTTTTTTTTTTT GGT TGG TGT GGT T GG-3′
2. Biotinylated secondary aptamer: 5′-biotin-GGT TGG TGT GGT TGG-3′

The primary aptamer is specific to fibrinogen binding site of the thrombin. This specific binding site is formed by the sequence GGT TGG TGT GGT TGG (Porfirieva et al., 2007), while the rest part of the aptamer is essential for its proper binding to the sensor surface.

2.2. Sensor fabrication

The cross-junction PHR sensors with an arm length of 100 μm and an active junction area of 50 μm × 50 μm were prepared on SiO_2 substrates using standard UV photolithography. Here, the sensor patterning was performed by photolithography using a spin coater (Spin-1200D, Midas System Co. Ltd. Korea) and a mask aligner (MDA–400s, Midas System Co. Ltd. Korea). In briefly, the SiO_2 substrate was cleaned in methanol and acetone solution using an ultrasonic cleaner for 30 min and dried using N_2 gas. Then, the substrate was coated with PR using a spin coater at 3000 rpm for 30 s. The PR thickness was ~1.6 μm, and soft baking was performed for 1 min at 120 °C to enhance the adhesion of the PR layer. The photolithography process is shown in Fig. S1, which used a Cr mask pattern aligned by a mask aligner, was used to form a sensor junction onto the PR coated substrate. After UV exposure, the PR layer was developed and rinsed with DI water, and the substrate was dried using N_2 gas. Then, a trilayer structure of thin films of Ta/NiFe/Cu/IrMn/Ta (3/10/0.26/10/3 nm) was sequentially deposited onto the substrate by using a DC magnetron sputtering system (Daeki hi-Tech. co. Ltd., Korea) under the working pressure of 3 mTorr with a base pressure of $\sim 1.0 \times 10^{-7}$ Torr. During the sputtering process, a uniform magnetic field of 200 Oe was applied parallel to the film plane to induce a magnetic anisotropy of ferromagnetic (FM) layer and to align the pinning direction of the antiferromagnetic (AFM) IrMn layer, which fixed the easy axis of the sensor to the field direction.

After the deposition of the multilayer thin film, the PR was removed using acetone and methanol and dried using a flow of N_2. Next, using a similar photolithography process and DC magnetron sputtering to those described above, Ta/Au (10/50 nm) electrodes were prepared to connect sensor junctions to the PCB. After that, the sensor junctions and electrodes were passivated with a SiO_2 (100 nm) layer deposited using RF magnetron sputtering to protect the sensor junctions and electrodes from corrosion in the fluidic environment during the experiment. Finally, an Au (10 nm) film was deposited using the above mentioned photolithography on the sensor surface for binding the thiolated aptamer to the sensor. The SiO_2 substrate containing the sensors was attached to the PCB. The sensors and the PCB were connected by using Au micro-wire bonding (7476D, West Bond Inc. USA), and the electrical connection was protected by coating it with epoxy. More details of the sensor fabrication process are described elsewhere (Hung et al., 2010).

2.3. Characterization of the magnetic labels and the sensor

Sensor characterization and measurements were performed using a function generator (HP/Agilent 8116A 50 MHz Programmable Pulse/Function Generator, USA) and a magnetic field generator (RPA–2A, www.magsen.com) prior to a hand-made Helmholtz coil and a Lock-In amplifier (SR830DSP; Stanford Research Systems, USA). The morphology of the magnetic nanoparticles was characterized using a field-emission scanning electron microscope (FE-SEM, Magellan400, FEI Company) at an operating voltage of 2 kV, and the magnetic properties were measured using a vibrating sample magnetometer at room temperature (VSM, Lakeshore 7407 series) with a sensitivity of 10^{-6} emu. The VSM measurement of the magnetic labels was done by drying the magnetic labels.

2.4. Sandwich structure of thrombin using aptamer on the sensor surface

In this study, thrombin was sandwiched between two aptamers, one covalently bound to Au on the sensor surface (primary aptamer) and the other bound to the target thrombin with biotin (secondary aptamer). A scheme of the detection of thrombin is shown in Fig. 1. The sensor chip was cleaned using methanol, followed by rinsing with distilled water, and then was dried by a gentle flow of N_2. Immobilization of thiolated 15-mer primary aptamer on the Au-covered sensor surface was followed by application of 5 μL of 70–ng/μl primary aptamer solution in Tris-EDTA buffer into the reaction well, which was allowed to sit overnight, allowing it to form Au–S bond with Au surface. Subsequently, the well was washed with Tris–EDTA buffer to remove any unbound aptamer. Then, for each sample studied, 2 μL of