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Detection of HIV-1 antigen based on magnetic tunnel junction sensors*

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We report a p24 (HIV disease biomarker) detection assay using an MgO-based magnetic tunnel junction (MTJ) sensor and 20-nm magnetic nanoparticles. The MTJ array sensor with sensing area of 890×890 μm^2 possessing a sensitivity of 1.39%/Oe was used to detect p24 antigens. It is demonstrated that the p24 antigens could be detected at a concentration of 0.01 μg/ml. The development of bio-detection systems based on magnetic tunnel junction sensors with high-sensitivity will greatly benefit the early diagnosis of HIV.

Keywords: MgO-based magnetic tunnel junction sensor, HIV bio-detection system, antigen p24, early disease diagnosis

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1. Introduction

Early disease diagnoses require detection and quantification of disease biomarkers in serum, urine, or other bloody fluids. The traditional fluorescent immunoassay method suffers from the fluorescent background of biological components, fluorescence quenching and photobleaching of the fluorescent probes which are photo-sensitive.[1,2] Benefited from the recent advancement of nanotechnology and thin-film technology, the magnetic immunoassay (MIA) utilizing magnetic nanoparticles and magnetoresistive sensors has attracted increasing attention in the biomedical area, due to its high sensitivity, low power consumption, low cost, and feasibility to be miniaturized. Since most biological systems do not exhibit significant magnetic background and the magnetic properties of magnetic nanoparticles are not subject to photo-bleaching, the magnetic immunoassay can exhibit stable and reliable performance with high signal-to-noise ratio.[3]

Magnetic tunnel junction (MTJ) sensors, one advanced kind of MR sensors, are recognized as an ideal sensor element for the biodetection due to their low cost, high sensitivity, and lab-on-chip compatibility. Compared with giant magnetoresistance (GMR) sensors, MTJ sensors offer even higher MR ratio (e.g., 604% at room temperature) and therefore potentially higher sensitivity at low magnetic field (especially for the magnetic field <1 Oe).[4,5] Besides the magnetic field detector, the magnetic iron oxide nanoparticles (IONPs) have been a popular choice as bio-labels due to their physical and chemical stability, low toxicity, environmental safety, and being inexpensive to produce.[6–8] The biodetection configuration utilizing IONPs and MTJ sensors has successfully been applied for bimolecular recognition and DNA detection in previous studies.[9–11] Recently, a dynamic range of 4 orders of magnitude and a limit of detection of 1 pM and 0.1 pM respectively for 15- and 18-cycle amplified synthetic GAPDH PCR products were realized in GMR sensor array platform with magnetic nanoparticles.[12] These developments show the reliability of magnetoresistive immunoassay techniques in biological detection area. In this work, we demonstrate feasibility of magnetic biodetection for p24 antigens using carboxyl-group functionalized magnetic IONPs with MTJ array sensors.

We take p24 antigens here as biotarget due to the fact that p24 antigen is the biomarker of HIV disease. Acquired immunodeficiency syndrome (AIDS) is the end-stage disease caused by human immunodeficiency virus (HIV) infection.[13] Early diagnoses of HIV and thus early treatment of patients have been confirmed to bring dramatic survival benefits, while unfortunately the early detection of HIV remains problematic. In recent years, it is evidenced that the individuals newly infected with HIV have transmitted the virus prior to knowing their HIV status. In the newly infected individuals, HIV antigen p24 is usually present in their serum or plasma 7–10 days before the HIV antibody. The detectable amount of p24 antigens in serum/plasma is in the pg/ml range.[14] Thus, the development of a rapid quantitative bio-detection system with

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high-sensitivity to detect the p24 antigen will greatly benefit the early diagnosis of HIV.

2. Experimental part

For the magnetic detection of p24, both the IONPs and MTJ sensors need to be surface biologically functionalized. The magnetite iron oxide nanoparticles synthesized with carboxyl groups were used for the immobilization of the detecting antibodies via covalent bonding.[15] Anti-p24 antibodies were purchased from Abcam. The iron oxide nanoparticles (IONPs) were characterized under TEM observation and their magnetic property measurement was carried out at room temperature using a vibrating sample magnetometer (Lakeshore, VSM 7400). The magnetization was measured over a range of applied field from around −10000 to 10000 Oe.

2.1. Preparation of biofunctionalized magnetic IONPs

The IONPs with carboxyl groups were conjugated with antibodies via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) coupling chemistry.[16,17] Then the nanoparticles are incubated with detecting antibody and resuspended in storage buffer (PBS, phosphate buffered saline). The detailed procedure to carry out the biofunctionalization of IONPs with detecting antibodies is illustrated in Fig. 1. Briefly, the carboxyl groups-coated magnetic nanoparticles are activated by adding EDC and NHS. Then the nanoparticles are incubated with anti-p24 antibody and resuspended in storage buffer (PBS, phosphate buffered saline). Through this procedure, biofunctionalized IONPs were prepared with a concentration of 1.00 mg/ml, which can be stored for a few months. All the buffers used here were purchased from Ocean Nanotech.

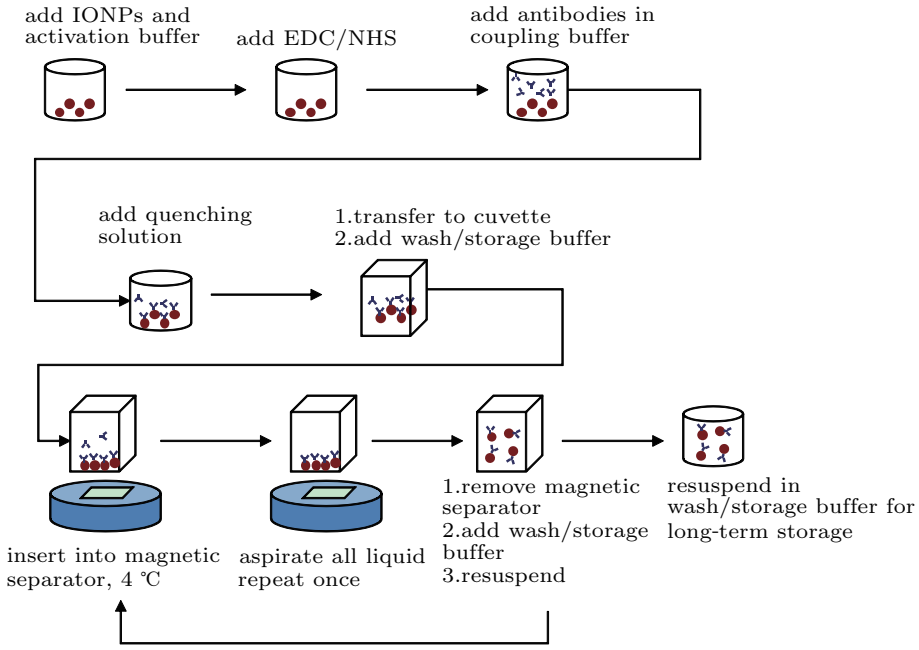


Fig. 1. Schematic illustration of the preparation of detecting antibody conjugated magnetic iron oxide nanoparticles (IONPs).

2.2. Preparation of surface-biofunctionalized MR sensor

An MR sensor was utilized in this platform to detect the magnetic signal of magnetic biolabels, including single MTJ sensor and MTJ array sensor (both obtained from Micro Magnetics), of which the surfaces were immobilized with capturing antibodies. An MTJ array sensor was bio-functionalized with anti-p24 antibodies. A 200-nm-thick gold layer was deposited on the top of MTJ sensing area, serving as bioactive area. The characterizations of a single MTJ sensor and the MTJ array sensor were carried out, respectively, through four-point probe measurement and two-point probe measurement. The detecting antibodies were immobilized on the MTJ sensing area through the procedures as shown in Fig. 2. The key point here is adding blocking solution (5% Skim Milk in PBS with 0.05% TWEEN 20) after the incubation of the MTJ sensor with antibody solution droplet. We performed this blocking step to reduce undesirable non-specific binding of protein molecules on the MTJ sensor sensing area surface which might induce false results.[18] After the immobilization of antibodies, the MTJ sensor surface was washed with PBST (phosphate-buffered saline with 0.05% TWEEN-20), and the capturing antibodies bio-functionalized MTJ sensor was prepared.

2.3. Magnetic immunoassay to detect biomarkers

After the biofunctionalization of the IONPs with detecting antibodies and the MTJ sensor with capturing anti-

bodies on its sensing area, the detection of target antigen biomolecules was carried out through a sandwich immunoassay configuration as illustrated in Fig. 3. Briefly speaking, the capturing antibody functionalized MTJ sensor sensing area was initially covered with a solution containing target p24 antigen biomolecules. Recombinant p24 antigen with original concentration of 1 mg/ml, purchased from Abcam, was diluted in serial concentration of 0.01–1 μg/ml with phosphate-buffered saline served as a target.[19]

Then the sensor surface was washed with PBST after the full binding of antigens with capturing antibodies immobilized onto the MTJ sensor surface. Subsequently, the detecting antibody functionalized IONPs were introduced, and bound to the target antigens at the different epitopes from the capturing antibodies. After removing the excess IONPs by flushing the sensor surface with PBST, a hard-axis field was applied for polarizing and magnetizing the IONP. The stray magnetic field of IONPs was detected by the MTJ sensor, and the signal acquisition was performed on the MTJ array sensor through two-point probe measurement.

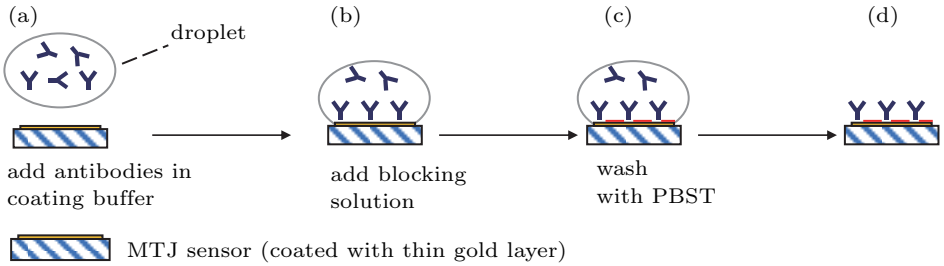


Fig. 2. Schematic illustration of the preparation of capturing antibodies biofunctionalized MTJ sensor. (a) One droplet of antibody solution was introduced onto the sensor sensing area surface. (b) Capturing antibodies were passively attached to the surface of MTJ sensor by incubation in buffer. (c) Blocking buffer was added to eliminate non-specific binding effect and block the remaining active area (blocking areas are represented by red color). (d) A biofunctionalized MTJ sensor was achieved.

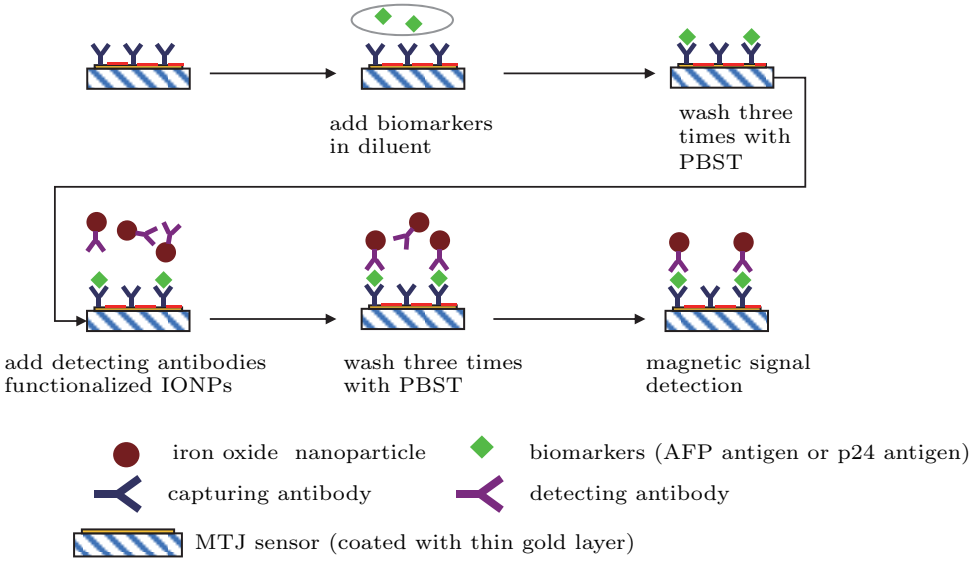


Fig. 3. Schematic illustration of magnetic sandwich immunoassay based on MTJ sensor and magnetic iron oxide nanoparticles (IONPs). This system exploits the capturing antibody functionalized MTJ sensor to capture biomarkers (antigens). The IONPs were labeled with detecting antibody. The target antigens were conjugated with both the capturing and detecting antibodies at different epitopes.

3. Results

3.1. Characterization of IONPs and MTJ array sensor

To acquire high sensitivity in magnetic biodetection, the IONPs as bio-labels are required to be superparamagnetic with low remanence ratio and high saturation magnetization. They need to generate strong enough magnetic signals to be detected by magnetic sensor, while the particle agglomeration can be avoided when there is no applied magnetic field. The IONPs with carboxyl groups were synthesized characterized as described previously.[20] They exhibited superparamagnetic behavior with a relatively higher saturation magnetization (Ms) of around 50 emu/g when displaying a spherical morphology with size of 20 nm.

The MTJ array sensor (purchased from Micro Magnetics) consists of 76 junctions, and every junction stack was prepared by magnetron sputtering on thermally oxidized silicon wafers with a base pressure of 2×10^{-10} Torr. The thin-film stack structure was (in units of nm): substrate/Ta (5)/Ru (30)/Ta (5)/CoFe (2)/IrMn (15)/CoFe (2)/Ru (0.8)/CoFeB (3)/MgO (1.9)/CoFeB (3)/Ta (5)/Ru (10). The MTJ array sensor was schematically drawn in Fig. 4(a). The MTJ array sensor was

placed on the sample stage, shielded in a mu-metal shielding box to avoid the disturbances of ambient magnetic field. Two pairs of Helmholtz coils were utilized here. One pair of the coils provided a changeable magnetic field from −100 Oe to 100 Oe along the easy-axis and the other pair provided a 10-Oe hard-axis magnetic field on the MTJ array sensor, respectively. Figure 4(b) shows the MR transfer curves of the MTJ array sensor, with a sensitivity of 1.39%/Oe. The sensor resistance is normally measured at a four-point probe for high precision. However, it is noted that the results obtained by four- and two-point probe measurements are the same in our experiment, which is probably due to the fact that the typical resistance values of the MTJ array sensor used here (in the range of tens of thousands of Ω) were much larger than the lead resistance attributed to the corresponding gold electrical leads.[21]

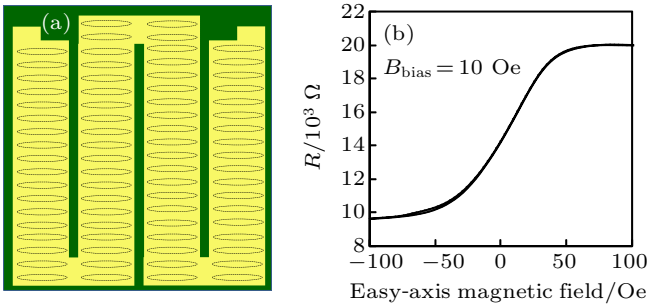


Fig. 4. (a) Schematic drawing of the MTJ array sensor, consisting of 76 tunneling junctions indicated by ellipses. (b) MR loop of the MTJ array sensor with 10 Oe of hard-axis bias field along the hard-axis.

3.2. Detection of p24 antigens

The detection of target p24 biomolecules was realized through the two-point probe measuring configuration with the MTJ array sensor and biofunctionalized IONPs. As illustrated in Fig. 5, it is a sandwich assay and the stray field of IONPs magnetized was measured by the MTJ array sensor for in-plane mode. The signal from IONPs labeled was obtained by comparing differentially the signal of sensor to its reference as described before.[22] A 10-Oe magnetic field was applied along the hard-axis of the sensor to reduce the sensor hysteresis. The MR transfer curves of the MTJ array sensor were measured before and after the binding of biofunctionalized IONPs through the conjugation with p24 on the MTJs array sensing area surface.

For the p24 with concentration of 1 μg/ml, the resistance variations ΔR of the sensor against easy-axis field changed is shown in Fig. 6(a). A reference measurement was carried out where no p24-biofunctionalized IONPs were introduced. The maximum resistance deviations appear at approximately 50 Oe, with value of 1190 Ω, demonstrating the detection of p24 with concentration of 1 μg/ml. The maximum ΔR are 540 and 74 Ω, respectively, for the p24 with concentrations of 0.1 and 0.01 μg/ml through similar procedure. Figure 6(b) illustrates the relation between sensor maximum ΔR and p24 concentration, and the maximum ΔR increases with the logarithm of p24 concentration. The error bars represent the standard deviations for the ΔR between 50 ± 10 Oe, where the MTJ array sensor exhibits the maximum ΔR. The ΔR of the reference curve between 40 Oe and 60 Oe was measured to be 24 ± 7 Ω, while the limit of p24 concentration detected in our work was 0.01 μg/ml with $\Delta R = 74 \pm 6$ Ω.

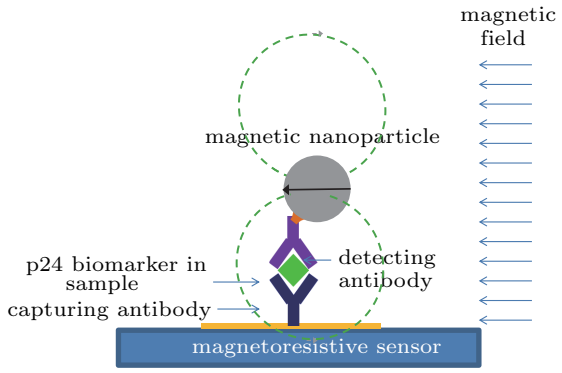


Fig. 5. Schematic drawings of p24 detection with a sandwich-assay configuration. The stray magnetic field produced by IONPs magnetized by the magnetic field in the parallel direction to the MTJ array sensor (magnetoresistive sensor) surface.

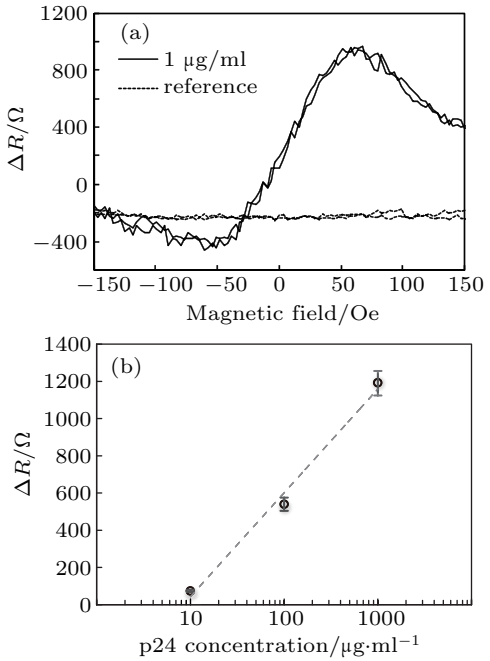


Fig. 6. (a) Diagram of the MTJ sensor resistance variation ΔR after binding with the target p24 antigens with a concentration of 1 μg/ml. (b) Relation between sensor maximum ΔR and p24 concentration. Generally, the maximum ΔR increases with the logarithm of p24 concentration.

Thus, here we demonstrated the feasibility of sensitive detection of p24 antigens using carboxyl-group functionalized IONPs with the MTJ array sensor. This logarithm relation is consistent with the previous works reported for biosensing using magnetic nanoparticles and MR sensors.[23]

4. Conclusion and perspectives

In summary, we have demonstrated the feasibility of HIV disease immunoassay with carboxyl-group functionalized IONPs and an MTJ sensors by a sandwich-assay configuration. The MTJ sensors and IONPs were biologically treated with capturing antibodies and detecting antibodies before the magnetic biodetection experiments. The MTJ array sensor with a sensing area of 890×890 μm^2 (almost the whole surface area of sensor) possessing a sensitivity of 1.39%/Oe was used to detect p24 antigens, HIV disease biomarker. It is demonstrated that the p24 antigens could be detected at a concentration of 0.01 μg/ml (10 ng/ml).

Furthermore, the magnetic biodetection of p24 antigens for health care is firstly realized here based on the configuration of MTJ sensors and carboxyl-group functionalized IONPs. The lower limit of p24 tested in our experiment is 0.01 μg/ml. However, the lower limit of detection in the regular HIV-1 p24 antigen test is reported to be 10 pg/ml, a concentration approximately reaches between three and four weeks after infection.[24] The commercially available fluorescent assay kits to detect p24 antigen with limits of detection is reported to be from 11.9 pg/ml to 33.5 pg/ml.[25] It has been demonstrated by researchers that the sensitivity of biodetection based on GMR sensor is superior to fluorescent immunoassay, and MTJ sensors offer higher sensitivity at low magnetic field than GMR sensors.[22,26] Our future work is to optimize this detection configuration with MTJ sensors and to improve its detection sensitivity for p24 antigens.

In addition, the multiplex sensing and miniaturization are also a very valuable future work for our magnetic biodetection configuration. The multiplex sensing of different bioanalytes can be realized by pre-mobilizing different capturing molecules on different working areas on an MR sensor array.[27] The compatibility of MR sensors with the standard CMOS fabrication technology enables great promise for our magnetic biodetection platform to be miniaturized in lab-on-chip applications for point of care with high sensitivity and low cost.[28]

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