# Liver cancer immunoassay with magnetic nanoparticles and MgO-based magnetic tunnel junction sensors

Z. Q. Lei, L. Li, G. J. Li, C. W. Leung, J. Shi et al.

Citation: J. Appl. Phys. **111**, 07E505 (2012); doi: 10.1063/1.3676212
View online: http://dx.doi.org/10.1063/1.3676212
View Table of Contents: http://jap.aip.org/resource/1/JAPIAU/v111/i7
Published by the American Institute of Physics.

# Liver cancer immunoassay with magnetic nanoparticles and MgO-based magnetic tunnel junction sensors

Z. Q. Lei,[1] L. Li,[1] G. J. Li,[1] C. W. Leung,[2] J. Shi,[3] C. M. Wong,[4,5] K. C. Lo,[6] W. K. Chan,[6] C. S. K. Mak,[6] S. B. Chan,[1] N. M. M. Chan,[7] C. H. Leung,[1] P. T. Lai,[1] and P. W. T. Pong[1,a)]
[1]*Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong*
[2]*Department of Applied Physics, The Hong Kong Polytechnic University, Hong Kong*
[3]*Department of Physics, Hong Kong Baptist University, Hong Kong*
[4]*Department of Pathology, The University of Hong Kong, Hong Kong*
[5]*State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong*
[6]*Department of Chemistry, The University of Hong Kong, Hong Kong*
[7]*Department of Surgery, University of Cambridge, United Kingdom*

(Presented 1 November 2011; received 23 September 2011; accepted 9 November 2011; published online 5 March 2012)

We have demonstrated the detection of alpha-fetoprotein (AFP) labeled with magnetic nanoparticles (MNPs) using MgO-based magnetic tunnel junction (MTJ) sensors. AFP is an important hepatic tumor biomarker and the detection of AFP has significant applications for clinical diagnostics and immunoassay for early-stage liver cancer indications. In this work, MgO-based MTJ sensors and 20-nm iron-oxide magnetic nanoparticles (MNPs) were used for detecting AFP antigens by a sandwich-assay configuration. The MTJ sensors with a sensing area of $4 \times 2\ \mu m^2$ possess tunneling magnetoresistance (TMR) of 122% and sensitivity of 0.95%/Oe at room temperature. The target AFP antigens of three concentrations were successfully detected, and the experimental data indicate that the resistance variations of the MTJ sensor increased with the AFP concentration ratios proportionally. These results demonstrate that MgO-based MTJ sensors together with MNPs are a promising biosensing platform for liver cancer immunoassay. © *2012 American Institute of Physics*. [doi:10.1063/1.3676212]

## I. INTRODUCTION

Liver cancer immunoassay has long been studied because of the poor survival rate of cancerous patients and the lack of effective methods for cancer treatments. Early-stage detection of hepatocellular carcinoma (HCC) benefits cancer patients with asymptomatic hepatitis by helping them avoid surgical resection or liver transplantation. Currently, cancer immunoassay is dominated by fluoroimmunoassay (FIA), enzyme immunoassay (EIA), and radioimmunoassay (RIA) technology. However, these diagnostic methods are confronted with disadvantages, such as high cost, they are time-consuming, and there are hazards for handling the radioactive antigen.[1] An alternative approach for bioanalytical assay is magnetic immunoassay (MIA), which utilizes ultra-sensitive spintronic magnetometer as a biosensor to detect the biomolecules labeled with functionalized magnetic nanoparticles (MNPs). In biodetections, the dimension of MNPs is comparable to biomolecules and thus capable of realizing single binding with the analytes. The presence of analytes can be identified by the magnetic field emanated from the binding MNPs, and the field intensity can be subsequently measured by the ultra-sensitive magnetometer. This MIA assay method takes advantage of high-sensitivity, low-cost, and portable capabilities of spintronic sensors.

Magnetic tunnel junctions (MTJs) are recognized as an ideal sensor element for biosensors and biochips because of their high tunneling magnetoresistance (TMR) ratios, small size, low cost, and lab-on-chip compatibility.[2–5] MgO-based MTJs experimentally demonstrated TMR ratio of 604% at room temperature,[6] and picotesla-sensitivity MTJ sensors are under intensive research.[7,8] The sensing platform of MTJ sensors together with MNPs has successfully been applied for detecting protein binding and DNA hybridization in previous studies.[2,4,9] In this work, we demonstrate the feasibility of detecting alpha-fetoprotein (AFP) tagged with 20-nm monodisperse iron-oxide MNPs using MgO-based MTJ sensors. Because the carriers of hepatitis B and C viruses suffer a high risk of developing HCC and AFP as a crucial hepatic tumor biomarker, it is very useful for early-stage liver cancer indications, and the biodetection of AFP will bring a revolutionary impact to clinical diagnostics and immunoassay for HCC biorecognition.[1,9,10]

## II. EXPERIMENT

The MTJ stack (purchased from Micro Magnetics) was deposited on thermally oxidized silicon wafers by a magnetron sputtering system with a base pressure of $2 \times 10^{-8}$ Torr, and it is composed of the following structure (units in nanometers): 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 sensing area of the MTJ junctions is $4 \times 2\ \mu m^2$ and the junctions were patterned with optical lithography and ion milling. A layer of gold with a thickness of 200 nm was deposited on top of the MTJs and serves as both

a)Electronic mail: ppong@eee.hku.hk.

 

electrical contacts as well as a biologically active area. This biologically active interface can provide a platform for selectively immobilizing anti-AFP antibodies onto the sensing area of MTJs. The TMR transfer curve was characterized with a standard four-probe method. Both the MTJ sensors and the sample stage were shielded in a mu-metal shielding box to exempt them from the disturbances of an ambient magnetic field. The easy-axis and hard-axis magnetic fields were provided by a pair of Helmholtz coils and all the measurements were carried out at room temperature.

Prior to the AFP-MIA experiment, MTJ sensors and MNPs need to be biologically treated. Monoclonal anti-AFP antibodies from human cord blood (purchased from Fitzgerald Industries International) were immobilized onto the biologically active surface of the MTJ sensor to be the capturing anti-AFPs for capturing the target AFP antigens. After the incubation, blocking solution (5.00% Skim Milk in phosphate-buffered saline with 0.05% TWEEN-20) was introduced to reduce undesirable non-specific binding of protein molecules onto the MTJ sensor surface, which might induce false results.[11] Iron-oxide MNPs ($Fe_3O_4$, purchased from Ocean Nanotech) with carboxyl groups were used for biomagnetically labeling the detecting anti-AFPs because of their high magnetization and biocompatibility.[12] The surfaces of the MNPs were coated with carboxylic acid groups for the purpose of functionalizing the MNPs with exterior modification, so that the MNPs are capable of binding with the detecting anti-AFP antibodies. The biofunctionalized MNPs were prepared with a constant concentration of 1.00 mg/ml. Figure 1 depicts the vibrating sample magnetometry (VSM) measurement result of these MNPs in the solvent of de-ionized (DI) water. The MNPs exhibit superparamagnetism and they possess a magnetization of 50 emu/g.

After the biofunctionalizations of the MgO-MTJ sensors with capturing anti-AFP antibodies and the MNPs with detecting anti-AFP antibodies, the detection of target AFP antigens was carried out with a sandwich-assay configuration, which is schematically shown in Fig. 2. The monodisperse MNPs conjugated with the detecting anti-AFPs possess a diameter of 20 nm and exhibit no aggregation as shown in the TEM image (inset of Fig. 2). The antibody-functionalized MTJ sensor surface was initially covered with target AFP antigens and washed with phosphate-buffered saline with 0.05% TWEEN-20 (PBST) after the full binding of the target AFP antigens to the capturing anti-AFP antibodies attached to the MTJ sensor surface. Subsequently, the MNPs conjugated with the detecting anti-AFP antibodies were introduced, and the capturing and detecting anti-AFPs bound to the target AFP antigens at the different epitopes. Magnetotransport characterization was performed on the MTJ sensor after flushing the sensor surface with PBST solution for removing excess MNPs.

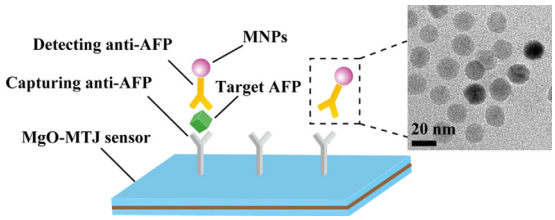


FIG. 2. (Color online) Schematic drawings of AFP detection with a sandwich-assay configuration. The MgO-based MTJ sensor surface is bonded with the capturing anti-AFP antibodies, and the carboxylated MNPs are covalently coupled to the detecting anti-AFP antibodies. The target AFP antigens can conjugate with both the capturing and detecting anti-AFP antibodies at different epitopes. The inset TEM image shows the monodisperse biofunctionalized MNPs. The isotropic MNPs possess a diameter of 20 nm.

## III. RESULTS AND DISCUSSION

The target AFP antigens with three different concentrations of 0.002, 0.010, and 0.050 mg/ml were successively detected by the same MgO-MTJ sensor. A hard-axis field of 60 Oe was applied for polarizing and magnetizing the MNPs. Compared to other biasing modes, this in-plane dc magnetic field is also effective for reducing the hysteresis as well as sensor magnetic noise by stabilizing the magnetic layers in MTJs.[13–15] The TMR transfer curves of MTJ sensors were measured before and after the binding of biofunctionalized MNPs. Figure 3 presents the TMR transfer curve of MgO-based MTJ sensor without any MNP binding. The MTJ sensors exhibited TMR ratio of 122% and sensitivity of 0.95%/Oe. The inset is an optical image of the MTJ sensing area. This sensitivity is compared with the MTJ biosensors used

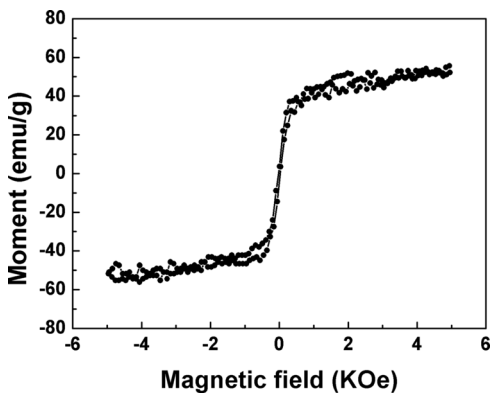


FIG. 1. VSM measurement showing the magnetic moment as a function of applied magnetic field. The samples of the iron-oxide MNPs with carboxyl groups were measured in the solvent of DI water. The saturation magnetization is 50 emu/g.

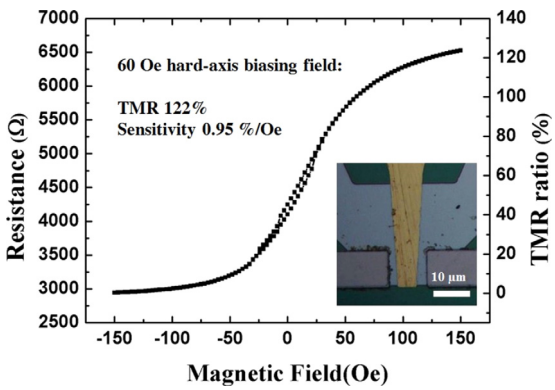


FIG. 3. (Color online) TMR transfer curve of the MTJ sensor. The sensor exhibits a TMR ratio 122% and a sensitivity of 0.95%/Oe under 60-Oe hard-axis biasing field. The inset shows the optical image of the MTJ sensor.

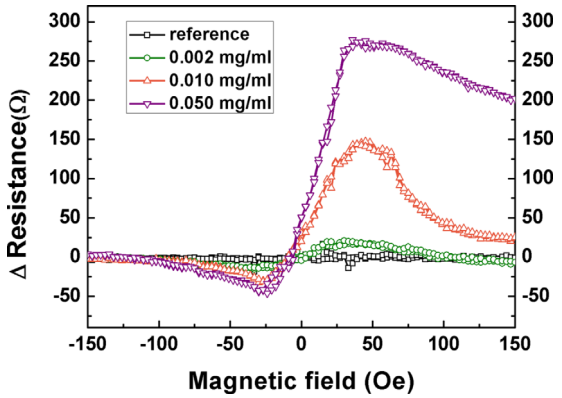


FIG. 4. (Color online) Diagram of the MTJ sensor resistance variations ΔR after binding with three different concentrations of target AFP antigens. The maximum resistance deviations appear at approximately 40 Oe, with the maximums of 17, 143, and 271 Ω, respectively.

previously in other groups. Cardoso *et al.*[3] obtained a sensitivity of 0.73%/Oe with the $Al_2O_3$-barrier MTJ-biochips. Shen *et al.* applied the arrays of MgO-MTJ with sensitivities of 0.91%/Oe (Ref. 4) and 1.00%/Oe (Ref. 5) for detecting DNA labeled with MNPs. Compared with these results, the MTJ sensors in this work exhibited a reasonably high sensitivity for biodetection. The resistance of the MTJ sensor changed with the concentration of the target AFP antigens, and the relation of resistance variations ΔR against easy-axis fields is shown in Fig. 4. Piranha solution was employed to effectively clean the sensor surface before a new experiment. To guarantee that there are no residual binding MNPs after the washing out, a reference measurement was carried out, and it is plotted in Fig. 4 as well. The maximum ΔR after binding the MNPs are 17, 143, and 271 Ω, respectively, for the target AFP antigen concentrations of 0.002, 0.010, and 0.050 mg/ml. Because the concentration of MNPs conjugated with anti-AFP remained the same in these three measurements, the variations of ΔR corresponding to the different target AFP antigen concentrations can be attributed to the different number of biomolecular binding events between the target AFPs and the detecting anti-AFPs. Figure 5 illustrates the relation between maximum ΔR and the concentrations of target AFP antigens. The error bars represent the standard deviations for the ΔR between $40 \pm 10$ Oe, where the MTJ sensor exhibits the maximum ΔR. We can observe that the resistance of MgO-based MTJ sensors is sensitive to the MNPs and the maximum ΔR changes logarithmically with the increase of AFP concentrations. Consequently, the AFP antigen concentration can be deduced through the ΔR of MTJ sensors. This logarithm relation consists of the previous works reported for detecting MNPs[15] and DNA[4,16] using other MR sensors. The AFP concentration of 0.010 mg/ml is recognized as the threshold of screening tests for HCC in patients with chronic hepatitis C.[17] The demonstration of AFP detection at this and even lower concentration levels in this work indicates that this biosensing platform of MgO-based MTJ sensors and MNPs is capable of liver cancer immunoassay for clinical applications.

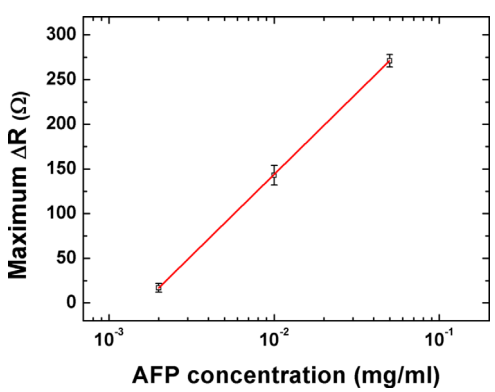


FIG. 5. (Color online) Plot of maximum ΔR vs AFP antigen concentration. The error bars represent the standard deviations for the ΔR between 30 Oe and 50 Oe. The fitting curve (solid line) illustrates that ΔR changes logarithmically with the concentration of target AFP antigens.

## IV. CONCLUSIONS

In summary, we have demonstrated the feasibility of liver cancer immunoassay with MNPs and MgO-based MTJ sensors by a sandwich-assay configuration, in which both detecting and capturing anti-AFP antibodies were employed for detecting the target AFP antigens. The MTJ sensors and $Fe_3O_4$ MNPs were biologically treated before the AFP-MIA experiment. The detection of target AFP antigens caused an increase of resistance variations of the same MTJ sensor in proportion to the logarithm of the AFP antigen concentration. This logarithm relation can be applied for identifying the presence of target AFPs and further deducing their concentrations.

## ACKNOWLEDGMENTS

The authors wish to thank X. Chen, Y. Zhou, and H. K. Y. Mak for their valuable assistance. This work was supported by the Seed Funding Program for Basic Research from the University of Hong Kong and the RGC-GRF grant (HKU 7049/11P).

[1]B. Zhang *et al.*, Biosens. Bioelectron. **23**, 19 (2007).
[2]S. G. Grancharov *et al.*, J. Phys. Chem. B **109**, 13030 (2005).
[3]F. A. Cardoso *et al.*, J. Appl. Phys. **99**, 08B307 (2006).
[4]W. Shen *et al.*, J. Appl. Phys. **103**, 07A306 (2008).
[5]W. Shen *et al.*, Appl. Phys. Lett. **93**, 033903 (2008).
[6]S. Ikeda *et al.*, Appl. Phys. Lett. **93**, 082508 (2008).
[7]R. C. Chaves *et al.*, J. Appl. Phys. **103**, 07E931 (2008).
[8]H. Duan *et al.*, J. Appl. Phys. **109**, 113917 (2011).
[9]V. C. Martins *et al.*, J. Magn. Magn. Mater. **322**, 1655 (2010).
[10]B. Srinivasan *et al.*, Angew. Chem. Int. Ed. **48**, 2764 (2009).
[11]M. L. Chan *et al.*, IEEE Trans. Magn. **45**, 4816 (2009).
[12]L. A. Harris *et al.*, Chem. Mater. **15**, 1367 (2003).
[13]D. Mazumdar *et al.*, Appl. Phys. Lett. **91**, 033507 (2007).
[14]X. Liu *et al.*, J. Appl. Phys. **92**, 4722 (2002).
[15]Z. Q. Lei *et al.*, IEEE Trans. Magn. **47**, 2577 (2011).
[16]J. Schotter *et al.*, Biosens. Bioelectron. **19**, 1149 (2004).
[17]K. A. Gebo *et al.*, Hepatology **36**, s84 (2002).