31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Biomarkers Identification and Detection Based on GMR Sensor and Sub 13 nm Magnetic Nanoparticles

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***Abstract*—In this paper, we present a ultra high sensitive (Zeptomole, 10^{-21}) technique to enable the detection of any potential low abundance biomarkers. We demonstrated for the first time the detection of sub 13nm high-moment magnetic nanoparticle and the implementation of a novel magnetoresistive (GMR) biosensor concept with higher sensitivity and 10 times lower external field in real biomarker sensing schemes. A potential lung cancer biomarker, interleukin-6 (IL-6), was successfully detected with extremely low concentration (as few as only 200 pieces of IL-6). Together with other features of GMR sensor systems like low-cost, portability, easy-to-use, our demonstrated device may lead to future family-based personalized medicine for cancer prevention.**

I. Introduction

Ultra-low concentration detecting of biomolecules, such as proteins and DNAs, has gain increasing interest since various protein biomarkers for cancer or chronic diseases are present at very low levels at the early stages of the disease development [1]. Moreover, longitudinally monitoring the changes of protein biomarkers is expected to help design medical treatment for specific individuals, which requires a low-cost and easy-to-use medical device [2]. Traditionally, fluorescence-based biosensors, such as enzyme-linked immunosorbent assays (ELISAs), have been widely used in biomedical research and clinic use for biosensing, which are only able to detect abundant of proteins. The main problems associated with fluorescence-based biosensors include lack of quantitative analysis, long processing time, high cost equipment and low signal-to-noise ratio due to the large optical background which limits the sensitivity [3]. Over the past ten years, magnetoresistive biosensors incorporated with magnetic labels (microbeads or nanoparticles) have been proposed as a promising candidate for biosensing due to the advantages of high sensitivity and low-cost. In addition, magnetic sensors can be compatible with today's integrated circuit fabrication technology, which leads devices on chip suitable for portable diagnostic. Moreover, this technology offers the low magnetic noise in the biological sample and the capability to manipulate biomolecules through magnetic labels.

The discovery of GMR effect by Fert [4] and Grunbery [5], who won the 2007 Noble Prize in physics, boosts the development of magnetic storage industry, as well as creating a new emerging area of spintronics. In recent years, GMR or magnetic tunneling junction (MTJ) based biosensors have attracted increasing interest in the world. This technology, detects the stray magnetic fields associated with the magnetic labels bonded with the biomolecules using GMR/MTJ sensors. The first demonstration of magnetic biosensing system was done by Baselt et al. from Naval research lab [6]. This unit, which is called Bead ARray Counter (BARC), detected microsized magnetic beads immobilized on the GMR sensor surface. Bruckl et al. employed a spiral-shaped GMR biosensor and concluded that the GMR biosensor has higher sensitivity than fluorescence-based biosensor [7]. Tondra et al. examined the theoretical signal to noise ratio of this type of assay and predicted the possibility of detection of magnetic nanoparticle labels [8]. Freitas et al. developed a prototype of hand-held microsystem based on fully integrated magnetic biosensors for the first time [9]. Prins et al. [10] and Boeck et al. [11] first demonstrated the real-time on chip detection and manipulation of microsized magnetic particle using microfabricated current wires. Wang et al demonstrated a multiplex protein assay using 50 nm magnetic nanotag sensing [12]. Besides GMR biosensor, MTJ biosensor is another promising candidate due to its high MR ratio. Xiao et al. has shown the detection of DNA labeled magnetic nanoparticles using MgO based MTJ sensor [13]. Recently our group developed a GMR sensing system and demonstrated for the first time a detection sensitivity of Zeptomole (10^{-21}), detection of 600 pieces of Streptavidin [14] and 200 pieces of IL-6 molecules (potential biomarker of lung cancer) [15], which will enable the detection of low abundance biomarkers in real serum samples that can't be detected by using traditional magnetic testing scheme before.

High sensitivity, rapid detection and on chip integration suitable for point-of-care device is the future for magnetic biosensors or biochips, which has been paid tremendously attention before and we will present our new design in this

Manuscript received April 23, 2009. This work was partially supported by the Center for Nanostructured Application and Nanobiotechnology Initiative at University of Minnesota and NSF BME 0730825. Parts of this work were carried out in the Minnesota Characterization Facility which receives partial support from NSF through the NNIN program.

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paper too. On the other hand, magnetic labels, including magnetic microbead and nanoparticle, play the most important role in magnetic biosensing. The size of magnetic labels is becoming very critical, especially for identifying and screening potential biomarkers that are typically much smaller than the magnetic labels used in most published results so far. Most of the magnetic labels used by groups mentioned above are commercially available, and vary in size from 50nm to 3μm. Due to the low diffusivity and poor binding selectivity, microsized magnetic labels are not favorable for biomolecule detection. In order to overcome these limitations, application of nanosized magnetic labels, similar to the size of biomarkers, in the magnetic biosensing system is needed. Fig. 1 demonstrates the size of magnetic labels, being used in magnetoresistive sensor for biomolecule detection, in the past ten years. The arrow drawn for the guide of eye shows a clear trend indicating that magnetic labels with smaller size are more preferred in the magnetic biosensing scheme. In recent two years (shown in the insert of Fig. 1), sub 50nm magnetic labels have been integrated into the magnetic sensing system. Table I briefly summaries, up to date, the application of magnetoresistive sensors and magnetic labels for biomolecule detection. In this paper, we report the detection of sub 13nm high moment FeCo magnetic nanoparticles, using a GMR biosensor for the real biomolecule detection.

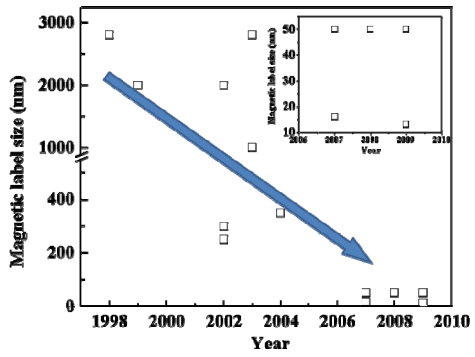

Fig. 1. Summary of size of magnetic labels used in the past ten years

Group, location	Sensor type, size (μm*μm)	Sensor working range	Magnetic label, size	Biomolecule detection?	Biomarker detection?	Reference
Baselt et al., Naval research lab	GMR strip, 80*20	50 Oe	Dynal M280, 2.8um	Yes	No	[6]
Tondra et al., NVE	Wheatstone bridge GMR spin valve, 11*2	150 Oe	N/A	Yes	No	[16]
Freitas et al., Portugal	MTJ, 6*2	30 Oe DC + 13.5 Oe rms AC	Nanomag-D, 130nm	Yes	No	[9]
Wang et al., Stanfrod	GMR spin valve, 100*1.5	80 Oe rms AC + 50 Oe bias	MACS, 50nm	Yes	Yes	[10]
Prins et al., Netherlands	GMR, 100*3	20 Oe rms AC	Dynal Myone, 1um	Yes	Yes	[11] [17]
Boeck et al., Belgium	GMR spin valve, 10*1.4	N/A	Micromer-M, 2um	No	No	[12]
Druck et al., Germany	GMR spiral-shaped, 1800*1	-500 Oe ~ 500 Oe	Bangs CM01N, 350nm	Yes	No	[7]
Xiao et al., Brown	MTJ, 18*6	-60 Oe ~ 60 Oe + 50 Oe DC bias	MACS, 50nm	Yes	No	[13]
Wang et al., U of Minnesota	GMR spin valve, 80*40	0 ~ 15 Oe	FeCo, 12.75nm	Yes	Yes	[15]

Table I. Summary of magnetoresistive sensor and magnetic labels

II. Biomarker Detection Scheme

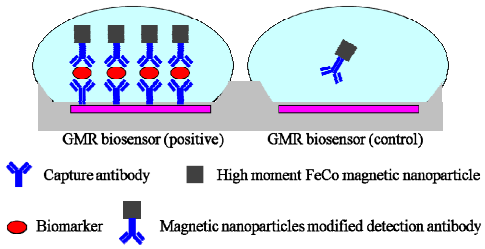

Fig. 2. A novel biomarker detection scheme using GMR biosensor and high moment FeCo magnetic nanoparticle

As discussed in section I, the detection of various biomarkers are of importance for chronic diseases including cancers. Fig. 2 demonstrates a novel, sandwich structure based, biomarker detection scheme using GMR biosensor and high moment FeCo magnetic nanoparticles. The capture antibodies are first immobilized on the positive GMR sensor surface, not on the control GMR sensor surface. Then the samples with biomarkers are applied on both positive and control GMR sensor surface under the same condition. Subsequently, high moment FeCo magnetic nanoparticles modified detection antibodies are applied on both positive and control sensors. After thorough washing, high moment FeCo magnetic nanoparticles modified detection antibodies will only bond to the capture antibody-modified positive GMR biosensor, contributing to the sensing signal picked up the GMR biosensor underneath.

III. Highly Sensitive GMR Biosensor

The sensitivity of the GMR biosensor is one of the key factors for the low concentration biomarker detection. Currently, most of the GMR sensors reported have a 90 degree ground state for magnetization directions between free

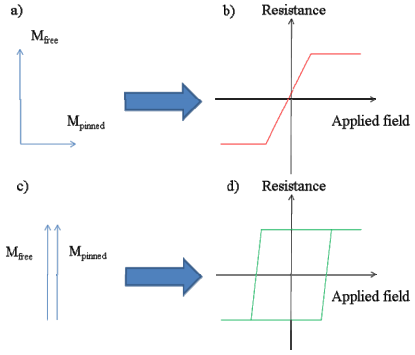

Fig. 3. a) Traditional GMR configuration: 90 degree ground state for state for magnetization directions between free layer and pinned layer. b) Linear transfer curve for traditional GMR configuration. c) New GMR configuration: zero degree ground state. d) High sensitive transfer curve from zero degree ground state. (M_{free} and M_{pinned} represent the magnetization directions of free layer and pinned layer, respectively)

layer and pinned layer. The transfer curve for this traditional configuration of the GMR sensor is shown in Fig. 3(b) as a red line. While this design shows good linear signal output, the drawback is the requirement of large applied field (100 Oe or above) which limits its sensitivity and applicability. To solve these problems, recently we proposed, designed and fabricated GMR sensors with zero degree ground state for magnetization direction between free layer and pinned layer shown in Fig. 3(d) as a green line. The new design enables a higher sensitivity (dR/dH) and requires a much lower applied field compared to the traditional 90 degree configuration. Specially, the unique feature of low applied field makes this design more suitable for lab-on-chip and portable diagnostics.

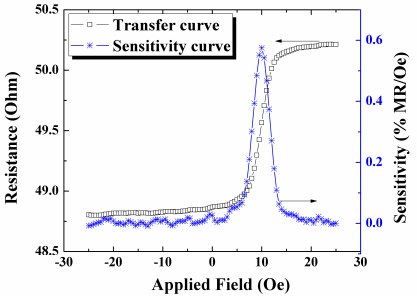

Fig. 4. Transfer curve and sensitivity curve of GMR

The GMR sensors were fabricated using thin film deposition and standard photo lithography technology. A silicon wafer with 100nm of thermal grown SiO_2 was used as a substrate. The multilayer films ($Ir_{0.8}Mn_{0.2}$ 10nm /$Co_{0.9}Fe_{0.1}$ 2.5nm /Cu 3.3nm /$Co_{0.9}Fe_{0.1}$ 1nm /$Ni_{0.82}Fe_{0.12}$ 2nm) were deposited on the substrate by a six-target Shamrock sputtering system. The GMR multilayers were then patterned into rectangular shape with different size using photolithography and ion milling. An 11 nm silicon dioxide layer was deposited on the sensor area (80μm*40μm) using lift-off process to get a suitable biofunctionlization surface. The magnetoresistance of GMR sensor was measured using a four-probe working station. A model Keithely 2400 sourcemeter was used to measure the resistance of the GMR sensor by four wire configuration. The sensing current which runs through the GMR sensor is 1mA, while the voltage of the GMR sensor was monitored by the Keithely 2400 sourcemeter at the same time. An external magnetic field generated by an electromagnetic coil was applied in plane to change the magnetization direction of the free layer. The second function of the external magnetic field is to polarize the magnetic nanoparticles on the GMR sensor which could generate the stray magnetic field for sensing. A self-coded labview program (National instruments, USA) was used to control the measurement system by a computer. The GMR sensor has 3% MR ratio and maximum sensitivity of 0.6 % MR per Oe near 10 Oe applied field as shown in Fig. 4. This sensitivity is three times higher than any traditional GMR sensor design that has been reported.

IV. Sub 13 Nm High Moment Magnetic Nanoparticle

Most of research groups use commercial iron oxide nanoparticles with low magnetic moments. We proposed and synthesized high moment FeCo-(Au) magnetic nanoparticles for biomedical applications [18]–[20]. The net magnetic moment of one 12.75 nm FeCo nanoparticle is 7 times higher than that of one γ-Fe2O3 nanoparticle (commercialized) at external field of 10 Oe, assuming the same nanoparticle volume and 1.5 nm oxide shell for FeCo nanoparticles. Fig. 5(a) shows TEM image of 12.8 nm FeCo magnetic nanoparticles with a composition of 70:30 which were synthesized using a sputtering gas condensation technique. The particle morphology and phase is precisely controlled by modifying the magnetic flux in the sputtering target. The prepared FeCo nanoparticles are dominantly cubic shaped. The nanoparticles are highly homogeneous as shown in Fig. 5(b), which is crucial for accurate biomolecule quantification.

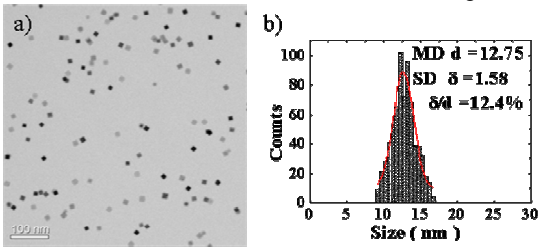

Fig. 5. a) TEM image of FeCo magnetic nanoparticles. b) Size distribution of FeCo nanoparticles

V. Zeptomole Sensitivity and Biomarker Detection

To demonstrate the sensitivity and specificity of this GMR sensor- and magnetic nanoparticle-based detecting platform for biomolecule detection and quantification, we have used this platform to successfully detect and quantify streptavidin (bi-molecule detection: sensor surface modified by biotin and streptavidin modified by nanoparticle) and IL-6 (Sandwich-based detection: sensor surface modified by capture antibody and detecting antibody modified by nanoparticle).

Fig. 6(a) shows the sensor signals for different amounts of magnetic nanoaprticle modified streptavidin molecules on the sensor surface. The figure indicates that GMR sensor detected signals from as low as 600 copies ($<10^{-21}$ mol, zeptomol) of streptavidin. More importantly, there is a linear dose-response relationship of the amount of streptavidin applied and the magnetic signals detected by the GMR sensors. Such a dynamic range of linearity outperforms most other GMR based detecting systems reported to date, making accurate quantification possible.

To demonstrate the real application of high sensitive GMR biosensor for the biomarker detection, we performed the detection of human IL-6 using a sandwich approach, which follows the same principle used in ELISA. As shown in Fig. 6(b), as low as $2.08*10^6$ molecules of human IL-6could be detected by the high sensitive GMR biosensor. This result is

13 times more sensitive than that of IL-6 ELISA assay (R&D). The low signal level of the control sensors demonstrates that there is no significant non-specific binding using this sensing scheme. Moreover, a linear trend, between the sensing signal and the number of IL-6 molecules on the sensor surface, is observed in Fig. 6(b), which suggests the application of GMR biosensing system for quantifying very small amount of the biomarker in the biological sample. More recently, we further optimized our testing platform and demonstrated that as few as 200 pieces of IL-6 molecules could be detected [15].

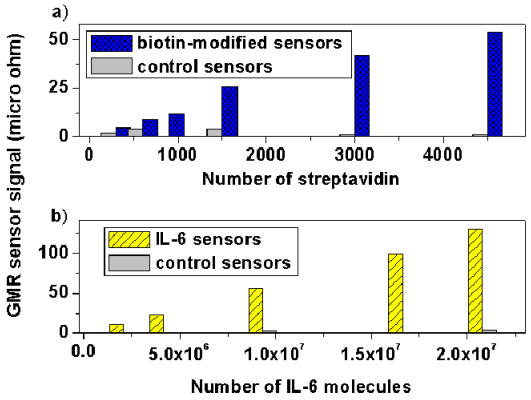

Fig. 6. a) Resistance change for different amounts of streptavidin molecules on the GMR sensor. Blue: GMR sensors with surface modified by biotin. Gray: GMR sensors with no biotin modification. b) Resistance change detected by sensors for different amounts of IL-6 molecules modified on the sensors using a sandwich structure. The same amount of capture antibody and detection antibody-modified magnetic nanoparticles was applied to each sensor with varied numbers of IL-6 molecules. Yellow: IL-6 modified GMR sensors. Gray: GMR sensors with no IL-6 modification.

VI. Conclusion

Sub 13nm high-moment magnetic nanoparticles have been detected using highly sensitive GMR biosensor for biomolecule detection, both using streptavidin-biotin interaction and IL-6 sandwich binding structure. The usage of ultra-small size of magnetic nanoparticles enables the detection of 600 copies of streptavidin molecules and later 200 pieces of IL-6 molecules. We demonstrated in real bio-recognitions, for the first time, the usage of sub 13 nm nanoparticles as magnetic labels and the implementation of a novel GMR sensor concept with 3 times higher sensitivity and 10 times low working field compared to traditional GMR sensors. This demonstration will facilitate the identification and vilification of various biomarkers and eventually lead to the low-cost and family-based personalized medicine devices for early cancer prevention.

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