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Giant Magnetoresistive Sensors for DNA Microarray

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Abstract

Giant magnetoresistive (GMR) sensors are developed for a DNA microarray. Compared with the conventional fluorescent sensors, GMR sensors are cheaper, more sensitive, can generate fully electronic signals, and can be easily integrated with electronics and microfluidics. The GMR sensor used in this work has a bottom spin valve structure with an MR ratio of 12%. The single-strand target DNA detected has a length of 20 bases. Assays with DNA concentrations down to 10 pM were performed, with a dynamic range of 3 logs. A double modulation technique was used in signal detection to reduce the 1/f noise in the sensor while circumventing electromagnetic interference. The logarithmic relationship between the magnetic signal and the target DNA concentration can be described by the Temkin isotherm. Furthermore, GMR sensors integrated with microfluidics has great potential of improving the sensitivity to 1 pM or below, and the total assay time can be reduced to less than 1 hour.

Index Terms

Giant Magnetoresistive sensor; spin valve; DNA microarray; biosensor; microfluidics

I. INTRODUCTION

In recent years, Giant Magnetoresistive (GMR) sensors have shown great potential as sensing elements for biomolecule detection [1–5]. GMR sensors are sensitive, can be easily integrated with electronics and microfluidics, and can generate fully electronic signals. The GMR detection system is also cheap and can be easily made portable. All these advantages make the GMR sensors attractive over the conventional fluorescent sensors. In this work, we successfully deployed GMR sensors in a prototype DNA microarray. Prior researches reported detection of DNA with concentrations above 1 nM using GMR sensors [1–5]. However, in order to be of use in most biomedical applications, the GMR sensors have to achieve a concentration sensitivity down to 1 pM or less. With the help of the “double modulation” detection technique and better surface chemistry, we accomplished detection of DNA samples down to ~10 pM. A dynamic range of at least 3 logs was achieved.

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To further improve the sensitivity and reduce total assay time, GMR sensors integrated with microfluidics are being developed. Since 1980s microfluidics has been used in many applications including inkjet printheads, Lab-On-A-Chip technology, micro-propulsion, etc. Microfluidics used in industry is often based on silicon or glass because of their high stability. Polydimethylsiloxane (PDMS) has also been widely used to make microfluidic systems in industry and research labs because it is much cheaper and easier to process [6]. In this work, we fabricated PDMS based microfluidic chips and integrated them with the GMR sensors. Preliminary results show that the microfluidics-integrated GMR sensors can detect DNA samples at a concentration of 1 pM or below.

II. MATERIALS AND METHODS

The GMR sensors used in this experiment are the same as or similar to a bottom spin valve structure: Ta(5)/seed layer/IrMn(8)/CoFe(2)/Ru(0.8)/CoFe(2)/Cu(2.3)/CoFe(1.5)/Ta(3), all numbers in parenthesis are in nanometers. Each sensor consists of 32 spin valve strips in serial connection and each strip has a dimension of $93\text{ }\mu\text{m} \times 1.5\text{ }\mu\text{m}$ (Fig. 1). The sensor has an MR ratio of 12% after patterning. Each chip has an array of 8 by 8 sensors, which are connected to the bonding pads on the peripheral by a 300 nm thick Ta/Au/Ta lead. Of the total 64 sensors on each chip, 32 are active sensors and 32 are reference sensors. Reference sensors are used in the electronics to cancel out the common mode noise and interference. To protect the sensors from corrosion, a thin passivation layer of $\text{SiO}_2(10\text{nm})/\text{Si}_3\text{N}_4(20\text{nm})/\text{SiO}_2(10\text{nm})$ was deposited everywhere except on the bonding pads by ion beam deposition. A thick passivation layer of $\text{SiO}_2(100\text{nm})/\text{Si}_3\text{N}_4(150\text{nm})/\text{SiO}_2(100\text{nm})$ was deposited on top of the reference sensors and leads whereas the active sensors and bonding pad area was exposed. This thick passivation layer protects the lead from corrosion and also separates the reference sensors from the magnetic nanoparticles to insure that no signals are generated in these sensors.

The protocol of the detection process with GMR sensor assay in an open well is as follows: the GMR sensors were coated with polyallylamine during the fabrication process. A layer of partially hydrolyzed polyethylene-maleic anhydride (polyacid) was coated on top of the polyallylamine layer by exposing the sensor chip to an aqueous polyacid solution. The chip was then heated at $100\text{ }^\circ\text{C}$ for one hour to cyclize maleic acid groups. This step crosslinked and strengthened the underlying polyallylamine layer and provided functional groups to covalently couple to probe oligonucleotides. The oligonucleotide probes bearing amino groups were then spotted onto the activated sensors and incubated overnight; the immobilization process finished after the surface was blocked with aminoethanol. Each chip was spotted with four types of probes with different sequences. Target DNA sample was then applied to the GMR sensors and incubated overnight. The target DNAs were single strands with 20 bases. Complementary target DNAs hybridized with the probe oligonucleotides and stayed on the sensor surface, whereas noncomplementary DNAs were washed away by rinsing. One end of the target DNA was modified to have a biotin group, which could bind to the streptavidin group attached to the magnetic nanoparticles. The stray field from the magnetic nanoparticles then generated signals in the GMR sensors.

A “double modulation” technique was used for signal readout [8]. In this technique an AC magnetic field with a peak value of 100 Oe and a frequency of 208 Hz was applied to excite the signal and an AC current (frequency = 500 Hz) was passed through the sensor for signal readout. A DC magnetic field of 50 Oe was applied to stabilize the spin valve sensors in the single domain state. The interaction between the AC field and AC current generated a peak at 708 Hz in the frequency domain. When the magnetic nanoparticles were in the vicinity of the GMR sensors, their stray fields decreased the AC field sensed by the sensors, and therefore generated a change in the 708 Hz peak. The voltage signals of two adjacent sensors on the same chip, one spotted with probe oligonucleotides (active sensor) and the other not spotted

(reference sensor), were fed into a differential amplifier, so that the common mode noise and interference was rejected. The amplified signal was then fed into a multiplexer for readout. With this technique we effectively reduced the 1/f noise while circumventing the electromagnetic interference at the same time.

In the experiments with microfluidics-integrated GMR sensors, the PDMS microfluidic chips were fabricated using soft lithography technology by the Stanford Microfluidics Foundry [6]. The microfluidic chip was then bonded to the GMR chip by pressing and heating at 50 °C on a hot plate. The alignment between the microfluidic chip and GMR chip was done in a probe station. There were four micro-channels on each integrated chip. Each channel was 110 μm wide and 10 μm high, and had its own inlet and outlet, which were 1 mm circular holes drilled in the PDMS layer on the periphery of the chip. The inlets and outlets were connected to 1.1 mm stainless steel needles and polystyrene tubes. All biochemistry steps were the same as the open well experiment except the target DNA hybridization step. For this step, the target DNA sample was injected into the micro-channels by a syringe pump with a flow rate of 5 μl/min. The incubation time was only 15 minutes. The total assay time was thus reduced to below one hour.

III. RESULTS

We performed assays with various concentrations of target DNAs. A plot of signal vs. target DNA concentration is shown in Fig 2a. The lowest concentration was 10 pM, which gave signals of 2–4 μV. A dynamic range of 3 logs was achieved. Scanning Electron Microscopy (SEM) images of these assays were shown in Fig 2b. The particle coverage corresponds well with the magnetic signal and the nominal DNA concentration.

One limiting factor to the biomolecular sensitivity of GMR sensors is the slow diffusion of target DNA to the sensor surface, a common problem in all biosensors. To further improve the sensitivity, we have been developing microfluidics integrated GMR sensors. Microfluidics can confine the DNA solution within the vicinity of the sensor surface. Therefore, the target DNAs do not need to travel a long distance before binding to the probe oligonucleotides. This can potentially improve the sensitivity and decrease the assay time [8]. Fig 3 shows SEM images of some preliminary experiments. The nanoparticle coverage of the sensor surface after incubation with 10 pM or 1 pM of target DNA with microfluidics (followed by magnetic nanoparticle solution incubation) was higher than the coverage resulting from the corresponding assays without microfluidics. In particular, the 1 pM assay with microfluidics had decent nanoparticle coverage, indicating that microfluidics integrated GMR sensors could detect DNA concentration at 1 pM or less.

IV. DISCUSSION

Some interesting feature was observed in the signal vs. concentration curve. According to the Langmuir isotherm, the surface coverage (θ) of hybridized DNA and the concentration (C) of the target DNA sample follow the Langmuir equation:

$$\frac{\theta}{1 - \theta} = KC$$

where the isotherm constant $K = B \exp(-\Delta G_{ads}/RT)$, ΔG_{ads} is the free energy of adsorption, B is a constant, R is the gas constant, and T is the temperature. When surface coverage is low, $\theta \approx KC$. The magnetic sensor voltage signal V is proportional to the surface coverage of magnetic nanoparticle, which is proportional to the coverage of hybridized DNA θ . Therefore, if Langmuir isotherm was valid, the voltage signal should be proportional to the target DNA

concentration C . However, the data in Fig 3 showed a logarithmic relationship. Other researchers also reported a logarithmic relationship between the signal and the analyte concentration [2,6].

Many factors may contribute to the deviation from the Langmuir isotherm. Here we propose one possibility. A closer look at the Langmuir isotherm reveals that the free energy of adsorption is assumed to be independent of the analyte surface coverage. This may not be true in the case of DNA analyte because DNA molecules are negatively charged. As the surface coverage increases, the electrical repulsion from the hybridized DNAs will increase the energy required for additional DNAs to get close to the surface. As a result, the free energy of adsorption increases with surface coverage. If we assume a linear relationship between ΔG_{ads} and θ , $\Delta G_{ads} = r\theta$, we can get the Temkin isotherm [10]:

$$\theta = \frac{RT}{r} \ln(KC)$$

Similar to the Langmuir isotherm, the Temkin isotherm is also a well-known model used in gas adsorption studies. Some prior studies suggested that the Temkin isotherm may be used to describe heterogeneous protein adsorption [11].

V. CONCLUSION

A sensitive GMR biosensor was developed and used for DNA detection. Microfluidics has a great potential to improve the sensitivity to < 1 pM and reduce the total assay time to < 1 hour. The GMR sensors, with some modification in biochemistry, can also be used in the detection of other biomolecule, e.g. proteins, microorganism, etc [12,13].

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Fig. 1. Scanning Electron Microscopy (SEM) image of GMR sensor. Each sensor consists of 32 spin valve strips in serial connection. Each strip has a dimension of $93 \mu\text{m} \times 1.5 \mu\text{m}$.

(a)

(b)

Fig 2.

(a) Signals of DNA assays with various DNA concentrations; (b) Scanning Electron Microscopy images of the DNA assays. The small white dots are nanoparticles.

Fig 3. Scanning Electron Microscopy images of 10 pM and 1 pM DNA assays with and w/o microfluidics. The small white dots are nanoparticles. Both assays with microfluidics were finished within one hour.