Giant Magnetoresistive Biosensors as Robust, Sensitive, and Cheap Multiplexable Biosensors

Giant magnetoresistive (GMR) sensors have been put to many good uses since its discovery and invention in the early 2000s. Ranging from hard disk information storage to automotive speed and angle sensing, to biosensing, the applications for GMR biosensors are seemingly boundless. In recent years, GMR biosensors have been gaining traction especially in its use as biosensors among the research community for various bioapplications, such as biomarker discovery and biomarker validation in disease diagnostics.

GMR biosensors

The GMR effect is a relatively new phenomena that was discovered independently by both Baibich *et al*.[1] and Binasch *et al*.[2] in 1988. It was awarded the Nobel Prize in 2007[3,4]. GMR biosensors operate on the principle of localized proximity sensing, whereby binding of a magnetically labeled biomolecule to an analyte of interest on the GMR biosensor surface generates a localized change in the magnetic field that transduces into a change in the sensor resistance. The term "giant" is used to highlight the ability to transduce small changes in magnetic resistivity, parts per million (ppm), into large changes in electrical resistance.

In 1998, Baselt *et al*.[5] proposed the first GMR-based biosensor, the Bead Array Counter (BARC). Since then, extensive research has been conducted to develop GMR sensors to perform various biomolecule detection, including DNA[6,7], proteins[8,9], and small molecules[10]. In addition, GMR sensors can be coupled with other technologies, such as microfluidic channels, to answer interesting biological questions or to understand binding kinetics.

GMR biosensors are usually composed of nanostructured multilayer materials consisting of a non-magnetic noble metal

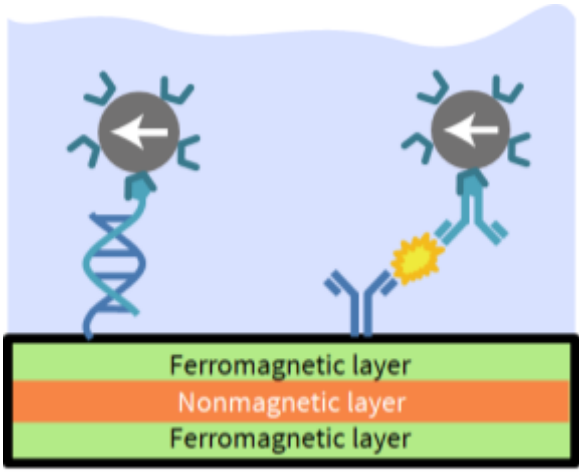

Figure 1. Schematic of a typical GMR biosensor.

[1] Baibich, M.N. *et al*. *Physical Review Letters* **1988**, d*61*, 2472-2475.
[2] Binasch, G. *et al*. *Physical Review B* **1989**, *39*, 4828-4830.
[3] Fert, A. *Reviews of Modern Physics* **2008**, *80*, 1517-1530.
[4] Grünberg, P.A. *Reviews of Modern Physics* **2008**, *80*, 1531-1540.
[5] Baselt, D. *et al*. *Biosensors and Bioelectronics* **1998**, *13*, 731-739.
[6] Nesvet, J. *et al. Clinical Chemistry* **2021**, 67, 534-542.
[7] Nesvet, J. *et al. Biosensors and Bioelectronics* **2019**, 67, 534-542
[8] Ng, E. *et al*. *ACS Sensors* **2020**, 5, 3049-3057.
[9] Ng, E. *et al. Nanomedicine* **2019**, 16, 10-19.
[10] Lee, J.R. *et al. Scientific Reports* **2016**, 88, 7457-7461.

spacer layer sandwiched between two ferromagnetic layers. When an in-plane current is passed through the sensor, the electrons pass through the sensor sandwich structure and all three layers. As electrons transition between layers, they are scattered in a fashion dependent on the magnetic orientation of the ferromagnetic layers. If the magnetic orientation of the ferromagnetic layers is oriented in a parallel manner, the electrons experience less scattering, and resistance is low. If layers are oriented in an antiparallel manner, the electrons experience more scattering and resistance is high.

The particular type of GMR biosensors utilized in the Magic Lifescience platform is spin-valve GMR biosensors. In spin-valve GMR biosensors, the top ferromagnetic layer is a “free layer” in which its magnetic orientation can be changed with a magnetic field. The bottom ferromagnetic layer is a “fixed layer” in which its magnetic orientation is held in place. In the presence of an external magnetic field, the magnetization of the top free layer is aligned parallel to the fixed layer. When magnetic nanoparticles, which generate their own local magnetic fields, come close to the sensor surface, they perturb the local magnetic field sensed by the sensor. This perturbation shifts the magnetic orientation of the free layer and changes the amount of scattering experienced by the electrons as they travel through the sensor stack, thereby changing the electrical resistance of the sensor. Spin-valve GMR sensors are attractive because only small magnetic fields are needed to change the resistance. In other typical antiferromagnetically coupled multiplayer stacks, the GMR effect is not observed until a large external magnetic field on the order of kOe is applied. Spin-valve GMR stacks are weakly coupled, and can therefore achieve the GMR effect at much lower fields on the order of 10 Oe.

On-chip PCR: MagIC on GMR sensors

MagIC Lifescience brings novelty to the GMR biosensing field by embedding temperature sensors, magnetic field- and heat-generating wires into the GMR sensor chip itself. This first-of-its-kind GMR technology enables an all-in-one workflow of performing multiplexed PCR and DNA detection on a single GMR sensor chip[11].

MagIC’s thermocycling GMR chip for multiplexed on-chip PCR

MagIC’s GMR chip is designed with embedded heating wires and temperature sensors that allow the chip to function as a thermocycler. PCR is conducted as usual. Briefly, sample with DNA target(s) is added and mixed into a MasterMix cocktail consisting of biotinylated primers, dNTPs, Mg+ ions, and Taq polymerase. The embedded heating wires can be cycled through and held at various temperatures. PCR amplification – denaturing, annealing, and extension – of DNA target(s) present in the sample is therefore performed on the GMR chip, resulting in copies of biotin-labelled double-stranded DNA (dsDNA) target(s).

[11] Wang, S. X., et al. (2021). *PCR meets GMR – integrated biosensors for POCT*. 14.

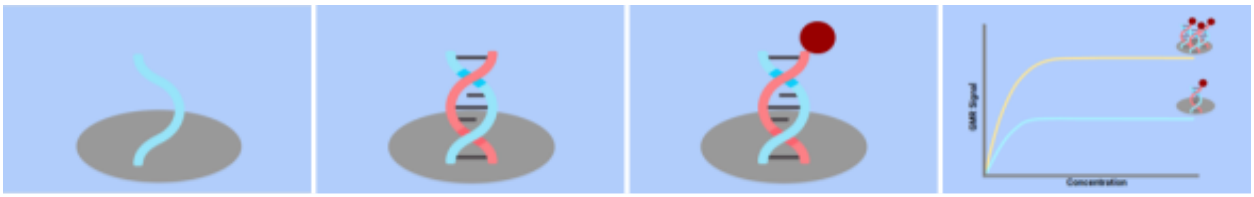

Figure 2. GMR biosensors for DNA detection. Oligonucleotides complementary to the target DNA sequence are functionalized onto the sensor surface and act as capture probes. Sample, usually labeled PCR product, is added and target DNA sequences, if present, hybridizes to the capture probe. Magnetic nanoparticles bind to the hybridized structure on the sensor and a change in resistance is measured. Real-time binding curves are generated, and the saturated signal can be correlated to the concentration of the target DNA sequence in the sample.

One challenge that occurs with traditional thermocyclers and PCR bioassay design, is the inhibition of optical signal due to interference of sample matrix with certain fluorescent dyes that are used in a PCR reaction. This often limits the bioassay's multiplexing capability as well. This is not the case for the GMR biosensor system as the magnetic field signal is not affected by biological sample matrix.

By placing N sets of primers into the reaction, an N-plex PCR reaction is performed. In addition, the super close proximity of the heating elements to the reaction solution – literally in contact right below – enables precise temperature control for faster and more efficient PCR reaction times.

MagIC's embedded magnetic field generator for multiplexed DNA detection

MagIC's GMR sensor chip can be utilized in the detection of DNA fragments, similar to DNA microarrays. In addition, the chip is embedded with a magnetic field generator wire that allows for the generation of magnetic fields without the need for an external magnet. This significantly reduces the number of device components and device complexity and footprint. Conventional PCR thermocyclers do not allow for highly multiplexed detection and/or readout upon finishing the PCR reaction, usually limited by the number of fluorescence channels of the imaging system. Optical components and fluorescent detectors lead to the larger footprint and bulkiness of thermocyclers.

Figure 2 illustrates how GMR biosensors can be used for molecular testing. Oligonucleotides complementary to target DNA strands of interest act as capture probes and are attached to the sensor surface. Surface chemistry treatment of sensors prior to the addition of the capture probes enable covalent binding of the capture oligonucleotide to the sensor surface. Sample, usually labeled polymerase chain reaction (PCR) product, containing the target DNA sequence of interest is added to the sensor surface.

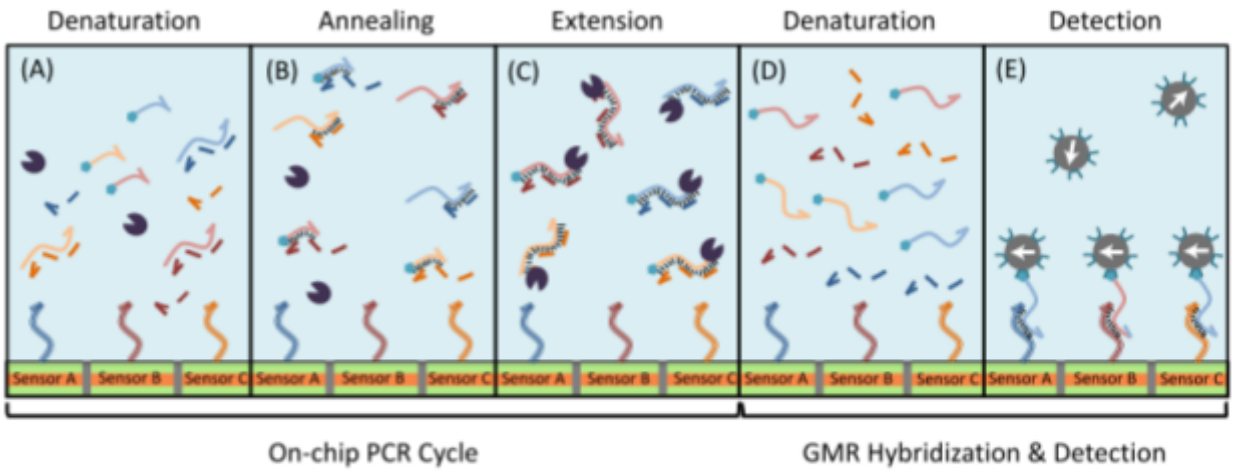

Figure 3. MagIC's complete multiplexed on-chip PCR and detection workflow. (A) – (C) Multiplexed PCR reaction for multi-target DNA amplification is performed on top of the GMR chip. The embedded heating wires enable the GMR chip to function as a thermocycler, cycling through various temperature ranges for the different stages of the PCR reaction – denaturation, annealing, extension. (D) Resulting target dsDNA is denatured into ssDNA and then (E) hybridized onto the appropriate DNA probes functionalized on the surface of dedicated GMR sensors for a multiplexed GMR signal readout.

The set of embedded wires in the chip generate a magnetic field and aligns the magnetic moments of the sensors. When magnetic nanoparticles are added to the sensor, they are brought close to the sensor surface as they bind to the labeled target sequence. This binding event perturbs the magnetic field and changes the magnetic moments of the sensors, generating resistance changes that is read out as a signal in parts per million (ppm). As more and more nanoparticles bind to hybridized capture probe-target DNA structures on the sensor surface, sensor resistance and signal increases until saturation, resulting in a real-time binding curve. The signal at saturation can then be used to quantitatively determine the concentration of the target DNA sequence in the sample.

Since each chip contains an array of 64 sensors, MagIC's GMR chip can effectively detect and quantify up to 64 targets. In addition, with finer temperature control, we can perform further melt analysis for genotyping, mutation detection, and/or antibiotic resistance detection, with just an extra 5-10 min. It has previously been demonstrated by de Olazarra, *et al*. that our GMR biosensors are capable of performing automated point-of-care genotyping of single nucleotide polymorphisms (SNPs)[12].

Advantages of GMR biosensors

GMR magneto-biosensors offer several advantages that allow them to serve as excellent quantitative biosensors. Several GMR biosensors can be placed together to form an array on a single silicon chip. This enables multiplexing capabilities, the detection of several different analytes simultaneously from a single sample on a single GMR biosensor chip, with little to no cross-reactivity. The ability to multiplex is quite a powerful and useful attribute

[12] de Olazarra, A. S., et al. *Lab on a Chip*, 2022, *22* (11), 2131.

because it allows for the collection of more information and data from a single sample within a reduced amount of time and with reduced reagent and sample volume. The sensor chips currently used by Magic Lifescience consist of an array of 64 sensors, and therefore, has the potential to detect up to 64 unique analytes. The sensors are highly versatile and, as previously mentioned, can be used to detect various types of biomarkers, including DNA, proteins, and small molecules. Given that most biological fluids (e.g., whole blood, plasma, serum, saliva, etc.) do not contain magnetic components, there is less interference with detection. As a result of low background noise and high signal-to-noise ratio, GMR biosensors tend to exhibit low limits of detection (LODs) down to femtomolar levels, large dynamic ranges of up to 6 logs, and therefore, high sensitivity and specificity. GMR biosensor array chips are fabricated and produced using standard clean-room processes on silicon wafers. This allows them to be easily mass-produced in large quantities, and therefore, makes them more cost-effective than typical methods of molecular testing (i.e. real-time PCR, next generation sequencing (NGS), etc.). A single chip costs around $2 - $3. The chips are also small and disposable, and do not require large, complicated, and bulky instrumentation to generate or record signal changes. Having such a light footprint allows easy portability, making them excellent for clinical point-of-care (POC) applications.

Future of MagIC: Single Copy DNA Detection

In the future, we imagine designing and producing extremely sensitive biosensors – ones that allow for single copy DNA detection. With this design, we can detect nucleic acids in raw sample without the need for amplification. Turnaround time can be less than 5 minutes and cost of goods (COGs) would be less than $5 per test. Our vision at MagIC is build a molecular diagnostic test that is as easy as a lateral flow test but with absolute DNA quantification in crude samples.