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Journal of Magnetism and Magnetic Materials 293 (2005) 702–708
Journal of magnetism and magnetic materials
www.elsevier.com/locate/jmmm

Magnetic biochips: a new option for sensitive diagnostics

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Available online 5 March 2005

Abstract

We present an overview of miniaturized biosensor devices based on the sensitive detection of magnetic nanoparticles using the giant magneto-resistance effect. This detection principle has advantages over established methods, and the sensitivity is promising. In our opinion, the challenge is now in the integration—of sample pretreatment, in a cartridge, of nanoparticles with proper stabilization, magnetic properties, and surface functionalizations.

Keywords: Biosensor; Medical diagnostics; Giant magneto-resistance effect; Magnetic actuation; Integration; Microdevices; Assays; Magnetic sensor; BARC; Dynabeads; Microchip

Medical diagnostics, both in the central laboratory and at the bed side, is characterized by a drive towards integration and automation. The reason is that tests need to be easy to perform, in a reliable and cost effective way, with minimum human intervention. At the same time, there is an ever increasing need for higher sensitivity and specificity of detection. Magnetic biochips have been proposed as a new means to sensitively detect low concentrations of targets in body fluids for diagnostics. In 1998, Baselt et al. [1] suggested to take advantage of the by then well-developed field of giant magneto-resistance (GMR) sensors for magnetic bead label detection. Magnetic beads are already used in other assays, such as those based on electrochemiluminescence [2], to speed up tests by actively directing the targets to a sensing zone, and for purifying and preconcentrating; magnetic bead labels are thus readily available commercially. The field has developed rapidly since, and one could say that the principle of magnetic sensing is now firmly established. However, to make magnetic biochips a practical option for diagnostics, it is desirable to take a next step in integration, i.e. to combine the biochip with actuation, fluidics and biochemistry in a cartridge, to establish a complete assay.

In the following, we will give an overview of the various sensors that have been developed and their special features (see Fig. 1). But first, we will give a brief introduction to the GMR effect.

GMR was discovered in 1988 by Fert et al. [3], and, independently, by Grünberg et al. [4]. The effect quickly found application in computer

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0304-8853/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jmmm.2005.02.046

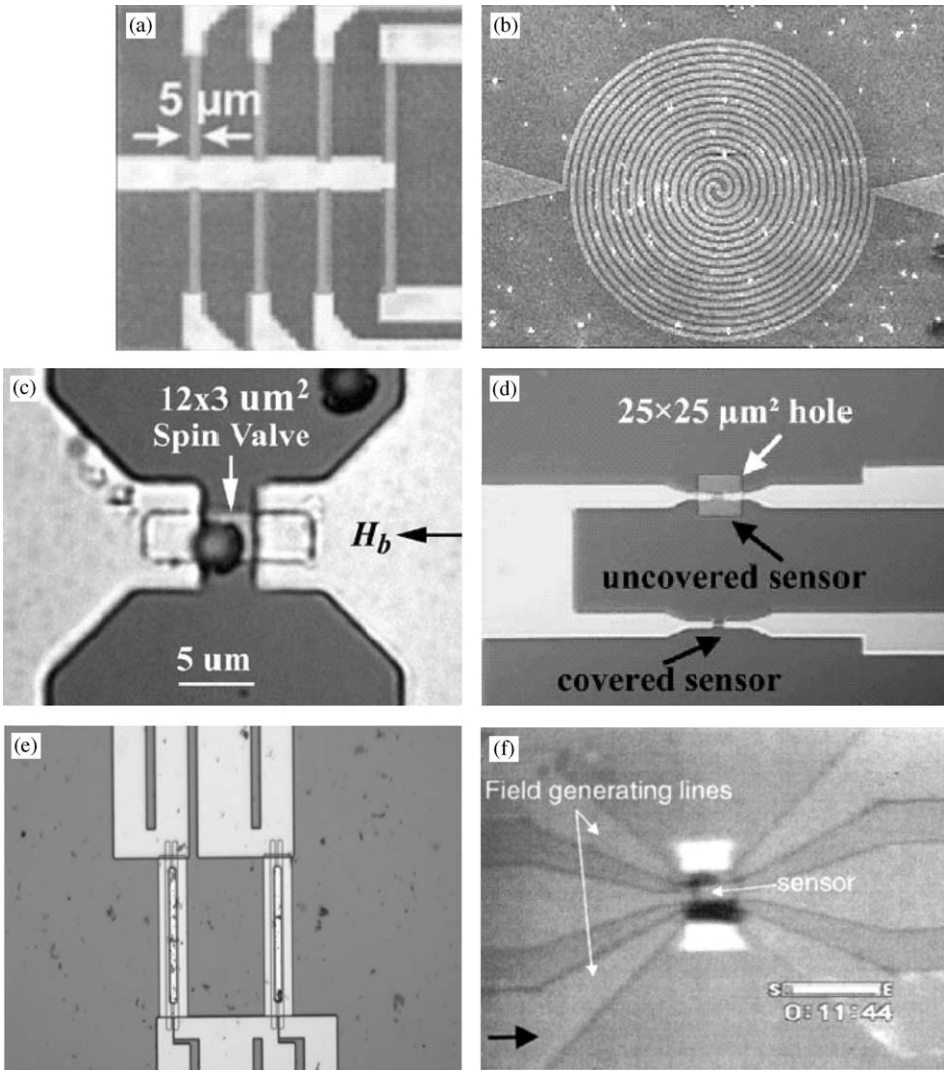

Fig. 1. Various magnetic biosensors: (a) BARC II from Naval Research/non-volatile electronics [11]; (b) large area sensor, from Universität Bielefeld, Germany [14]; (c) single bead detection, from Stanford [16]; (d) sensors that measure progression with time in fluid, from INESC, Lisbon, Portugal [20]; (e) sensors that can distinguish between bulk fluid and surface concentration, from Philips Research; (f) sensor combined with actuation, from IMEC, Leuven, Belgium [26].

magnetic hard disk read heads, and in the near future it is expected to replace position and rotation sensors in automotive applications. The related tunneling magneto-resistance effect is a promising candidate for future non-volatile memories (MRAM). GMR arises in stacks of alternating magnetic and non-magnetic thin films, e.g., Fe and Cr [5]. The electrical resistance of the layers depends on the scattering of the electrons in the layers, which is influenced by the orientation of the electron spin with respect to the magnetization direction of the magnetic layers. The interaction among the layers normally aligns the magnetization of adjacent layers in opposite directions, and electrons of both spins are scattered equally. An external applied field aligns the magnetizations of

all the layers in one direction, and thus the scattering for electrons of one of the spins is reduced. As a consequence, the resistance of the sensor drops. The sensor is sensitive mostly to the field components in the plane of the sensor; any in-plane field decreases the resistance.

A slightly different effect can be achieved by using an antiferromagnet next to a pair of spaced ferromagnetic layers [6]. The antiferromagnet serves as exchange biasing for the adjacent magnetic layer, pinning its magnetization direction. The other magnetic layer is free to rotate. This leads to a linear magnetic field dependence of the resistance. The resulting device is known as a spin valve, since applying an external field effectively acts as a valve for one of the electron spins. Spin valves are sensitive not only to the magnitude but also to the direction of the field in the plane. GMR sensors and spin-valves are the most common sensor types; biosensors based on tunneling magneto-resistance [7] and giant magneto-impedance [8,9] are still very new [10].

Sensitive GMR magnetic field sensors can be combined with suitable biochemistry to selectively attach magnetic beads, resulting in a miniaturized biosensor that is suitable for detection in an array format. The sensitivity and specificity of rapid tests is usually provided by antibody–antigen affinity. In such an immunoassay, the target molecules become sandwiched between antibodies on a solid support and a label that is detected by the sensor. Conventionally this label is a fluorophore, and a plate reader is used for detection. In the most sensitive assays, the test is performed on magnetic bead carriers that can be actuated so the reaction rate is no longer limited by diffusion and the test is speeded up. Magnetic detection naturally combines actuation and detection by using the magnetic beads as both label and carrier. Besides this natural integration, magnetic labeling has several other advantages: body liquids do show autofluorescence, but are by nature hardly magnetic, which helps to improve the detection limit; the detection of magnetic particles requires no expensive optics, yet is fast and sensitive; and furthermore, it is well suited for miniaturized diagnostic sensing, due to the direct availability of electronic signals and the small size of the required instrumentation.

As labels it is desirable to use superparamagnetic beads, i.e. ferromagnetic beads so small that they quickly lose their magnetic moment in absence of an external magnetic field. Superparamagnetic beads are readily magnetized to large magnetic moments, facilitating detection, yet the mutual magnetic attraction can be switched off, preventing irreversible aggregation. As a consequence of the fast superparamagnetic relaxation, it is necessary to apply an external magnetizing field to detect the beads. It is expeditious to apply this field perpendicular to the GMR or spin valve sensor, since their thin magnetic layers are sensitive mostly to in-plane field components. With this arrangement, a large magnetizing field can be applied to the beads, while the sensor can still detect very small fields from the magnetized labels.

A realization of such a sensor was demonstrated for the first time by Baselt et al. in 1998 [1]. Using 2.8 μm diameter Dynabeads on $80 \times 5\,\mu m^2$ GMR strips, they estimated a signal-to-noise ratio corresponding to a detection limit of about one bead per strip. The concept resulted in the development of the Bead ARray Counter, or BARC, that uses DNA hybridization to detect biological warfare agents [11,12], see Fig. 1a. It contains 64 sensors, grouped as eight DNA spots with eight narrow sensor strips each.

The concept was quickly followed by a number of groups, demonstrating a variety of sensors and focusing on different aspects [13], see Fig. 1. Recently, Schotter et al. [7,14] from the University of Bielefeld developed similar sensors, but with a larger area. They realized that the quantity of primary interest for a test is the coverage of the sensors, not the distribution over the sensor strips, so it is expeditious to enlarge the sensor size to the typical size of a probe DNA spot. This resulted in sensors with 75 μm diameter spiral shape for pen spotted or ink-jetted DNA probes, as shown in Fig. 1b. They also used smaller beads, down to 0.35 μm diameter, to improve the counting statistics. In the new BARC-III system, the Naval Research group now also uses circular spots,

200 μm in diameter, consisting of GMR strips connected in series [15].

In contrast, the group of Wang et al. at Stanford focuses on the detection of a single bead, with the aim of eventually detecting a single DNA fragment [16,17]. They use small sensors, since the highest sensitivity for single bead detection is achieved when the size of the sensor matches the bead size (Fig. 1c). The sensors are of the spin valve type; the first realizations used magnetic fields in the plane of the spin valve to magnetize the beads, while at the same time biasing the spin valve to its optimal linear operating point. The signal is generated by applying an additional modulated in-plane field perpendicular to the bias field and detecting the resulting resistance modulation, either at the fundamental or harmonic frequency. In a recent analysis, they suggest that a perpendicular field configuration could be more favorable than this somewhat complicated scheme. Nevertheless it proved possible to measure single 2.8 μm Dynal beads using this setup. Simulations suggest that similar sensors would be able to detect even the 11 nm Co and other nanoparticles synthesized by Sun from IBM Watson Research Center [18,19].

Most of the measurements on the sensors discussed so far actually have been performed in a dry state. Naturally, the binding of the magnetic beads takes place in liquid, but the binding has been a separate step from the sensing, and the measurement yields only an end result. In contrast, the group of Freitas et al., in Lisbon measured the progression of magnetic marker binding in time as it takes place in the sample fluid [20,21]. Using optical microscopy, they could even correlate the electrical signal with the specific arrangement of beads on the sensor. They use a spin valve sensor (Fig. 1d), and the beads are magnetized in plane, across the sensor strip. With this arrangement, it was possible to measure single 2 μm micromer beads even without modulating the magnetic field.

Magnetizing the beads in the plane of the sensor limits the magnetizing field since the sensor should not be saturated; as the sensors are only sensitive to in-plane fields, it is thus advantageous to apply the magnetizing field perpendicular to the sensor plane, as already mentioned. However, when a bead is magnetized perpendicular to the sensor, the field around the bead is axially symmetric in the plane of the sensor, i.e. the field components in the plane point in opposite directions on either side of the bead, see Fig. 2. For the GMR type sensors this is not a concern, since such sensors are primarily sensitive to the magnitude and not to the direction of the in-plane magnetic field. However, for the spin valve sensor, the resistance changes due to opposing fields cancel out due to the sensor's linearity. In the Philips biosensor shown in Fig. 1e, this obstacle is circumvented by using not just one but a combination of two spin valve sensors, configured as a gradiometer, see Fig. 2c. The gradiometer configuration also compensates for temperature effects and drift, and it makes the system insensitive to small external magnetic fields, like that of the Earth.

Our first sensor devices consisted of eight pairs of typically 100 μm long, 3 μm wide spin valve strips, situated at the sides of a 6 μm wide gold binding area. The $20 \times 10\,\mathrm{mm}^2$ chips were mounted directly onto a printed circuit board, the electrical connections were wire bonded, and a glass flow cell was sealed with a silicone spacer ring. The gold binding area is recessed, forming a 3 μm deep trench. This enables us to measure not only the areal density of beads attached to the gold, but also the bulk density in the trench. Without the trench, a homogeneous bulk density would not give any signal, since it does not give rise to a gradient in the gradiometer. We have determined the sensitivity in this arrangement by flushing the flow cell with suspensions of beads at

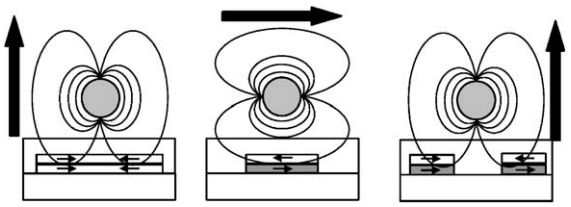

Fig. 2. Magnetic field arrangement in various detection schemes: applied field perpendicular to a GMR sensor (Naval Research/NVE; Bielefeld); in-plane with modulation across a spin valve (Stanford) or without modulation (Lisbon); perpendicular to spin valve gradiometer (Philips). Note that the figure is not to scale.

various concentrations and measuring the resulting signal. The detection limit for 300 nm Ademtech beads is presently around 10 beads in the trench. The sensitivity is limited by the Barkhausen noise of the sensors; it is expected that modulating the magnetic field will improve the detection limit significantly.

Determining the nanobead sensitivity is generally not a straightforward task, since it requires accurate information about the number of small beads on the sensor. Replacing a suspension takes time since it requires diffusion of the nanobeads away from the surface, and at the same time the beads are sedimenting, which modifies their concentration. Thus nanobead sensitivity curves are uncommon. The most extensive published results are from Schotter et al. in Bielefeld [14], who systematically measured the signal versus bead coverage for three different types of beads. The bead coverage was determined from scanning electron microscopy. From the images, it is clear that the suspensions are quite polydisperse, and the beads tend to cluster, presumably due to the drying. Nevertheless the signal is fairly linear with coverage, especially for the smallest bead size.

Even more challenging is establishing a dose–response curve for a real biochemical assay. This requires a whole series of tests, whereas there is typically only a limited number of magnetic biochips available. Therefore, publications typically describe a single test [12], or models such as biotin–streptavidin binding [20]. The only published systematic quantitative investigation that we are aware of is again from the Bielefeld group [14]. They also performed a comparison with fluorescence detection. The assay is as follows: 1 kb double stranded denatured probe DNA is spotted on the sensor surface, a fixed volume of 0.2 μL at five concentrations, ranging from 16 to 10 ng/μL. A spot of unspecific 100 ng/μL DNA is used as a reference. After covalent attachment and washing, the sensor is incubated in a solution of 10 ng/μL complementary specific DNA for 12 h. The biotinylated complementary DNA is then allowed to react with either 350 nm streptavidinated magnetic beads, or a fluorescent streptavidin marker. The format of the test is rather peculiar, in that the concentration of probe DNA on the surface is varied rather than the concentration of DNA in the incubation step. It is thus more an assay of the likelihood of DNA attachment than of concentration in a test sample. Perhaps this is the reason why the measured signal varies only by a factor of ~5 while the concentration of spotted DNA varies over three orders of magnitude; and why the results differ significantly from the corresponding fluorescence assay. Nevertheless, this test is a laudable first attempt.

The test described above relies on diffusion to bring the target molecules to the sensor. As a consequence, the detection of very low concentrations will take a long time, which is not acceptable for a rapid test. The assay can be speeded up and the sensitivity enhanced by performing the first binding step homogeneously in solution, and then actively transporting the magnetic beads to the sensing zone. Present-day laboratory immunoassays reach the sub pmol/L level in this way [2]. Such high sensitivities require careful measures to reduce non-specific adsorption. Magnetic actuation can be used advantageously to detach non-specifically adsorbed labels or replace a washing step [22]. Such a function has already been included in the first BARC reader from Naval Research. Part of the permanent magnet used to magnetize the beads can be extended by a plunger. This creates the required field gradient [28]. Magnetic actuation readily lends itself to integration, as demonstrated by an experiment using 3.5 mm diameter coils on printed circuit board at EPFL [23]. By energizing consecutive coils, beads can be transported. The beads are magnetized using a permanent magnet in this scheme. The force on the particles is proportional to the strength of the magnetic gradient, so it is advantageous to use small coils. However, when the coils also have to provide the field to magnetize the particles, heating of the wires becomes a challenge, as shown by the experiments of Lee and Westervelt [24]. They use arrays of crossed wires on sapphire to magnetize and transport beads. Such an elaborate system provides considerable flexibility. For simple transport of magnetic beads, ingenuous methods have been devised recently [25].

The devices just mentioned focus on actuation only. The first steps toward integration of actuation and detection have already been taken: the group at IMEC in Leuven and also the group in Lisbon demonstrated spin valve sensors combined with tapered current wires, as shown in Fig. 1f [26,27]. The field gradient around the current wires is used to draw the beads toward and across the sensor. The sensors of these groups are quite similar and both yield good sensitivity, 300 nm beads can be detected on a small area, but in both cases the biochemistry is still in an embryonic stage.

Looking at the sensors developed so far, two application areas emerge. On the one hand, there are very small sensors that are able to detect a single bead. Such sensors could have application in research, for single molecule detection, investigation of binding forces, pulling with magnetic tweezers, etc. On the other hand, there are sensors that focus on determining areal densities of beads rather than counting beads individually. These sensors have larger areas so that sufficient beads will bind to obtain a statistically meaningful result, i.e., not hampered by the counting of small numbers. The latter type of sensors is probably closer to application in diagnostics, e.g., as a point-of-care test device.

For both applications, it is desirable to have nanobeads with larger magnetic moments. In single bead experiments, this will enlarge the force range, and in diagnostics applications, it will permit the use of smaller particles. More beads will fit on the sensor surface, improving the statistics, and non-specific adsorption will be reduced for small beads. It is also desirable to reduce the bead polydispersity, i.e., variations in bead size and magnetic moment, so measurements of bead numbers do not have to rely on averaging [14].

Future trends will be the advancing integration of various aspects of magnetic biochip detection, and for medical diagnostics in particular. First steps in this direction have already been taken, as demonstrated by the BARC reader from Naval Research, which includes many elements, valves, pumps, and packaging, but in a still rather macroscopic system, with a large one square inch silicon die, and expensive quartz glass for the fluidic channels [28]. This is satisfactory for a demonstration or specialty application, however. Since the cartridge will have to be disposable for medical applications, it is essential to minimize cost and reduce silicon area to a minimum. Fortunately, there is a growing tendency in semiconductor manufacturing to provide a full solution rather than single silicon chips, combining various technologies to take advantage of their particular strengths. This philosophy is referred to as system-in-package (SiP) technology, for example in memory cards, or camera modules in mobile phones. Fluidics are a natural next step, and in this way biochips on silicon will progress from academic research to mass production. In line with these expectations, the detection part in the next generation of Philips biochips therefore measures only $2\,mm^2$ rather than the previous $200\,mm^2$, and is integrated in an injection moulded plastic cartridge, including electrical connections and simple fluidics.

Summarizing, we can say that magnetic detection as a biochip principle has been firmly established by now; various sensors have been constructed and the field is developing rapidly. The sensitivity of these sensors is promising, but the characterization is still in an early phase. Basic questions such as linearity and recovery of known concentrations of spiked samples still have to be addressed. Likewise, work on actuation to speed up the tests has been started. In the near future, we can expect actuation to be linked to biochemical assay formats, and systematic comparisons with existing fluorescence and chemiluminescence assays.

To our understanding, the challenge for the future is in the integration to establish a complete assay—in a cartridge, including sample pretreatment, (micro) fluidics, actuation, and suitable magnetic nanoparticles, properly stabilized, easily magnetized, with large saturation moment, monodisperse in size as well as magnetically, and with biological surface functionalization. The first results are promising—we believe that the future is bright for magnetic particle-based biochips in diagnostic tests.

We would like to express our gratitude to groups working in the magnetic biochip field for kindly sending their latest preprints and for helpful discussions.

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