Biomolecular diagnostics by a magnetic lab-on-a-chip

H. Brückl*, M. Eggeling*, R. Heer*, C. Nöhammer** and J. Schotter*

*ARC Seibersdorf research GmbH, Nano-System-Technologies
Donau-City-Strasse 1, 1220 Vienna, Austria, hubert.brueckl@acrs.ac.at
** ARC Seibersdorf research GmbH, Life Sciences, 2444 Seibersdorf, Austria

ABSTRACT

Compared to the established fluorescent labeling method, the use of magnetic markers in biochip sensors has important advantages with respect to the detection of biomolecules at low concentrations. The direct availability of an electronic signal allows the design of inexpensive integrated detection units. In addition, the magnetic beads can be used as carriers for biomolecules. They can be manipulated on-chip via currents running through specially designed line patterns on a chip platform. An obvious benefit is a much shorter incubation time of the marker binding in biochip applications. Therefore, magnetic markers in combination with magnetoresistive sensors are a promising choice for future integrated "magnetic lab-on-a-chip" systems.

Keywords: biochip, magnetoresistance, bead, sensor

1 INTRODUCTION

Magnetic micro- and nanoparticles are gaining a growing interest in the field of biology, biotechnology and medicine. While the application of magnetic beads for cell or molecule separation is well established since decades, new ideas came up in the recent years to use magnetic particles in diagnostics and therapy, too. The selective transport and specific enrichment of magnetic nanoparticles in vivo are remarkable benefits for magnetic drug targeting or hyperthermia. The latter applies a local particle heating by an external ac-field for cancer treatment. For diagnosis, nanoparticles are adopted for contrast enhancement in imaging methods, or for molecular recognition in assays or on a chip platform.

The idea of integrating standard laboratory diagnostics into easy-to-use portable devices has received growing attention both by researchers and biotechnology companies. A recent development is to combine magnetic markers and magnetoresistive (MR) sensors in a magnetic biochip [1-9]. Such systems promise a number of advantages. First of all, the MR sensors are compatible with the established semiconductor process technology and directly provide an electronic signal suitable for automated analysis. They are scaleable and can be tailored to meet any desired functionality. Furthermore, there is no disturbing background signal like in the case of fluorescent methods. Contrary to fluorescent markers, magnetic markers are stable, so that measurements can be repeated many times. By applying magnetic gradient fields, the magnetic markers can also be manipulated on-chip, which for example can be utilized to pull the analyte molecules to specific binding sites or to test the binding strength and distinguish between specifically and unspecifically bound molecules. These fields can be generated on-chip using either conducting lines [4,10-13] or static traps [14]. Furthermore, a strong magnetic gradient field can also remove the hybridized analyte DNA and ensure reusability of the biosensor.

2 MAGNETIC LAB-ON-A-CHIP

Magnetic particles and so-called beads are commercially available in a wide range of sizes, functionalities and magnetic properties. They can be used as markers and carriers for the detection and manipulation of biomolecules, e.g. DNA, on a chip platform. Like in the case of standard fluorescent DNA microarrays, the magnetoresistive biochip is based on the principle of molecular recognition between specific known DNA sequences immobilized locally on the sensor surface (so-called probe DNA) and the DNA sequences which are to be analyzed (so-called analyte DNA). The only difference between the fluorescent and the magnetoresistive biosensor are the markers (magnetic instead of fluorescent) and the method of detection. The principle and the underlying physical mechanisms are briefly illustrated and described in figure 1. The platform components, i.e. the sensors and the manipulators, are prepared by modern thin film and lithography technology on a Si wafer or glass.

2.1 On-chip detection

For detection, thin film stacks exhibiting the giant magnetoresistance (GMR) effect [15,16] are developed as sensor elements for magnetic bead detection. They consist of multilayers in the second antiferromagnetic coupling maximum ($Si/(Ni_{80}Fe_{20})_{1.6nm}/ [Cu_{1.9nm}/(Ni_{80}Fe_{20})_{1.6nm}]_{10}/ Ta_{3nm}$). The patterned sensors consist of lines with a thickness of 1 µm and a total length of about 1,8 mm which are wound into spirals with a total diameter of 70 µm and an electrical in-plane resistance at zero magnetic field of about 10 kΩ. At a saturation magnetic field of about 12 kA/m, the parallel configuration of the magnetization directions is reached, and the resistance drops by about 7,4 % relative to the high resistance state. Thus, the overall sensitivity to in-plane magnetic fields for this type of GMR-

sensor is about 0,6 % per kA/m, which is comparable to the magnitude obtained by ref [1].

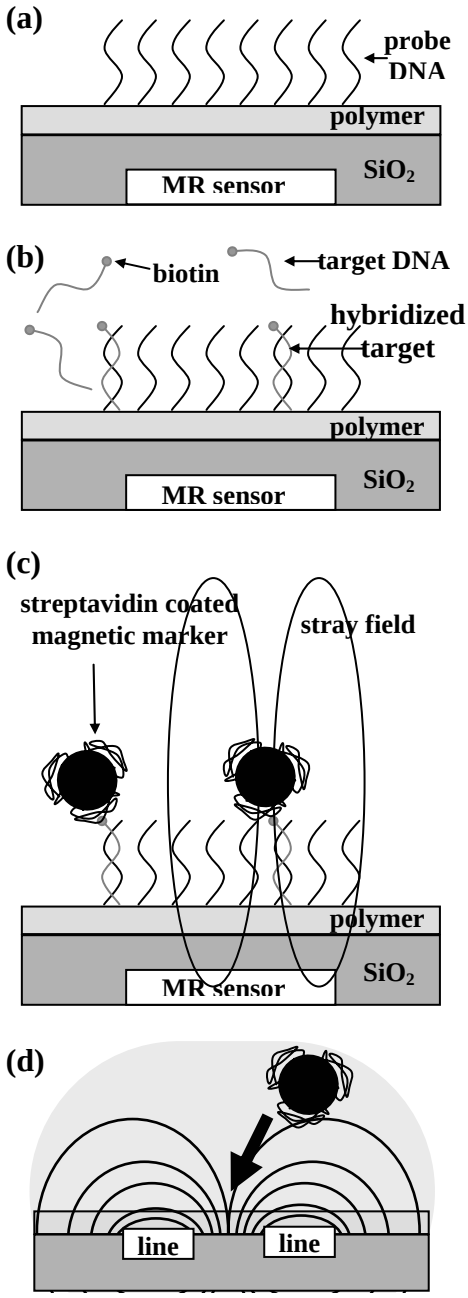

Figure 1: Principle of the magnetoresistive biosensor. (a) Immobilization of the probe DNA. (b) Hybridization of the analyte DNA. (c) Binding of the magnetic markers and detection of their stray field by the GMR-sensor. (d) Manipulation of particles by on-chip magnetic field gradients

GMR or spin-valve type sensors are state-of-the-art and best suited for MR biosensors with respect to costs and today's technology. Although the performance tunneling magnetoresistance is superior, the sensor fabrication is more expensive due to additional lithographic steps. GMR and spin-valve sensitivity is sufficient for the commercially available magnetite bead generation of smallest diameter of around 100 nm.

In principle, two distinctly different setups are thinkable: the magnetizing field for the paramagnetic beads could be applied perpendicular or parallel to the film plane of the magnetoresistive sensor. Although the in-plane configuration generates the larger signal amplitude, geometrical and practical reasons favor the situation that the magnetizing field is usually applied in the out-of-plane direction [12]. The situation which is sketched in figure 1 (c) may turn to the opposite in case of ferromagnetic particles.

2.2 Magnetic markers

Another important issue is the particles themselves. First, from the biological point of view, magnetite is proven to be biocompatible and the material of choice. Whereas Ni is known to cause allergic reactions, the usability of Fe, Co and their alloys are attractive alternatives if health hazard risks can be excluded in forthcoming approvals. The smallest available diameters of functionalized magnetite beads are around 100 nm (up to a few microns). Even if the magnetite content is large (up to 80%), the magnetization of magnetite is lower by a factor of 2-3 than for other ferromagnetic materials (e.g. cobalt or iron). In order to compensate the clear disadvantages of low moment and large external magnetizing fields, the wish and the research developments are to reach the ten or a few tens nanometer range with a high magnetic moment material, preferably ferromagnetic. Of course, one has to be aware that ferromagnetic particles have to be packed into a suitable functionalized coating in order to prevent agglomeration already in the solvent. Although such markers suitably functionalized are not yet available - at least commercially - it would be quite attractive to test the MR sensor response. For example, a magnetic force tip can be utilized to create a dipole-like magnetic stray field and to model a magnetic nanoparticle on top of a magnetoresistive sensor. It offers the great advantage that the tip can be placed at any desired site on top of the MR sensor element in a specific distance. Such experiments provide the principle evidence that the MR detection of even a single molecule by means of a magnetic nanoparticle is possible [8,12,17,18]. A detailed understanding of the sensor response can be gained by additional micromagnetic simulations [19,20].

2.3 On-chip manipulation

Further on, the manipulation and positioning are also affected if the particle size downscales. Since the force scales by the cube of the radius, its range becomes narrower and the movement slower for a constant current and the

same layout. On the other hand, the positioning of particles is as exact as the according lithographic pattern and the size of the particles [12]. The force may be improved by implementing additional magnetic layers as flux concentrators in the conducting lines. Finally however, if the sensor area decreases for low concentration measurements, it is indispensable that active manipulators accelerate the dwell time of the hybridization step. Otherwise one can easily calculate and imagine that it would take years for a single molecule/marker to find a nanoscale sensor only by diffusion.

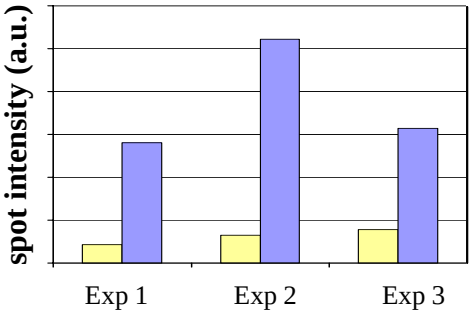

Figure 2: Fluorescent signal after 30 minutes hybridization: left bar (yellow): standard; right bar (blue): with magnetic particle motion.

Therefore as a second tool in a magnetic lab-on-a-chip, manipulators for magnetic particles are fabricated by embedding Au conducting lines into the chip (figure 1d). For this, a positive resist is spin-coated and exposed by laser lithography. Due to the flexibility of laser lithography, the patterns can be easily adjusted to the corresponding tasks [13]. After resist removal, the whole plain surface is covered by about 100 nm SiO_2 excluding the Au contact pads. The contact pads are bonded to a usual IC-socket.

As an alternative method, an acceleration of DNA hybridization can be also achieved by motioning magnetic particles via externally applied fields. Magnetic beads which are immersed in a hybridization solution, e.g. in a fluidic channel, may be moved around and cause a local whirling of the fluid. Figure 2 show the positive effect on hybridization. Two standard hybridizations were carried out in parallel: in one experiment, paramagnetic beads of 250 nm diameter were added and actively moved around. Both experiments were stopped after 30 minutes, and the hybridization degree was measured by fluorescence of the target DNA. The magnetic bead supported hybridization was more than five times as effective as the standard procedure if only diffusive motion is present. Further experiments will clarify the dependence on bead size, or concentration [21].

3 CONCLUSION

In conclusion, magnetic particles as markers in combination with magnetoresistive sensors have proven to be more sensitive than a comparable standard fluorescent DNA-detection method at low concentrations [9]. Due to the direct availability of an electronic signal and the small size of the required instrumentation, MR sensors are a promising choice for a magnetoresistive biochip. Additionally, magnetic particles provide the unique possibility to manipulate biomolecules on-chip in a defined way. Further experimental progress towards the stated key issues will help in the final integration of the two components of detection and manipulation into an integrated “magnetic lab-on-a-chip”.

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