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Short communication

GMR sensors: Magnetoresistive behaviour optimization for biological detection by means of superparamagnetic nanoparticles

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ABSTRACT

An immunomagnetic method for the selective and quantitative detection of biological species by means of a magnetoresistive biosensor and superparamagnetic particles has been optimized. In order to achieve this, a giant magnetoresistive [Co (5.10 nm)/Cu (2.47 nm)]$_{20}$ multilayer structure has been chosen as the sensitive material, showing a magnetoresistance of 3.60% at 215 Oe and a sensitivity up to 0.19 Ω/Oe between 145 Oe and 350 Oe. The outward gold surface of the sensor is biofunctionalized with a Self-Assembled Monolayer (SAM).

In addition, three different types of magnetic labels have been tested. 2 μm diameter magnetic carriers (7.68 pg ferrite/particle) have shown the best response and they have induced a shift in the magnetoresistive hysteresis loops up to 9% at 175 Oe.

1. Introduction

The idea of incorporating standard laboratory diagnosis techniques for biological agents into a hand-held, easy-to-use, portable and economical lab-on-a-chip type device has attracted the interest of biotechnological companies and the scientific community in recent years. Since then, Biological Micro-Electro-Mechanical-System (BioMEMS) technologies have been under intense development. BioMEMS are used for many applications such as medical diagnosis, drug-delivery, therapeutics and tissue engineering. For biosensing applications, these microsystems normally consist of a biologically sensitive element and a transducer which together, are able to detect the target compound in a selective and a quantitative way. The target compound can be any kind of biologically relevant species (bacteria, virus, proteins, DNA, disease markers). The sensing element is based on a physical or chemical phenomenon; in this manner several different designs have been reported depending on the method to detect the target element. The detection system can be potentiometric (Hintsche et al., 1995), mechanical (Ilic et al., 2000), amperometric (Martelet, 1998), optical (Stokes and Vo-Dinh, 2001), conductometric (Park et al., 2002), piezoelectric (Tombelli et al., 2005), or based on any other physico-chemical phenomenon. In this sense, magnetoresistive sensors have gained importance in the last decades (Baselt et al., 1998; Ejsing et al., 2005; Li et al., 2006; Vopálenský et al., 2004). Nowadays, such transducers are well-stabilized in various industrial sectors for a wide range of applications due to their many advantages. One of the most important features is that these sensors are scaleable. This property implies huge increase in their sensitivity, even to make them suitable for single molecule detection (Brzeska et al., 2004; Janssen et al., 2008). On the other hand, they can be fabricated with semiconductor processing technologies and supply an electric signal, which makes them feasible for electronic automated analysis. Furthermore, as opposed to other detection markers such as fluorescent labels, magnetic particles used for magnetoresistive sensors are highly stable and they cause almost no noise or disturbing background signal. Due to all these benefits in this article we have reported an optimized method for the detection of biological species by means of a magnetoresistive biosensor and superparamagnetic particles.

Nowadays, one of the main biomedical research topics in developed countries is the detection of biological species. The basic research in this field will lead to many direct applications in the near future, such as food borne pathogen detection, biological warfare defense or biodiagnosis. Under this scenario it is necessary to develop an easy, cheap and rapid method for the detection of these agents. One way to detect target species is to combine superparamagnetic particles with a magnetoresistive sensor. Since they were developed for magnetic bioseparation systems, magnetic labels (so-called magnetic particles or magnetic microspheres) have been used in many industrial processes. In recent years, these particles have gained importance in BioMEMS technologies due to their capability to be functionalized and to mark target species. They normally consist of a core magnetic material

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(ferrite, magnetite) embedded in a matrix, which can be made up of different kinds of materials (polymeric, ceramic). Although they have been widely used in medical and bioscientific procedures, these kinds of microbeads have to satisfy some specific requirements for biosensing applications. In this article, a sandwich-like structure has been proposed in order to detect the target pathogens. In such immunomagnetic detection method, immobilization procedures play an essential role in the detection process of the biological species due to the fact that the biofunctionalization of the sensing surface allows the capture of the target species. Thus, the outward surface of the sensor is biofunctionalized with a SAM in order to immobilize the specific antibodies, trap the pathogen between them and, due to the magnetic field that the microspheres generate, detect the antigen in further researches. Therefore, the sandwich-like structure will have the following organization: gold–antibody–antigen–antibody-magnetic particle. Once the target microorganism is sandwiched between the sensing surface and the magnetic microbeads an external magnetic field is applied to magnetize the microspheres. Superparamagnetic particles act as magnetic field concentrators and induce a change in the resistance of the multilayered magnetic material. Hence, the magnetic microbeads and, consequently, the target element can be detected.

Moreover, magnetoresistive sensors change their electrical resistance when a magnetic field is applied. Although there are different kinds of magnetic sensors, transducers based on giant magnetoresistances (GMR) have become more popular in the last years. It has been proved that multilayered magnetoresistive materials (Vovk et al., 2007) exhibit higher sensitivity at low fields than nanogranular alloys (Mujika, 2008). This fact implies that these kinds of sensors are especially suitable for the detection of biological target species by means of immunomagnetic assays due to the fact that the change induced by the particles in the sensor is very small and, therefore, can be detected easier at low fields.

In this article a giant magnetoresistive Co/Cu multilayer has been selected as a transducer. Such metals oxidize quickly even in friendly environments. In order to avoid this oxidation a silicon nitride film has been deposited as a passivation layer. Finally, a gold layer has been posited on the top of the structure due to its high affinity to make strong links with thiols and other chemicals. Different biochemical reagents are added to the gold outward surface. Thus, a Self Assembled Monolayer (SAM) is formed in order to attach the antibodies to the gold layer.

2. Materials and methods

2.1. Magnetoresistive sensor fabrication and layout

The multilayered copper/cobalt GMR sensor fabrication has been carried out by means of lithography, DC magnetron sputtering (Edwards ESM-100 for metallizations and Pfeiffer Classic 500 for multilayered magnetic stacks) and lift-off techniques. Thereby, 200 nm thick platinum contacts and $[Co\ (5.10\ nm)/Cu\ (2.47\ nm)]_{20}$ meander-like magnetoresistances are deposited onto thermally oxidized 4″ diameter 450 μm thick silicon wafers. Immediately afterwards an annealing treatment is applied to the wafer for 1 h at 300 °C in order to improve the magnetic properties of the multilayer. A 200 nm thick Si_3N_4 passivation layer has been defined by PECVD (Plasmalab 80+, Oxford) and RIE (Plasmalab 80+, Oxford) processes to protect the sensing material and prevent its oxidation. Finally, a 200 nm gold outward replica of the meanders is deposited. All magnetic measurements have been carried out by means of an electromagnet (GMW mod. 5070) connected to a programmable DC power supply (Amrel MPS 60-5).

A Self Assembled Monolayer (SAM) is formed onto the outward gold surface. For that purpose three different biochemical reagents have been used. First of all 1 mM 3-mercapto-propionic acid (3-MPA) is incubated on the gold layer for 2 h. 3-MPA is a thiolated acid that creates a strong link with the gold outward surface. Shortly afterwards 46 mM ethylene dichloride (EDC) is added for 1 h. Eventually, 46 mM N-hydroxysuccinimide (NHS) is also added for another hour. These two reagents are used in order to activate the SAM and permit the biofunctionalization of the surface. EDC bonds to the carboxyl functional group of the acid and NHS replaces EDC and acts as a good leaving group, facilitating the subsequent binding between the carboxyl group of 3-MPA and the amine group of the biofunctionalized magnetic particle. The magnetic particles used for this research were already biofunctionalized with amine groups. Amine groups do not show a high affinity to gold surfaces but if the gold surface is biofunctionalized with a carboxyl functional group a strong link is created between the gold surface and the magnetic particles. It has to be taken into account that the SAM formation and the binding of the magnetic particles onto the sensor surface is a basic preliminary step for the future use of antibodies in order to achieve a successful immunomagnetic assay.

In order to analyze the influence of the design in sensor response, several meander layouts have been designed. Spiral shaped meander designs (Schotter et al., 2004) and serpentine-like meanders inspired by BARC (Bead ARray Counter) design (Rife et al., 2003) have been developed as well as typical rectangular geometries. Spiral shaped meanders were developed in order to avoid anisotropic effects that could diminish the magnetoresistive performance of the sensor. In these meanders, the paths are 25 μm wide and the gap between paths is 100 μm. On the other hand, serpentine meander geometry was designed with the purpose of taking advantage of the outward gold circular surface. This geometry performs with a higher anisotropy compared to the spiral design geometry, but the isotropy is much higher than in other rectangular geometries in longitudinal configuration. Serpentine paths are 150 μm high, 25 μm wide, with a gap between paths of 100 μm and variable length. Eventually, various rectangular meander shapes have been manufactured with different features. While path length, height and width vary between 175–450 μm, 75–150 μm and 25–50 μm respectively, the gap between paths oscillates from 25 μm to 100 μm. These kinds of designs suffer from high anisotropy due to their geometries, but their layout makes them more suitable for future microfluidic channel designs for continuous flow processes.

2.2. Magnetic labels

Magnetic carriers have to fulfill a series of requirements for their use in biosensing applications. First of all, they have to be able to create strong links with biochemical reagents in order to bind specific antibodies onto their surface and to be capable of attaching the target species. On the other hand, this surface has to show an affinity as low as possible to the sensor outward layer to avoid unspecific bindings. Moreover, the magnetic moment per particle must be as high as possible to facilitate the detection by means of the magnetoresistive sensor. Finally, in order to have a proper reproducibility in the measurements, the microbeads have to be uniform in shape and magnetic material content, they should have a narrow size distribution and they must be biocompatible (Martins et al., 2010). All these requirements implicate that a compromise has to be found between binding capability, detection simplicity, shape and size features and biocompatibility. In this work ferrite-polystyrene superparamagnetic particles have been selected as magnetic labels.

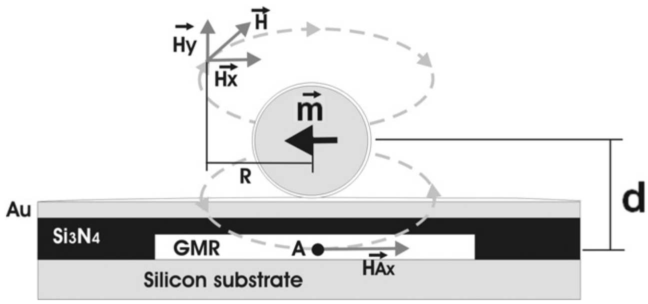

Fig. 1. Particle magnetic behaviour. Magnetic moment induced by superparamagnetic particles in the magnetoresistive stack.

Three kinds of ferrite-polystyrene superparamagnetic particles have been chosen. The diameter of these particles varies from 0.2 μm to 2 μm while the magnetic pigment content goes from 35% to 65%. All the particles were supplied by Merck-Estapor and they were biofunctionalized with amines as terminal functional group. The amount of $-NH_2$ oscillates between 15 μeq/g and 245 μeq/g. In order to understand how superparamagnetic microbeads are detected by magnetoresistive sensors, it is necessary to take into account the magnetization of the particle. As it is known, a uniformly magnetized sphere can be considered as a pure dipole at the center of the sphere under an external magnetic field. Since giant magnetoresistive sensors exhibit a variation in the resistance with in-plane components, the magnetic field is applied parallel to the sensor plane. Hence, the magnetic dipoles created by the microbeads are also located parallel to the sensor plane.

In this context, there are many factors that affect the correct detection of the superparamagnetic particles. Two of the most important ones are the magnetic content of the particles and the distance between the particles and the magnetoresistive material. On one hand, these particles must have a magnetization as high as possible when they are under a magnetic field in order to induce larger changes in the magnetoresistive response of the sensor. In this sense, the amount of magnetic material embedded in the particles plays a crucial role in the detection process. On the other hand, the distance between the sensing material and the particles should be adjusted to maximize the in-plane component of the stray field induced by the microbeads (Miller et al., 2001). Therefore, the magnetic field created by the microspheres can excite the sensitive material and, thence, provoke the highest variation in the behaviour of the sensor. All these issues are schematically presented in Fig. 1, where **d** is the sum of the half thickness of the sensitive material, the total thickness of the passivation and the gold layer and the microsphere radius, **m** is the magnetic moment and **Hx** and **Hy** are the in-plane component and the perpendicular component respectively at a distance **R** from the dipole center. Note that when **R** is 0 (**A**) there is only a planar component.

3. Results and discussion

3.1. Magnetoresistive results

Several sensor series have been manufactured and tested with the aim of improving their magnetoresistive response. The optimal thicknesses for copper/cobalt multilayers are 5.10 nm and 2.47 nm, respectively (Giron et al., 1993; Heitmann, 2004). Thus, as mentioned before, the sensing material is a multilayered $[Co\,(5.10\,nm)/Cu\,(2.47\,nm)]_{20}$ structure. The magnetoresistive values of the sensors oscillate between 1.80% and 3.58% at 215 Oe, whereas the linear measurement sensitivity varies from 0.03 Ω/Oe to 0.19 Ω/Oe in a range between 145 Oe and 350 Oe. These results are consistent with previous researches carried out by our group (Mujika et al., 2009).

The sensors have been subjected to a cyclical magnetic field in order to obtain their magnetic hysteresis loops. Rectangular meander geometries show their peak at approximately 210 Oe, but in spiral and serpentine shapes this peak is located roughly at 180 Oe. Furthermore, it has to be taken into account that rectangular meander-like geometries suffer from a higher anisotropy than the other geometries. This phenomenon can cause some changes in the magnetoresistive performance of the sensors (Schotter et al., 2004). Fig. 2 exhibits the shape of the meanders, the typical hysteresis loop for these kinds of sensors and the displacement of the peaks depending on the geometry of the meander.

3.2. Magnetic label detection

Superparamagnetism is a magnetic phenomenon that takes place in ferromagnetic and ferrimagnetic materials. When no magnetic field is applied their overall magnetic behaviour seems to be zero, if they are small enough, but when they are subjected to a magnetic field they are magnetized. This phenomenon implies that when they are magnetized, they can induce a change in the resistance of a magnetoresistive transducer. Hence, superparamagnetic carriers can be quantitatively detected by means of this type of sensors. Due to the fact that these particles are relatively new and their fabrication can be considered an emerging market, it is not easy to find commercially available particles that have the same content in ferrite and different sizes. That is why this study is focused on the influence of ferrite content in the magnetoresistive behaviour of the sensor.

All measurements involving magnetic particles were carried out with "spiral" design sensors. The particles have been subjected to a variable magnetic field with the aim of examining their associated magnetic moment and, hence, the changes induced in the magnetoresistive sensor. Results reveal that bigger ferrite content in the particle induces more remarkable changes in the magnetoresistive behaviour. Concretely, 2 μm diameter superparamagnetic microbeads (7.68 pg ferrite/particle) induce a shift in the magnetoresistive hysteresis loops up to 9% at 175 Oe, whereas with 1 μm (2.23 pg ferrite/particle) and 0.2 μm (0.02 pg ferrite/particle) diameter microspheres this value decreases to 5.1% and 3.7% at 175 Oe respectively as shown in Fig. 3. A linear relationship between particle ferrite content and magnetoresistive displacement has been found. The concentration of all particle solutions was 100 μg/mL. The highest displacements between sensors with and without microbeads occur in the linear range zone. Moreover, it has to be mentioned that this phenomenon is much more noticeable at low fields and it becomes less remarkable at higher fields.

The sensors have been fabricated following the same process, so the thickness for the silicon nitride layer and the gold outward layer is approximately the same for all the samples (200 nm each). This proves that these differences in the magnetoresistive displacements are only induced by the superparamagnetic microbeads because the distance between the sensing element and the microsphere is kept constant. 2 μm particles can create a bigger magnetic field and, therefore, the in-plane component of this field can cause a higher change in the magnetic behaviour of the sensitive material than in magnetic fields induced by smaller particles. Although ferrite content of 2 μm particles has a lower proportion (36%), as such microbeads are much bigger than the others, the total amount of ferrite in these particles is higher than in other microspheres. That is why they show the biggest shift in the magnetoresistive measurements.

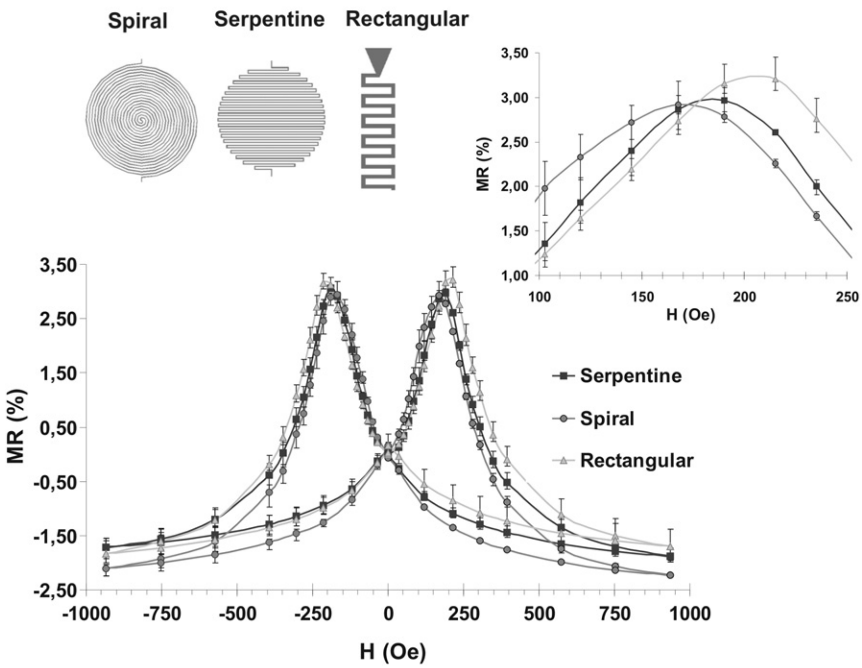

Fig. 2. Meander features and magnetoresistive behaviour. Principal meander features of spiral, serpentine and rectangular geometries. Hysteresis loops and peak displacement in different designs.

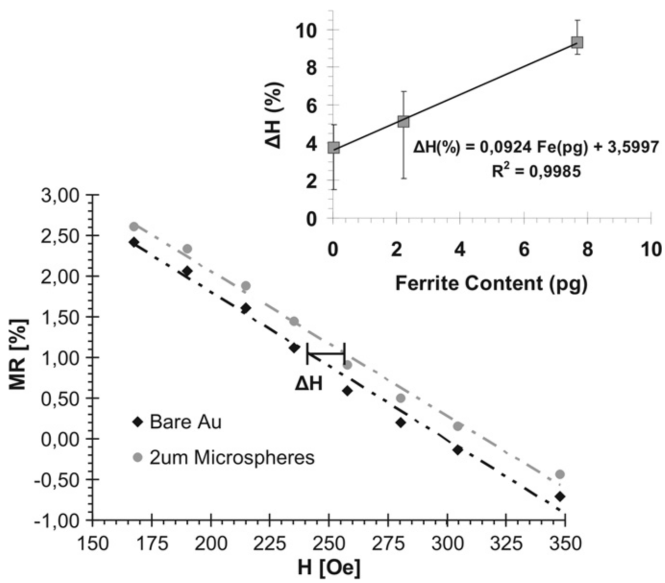

Fig. 3. Magnetoresistive displacement. Shift induced by different superparamagnetic microspheres in magnetoresistive sensors depending on ferrite content.

4. Conclusions

An optimized magnetoresistive method for the detection of biologically relevant agents has been developed. In order to achieve this objective, a multilayered thin film structure [Co (5.10 nm)/Cu (2.47 nm)]$_{20}$ has been fabricated by means of lithography, DC magnetron sputtering and lift-off techniques, PECVD and RIE processes. These transducers have shown magnetoresistance values up to 3.5% at 210 Oe and sensitivity values higher than 0.19 Ω/Oe between 145 and 350 Oe. Clear changes have been observed in the magnetic behaviour of the sensor due to the presence of the magnetic particles attached to the outward gold layer.

Geometry of the meanders changes the magnetoresistive properties of the sensors. Whereas rectangular designs show the linear range between 210 and 350 Oe, circle-like geometries (spiral, serpentine) exhibit a magnetoresistive linear range zone at lower fields (peak at 180 Oe), which is more appropriate for biosensing applications.

Results with 2 μm diameter superparamagnetic particles (7.68 pg ferrite/particle) attached to the sensor have shown the highest shift compared to bare sensors (approximately 9% at 175 Oe) while smaller microbeads induce smaller displacements. Concretely 1 μm and 0.2 μm microspheres have induced changes of 5.1% and 3.74% at 175 Oe. The ferrite content for these particles was 2.23 and 0.02 pg ferrite/particle, respectively. Therefore, 2 μm particles seem to be more suitable for the detection of target species by means of magnetoresistive sensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.02.013.

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