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Photolithographic bio-patterning of magnetic sensors for biomolecular recognition

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ABSTRACT

In the last years, magnetoresistive biosensors arrays have drawn a great interest due to their high sensitivity and integrability in lab-on-chip platforms. In such devices, the selective functionalization of the sensor active area is a major issue, in order to achieve high sensitivity and quantification capability. Here, we present a straightforward photolithographic procedure to create patterns of bio-reactive polymer regions on the sensor's surface, with micrometric resolution. The effectiveness of the procedure in providing high specificity and improved sensor performance is demonstrated in the case of magnetoresistive biosensors based on magnetic tunneling junctions (MTJs). On-chip biomolecular recognition assays demonstrate an enhanced sensitivity in selectively functionalized sensors with respect to non-patterned sensors, eventually leading to a limit of detection below the pM range, without target pre-concentration or chemical amplification.

1. Introduction

Since the first pioneering work by the Naval Research Laboratory group in 1998 [1], magnetoresistive biosensors based on the detection of biological entities labeled with magnetic beads have emerged as a promising new platform technology for biosensing. Giant magnetoresistance (GMR), tunneling magnetoresistance (TMR), anisotropic magnetoresistance (AMR) and Planar Hall Effect (PHE) sensors have been successfully applied to the detection of single magnetic particles [2–5] as well as to the focusing and detection of magnetic beads labeling target molecules in a biological sample [6]. In case of GMR devices, the detection of biomolecules with concentration down to the femtomolar [7] and zeptomolar [8] ranges has been achieved employing different techniques. The achievement of such extremely low limit of detection (LOD) essentially relies on two factors: (i) the optimization of the biochemistry connected to molecular recognition on the surface area, including the process of labeling with magnetic beads, and (ii) the optimization of the sensor sensitivity to magnetic beads.

The first issue deals with the implementation of a suitable biological assay for the target molecule (analyte) to be detected: sandwich immunoassay for proteins, direct single strand recognition for DNA or even competitive assays such as in the case of ELISA. More relevant for magnetoresistive biosensors is the detection scheme. In the so-called "post-hybridization method", initially used by the NRL group and adopted also in the present work, target biomolecules are labeled with biotin. First, molecular recognition between probes and targets takes place on the sensor surface, sometimes using a sandwich assay with the biotin attached to the second molecule sandwiching the analyte [9]. Then, magnetic beads coated with streptavidin are flushed over the sensors, allowing for a selective labeling of the sensor only where molecular recognition has taken place. In the magnetically assisted hybridization method, instead, target molecules are first labeled with beads which can then be used to magnetically concentrate the targets over the sensor area [10]. In this way lower LODs can in principle be achieved, thanks to pre-concentration, but the perturbation of the magnetic bead on the molecular recognition imposes the use of small nanoparticles. In this work, we will not deal with these biochemical aspects of the assays. Instead we will present an innovative method for patterning DNA probes only over the sensor area. This is related to the second fundamental issue for achieving a low LOD: the optimization of the sensor sensitivity to magnetic beads while keeping an almost linear response to their concentration.

In order to achieve such a high sensitivity, different aspects must be taken into account. First of all, the working point of the sensor must be carefully chosen in connection with the shape of the resistance vs. external field sensor transfer curve $R(H)$. The parameter

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to be maximized in case of lock-in detection and bead excitation by a small AC magnetic field does not coincide with the sensor sensitivity: $S_0 = (R\mu_0)^{-1}(dR/dH)$. The highest sensitivity to magnetic beads is indeed achieved by biasing the sensor in the region of its characteristics where the product between the DC bias field and the second derivative of $R(H)$ is maximum [11]. The second crucial aspect to be taken into account is the position and size of the biologically active area (i.e. the region where the biological probes are immobilized) with respect to the sensor. As it will be discussed in the following paragraphs, the average magnetic field generated by the beads over the sensor area strongly depends on their position, changing even its sign if generated by a particle inside or outside the sensor [12]. This raises issues in quantifying the immobilized beads upon biomolecular recognition, and thus in determining a straightforward relationship between the sensor signal and the analyte concentration. For this reason, controlling the beads distribution over the sensor, via selective patterning of probes, is critical for achieving a high and linear sensitivity to beads, which in turns ensures a low LOD.

Over the last decade, several methods for patterning biomolecules in micrometer size structures have been developed, such as micro contact printing [13], capillary force lithography [14], dip pen nanolithography [15] and nano-spotting [16]. A popular method for defining bio-active regions on magnetoresistive sensors is based on the selectiveness of thiol chemistry [7]. A gold layer is microfabricated and properly aligned over the sensor surface, and then selective gold functionalization is achieved thanks to spontaneous assembly of chemically reactive alkene thiols only on gold, thus leading to the formation of so called self-assembled monolayers (SAM) [17–19]. However, the formation of a well-assembled monolayer is not always straightforward, as it strongly depends on the purity of the alkanethiol being used. The presence of even low levels of contaminants can result in a disordered, non-ideal monolayer [20]. Furthermore, the additional step consisting in the microfabrication of a gold overlayer can be critical in terms of cost and compatibility with the sensor layout. In the great majority of cases, the gold layer constitutes an additional film above the insulating protecting layer which ensures biocompatibility and protects the sensor from the wet biological environment. This contributes to move the beads away from the active sensing layer, thus reducing the overall sensitivity.

Alternative strategies, not requiring the physical deposition and patterning of gold, consist in the use of photo-activated esters derived from N-hydroxysuccinimide (NHS) [21]. Patterning of aminohexyl modified DNA oligonucleotides is achieved through the selective photochemical reaction of a hydrogen-terminated silicon surface with alkenes functionalized with N-hydroxysuccinimide ester groups [22]. However, this technique is suitable only to silicon surfaces and the absence of a physical mask increases the risk of unspecific binding outside the patterned area.

In this paper, we present a novel approach to surface functionalization with micron or sub-micron resolution, which consists in the photolithographic patterning of a polymeric layer suitable for probe immobilization deposited by dip coating. The method presents relevant advantages with respect to state of the art techniques. Being optical lithography a widely accessible process, available in all microfabrication facilities, and providing sub-micron resolution, an easy scale up to mass production is possible. On the other hand, the use of a functional polymeric coatings compatible with many different substrates (e.g. SiO_2, Au, SiN, TiO_2, ...) [23] allows for a high flexibility and versatility, at variance with thiol-based chemistry which is strictly limited to gold. Finally, the use of an entirely wet process, including the polymer dip-coating and wet chemistry associated to photolithography, is fully compatible with low-cost massive production.

In our method a photoresist pattern acts as a mask for a peculiar self-adsorbent bioreactive polymer, which binds only to the areas of the chip surface not covered by the photoresist upon development in the organic solvent. In this way the subsequent DNA probe immobilization by spotting is effective only in the area coated by the polymer. The critical point of this approach is the need for organic solvents during the lithographic process which can denature probes immobilized on the polymeric coating. In case of DNA probes, however, their high stability even in organic solvents allows to easily overcome this issue. Being the polymer stable in acetone, its bioconjugation properties are not altered by the photoresist lift-off process which removes the photoresist while not affecting the DNA probe functionality for subsequent molecular recognition. In case labile probes are used, the spotting process can be performed after the lift-off procedure, so that any harsh chemical treatment on the probes is avoided. In principle this allows to extend this method to any kind of probe molecules.

The polymer used in this work is a reactive ter-copolymer obtained by radical polymerization of *N*,*N*-dimethylacrylamide (DMA), N-acryloyloxysuccinimide (NAS) and 3-(trimethoxysilyl) propyl methacrylate (MAPS), (DMA-MAPS-NAS).[24] This copolymer, introduced initially to immobilize oligonucleotides on the surface of microarray slides, suits the present application as (i) it forms a selective coating on the SiO_2 substrate by simple adsorption from an aqueous solution, not being affected by the photoresists proximity, (ii) it perfectly withstands lift-off in acetone.

The effectiveness of the proposed bio-patterning technique is demonstrated in DNA recognition experiments run in a microfluidic cell. The magnetic signal arising from molecular recognition events in bio-patterned sensors is found to be more than two times higher than in non-patterned sensors. This work demonstrates the huge potential of optolithographic bio-patterning for improving the quantification capability of magnetoresistive biosensors for molecular recognition. Besides, the very same technology for bio-patterning could be of high interest for many other biosensing approaches requiring on-chip probe immobilization over selected areas.

2. Materials and methods

2.1. Sensor and microfluidics

MTJ stacks with the structure (thicknesses in nm from now on) $Si/SiO_2(1000)/Ta(5)/Ru(18)/Ta(3)/Ir_{22}Mn_{78}(20)/Co_{60}Fe_{40}(2)/Ru(1.1)/Co_{40}Fe_{40}B_{20}(3)/MgO(2)/Co_{40}Fe_{40}B_{20}(1)/Ru(5)/Ta(5)$, were deposited by magnetron sputtering in an AJA Orion8 system with a base pressure of 2×10^{-9} Torr. CoFe and MgO layers were deposited in RF mode, while all the other layers were deposited in DC mode.

After the stack deposition, arrays of 8 MTJ sensors were fabricated using optical lithography and the same layout as in [11,25]. The junction areas were defined by ion milling in the shape of rectangles, with lateral dimensions of $2.5 \times 120\,\mu m^2$, where the shorter side is parallel to the magnetic easy axis of the pinned bottom reference layer, oriented along the *y*-axis in Fig. 1A. After e-beam evaporation of Cr(7)/Au(300) contacts, the samples were annealed at 330° C at a 10^{-6} Torr pressure for 1 h in a 400 mT magnetic field applied along the positive *y*-direction. Then, a $SiO_2(50)/Al_2O_3(80)/SiO_2(200)$ multilayer was deposited in RF mode from SiO_2 and Al_2O_3 targets to electrically insulate the sensor stack and protect it against fluids dispensed on the chip during the experiment. The $R(H)$ curve of a MTJ sensor is shown in Fig. 1B, for H applied parallel to the *y*-axis. The tunneling magnetoresistance is 50%, while the low-field sensitivity $S_0 = (R\mu_0)^{-1}(dR/dH)$ is 12%/mT in the linear region.