DETECTION AND QUANTIFICATION OF ALGINATE FUNCTIONALIZED MAGNETIC NANOPARTICLES ON A SURFACE MODIFIED MAGNETORESISTIVE BIOSENSOR

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ABSTRACT

In this paper we present a simple, versatile, portable microfluidic biosensor for the detection and quantification of biomarkers such as proteins, antibodies or anti-cancer drugs conjugated with iron oxide (Fe_3O_4) magnetic nanoparticles (MNs) and suspended into a static fluid. The designed system utilizes Giant Magnetoresistance (GMR) – spin valve sensors and microconductors for the manipulation of the nanoparticles, integrated on a microfluidic chip with a modified surface for preventing biofouling. In this study we used MNs encapsulated into a polymeric matrix of Alginate, which are perfect candidates for applications in biomedicine and for being an important carrier for different biological agents.

KEYWORDS: Biosensor, Magnetic Nanoparticles, Alginate, GMR

INTRODUCTION

Magnetic nanoparticles play an increasingly important role in biotechnology due to the vast possibilities for use in medical applications that range from magnetic separation and contrast enhancement for imaging to diagnostics and quantification platforms for genomics and proteomics [1-3]. Among the different functionalizations of magnetic nanoparticles, lately, alginate encapsulated iron oxide nanoparticles draw attention due to their biocompatibility, the straightforward gelation with divalent cations [4] and for being an important carrier for different biological agents [5].

In this paper we present a microfluidic chip (figure 1), with integrated magnetic field inducing microconductors for the manipulation and magnetization of the MNs, integrated GMR sensors for the detection of the MNs and a modified surface to prevent biofouling, non-specific binding and enabling the possibility for multiple uses of the chip. All the operations on chip are held in a static fluid and the channels are filled with capillary forces alone restraining the need for pumps or other integrated microfluidic components. This in combination with a signal generation and acquisition system realized on a laptop's soundcard proclaims the use of the proposed system as a point of care, portable, biosensor.

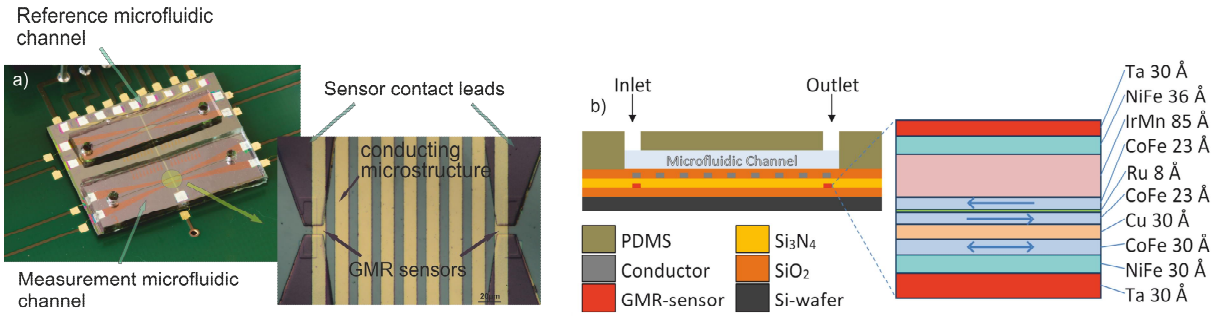

Figure 1: (a)Photograph of the proposed microfluidic chip mounted on a PCB board. In the accompanied microscope image the GMR sensors with their connecting leads and the micro conductors' area are shown. (b) Cross section of the chip with a detailed GMR, spin valve sensor element.

EXPERIMENTAL

The biosensor's operation is realized in two steps; once the sample is loaded a pre-concentration step is essential as it significantly lowers the LOD (Limit Of Detection) of the biosensor and the amounts of reagent needed thus improving the overall performance. Sequentially switching ON and OFF a current through microconductors (MCs) induces a "moving" magnetic field gradient, which attracts the MNs from one MC and moves them to the next (figure 2). At the end of the sequential actuation all the MNs – previously scattered over the area of the MCs - are concentrated on one conductor, which is the last MC to be turned ON.

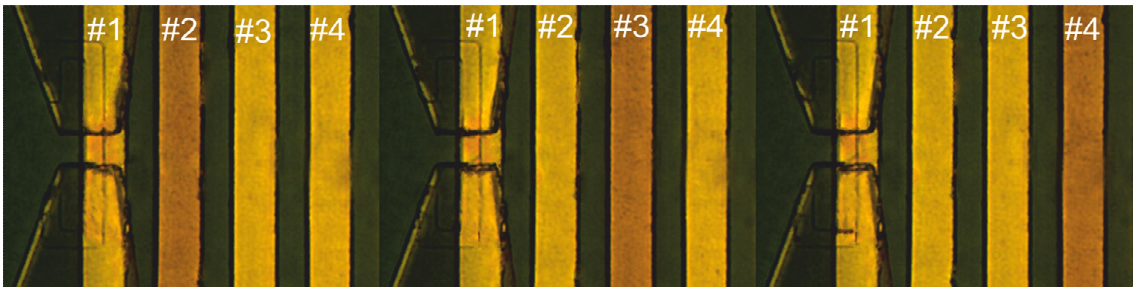

Figure 2: Sequential actuations of the MCs and manipulation of the MNs as a pre-concentration step. MNs are attracted to the current currying MCs and are finally concentrated on MC #2.

Then the detection itself takes place; the last MC (#2) that is actuated during the pre-concentration step is situated a few micrometers away from a magnetic field sensor. Once the MNs are concentrated on it, the MC right above the sensor (MC #1, figure 2) is actuated with an AC current. This AC current both induces a magnetization to the paramagnetic iron oxide particles and attracts them to the vicinity of the sensor which then detects their stray fields. The time the particles need to accumulate on the sensor's surface and the magnitude of the sensor's output determine their concentration. Furthermore, the time required until the saturation of the sensor's signal can be used to define the size difference between the conjugated (with a biomolecule) MNs and plain MNs that are measured in a reference channel (figure 1). That is because conjugated MNs exhibit greater hydrodynamic friction (Stoke's drag force) with respect to the plain MNs and/or greater friction force when moving in contact with the chip's surface as in the proposed system [6].

The MNs used in this study are magnetite nanoparticles synthesized by co-precipitation of ferric and ferrous hydroxide in alkaline conditions. In order to be coated with alginate, the MNs were dispersed in an alginate solution, resulting in spherical MNs with an average size of 10 nm $\pm$ 2.5 nm. More details about the studied MNs and their magnetic characterization are reported by Devkota et al. [5].

Alginate exhibits adhesion to commonly used passivation materials i.e SiO_2 and Si_3N_4. Thus, a surface modification of the biosensor was necessary. Since alginate is a hydrogel, in which the magnetic iron oxide core of the particles has been engulfed, it repels hydrophobic surfaces. For that reason we developed such a hydrophobic surface modification by immobilizing a monolayer of perfluorinated alkoxysilane [7] on the SiO_2 surface of the biosensor. The monolayer was evaluated with a spectral reflectometer and it was found to be 2.17 nm thick, marginally influencing the sensor's performance.

RESULTS AND DISCUSSION

The exact acquisition scheme and experimental set-up have been described in detail elsewhere [8][9]. Three different concentrations of alginate encapsulated iron oxide nanoparticles suspended in deionized water were prepared, injected into the microfluidic channel and detected using the aforementioned procedure; when the MC on top of the sensor was turned ON, the MNs, previously concentrated on the MC #2 (fig. 2), started moving towards the sensor changing its voltage output. The change of the sensor's output is reported in figure 3, where the response of the sensor to the motion of the MNs has been plotted

over time for the three concentrations. Each time step corresponds to 120 msec. The curves were fitted to the $y = ax^b + c$ equation using an itinerary process in Matlab®. The initial value of the sensor's output is the same for all three concentrations and is due to the magnetic field of the MC #1. The final value and thus the $\Delta V = V_{fin} - V_{init}$ follows a linear increase with goodness of fitting $R^2 = 0.95$ (figure 3, inset).

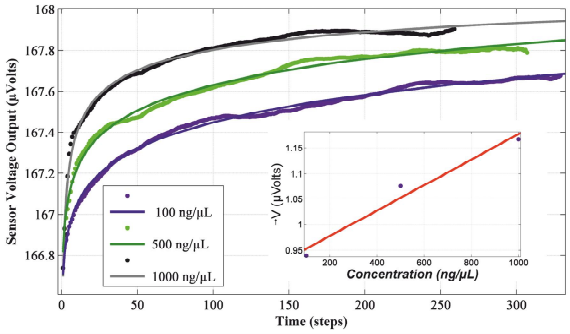

Figure 3. Change of the sensor's output voltage with the accumulation of the MNs on its surface versus time. Inset shows the linear ascent of ΔV with concentration.

CONCLUSION

The linearity of the GMR sensor's ΔV over concentration supports the argument that the proposed biosensor can be used for the detection and quantification of alginate functionalized MNs. Further experiments are carried out in order to define the sensor's range and LOD.

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