Investigation of Microfluidic Integration in Magneto-Nanosensor Based Protein Biomarker Detection

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Abstract:

The use of magnetic nanoparticles in conjunction with a spin-valve type giant magnetoresistive (GMR) sensor provides a method for the detection and quantification of picomolar concentrations of proteins in biological samples. The magnetic nanoparticles act as a magnetic tag for proteins which allow the quantification of targeted proteins. Recent developments in the lab's custom microfluidic integration approach with the spin valve sensor array allow for simultaneous testing for multiple samples on a single chip and a variety of proteins within a given sample. In this work, testing is being conducted to study the distribution of magnetic nanoparticles following the completion of microfluidic assay experimental procedures. Specifically, the final locations and local densities of the magnetic nanoparticles and the presence of washing buffer fluid following the assay are being compared to the geometry of the microfluidic channels. The comparison of these patterns may lead to improvements in the efficiency and reproducibility of the bioassays.

An SEM was used as the primary source of images in this study. Some potential applications for this technology include multiplexing of surface interface sensors within automated point-of-care diagnostic devices.

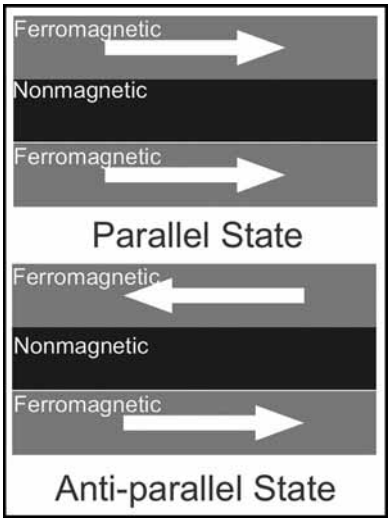

Figure 1: View of the magnetic parallel and antiparallel states.

Experimental Procedures:

Lab-on-a-Chip Devices. Lab-on-a-chip tools are self-contained devices that can run immunoassays on protein samples. These devices are useful for many reasons. One major benefit is their portability, which permits their use in remote locations that would otherwise require the transportation of samples for diagnostic tests. Another major point is that with small devices, very little material is required during a single test, leading to both lower material costs and the generation of less waste. Finally, there is often minimal training required for operators of such tools, as they are primarily self-contained, and highly automated.

Giant Magneto Resistance Sensors (GMR). GMR is a large change in resistance in certain materials and structures upon the application of a magnetic field. With the example of our sensors as pictured in Figure 1, the bottom ferromagnetic layer has a fixed magnetic field, while the field in the top ferromagnetic layer is switched between the parallel and anti-parallel states in order to induce a change in the magnetic field. The observed change in resistance is due to more electrons being scattered when they pass through the material in the anti-parallel state as opposed to the parallel state.

When magnetic nanoparticles, which act as tags for proteins, are added to the top of the GMR, the strength of the magnetic field is affected. This leads to a change in the magnetoresistance which is measured and normalized, resulting in the GMR acting as a sensor to analyze the sample composition.

Bioassays. Bioassays can provide information on the concentration of targeted proteins in samples. In these experiments, BSA was used as a negative control that produced no change in the magnetic resistance, biotin was the positive control that produced a very high signal, and test proteins were applied to sensors in a range of concentrations, resulting in a signal bands located between the controls.

Standard Well Assays. Standard well assays were performed by applying reagents in a preset order to a chip, and adding magnetic nanoparticles while the chip was located in the GMR sensor. These reagents included proteins and matching antibodies, magnetic nanoparticles, biotin, and BSA. Data from bioassays can be plotted in the format of change in

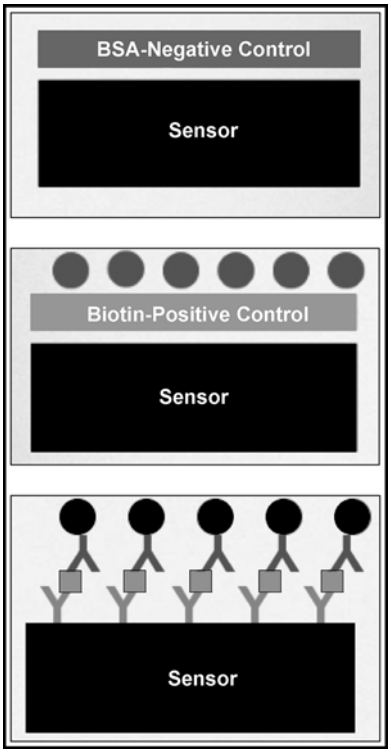

Figure 2: Negative control, positive control, and test biosensors with reagents attached.

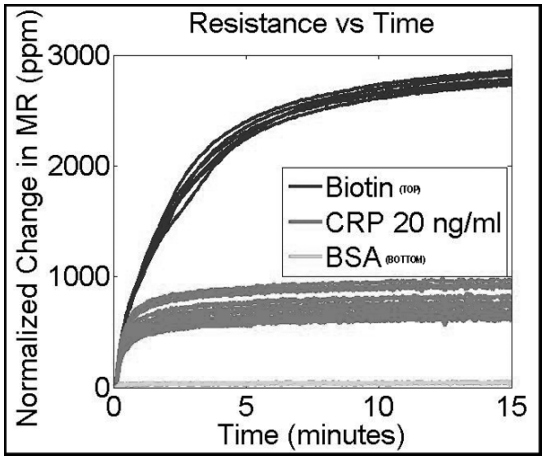

Figure 3: Data plot of corrected resistance versus time from standard well bioassay.

magnetic resistance versus time. In Figure 2, the test protein was C-reactive protein, which is a common protein released as a part of the complement system in the inflammatory response. Throughout the summer, C-reactive protein and FLT3LG were both used as test proteins in various concentrations.

Following the bioassay, the results were recorded in the form of corrected change in magneto-resistance versus time. An example of this data can be seen in Figure 3.

Microfluidic Assays. When used in conjunction with GMR sensors, microfluidics could improve both the reliability and efficiency of bioassays. Some reasons for this are that the use of microfluidics can automate much of the bioassay procedure, it directs reagent material to the sensors, and it can provide separate channels, allowing for concurrent testing of multiple samples. The reliability of microfluidic testing depends on factors including the physical structure of the channels, the surface chemistry of the sensor, and the alignment of the chips.

Conclusions:

Multiple proteins were detected at picomolar concentrations through the use of GMR sensors and magnetic nanoparticles. Microfluidic channels were found to be an effective means of directing reagents along the surface of sensor chips. No cross-contamination was witnessed between the channels, meaning that a variety of tests could be run simultaneously on a single chip.

Future Work:

This research will be continued by the addition of tests involving new proteins, as well as experiments with an expanded range of concentrations, in order to better evaluate the sensitivity of the tool. More research and development will be done to increase the portability of the total diagnostic device, thus increasing its functionality as a Lab-on-a-Chip.

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References:

[1] Gaster, R., et al.; "Matrix-insensitive protein assays push the limits of biosensors in medicine"; Nature Medicine, 15, 1327-1332 (2009).

[2] Gaster, R., et al.; "nanoLAB: An ultraportable, handheld diagnostic laboratory for global health"; Lab Chip, 11, 950-956 (2011).

[3] Osterfield, S., et al.; "Multiplex protein assays based on realtime magnetic nanotag sensing"; PNAS, 105, 20637-20640 (2008).