Early Liver Cancer Diagnosis Using Magnetoresistive Biosensors

By Andy Tay

Liver cancer is a leading cause of deaths worldwide. It was ranked third in terms of cancer-related deaths with a toll of 830,000 according to the World Health Organization. The most common type of liver cancer is hepatocellular carcinoma (HCC) which makes up 80% of total cases, mostly in resource-scarce settings. Unfortunately, there are no obvious symptoms in early-stage HCC so an urgent need exists to develop inexpensive methods to detect and diagnose for HCC early to improve prognosis and survival rates in developing nations.

There have been multiple publications demonstrating point-of-care tests using biomarkers like elevated levels of alpha-fetoprotein (AFP) and C-reactive protein (CRP), but these studies often provide lower sensitivity and poorer multiplexing capability than standardized lab assays.

In a recent publication authored by Dr. Chengyang Yao and colleague in the lab of Professor Shan Xiang Wang in the Department of Electrical Engineering at Stanford University, the team developed a biosensor to detect for HCC using a panel of clinically-validated biomarkers which is disposable, automated, portable, fast (requiring only 28 mins for results) and sensitive (Yao et al., 2022).

Giant magnetoresistive effect for better biosensor performance

Enzyme-linked immunosorbent assay (ELISA) is the gold-standard for many analytical detection tests. However, giant magnetoresistive (GMR) effect has been previously found to provide lower limit of detection, expand dynamic range for detection and enable multiplexing of biomarkers with negligible cross-reactivity.

GMR refers to a larger or 'giant' change in resistivity in the magnetic field when magnetizations in a sensor material are changed from anti-parallel to parallel orientations. GMR sensors use the principle of localized proximity sensing. When a magnetically labelled biomolecule binds to an immunoassay structure on a GMR sensor surface, a localized magnetic field is generated and that changes the sensor resistance for detection.

In the case of Yao's sensor, the team functionalized their GMR sensor surface with capture antibody for the antigens of choice, namely AFP and CRP. The antigens then bind to biotinylated detection antibodies, forming a sandwich immunoassay (**Fig. 1**). Later, streptavidin-conjugated magnetic nanoparticles are added. Streptavidin and biotin bind specifically, leading to the indirect binding of the magnetic nanoparticles. This generates a local magnetic field, causing a change in electrical resistance of the sensor in real time. The change in resistance is also correlated to the concentration of antigens.

"The GMR sensors are extremely sensitive biosensors that can be used to detect both proteins and nucleic acid biomarkers. They are compatible with most sample matrices and can detect multiple targets simultaneously. Utilizing standard, micro-electro-mechanical (MEMs) fabricating technologies, these biosensors are easily scalable, and can be made at an affordable cost," says Yao.

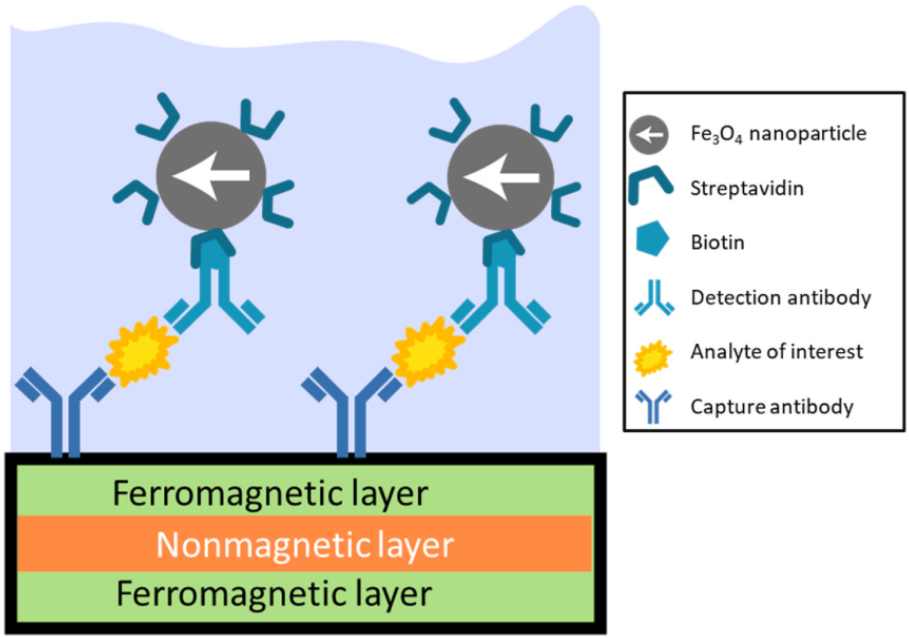

Fig. 1 *Mechanisms of GMR biosensor. Indirect binding of magnetic iron nanoparticles through antibodies and antigens triggers changes in local magnetic fields, leading to electrical resistance change in GMR biosensor in real time and correlated to amount of target antigens. Image credit: Chengyang Yao, Elaine Ng, Shan X. Wang, An automated and mobile magnetoresistive biosensor system for early hepatocellular carcinoma diagnosis, Biosensors and Bioelectronics, Volume 202, 2022, 113982, ISSN 0956-5663, https://doi.org/10.1016/j.bios.2022.113982*

An automated system

After working on the GMR sensor, Yao and colleagues further enhanced the translational potential of their work by adding an automation functionality. The authors decided to use DC-powered peristaltic micropumps as they have lower power consumption than piezoelectric diaphragm micropumps. By reversing the voltage (+3V to -3V) repeatedly, the flow direction can also be reversed, making reagent mixing, flushing, and cleaning of the system easier. Although DC-powered peristaltic micropumps slow the flow rate, which can lengthen the washing steps in their detection process, the authors argue the increased time is acceptable clinically as most assays do not require a very fast wash. Importantly, the authors also found fluid can be trapped inside piezoelectric diaphragm micropumps. This is not ideal as it can lead to contamination, gradual corrosion, and clogging of pumps overtime.

The assay can be run in a user-friendly manner. The user only needs to add the sample into a reaction well and press the start button on a smartphone app. The phone then communicates to the micro-controller unit in the biosensor analyzer to perform various steps like mixing, washing, and detecting according to predetermined action sequences. The authors also custom-made an electromagnet to drive magnetic signals to excite the GMR biosensor.

Biosensor performance

To test the performance of their biosensor, the authors spiked different amounts of AFP and CRP into water and found that their sensor is sensitive enough to measure clinically relevant levels of biomarkers, i.e., 20 ng/mL of AFP and 1000 ng/mL of CRP. The dynamic ranges for both biomarkers also cover three orders of magnitude, comparable to gold-standard ELISA. Importantly, the

standards curves showed excellent linearity, low background noise and low signal standard deviations to improve accuracy and precision of readings.

“The HCC detection was a successful demonstration of the automated GMR biosensing platform. We are including more biomarkers to the panel and have developed a machine learning algorithm to increase the diagnosing power. This is a collaborative work with Dr. Mindie Nguyen from Stanford Medical school, who provides clinical samples, medical insights, and guidance,” adds Yao.

“Additionally, we have expanded the sensing technology to detect DNA targets and are developing an automated way to perform on-chip polymerase chain reaction and GMR detection. With the unparalleled multiplexing capabilities, multiple DNA targets are being detected at once, from the same sample.”

GMR sensing has great potential to transform healthcare diagnosis, especially for point-of-care testing where there is a shortage of professional staff and expensive infrastructure. By incorporating automation into their biosensor, Yao and co-workers have demonstrated an excellent way that innovations in lab can be useful to reduce healthcare inequality. It can be used to detect diseases beyond HCC, but also other metabolic and brain diseases with established clinical biomarkers in biofluids.

Source article

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