Magnetic nanotags spot cancer in mice earlier than methods now in clinical use

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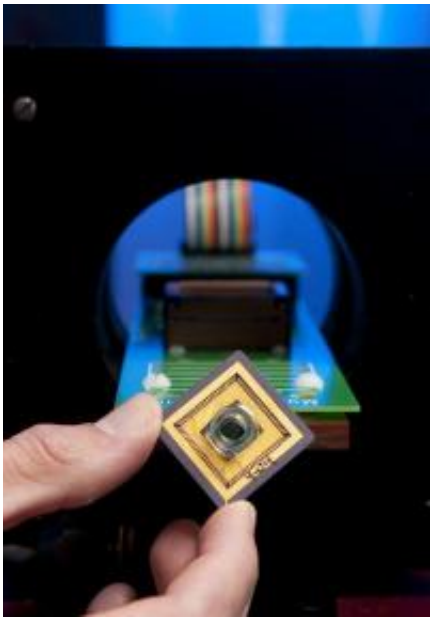

The magnetonanosensor has 64 sensors capable of detecting up to 64 different proteins. In the center of the chip is the well that holds the fluid of interest. The reader that measures the magnetic fields of the sensors is in the background. Thumb and fingers are courtesy of Richard Gaster, M.D./Ph.D. candidate in both bioengineering and the school of medicine. Credit: Linda Cicero, Stanford News Service

Searching for biomarkers that can warn of diseases such as cancer while they are still in their earliest stage is likely to become far easier thanks to an innovative biosensor chip developed by Stanford University researchers.

The sensor is up to 1,000 times more sensitive than any technology now in clinical use, is accurate regardless of which bodily fluid is being analyzed and can detect biomarker proteins over a range of concentrations three times broader than any existing method, the researchers say.

The nanosensor chip also can search for up to 64 different proteins simultaneously and has been shown to be effective in early detection of tumors in mice, suggesting that it may open the door to significantly earlier detection of even the most elusive cancers in humans. The sensor also can be used to detect markers of diseases other than cancer.

"In the early stage [of a cancer], the protein biomarker level in blood is very, very low, so you need ultra-sensitive technology to detect it," said Shan Wang, professor of materials science and engineering and of electrical engineering, and senior author of a paper describing the sensor, which was published online on Nature Medicine's website on Oct. 11. "If you can detect it early, you can have early intervention and you have a much better chance to cure that person."

Wang said the nanosensor technology also could allow doctors to rapidly determine whether a patient is responding to a particular course of chemotherapy. "We can know on day two or day three of treatment whether it is working or not, instead of a month or two later," he said.

The sensor Wang and his colleagues have created, which uses magnetic detection nanotechnology they had developed previously, can detect a given cancer-associated protein biomarker at a concentration as low as one part out of a hundred billion (or 30 molecules in a cubic millimeter of blood).

Although the basics of the magnetic detection technology used in the new biosensor were described last year in a paper in the Proceedings of

the National Academy of Sciences, the new sensor is not only more sensitive than the previous one by several orders of magnitude, it also outperforms its predecessor - and detection methods now in use - in several other ways.

Early detection of tumors in mice

The most impressive performance gain detailed in the *Nature Medicine* paper is that the researchers have now demonstrated that the magneto-nano sensor can successfully detect cancerous tumors in mice when levels of cancer-associated proteins are still well below concentrations detectable using the current standard methodology, known by the acronym ELISA.

"That is a critical finding for us because it says that in a realistic biological application - that of tumor growth in mice - we can actually see tumors before anything else could have detected them," said Sam Gambhir, professor of radiology at Stanford.

"I would say that the PNAS paper is proof of concept of the technology, and the *Nature Medicine* paper is proof of concept of the technology working in a real-world application," he said. "It is one thing to have the technology show that it can work in principle; it is quite another to actually utilize it with real mouse blood samples from a real mouse growing a real tumor."

In the *Nature Medicine* paper, the researchers show that the new magneto-nano sensor has a broad range of sensitivity, from the minute quantity described earlier to concentrations six orders of magnitude, or a million times, greater. The best existing analysis methods, or assays, in clinical use are able to detect proteins over a range of concentrations of at most two orders of magnitude.

Most of the sensing platforms currently in use are also limited to performing a single analysis at a time, but because the magneto-nano sensors are attached to a microchip in an array of 64 sensors, each of which can be set up to detect a different protein, the researchers can search for up to 64 different proteins simultaneously during a single analysis, which typically takes one to two hours - far less than most existing assays.

The researchers also demonstrated that the sensor is equally effective in every likely biological fluid, or matrix, that a doctor would want to analyze for cancer-associated proteins. Those fluids include urine, saliva, blood plasma (blood with the blood cells removed), serum (blood plasma with the factors that promote clotting removed) and cell lysates (the name applied to the cellular stew produced by dissolution of cells).

"The idea that you could essentially, on a single assay platform, measure a broad diversity of biomolecules that are at such a wide range of concentrations with such sensitivity is really, truly remarkable," said Charles Drescher, a professor of obstetrics and gynecology at the University of Washington in Seattle, who was not involved with the research. "I think we'll all be very excited if this really does pan out."

The key to the versatility of the magneto-nano sensor and the broad range of concentrations it can detect lies in the use of magnetism.

How magnetic nanotags reveal the quarry

The basic mechanism of detection employed in the magneto-nano sensors is to capture antigens - deleterious compounds produced and shed by the cancer cells - using antibodies that naturally tend to bond with the antigens. The antibodies, dubbed "capture antibodies," are applied to a sensor, so that when the matrix of interest is placed onto the sensor chip, the appropriate antigens bind.

While the antigens are held fast, another dollop of the antibodies is applied. These antibodies are attracted to the antigens held on the sensors, and in bonding with them effectively seal the antigens inside an antibody sandwich. The researchers then apply a wash containing magnetic nanoparticle tags that have been tailored to fit specific antibodies. The magnetic nanotags attach themselves to the outer antibody on the sandwich, where they alter the ambient magnetic field in a small but distinct and detectable way that is sensed by the detector.

The protein-detection assays that are currently in use rely on a variety of mechanisms, such as measuring electrical charge, fluorescent signals or pH, all of which are prone to interference from the biological matrix in which the desired proteins reside. While a particular assay may be fine for assessing a protein's concentration in urine, for example, it may perform poorly when applied to a blood sample, as differences in the composition of the matrix affect properties such as pH or electrical charge.

"Our sensors are shown to be rather insensitive to matrix, so that is another key element from a scientific point of view," said Wang. As an example, he said, "We know that in saliva and blood, they have totally different pH values and different chemistry, but they are all nonmagnetic. Magnetically they are just like air. So it does not interfere with our mechanism [of detection]."

Most of the assays currently in use are only able to detect proteins over a narrow range of concentrations before interference of some kind degrades the sensitivity of the assay. That can require a series of assays to be performed on a sample diluted to different strengths, in order to assemble a complete picture of a protein's concentration in the matrix. But again, by using magnetic detection, Wang and his colleagues are able to sidestep such signal degradation.

"With the high sensitivity and the broad range we can look at a big panel of proteins over a wide range of concentrations, and with the matrix insensitivity, we can look at them in different fluids," said Richard Gaster, MD/PhD candidate in bioengineering and medicine, and first author on the *Nature Medicine* paper. "We don't have to tailor where we are looking; we can look at everything simultaneously." That produces savings in time, which, once the sensor comes into commercial use, will also translate into monetary savings.

Another virtue of the technology, Wang said, is that it uses existing technology already in use in the data storage and semiconductor industries and because of that, he added, "It can be made relatively cheaply."

"It is the same sensor you are using in a hard disk drive to read a hard disk back," he said. "Very similar to that."

One of the next steps in the research, Wang said, is to test the magneto-nano sensors on human blood samples taken from a long-term study in which researchers drew blood samples from subjects prior to any of them being diagnosed with cancer. To this end, the Stanford team will be collaborating with the Fred Hutchison Cancer Research Center in Seattle and the Canary Foundation, a nonprofit organization that focuses on early diagnosis of cancer.

"We can actually use our technology to study all these samples and we may be able to tell a year before or half a year before or three months before the diagnosis," Wang said. "That work will be extremely interesting."

Source: Stanford University (news : web)