NanoBiotech News

The global nanobiotechnology intelligence source

Volume 4 • Number 6 • February 8, 2006

Carbon nanotube-derived device detects gene mutations without labeling

By Russell A. Jackson

University of Pittsburgh researcher Alexander Star, PhD, and colleagues at Emeryville, CA-based Nanomix Inc. have developed a carbon nanotube-based device that can detect gene mutations without use of more expensive and time-consuming fluorescent labeling. Once commercialized, the discovery could have a near-term market opportunity value of more than $2.5 billion.

In the research, the detection devices were fabricated using carbon nanotubes "grown by means of a chemical vapor deposition technique on four-inch silicon wafers," Star tells *NanoBiotech News*. "Each device was made of a random network of carbon nanotubes between interdigitated gold electrodes with 10-micron separation."

Specifically, an abstract of Star's published work[1] points out, the devices "were fabricated by using single-walled carbon nanotubes grown at 900 degrees C using dispersed iron nanoparticles as growth promoter and a methane/hydrogen gas mixture on doped Si 100-mm wafers with SiO2 at its surface." Electrical leads, the abstract continues, "were patterned on top of the nanotubes from evaporated Ti-Au films by using standard photolithography techniques."

Each wafer consisted of about 1,000 dies with 2.54 nm x 2.54 mm dimensions. On each die, a random network of SWNTs is patterned into several devices that consist of the interdigitated electrodes. Nanotubes outside the device area were removed with oxygen plasma to electrically isolate each device. Electronic characterization of the nanotube network field-effect transistor devices, such as current flow between source and drain electrodes as a function of applied gate voltage and bias voltage, were conducted by using automated test equipment similar to semiconductor industry set-ups.

Carbon nanotubes, Star says, are basically "rolled-up sheets of graphite only a few nanometers wide -- about the width of a molecule of DNA." The Nanomix team used the nanotubes' electrical properties to find a particular mutation in the gene that causes hereditary hemochromatosis, a disease in which too much iron accumulates in body tissues. "The size compatibility between the detector and the detected species -- DNA molecules in this case -- makes the approach very attractive for further development of label-free electronic methods," he notes.

Fluorescent labeling is eliminated

And that, he points out, "eliminates the necessity of DNA labeling with fluorescent labels -- including the cost of the reagents and of people's time -- and of state-of-the-art fluorescent microscopy." Star adds that the technology "can bring to market hand-held, field-ready devices for genetic screening, as opposed to laboratory methods using labor-intensive labeling and sophisticated optical equipment."

In the research, Star used the fact that carbon nanotubes integrated into a silicon chip "have a characteristic electrical conductance that can be measured by applying a voltage, thus providing a signal for a carbon nanotube-based field-effect transistor device -- our DNA sensor transducer." In particular, he notes, the conductivity of the carbon nanotubes as a function of applied gate voltage was measured.

The hybridization of the DNA molecules -- DNA duplex formation -- on the surfaces of the carbon nanotubes can change their electrical conductance. "In the case of a genetic mutation," he says, "DNA hybridization does not occur under our testing conditions, and the change in the carbon nanotubes' electrical conductance is insignificant compared to normal DNA molecules, which form DNA duplexes."

Because DNA molecules have similar diameter to carbon nanotubes -- about 2 nm -- he continues, "the presence of a DNA molecule and consequent hybridization on the side walls of the carbon nanotubes can readily affect their conductivity."

Star and his colleagues at Nanomix also tested fluorescently labeled DNA molecules to confirm that DNA had attached to the nanotube surfaces and was subsequently hybridized, or matched to its complementary DNA.

"For DNA hybridization experiments -- so-called 'mutation identification' -- we first attached unlabeled DNA molecules to carbon nanotubes and then added complementary DNA molecules that were fluorescently labeled," he explains. "When the hybridization took place, we observed fluorescence in the microscope. In the case of mutated DNA molecules, the hybridization did not take place, which can be observed independently by fluorescent microscopy, a 'conventional technique,' and by measuring carbon nanotube conductivity, which is our new detection method."

"Complementary DNA" is a DNA molecule that has a nucleotide base sequence that is complementary to a probe DNA sequence, which the researchers attached to carbon nanotubes first. "Complementary" means that two nucleotide bases form a strong bond with each other. If DNA molecules consist of complementary bases, they can form a duplex; that is, hybridization occurs.

"In the control experiments, we used fluorescently labeled DNA molecules," Star continues. "When you shine a light on them, they emit light of a characteristic color back. The emitted light can be isolated using filters and is easily visible under an optical microscope. Using fluorescence microscopy, we were able to confirm that DNA molecules actually bind to the side walls of carbon nanotubes."

As the abstract puts it: "The development of nucleic acids diagnostics has become the subject of intense research, especially in the post-genome era. Current methods have mainly focused on optical detection using fluorescence-labeled oligonucleotides with dyes, quantum dots or enhanced absorption of light by oligonucleotide-modified gold nanoparticles. On the other hand, label-free electronic methods promise to offer sensitivity, selectivity, and low cost for the detection of DNA hybridization."

Advantages for DNA detection

The abstract points out: "Using smaller nanowires with virtually all atoms on their surface, such as single-walled carbon nanotubes, will provide additional advantages in DNA detection. To date, there are several reports on electrochemical detection of DNA hybridization using multi-walled carbon nanotube electrodes. Whereas electrochemical methods rely on electrochemical behavior of the labels, measurement of direct electron transfer between SWNTs and DNA molecules paves the way for label-free DNA detection. SWNT-based field-effect transistors have excellent operating characteristics and they have already been explored for highly sensitive electronic detection of gases and biomolecules such as antibodies."

That flexibility, of course, is what has Nanomix executives excited about the prospects for the discovery. "We have found that electrical measurement of carbon nanotube devices produces sensor results that are comparable to state-of-the-art optical techniques," Star says. "The applications of our method for detection of other, more serious genetic diseases can be seen."

Commercial applications are already in the works. "Our internal development process is ongoing," reports Bill Perry, vice president, business development, marketing and sales, at Nanomix. "Commercial collaboration efforts will commence in the next nine to 12 months. The first commercial application for non-medical DNA detection is planned to be available within approximately two years."

The pay-off could be huge, he adds. "The total biomolecule detection [market] is approximately $15 billion and growing substantially," he tells *NanoBiotech News*. "The first three areas we have chosen to target -- genetically modified organisms, infectious diseases and pharmacogenomics -- have a current [market] of more than $2.5 billion."

From clinic to bedside

Adds Christian Valcke, vice president of research and development at Nanomix: "Our proprietary platform enables small, automated, hand-held devices that allow point-of-care detection of biomolecules. That moves testing from clinical laboratories to the bedside, making it faster, simpler and less expensive. Our expertise with nano-electric devices is backed by an extensive patent pipeline and represents a fundamentally new and advantageous approach to biomolecule detection."

Editor's Note: Contact Bill Perry at (510) 428-5302 and Alexander Star at (412) 624-6493.

Reference

1. Star A, Tu E, Niemann J, et al. Label-free detection of DNA hybridization using carbon nanotube network field-effect transistors. *Proc Natl Acad Sci USA* Jan. 24, 2006 103:4 921-26 ⊙