Nanotechnology: The Promise Tiny Technology Holds for

How getting small may have large potential for the field of oncology

BY BALAJI PANCHAPAKESAN, PHD

***IN BRIEF*:** Research in the field of nanotechnology holds much promise for the future of cancer diagnosis and treatment, from detecting specific markers in cells to magnetically guided photo-thermal nanoshells for killing cancer cells. Currently, cancer-related nanotechnology research is proceeding along two main fronts: laboratory-based diagnostics and *in vivo* diagnostics. While these research efforts have not yet been translated into clinical advances, here's a look at how nanotechnology and nano-tool research offers promise for the oncology community.

The State of Nanotechnology

For the first time in history, researchers are attempting to manipulate and control matter at the atomic and molecular scale. The development of detection systems capable of tracking a single cancer cell or even a cluster of cells offers priceless potential for cancer diagnosis and treatment. Nanotechnology research holds much promise in cancer care that includes:

- Early cancer detection technologies that are minimally invasive or non-invasive

NANO-TOOLS IN CANCER RESEARCH

Biological nanosensors, fabricated using techniques borrowed from the integrated circuits industry, are emerging for potential use in detecting specific tumor markers. Nanosensors are precisely constructed using a technique called "top-down" manufacturing. In this process, nanoscale materials are precisely patterned on silicon substrates using lithography. Ultraviolet and electron beams are focused on a specific substrate to sculpt features on the nanoscale using patterned masks. Biological entities such as antibodies, DNA, and proteins can be patterned on top of nano-materials such as nanowires, nanoparticles, and carbon nanotubes.

So how exactly do these nanosensors detect tumor markers? The answer is simple. When specific antigens bind to the biological species (such as an antibody) on top of the nano-material, they change the electronic properties of the nano-materials. This change can be detected with great precision.

Figure 1 is a single nanotechnology chip that can be used for multi-component detection strategies. Note the array of nanowires and nanotubes that consist of hundreds of tiny nanosensors. The array of nanowire and nanotubes are precisely patterned using "top-down" manufacturing techniques.

In this device, which is also called a field effect transistor, current flows from the source to the drain. The amount of current flow is modulated through the gate of the transistor. The nanowires and nanotubes act as small channels for the flow of current in this transistor. When biological entities such as an antibody or protein bind on the surface of this channel, the electrical properties of the device change significantly. This change can be precisely measured in real time. The advantages of this approach are that it can provide faster, cheaper, label-free analysis; run multiple tests in one device; and potentially could be administered by doctors in the clinic setting or even by patients themselves.

This nanotechnology offers the ability to control molecular depositions in the nanometer range, which means a million-fold increase in the information density that can be packed in "nano-arrays." These nano-arrays can detect multiple targets (or large number of molecules) simultaneously and are utilized for proteomic profiling in cancer diagnostics, prognostics, and in monitoring therapeutic efficacies. Further, these sensors could also be used to analyze body fluids in nano- and pico-liters—offering the tantalizing potential of taking the level of cancer diagnostics down to the level of a single protein, DNA, or cell.

Nanocantilevers. Mechanical engineers have made tiny nanoscale levers that bend when proteins bind on their surface. Figure 2 shows how tumor biomarker proteins bind to specific targets that are immobilized on the surface of the cantilever. The binding event creates a free energy change that causes a "stress" and makes the cantilever "bend." By reflecting a laser light off the cantilever and monitoring the reflected light using a position-sensitive photo detector, researchers are able to precisely monitor this "bend," or deflection in the lever.

Since the change in surface free energy associated with a tumor-protein-binding event is specific, the deflection caused by this binding event is also specific. In this way, nanometer-scale displacements of the

- High-contrast imaging capable of viewing cancer proteins inside the cancer cells
- Nanovectors for targeted guidance and killing of cancer *in vivo*
- Nanosurgery for pain management
- Nanomachines for cancer surveillance inside the human body.

In fact, nano-tools for *in vitro* diagnostic screening, drug delivery, high-contrast imaging, monitoring gene mutations, and targeting cancer cells have been demonstrated in laboratory settings with exceptional results. A very few of these technologies—ones involving small particles that are impregnated with radioactive materials and chemotherapy drugs—are currently undergoing Phase I and Phase II clinical trials for localized radiation and chemotherapies.

Small, Smaller, Smallest

Nanotechnology encompasses the creation, manipulation, and utilization of materials, devices, and systems through

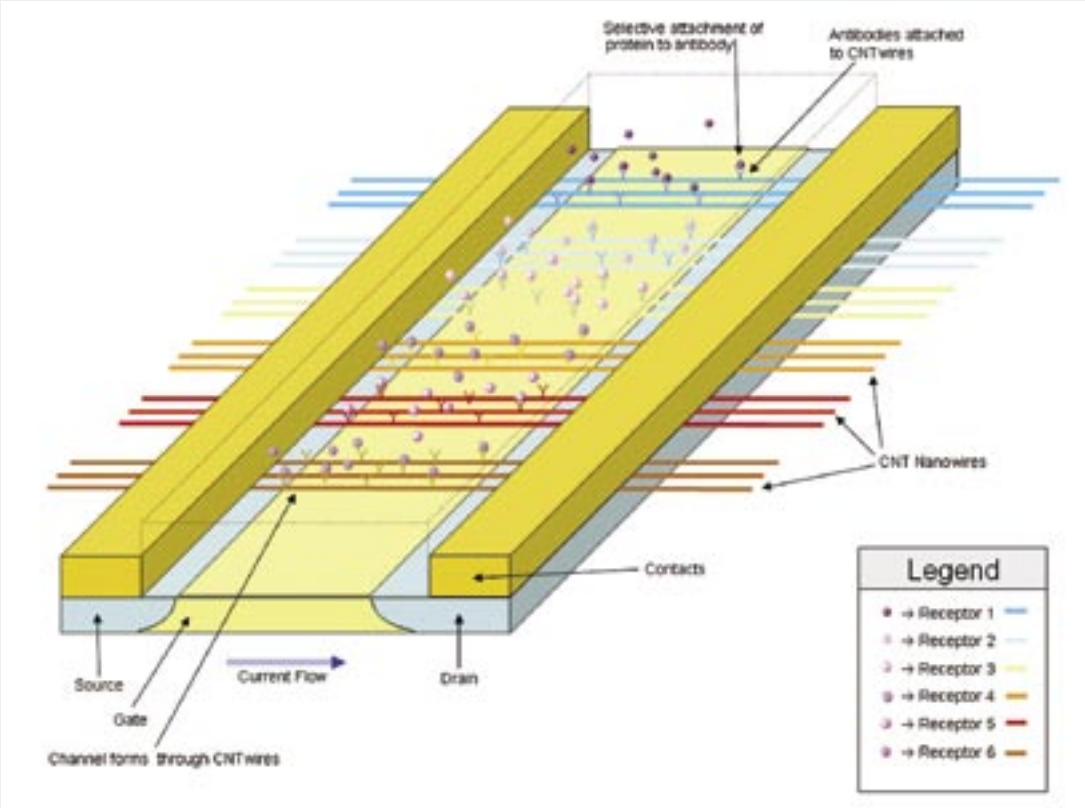

Figure 1: A Single Nano-technology Chip

Comprised of nanowire and nanotubes, it can be used for multi-component detection strategies.

ARTWORK COURTESY OF DR. PANCHAPAKESAN, UNIVERSITY OF DELAWARE

cantilever can be monitored with great accuracy. Further, thousands of cantilevers can be manufactured in the same micro-chip, making it possible to simultaneously monitor hundreds of tumor proteins. Both the nanowire and nano-mechanical arrays of cantilevers can be used for massive rapid multiplexing—without labeling these bio-molecules. In other words, these cantilevers have the ability to detect multiple proteins of bio-molecules that have primary importance in cancer.

continued on page 24

…***nanobeads*** have been used to detect DNA and proteins that may serve as **diagnostic or prognostic indicators of cancer**.

the control of matter at nanometer-length scales (i.e., at the level of atoms, molecules, and supra-molecular structures). Generally, these materials, devices, and systems are between 1-1,000 nm—or about 100 to 100,000 times smaller than a single human hair. Or to put it in another perspective, 20,000 nanodevices, which are a hundred times smaller than a human hair, could fit onto a penny.

Much of the interest in using nanoparticles for cancer detection came about with the use of semiconductor nanocrystals (or quantum dots of cadium selenide) as a tool for laboratory diagnostics of cancer.[1] These quantum dots change their optical properties with their size. When linked to an antibody or a molecule capable of binding to a substance of interest, quantum dots act like beacons that light up when binding occurs. These dots have the potential for creating assays capable of detecting multiple substances simultaneously such as Her2, actin, microfibril protein, and nuclear antigens.[2,3]

Examples of cancer-related nanotechnology include nanovectors[4] such as liposomes for breast cancer therapy,[5] nanoparticle contrast agents for high-contrast imaging of brain tumors,[6] paramagnetic nanoparticles for imaging of clinically occult lymph nodes metastases in patients,[7] nanomechanical cantilevers,[8,9] nanowires[10] and nanotube sensors for detecting cancer proteins,[11] and finally magnetically guided photo-thermal nanoshells for killing cancer cells.[12]

Nanowire and *nanotube* devices are designed in the laboratory. These devices are fabricated at micro- and nano-scale lengths using sophisticated printing techniques. Following fabrication, monoclonal antibodies directed against various tumor markers are adsorbed onto the devices against specific antigens. With minimal sample preparation, substrate binding to even a small number of antibodies produces a 100-fold change in the device sensitivity compared with current diagnostic techniques.[13-15]

A *nanocantilever* that has been coated with molecules capable of binding specific substrates—DNA complementary to a specific gene sequence—can detect a single molecule of DNA or protein. How? Changes in the surface stresses will actually bend the cantilever (see Figure 2). The changes are detected using an optical technique.[16]

Recently, DNA-labeled magnetic *nanobeads* have been used to detect DNA and proteins that may serve as diagnostic or prognostic indicators of cancer.[17]

Roadblocks and Roadmaps

Although most of the technologies discussed in this article are still in the laboratory, the hope is that eventually they will translate to the clinical setting. For example, because these devices are so tiny, fabrication of 100 to 1,000 devices onto a 1 mm chip with integrated electronics and software could—in the near future—lead to the development of hand-held cancer detection systems,

continued on page 26

Nano-atomic Tubes. Scientists at the University of Delaware, the Thomas Jefferson University in Philadelphia, Pa., and Christiana Care Hospital in Wilmington, Del., have been developing single-wall carbon nano-atomic tubes for monitoring cancer-specific-proteins. These nano-atomic tubes are hundreds of times smaller than nano-cantilevers, highly sensitive to single-protein binding events, and can be massively multiplexed with millions of tubes per chip for proteomic profiling. Additionally, the tubes have shown extraordinary strength, unique electronic properties, and the ability to tag cancer-specific proteins to their surface.

These nano-meter-sized tubes can be fabricated by decomposition of carbon-based gas in a furnace, using the presence of iron nanoparticles as catalyst material. These tubes (1 nm in diameter and 1 μm in length) are smaller than a single-strand of DNA. All of the atoms in these tubes are on the surface. In other words, this tube is an atomic arrangement of one layer of carbon atoms. Protein binding events occurring on the surface of these nano-atomic tubes produce a measurable change in the mechanical and electrical properties of these tubes.

Figure 2: A Nanocantilever Device

Tumor biomarker proteins bind to specific targets on the surface of the cantilever, causing the cantilever to "bend." This bend can then be measured by researchers.

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similar to the diabetes testing kits in use today.

In fact, within the next two to five years, some of these devices and tools may become a reality for early detection of cancer in the laboratory setting. Before clinical trials on nanotechnology-based sensors and therapies become available to patients, however, a number of protocols must first be established.

One roadblock to bringing nanotechnology to clinical trials is the lack of data to compare results from various laboratories for the same types of tests. Second, the biofluidic material interactions with nanostructures should be understood and standardized for *in vivo* applications. Third, unconventional pathways for clinical testing need to be developed, and clinical protocols based on nanotechnology should be established. Finally, a lack of clinicians trained in the field of nanotechnology means that strong emphasis must be placed on educating clinicians about nanotechnology protocols for cancer detection and therapeutics, including how to apply these technologies in the clinical setting.

While nanotechnology offers a roadmap to the future of cancer research, nanotechnologists face tremendous challenges enroute. For example, nanotechnology-based sensors work exceptionally well in the laboratory setting, but they are far less successful in detecting proteins *in vivo*. Molecular detection *in vivo* involves the use of sensors that must be implanted inside the body to relay information to external electronics. Despite years of research, the most important challenge still facing translation of these tiny sensors from bench to bedside is "bio-fouling," or non-specific adsorption of serum proteins on the sensing surface. Bio-fouling results in the rapid loss of the sensor's sensitivity. A challenge for nanotechnology researchers in this area may be to make "self-cleaning" sensors or to integrate tiny mechanical elements, such as nano-wipers, that could clean the sensor surface periodically.

When properly integrated with established cancer research, nanotechnology has the potential to make laboratory-to-clinical transfer of technology successful, holding the promise of potential breakthroughs in patient care. The potential exists for this research to be accelerated, provided that the advances of many research groups are properly integrated toward a common goal of eradicating cancer.

Looking far into the future of nanotechnology, tiny nano-bio-robots may one day be a reality. These miniscule nano-bots could potentially remove microscopic tumors in parts of the body that may be considered inaccessible using traditional surgery. Before such nano-robots can become a reality, however, scientists and researchers from different disciplines such as physics, chemistry, biology, electrical and mechanical engineering must come together to collaborate on a common mission.

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