NCI Alliance for Nanotechnology in Cancer

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Nanotubes Poised to Help Cancer Patients

As anyone who's ever gone to the doctor knows, having a needle stuck into your arm is no fun, but that thin hollow tube of metal is an essential tool of medicine, delivering drugs and vaccines—and eventually nanotechnology-enabled cancer therapies—into our bodies. Now imagine a needle 100,000 times smaller, one capable of poking not through your skin, but through the wall of a cancer cell. Though seemingly impossible, such nanoscale needles exist—they're called carbon nanotubes, and they are well on their way to becoming as essential a tool for 21st century oncologists as the metal needle is for today's physicians.

But the ultimate utility of carbon nanotubes should extend beyond their use as nanoscale needles that can deliver drugs directly into tumor cells (after being taken orally or injected using a standard needle). Already, researchers are using this novel form of the element carbon to create miniature x-ray machines that can snake through the bloodstream and deliver cancer-killing radiation directly to tumors. Nanotube heaters that bake tumors to death and nanotube-based sensors capable of spotting even the smallest tumors are also in the offing. "Carbon nanotubes are versatile structures that over the next decade are going to make a huge impact in medicine," says Kirk Ziegler, Ph.D., of the University of Florida.

Carbon nanotubes were discovered in 1991 by Sumio Iijima, Ph.D., an electron microscopy expert who was studying the various materials formed when carbon is vaporized by an electric current. They are related to buckyballs, the soccer ball-shaped form of carbon discovered that had been created six years earlier in 1985 by the late Richard Smalley, Ph.D., and fellow chemists Robert Curl Jr., Ph.D., and Harold Kroto, Ph.D.

Carbon nanotubes come in two basic forms—single-walled and multi-walled. Single-walled carbon nanotubes, which have the greatest potential for use in cancer research and clinical oncology, can be thought of as a sheet of graphite—the material used to make pencil lead—rolled into a cylinder of about 1.2 to 1.4 nanometers in diameter. As such, they resemble a piece of chicken wire rolled into a tube, with carbon atoms arranged as a series of connected hexagons. Multi-walled carbon nanotubes are similar except that they comprise multiple, concentric tubes that when viewed on end look like tree rings.

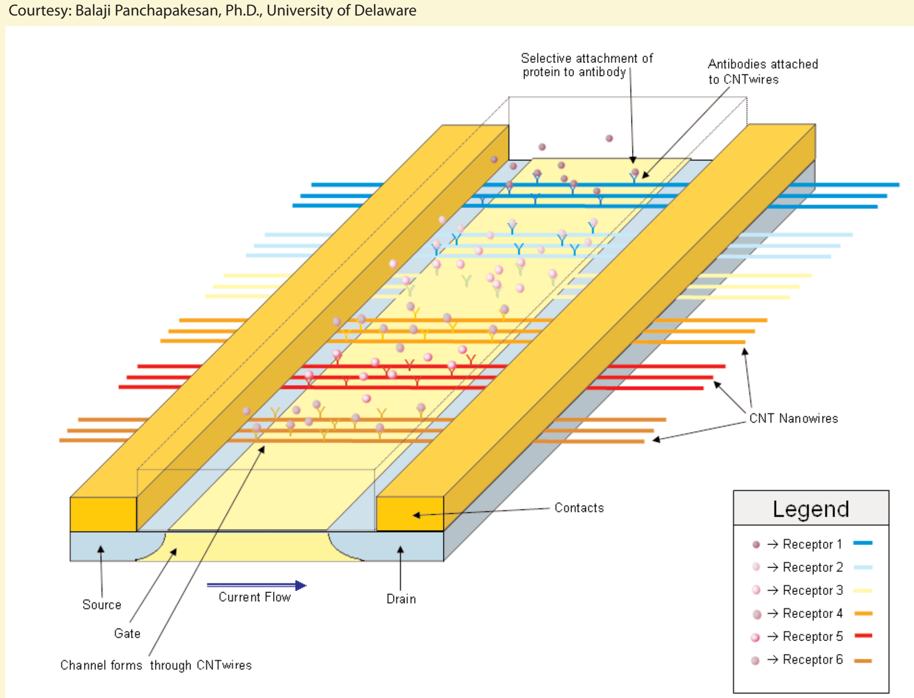
In the materials science world, carbon nanotubes are famous for their unique physical properties. Single-walled carbon nanotubes can behave like metals, and in fact, few metals conduct electricity or heat as readily as do carbon nanotubes. However, with a slight twist in their structure, carbon nanotubes become semiconductors. Carbon nanotubes are the strongest fibers ever created.

"Though we are getting a handle on the physical properties of carbon nanotubes, we're continually surprised by just what we can do with this material," says Lon Wilson, Ph.D., of Rice University. "And as we learn how to modify nanotubes chemically, we're creating materials with properties we've never seen before."

What's this stuff?

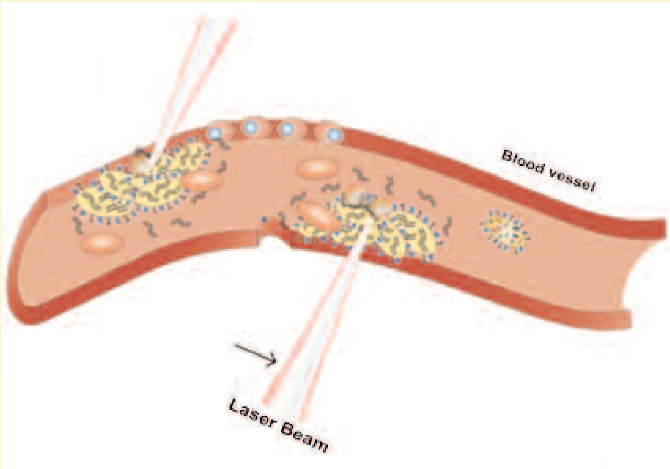
As with any new material, it has taken some time to learn how to make the best use of carbon nanotubes. Wilson, who was a colleague of Smalley, recounts the time in 1990 when someone from Smalley's lab

Courtesy: Balaji Panchapakesan, Ph.D., University of Delaware



Antibodies attached to carbon nanotube wires create nanoscale sensors. When the antibody binds to its target molecule—a specific cancer-related protein, for example—the carbon nanotube wire experiences a sharp drop in its electrical conductivity that changes the current flowing between two electrodes, known as the source and drain.

Courtesy: Balaji Panchapakesan, Ph.D., University of Delaware



Targeted killing of cancer cells in a blood vessel using nanotube nanobombs.

"plopped a sample down on my desk and said, 'do something with this.' Of course my first response was what is it, but eventually we got the idea of putting gadolinium in them with the idea of making a safer contrast agent for magnetic resonance imaging."

After spending some time with this novel material, Wilson and his colleagues found that the best nanotubes for the particular application they envisioned are ultrashort ones, in the range of 20 to 100 nanometers long, a thousand times shorter than the standard nanotube of the day. "In this size range, the nanotubes are readily taken up by cells and as importantly, they are also cleared rather rapidly from the body, which is important with an imaging agent in order to minimize the amount of nonspecific background signal you'd get from any agent that isn't binding to a tumor and is just circulating in the bloodstream," he explained.

Working with TDA Research, Inc., based in Wheat Ridge, CO, Wilson's group has developed methods for loading a variety of metal ions into these nanotube capsules. Nanocapsules loaded with gadolinium ions show promise as MRI contrast agents, while those with radioactive iodine or astatine (an element in the same family as iodine) are being developed as cancer-killing therapeutics. One formulation under development, for example, adds a monoclonal antibody that recognizes melanoma cells to the outside of the nanotube. Another nanotube formulation will target leukemia cells.

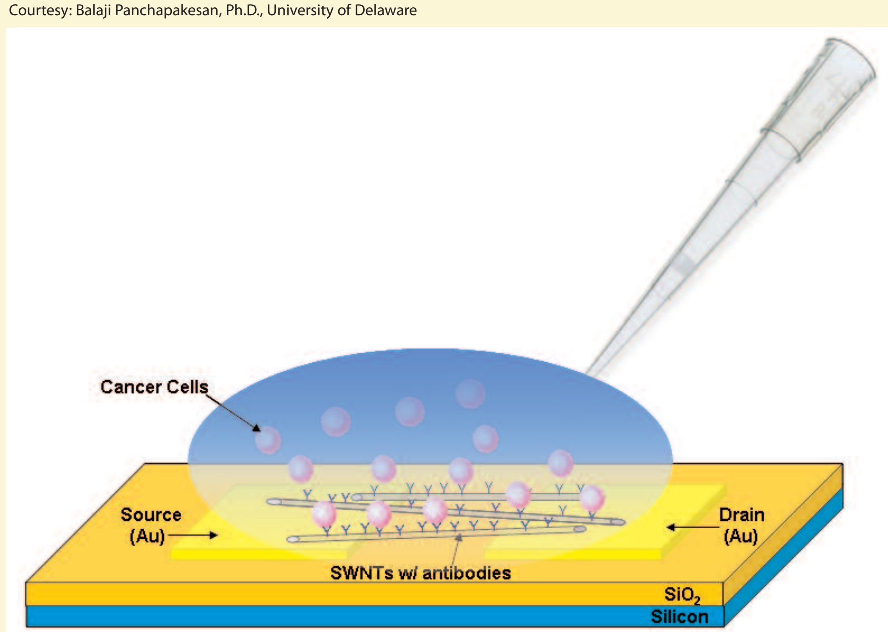
Several research groups are also developing carbon nanotubes as targeted delivery vehicles for anticancer drugs. One of the leading groups in this area of research is led by Hongjie Dai, Ph.D., at Stanford University, who has shown that carbon nanotubes can both ferry proteins and other anticancer drugs into cells and act as miniature thermal scalpels that can bake a cancer cell to death. In 2005, Dai and his colleagues reported a number of advances using nanotubes as drug delivery devices. In one experiment, for example, the investigators showed that the carbon nanotubes could ferry a protein known as cytochrome c across the cell membrane. More importantly, the transported protein, which can trigger cell death, retained all of its biological activity once it was inside the cell.

Several groups are also using carbon nanotubes as the therapeutic agent itself. For example, Balaji Panchapakesan, Ph.D., of the University of Delaware, and Eric Wickstrom, Ph.D., of the Kimmel Cancer Center at Thomas Jefferson University, found that carbon nanotubes can act as nanoscale bombs that literally blow apart a cancer cell. While their initial work was aimed at using carbon nanotubes as drug carriers, the investigators found that they could "trigger microscopic explosions of nanotubes in a wide variety of conditions," explained Panchapakesan.

Panchapakesan believes that carbon nanobombs hold great promise as a therapeutic agent for killing cancer cells, with particular emphasis on breast cancer cells, because the bomb's shockwave kills the cancerous cells as well as the small blood vessels that nourish the diseased cells. Once the nanobombs are exploded and kill cancer cells, immune system cells known as macrophages can effectively clear the cell debris and the exploded nanotube along with it.

Dai and his colleagues are also using carbon nanotubes as therapeutic agents. "An interesting property of carbon nanotubes is that they absorb near-infrared light waves,

Courtesy: Balaji Panchapakesan, Ph.D., University of Delaware



Antibodies attached to carbon nanotube wires can diagnose cancer by detecting specific proteins found only on the surface of cancer cells. Using more than one type of antibody, each of which recognizes a different cancer-related protein, increases the accuracy of the diagnosis. In this case, antibodies recognizing two cancer-related proteins, Her2 and IGF1R surface receptors, are incorporated on a chip designed to detect breast cancer.

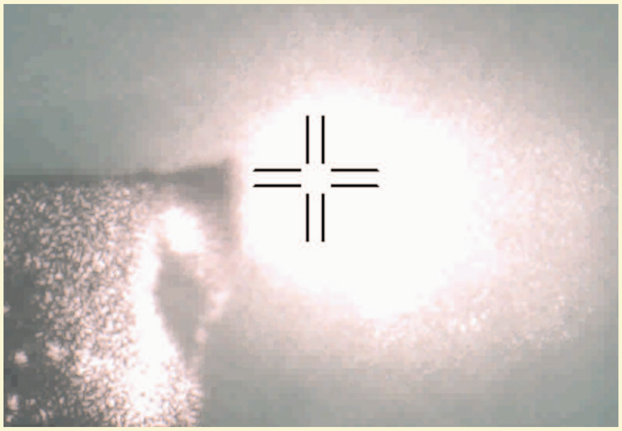
which are slightly longer than visible rays of light and pass harmlessly through our cells," Dai says. But shine a beam of near-infrared light on a carbon nanotube, and the results are dramatic. Electrons in the nanotube become excited and begin releasing excess energy in the form of heat.

In their experiments, Dai's team found that if they placed a solution of carbon nanotubes under a near-infrared laser beam, the solution would heat up to about 70° C (158° F) in two minutes. When nanotubes were placed inside cells and radiated by the laser beam, the cells were quickly destroyed by the heat. However, cells without nanotubes showed no effects when placed under near-infrared light.

The nanotubes were targeted to cancer cells by attaching folic acid to the surface of the nanotubes. Folic acid binds to a folic acid receptor protein found in abundance on the surfaces of many types of cancer cells. "Folate is just an experimental model that we used," Dai says. "In reality, there are more interesting ways we can do this. For example, we can attach an antibody to a carbon nanotube to target a particular kind of cancer cell."

One example is lymphoma, or cancer of the lymphatic system. Like many cancers, lymphoma cells have well-defined surface receptors that recognize unique antibodies. When attached to a carbon nanotube, the antibody would play the role of a Trojan horse. Dai and Dean Felsher, M.D., a lymphoma researcher in the Stanford University School of Medicine, have begun a collaboration using laboratory mice with lymphoma. The researchers want to determine if shining near-infrared light on the animal's skin will destroy lymphatic tumors, while leaving normal cells intact.

Courtesy: Balaji Panchapakesan, Ph.D., University of Delaware



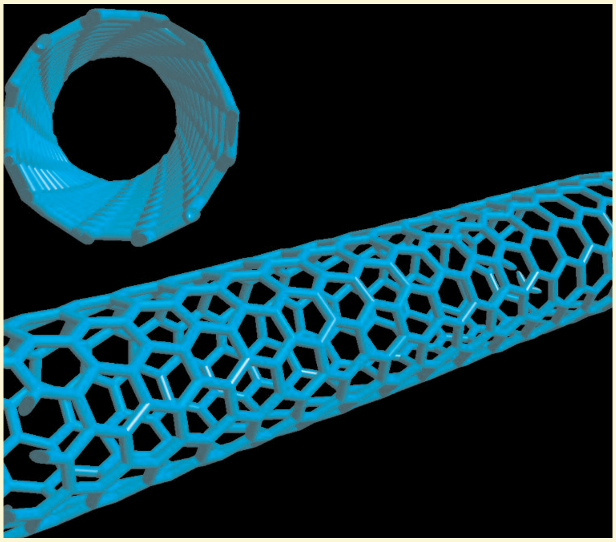
Nanobomb explosions when irradiated with NIR light.

Can't keep them out

What cancer researchers are finding from these and other studies is that carbon nanotubes are intrinsically intracellular agents. "Nanotubes almost can't help getting inside cells," explains Wilson.

Recent work by Dai and his colleagues appears to show how nanotubes force their way into cells, a process that requires the cell to expend energy to move the nanotubes across its outer membrane. But given that carbon nanotubes pass through the cell membrane so easily, some investigators have expressed concern that carbon nanotubes themselves could prove toxic if administered in sufficient amounts. And in fact, some experiments have found that under certain circumstances, carbon nanotubes can be toxic. The most well-known of these studies, conducted by investigators at the NASA Johnson Space Center, Wyle Laboratories in Houston, and The University of Texas Medical School at Houston, showed that inhaled carbon nanotubes could induce tumor-like growths in the lungs of mice. Other work by Govindrajan Ramesh, Ph.D., and his colleagues at Texas Southern University found that carbon nanotubes could damage cells by triggering proteins involved in responding to oxidative stress.

Courtesy: R. Bruce Weisman, Ph.D., Rice University



Single-walled carbon nanotubes are simple in structure, consisting of repeating hexagonal arrangements of carbon atoms attached to one another. The interior of a carbon nanotube is hollow, but can be loaded with a wide variety of molecules.

But Wilson believes that at the low levels of injected nanotubes used in biomedical applications, toxicity will not be an issue. "I think that the toxicity that some experiments have found are a result of contaminants that remain when the nanotubes are synthesized," he said. "When we make highly pure carbon nanotubes, we don't see any toxicities, even at relatively high doses." Indeed, work by Vicki Colvin, Ph.D., at Rice University, has shown that nanotubes modified to make them dissolve easily in water show very little toxicity even at very high concentrations. And work by R. Bruce Weisman, Ph.D., also at Rice University, found that cells that had taken up as many as 70,000 carbon nanotubes showed no signs of toxicity. Nonetheless, any imaging or therapeutic agents developed using carbon nanotubes would have to undergo the same rigorous toxicology studies that all agents for use in humans must undergo.

Weisman's work was aided by a non-destructive optimal method that his group developed to selectively and sensitively detect carbon nanotubes in biological surroundings. This method provides a valuable tool for tracing the locations of nanotubes in cells, tissues, and organisms, and it could play a critical role in studying how nanotubes become distributed in the body. This type of data on nanotube biodistribution will not only aid researchers to fine-tune nanotube-enabled cancer therapeutics and imaging agents, but will also help provide some of the data that will be needed to have such agents approved by the U.S. Food and Drug Administration for use in humans.

While many groups are working to develop such nanotube-based therapeutics and imaging agents, others are exploring cancer-related applications that will not involve getting nanotubes and their cargoes into cells. Because of their excellent electrical conductivity, carbon nanotubes are poised to become the key component of ultrafast, miniaturized diagnostic gear that may soon be able to detect the earliest signs of cancer from a pinprick of blood right in a doctor's office.

Joseph Wang, Ph.D., and his colleagues at Arizona State University, for example, are using electrodes modified with carbon nanotubes to create highly sensitive devices capable of detecting specific sequences of DNA, including those associated with the breast cancer gene BRCA1. Another group of investigators, led by Panchapakesan, have attached antibodies to the surfaces of carbon nanotubes that can bind molecules shed by tumors into the bloodstream. In both cases, the idea would be to incorporate the tumor-sensing nanotubes into a small electrical circuit, which would signal the presence of the tumor marker with a change in electrical conductivity.

Then there are the more futuristic applications for carbon nanotubes that researchers are only just starting to envision, such as using branched nanotubes as tweezers for manipulating molecules within a cell. Other researchers are starting to use carbon nanotubes as nanoscale electron sources that could form the heart of a fiber optic x-ray source—imagine snaking a tiny x-ray machine into the body directly to a tumor and blasting it with a lethal dose of energy without damaging surrounding tissue.

Of course, only time will tell which of the many proposed uses of carbon nanotubes will ultimately impact cancer research and clinical oncology. Indeed, the majority of work with carbon nanotubes is still in the early stages of development, and it will be several years before any of these applications will begin human clinical trials. But given that this field is only a few years old, the future of carbon nanotube-based cancer research seems as bright as the light given off by an irradiated nanotube itself. <

—Joe Alper

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