NATIONAL CANCER INSTITUTE
NCI Alliance for Nanotechnology in Cancer

Shining a Light on Cancer Research

Quantum dots and nanoshells are driving development of novel analytical and therapeutic approaches for cancer

In the early 1980s, researchers at Bell Laboratories in the United States and at the Yoffe Institute in Russia made an unexpected observation: as semiconductor crystals grew ever smaller, their optical properties began to change in what, at the time, seemed a mysterious fashion. Depending on their size, the crystals fluoresced at different wavelengths even though the chemical composition of the crystals stayed the same. Eventually, the researchers came to understand that the unusual behavior of these "quantum dots" resulted from their nanoscale size, which changed the electronic properties of the semiconductor materials in a fundamental manner.

Fast forward to the mid-1990s, when researchers at Rice University made a similar discovery about another class of materials. Working with gold-coated silica nanoshells, Naomi Halas, professor of chemistry and electrical and computer engineering at Rice University in Houston, determined that by varying the thickness of the gold coating relative to the diameter of the silica core, it was possible to tune the optical behavior of the resulting nanoshells. But instead of emitting light of defined wavelength, i.e. color, as do quantum dots, the nanoshells absorbed or scattered light at well-defined frequencies. Two different materials, two different mechanisms explaining a color change, and both relying on fundamental differences in the way matter behaves at the nanoscale.

Today, those initial discoveries and the research they fostered have led to the development of new technologies that are changing the way that cancer researchers, among others, are observing the fundamental molecular events that occur in and around cells. Using nanoscale semiconductor quantum dots and gold nanoshells of various diameters, and thus different colors, biomedical researchers are able to tag multiple different biological molecules with brightly colored beacons that they can easily track *in vivo* using a variety of imaging technologies, such as fluorescence microscopy.

As an example of this type of approach, a team from Quantum Dot Corp., a company based in Hayward, CA, and Genentech, a pharmaceutical company in South San Francisco, CA, used quantum dots to simultaneously label and visualize Her-2 on the surface of live cancer cells and nuclear antigens inside the cell.[1] More recently, investigators at Quantum Dot have used quantum dots that fluoresce at different colors to simultaneously label and track mammalian cells in culture using either standard fluorescence microscopy or a commercial cell sorter. To get the quantum dots into cells, the group used a ferrying peptide known as Pep-1 to carry the quantum dots through the cell membrane. The researchers estimate that they can tag and image over 100 different cells simultaneously using this method.[2]

The ability to keep track of multiple molecules and cells will undoubtedly be a boon for molecular and cell biologists who are trying to understand the interplay among multiple biochemical and genetic pathways that are involved in cancer. But that is just one of the many developing uses for tunable nanoscale beacons. "From their initial use as easily-tracked markers, these nanoscale beacons are proving to be quite versatile in what we can do with them," says Shuming Nie, associate professor of biomedical engineering at Emory University School of Medicine and director of nanotechnology at the Winship Cancer Institute.

Nie, for example, is heading a multi-institutional consortium that is attempting to develop second-generation quantum dots for use as tumor detection agents, and perhaps delivery vehicles for anticancer therapeutic agents. Recent recipients of a $7.1 million grant from the National Cancer Institute, Nie's team has modified the original cadmium selenide (CdSe) quantum dot with a coating of polymer that has two functions: the impermeable coating prevents highly toxic cadmium from leaching out of the quantum dots and it provides a means of chemically attaching tumor-targeting molecules and drug-delivery functionality to the molecular beacon.[3] His team plans on using these quantum dots to identify tumor markers from an extensive collection of archived tumor biopsies taken from hundreds of patients.

In the future, targeted quantum dots could also serve as *in vivo* imaging agents if Nie and others, such as John Frangioni, assistant professor at Harvard Medical School, and Massachusetts Institute of Technology chemistry professor Moungi Bawendi, are successful in their on-going efforts to extend the wavelength at which these nanoparticles emit light above 900 nanometers, the current upper limit. Nie explains that since there are no biomolecules that fluoresce above 1000 nanometers, quantum dots capable of fluorescing in that range would provide an unambiguous signal when used in imaging applications. He adds that computer calculations predict that fluorescence above 1000 nanometers should be capable of passing through more tissue and be detectable at far lower levels, which would boost the sensitivity of any test using such materials. As a corollary to this work, the Emory team is

also developing a new imaging camera that will be sensitive to emissions at 1000 nanometers.

Proof that imaging with quantum dots is not just a goal but a reality comes from recent work out of Harvard Medical School, where a team led by Frangioni and assistant professor Tomislav Mihaljevic, also of Harvard Medical School, used coated, water-soluble quantum dots to detect so-called sentinel lymph nodes – the first lymph nodes to accumulate metastatic cells shed by nearby tumors – in animals as large as a 35 kilogram pig. These coated semiconductor nanoparticles, developed by Massachusetts Institute of Technology chemistry professor Moungi Bawendi, are readily visible in lymph nodes up to one centimeter beneath the skin[4] and five centimeters in lung tissue.[5]

Meanwhile, Halas and Rice University colleagues Jennifer West and Rebekah Drezek, both professors of biomedical engineering, have used gold nanoshells to image tumors in mice using an imaging approach that can work as deep as 10 centimeters within an animal's body.[6] Halas and West have also pioneered nanoshells as miniature "thermal scalpels" that can literally cook cancer cells to death. The operating principle here is that these nanoshells will become hot when irradiated with relatively low-intensity near-infrared laser light, and tests in laboratory animals have shown that the nanoshells can transfer this thermal energy to tumor cells and kill them.[7] Thus, if the nanoshells are targeted to tumor cells, they may enable physicians to first image the tumors and then kill them by turning up the light intensity. Nanospectra Biosciences, based in Houston, TX, is currently conducting further animal tests and is hoping to begin human clinical trials with these nanoshells early in 2006. Halas and her team are trying to better understand how these particles turn light into heat in order to better predict what type of gold nanoshell is best suited as a thermal scalpel.

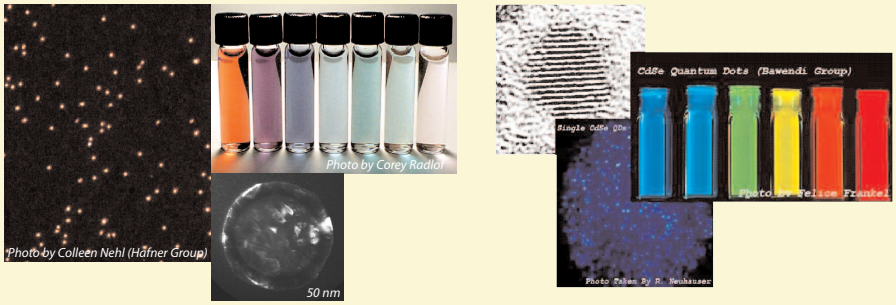
Different particles, different mechanisms

Though quantum dots and gold nanoshells are both visible in the near-infrared region of the optical spectrum, the two types of nanoparticles rely on different mechanisms for their ability to interact with light. Quantum dots owe their fluorescence to a property shared by all semiconductors. When light falls on a semiconductor, electrons are excited from what chemists call the valence band – where they are tightly bound to their parent atom – into the so-called conduction band, where they can move and contribute to current flow. Each electron leaves a positive hole in the valence band, and the electron stays close to this hole in a bound system called an exciton. Electrons emit their excess energy as light when they recombine with positive holes, and this means that excitons are the source of light in semiconductors. In contrast, gold nanoshells owe their optical properties to plasmons, ripples of waves in the ocean of electrons flowing across the surface of metallic nanostructures. The type of plasmon that exists on a surface of a nanoscale object is directly related to its geometric structure – the precise curvature of a nanoscale gold sphere or a nano-sized pore in metallic foil, for example. When light of a specific frequency strikes a plasmon that oscillates at a compatible frequency, the energy from the light is harvested by the plasmon, converted into electrical energy that propagates through the nanostructure and eventually converted back to light.

In research described in the journal *Science*,[8] Halas and colleague Peter Nordlander, professor of theoretical physics at Rice, show that the equations that determine the frequencies of the plasmons in complex nanoparticles are almost identical to the quantum mechanical equations that determine the energies of electrons in atoms and molecules. And just as quantum mechanics allows scientists to predict the properties of complex molecules, the work performed by the Rice team shows how the properties of plasmons in complex metallic nanostructures can be predicted in a simple manner. As a result of this advance in understanding plasmons, Halas explains that, "we can design nanoscale materials in advance on the computer and then create them with the predicted optical properties in the laboratory."

Figure 1: Comparing Nanoshells to Quantum Dots

Courtesy of Naomi Halas, Rice University



Nanoshells
Tunable plasmonic nanoparticles
~ 10-300 nm diameter
Quantum efficiencies ~10-4
Spectral range (extinction): 500(Ag)-9000 nm
Cross sections: ~10-13 m2

Quantum Dots
Tunable excitonic nanoparticles
~ 1-10 nm diameter(uncoated)
Quantum efficiencies ~0.1-0.5
Spectral range (emission): 400-2000 nm
Cross sections: ~10-19 m2

Given the way that science works, it should not be surprising that the research community is not content with having two different, versatile nanoscale tagging systems at their command. Among the up-and-coming nanoscale beacons are those developed by Weihong Tan, professor of chemistry at the University of Florida. He and his colleagues have worked out methods for incorporating a wide range of organic fluorescent dyes into the core of silica nanoparticles.[9] Florida colleague Shouguang Jin, associate professor of molecular genetics and microbiology, and his team recently used these nanobeacons to develop sensitive and rapid assays for a wide range of biological molecules by hooking them to monoclonal antibodies.[10] Tan, working with a group at China's Hunan University, has also demonstrated that these doped silica nanoparticles could form the basis of an assay for hepatitis G-positive liver cancer cells.[11]

Though there is still work to be done before any of these tunable nanoscale beacons make an impact on the detection and treatment of

cancer, researchers are optimistic that the future is bright for quantum dots, gold nanoshells and other fluorescent markers built using nanotechnology. Perhaps the biggest question remaining has to do with the potential toxicity of some of the materials used to make these nanoscale beacons. "Semiconductor metals are highly toxic, and not even gold has been proven safe in the quantities and formulations employed," cautions Frangioni. "Until more suitable formulations are discovered, and until extremely thorough toxicity studies are performed, the likelihood of any of these entities making it into the clinic are slim."

Not that researchers are daunted. "The technical challenges have been met one by one," says Nie, "and now that these materials are widely available to the research community, I have no doubt that we'll be seeing applications reach the clinic. This field is moving very rapidly." <

— Joe Alper

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