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ENGINEERED NANOMATERIALS FOR BIOPHOTONICS APPLICATIONS: Improving Sensing, Imaging, and Therapeutics

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■ **Abstract** Advances in chemistry and physics are providing an expanding array of nanostructured materials with unique and powerful optical properties. These nanomaterials provide a new set of tools that are available to biomedical engineers, biologists, and medical scientists who seek new tools as biosensors and probes of biological fluids, cells, and tissue chemistry and function. Nanomaterials are also being used to develop optically controlled devices for applications such as modulated drug delivery as well as optical therapeutics. This review discusses applications that have been successfully demonstrated using nanomaterials including semiconductor nanocrystals, gold nanoparticles, gold nanoshells, and silver plasmon resonant particles.

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INTRODUCTION

Recent advances in nanotechnology have provided a variety of nanostructured materials with highly controlled and interesting properties—from exceptionally high strength to the ability to carry and target drugs to unique optical properties. By controlling structure at the nanoscale dimensions, one can control and tailor the properties of nanostructures, such as semiconductor nanoscrystals and metal nanoshells, in a very predictable manner to meet the needs of a specific application.

These materials can bring new and unique capabilities to a variety of biomedical applications ranging from diagnosis of diseases to novel therapies. In particular, nanotechnology may greatly expand the impact of biophotonics, particularly optical imaging and biosensing, by providing more robust contrast agents, fluorescent probes, and sensing substrates.

In addition, the size scale of nanomaterials is very interesting for many biomedical applications. The fact that many nanoparticles are similar in size scale ($<$50 nm) to common biomolecules makes them interesting for applications, such as intracellular tagging, and also makes them appear to be ideal for bioconjugate applications, such as antibody targeting of contrast agents. In many cases, one can make modifications to nanostructures to better suit their integration with biological systems; for example, one may modify their surface layer for enhanced aqueous solubility, biocompatibility, or biorecognition. Nanostructures can also be embedded within other biocompatible materials to provide nanocomposites with unique properties.

Many of the biomedical applications of nanotechnology involve bioconjugates. The idea of merging biological and nonbiological systems at the nanoscale has been investigated for many years. The broad field of bioconjugate chemistry is based on combining the functionalities of biomolecules and nonbiologically derived molecular species for applications, including markers for research in cellular and molecular biology, biosensing, and imaging (1). Many current applications of nanotechnolgy, particularly in the area of biophotonics, are a natural evolution of this approach. In fact, several of the "breakthrough" applications recently demonstrated using nanostructure bioconjugates are in fact traditional applications originally addressed by standard molecular bioconjugate techniques that have been revisited with these newly designed nanostructure hybrids.

Why replace conventional molecular tags, such as fluorescent chromophores, with nanostructures? Typically, nanostructured materials possess optical properties far superior to the molecular species they replace—higher quantum efficiencies, greater scattering or absorbance cross sections, optical activity over more biocompatible wavelength regimes, and substantially greater chemical stability or stability against photobleaching. Additionally, some nanostructures provide optical properties that are highly dependent on particle size or dimension. The ability to systematically vary the optical properties via structure modification not only improves traditional applications, but also may lead to applications well beyond the scope of conventional molecular bioconjugates.

In this review, we introduce several successful examples of nanostructures that have been applied to relevant problems in biotechnology and medicine. The use of semiconductor nanocrystals, also known as quantum dots, as fluorescent labels is discussed. Bioassays based on the optical properties of bioconjugated gold nanoparticles and silver plasmon resonant particles, a new innovation based on a traditional bioconjugate technology, are also described. Last, a photothermally modulated drug delivery system based on a nanoshell-polymer composite is discussed.

QUANTUM DOTS AS FLUORESCENT BIOLOGICAL LABELS

Semiconductor nanocrystals, also referred to as quantum dots, are highly light-absorbing, luminescent nanoparticles whose absorbance onset and emission maximum shift to higher energy with decreasing particle size due to quantum confinement effects (2, 3). These nanocrystals are typically in the size range of 2–8 nm in diameter. Unlike molecular fluorophores, which typically have very narrow excitation spectra, semiconductor nanocrystals absorb light over a very broad spectral range. This makes it possible to optically excite a broad spectrum of quantum dot "colors" using a single excitation laser wavelength, which may enable one to simultaneously probe several markers in biosensing and assay applications. The variability in fluorescence of CdSe nanocrystals of varying sizes illuminated with a single excitation source is demonstrated in Figure 1. Although the luminescence properties of semiconductor nanocrystals have historically been sensitive to their local environment and nanocrystal surface preparation, recent core-shell geometries where the nanocrystal is encased in a shell of a wider band gap semiconductor have resulted in increased fluorescence quantum efficiencies ($>50\%$) and greatly improved photochemical stability. In the visible region, CdSe-CdS core-shell nanocrystals have been shown to span the visible region from approximately 550 nm (green) to 630 nm (red). Other materials systems, such as InP and InAs, provide quantum dot fluorophores in the near-infrared region of the optical spectrum, a region where transmission of light through tissues and blood is maximal (3a,b). Although neither II–VI nor III–V semiconductor nanocrystals are water soluble, let alone biocompatible, surface functionaliztion with molecular species such as mercaptoacetic acid or the growth of a thin silica layer on the nanoparticle surface facilitate aqueous solubility (4). Both the silica layer and the covalent attachment of proteins to the mercaptoacetic acid coating permit the nanoparticles to be at least relatively biocompatible. Quantum dots have also been modified with dihydrolipoic acid to facilitate conjugation of avidin and subsequent binding of biotinylated targeting molecules (5). Quatum dots can also be embedded within polymer nano- or microparticles to improve biocompatibility while maintaining the unique fluorescence.

Specific binding of quantum dots to cell surfaces, cellular uptake, and nuclear localization have all been demonstrated following conjugation of semiconductor nanocrystals to appropriate targeting proteins, such as transferrin or antibodies (2, 4). This could be useful in a variety of microscopy and imaging applications. Quantum dots may also be useful in a variety of in vitro diagnostic applications, particularly because concerns about semiconductor nanocrystal biocompatibility can be neglected in such uses. One example is the development of a fluorescent immunoassay using antibody-conjugated quantum dots (5); several protein toxins have been successfully detected using this system. In another example, quantum dots embedded in polymer microbeads have been used for DNA hybridization studies (6). Encasing the nanocrystals in the polymer beads allows for simultaneous

reading of a huge number of optical signals. The photon emission wavelength of different nanocrystal species can be tuned by varying the particle size. Microbeads can then be prepared with varying colors and intensities of quantum dots. Using ten intensity levels and six colors, one could theoretically code one million optically differentiated signals to mark different nucleic acid or protein sequences for high-throughput screening and diagnostics.

GOLD NANOPARTICLE BIOCONJUGATE-BASED COLORIMETRIC ASSAYS

The use of gold colloid in biological applications began in 1971 when Faulk & Taylor invented the immunogold staining procedure. Since that time, the labeling of targeting molecules, such as antibodies, with gold nanoparticles has revolutionized the visualization of cellular components by electron microscopy (7). The optical and electron beam contrast properties of gold colloid have provided excellent detection capabilities for applications, including immunoblotting, flow cytometry, and hybridization assays. Furthermore, conjugation protocols to attach proteins to gold nanoparticles are robust and simple (1), and gold nanoparticles have been shown to have excellent biocompatibility (7).

Gold nanoparticle bioconjugates were recently applied to polynucleotide detection in a manner that exploited the change in optical properties resulting from plasmon-plasmon interactions between locally adjacent gold nanoparticles (8). The characteristic red color of gold colloid has long been known to change to a bluish-purple color upon colloid aggregation due to this effect. In the case of polynucleotide detection, mercaptoalkyloligonucleotide-modified gold nanoparticle probes were prepared. When a single-stranded target oligonucleotide was introduced to the preparation, the nanoparticles aggregated due to the binding between the probe and target oligonucleotides, bringing the nanoparticles close enough to each other to induce a dramatic red-to-blue color change as depicted in Figure 2. Because of the extremely strong optical absorption of gold colloid, this colorimetric method can be used to detect $\sim$10 fmol of an oligonucleotide, which is approximately 50 times more sensitive than the sandwich hybridization detection methods based on molecular fluorophores.

A similar approach has been used to develop a rapid immunoassay that can be performed in whole blood without sample preparation steps. This assay utilizes a relatively new type of gold nanoparticle called a gold nanoshell. Gold nanoshells are concentric sphere nanoparticles consisting of a dielectric core nanoparticle (typically gold sulfide or silica) surrounded by a thin gold shell (9). By varying the relative dimensions of the core and shell layers, the plasmon-derived optical resonance of gold can be dramatically shifted in wavelength from the visible region into the mid-infrared, as depicted in Figure 3 (10). By varying the absolute size of the gold nanoshells, they may be designed to either strongly absorb (for particles $<\sim$75 nm) or scatter the incident light (10). The gold shell layer is formed using the same chemical methods that are employed

to form gold colloid; thus, the surface properties of gold nanoshells are virtually identical to gold colloid, providing the same ease of bioconjugation and excellent biocompatibility. To develop a whole-blood immunoassay, gold nanoshells were designed and fabricated for near-infrared resonance, and antibodies against target antigens were conjugated to the nanoshell surfaces (11). When introduced into samples containing the appropriate antigen, the antibody-antigen linkages caused the gold nanoshells to aggregate, shifting the resonant wavelength further into the infrared. This assay system was shown to have sub-nanometer/milliliter sensitivity. More importantly, this assay can be performed in whole-blood samples because the wavelengths utilized are in the near infrared, above the absorption of hemoglobin yet below the water absorption band, where penetration of light through blood is relatively high (3a,b). Additionally, because gold nanoshells have highly tunable optical properties that can be designed for the visible through mid-infrared regions of the electromagnetic spectrum, it may be possible to probe for several antigens simultaneously using nanoshells with varying optical resonances.

NANOCOMPOSITE HYDROGELS FOR PHOTOTHERMALLY MODULATED DRUG DELIVERY AND CELL ABLATION

Gold nanoshells, described above, can be designed to strongly absorb light at desired wavelengths, in particular, in the near infrared between 800–1200 nm where tissue is relatively transparent. Very few molecular chromophores are available in this region of the electromagnetic spectrum (800–1200 nm), let alone ones with low toxicity. When optically absorbing gold nanoshells are embedded in a matrix material, illuminating them at their resonance wavelength causes the nanoshells to transfer heat to their local environment. This photothermal effect can be used to optically modulate drug release from a nanoshell-polymer composite drug delivery system (12). To accomplish photothermally modulated release, the matrix polymer material must be thermally responsive. Copolymers of N-isopropylacrylamide (NIPAAm) and acrylamide (AAm) exhibit a lower critical solution temperature (LCST) that is slightly above body temperature. When the temperature of the copolymer exceeds its LCST, the resultant phase change in the polymer material causes the matrix to collapse, resulting in a burst release of any soluble material (i.e., drug) held within the polymer matrix. As demonstrated in Figure 4, when gold nanoshells that were designed to strongly absorb near-infrared light were embedded in NIPPAAm-co-AAm hydrogels, pulsatile release of insulin and other proteins could be achieved in response to near-infrared irradiation.

In another application based on the photothermal effects that can be achieved with gold nanoshells in the near infrared, bioconjugates of nanoshells were prepared with an antibody against the HER-2 oncoprotein (13). These bioconjugate nanoshells were capable of specifically binding to human breast epithelial carcinoma cells, and upon near-infrared irradiation, the photothermal effects resulted in ablation of all carcinoma cells within the laser spot, as shown in

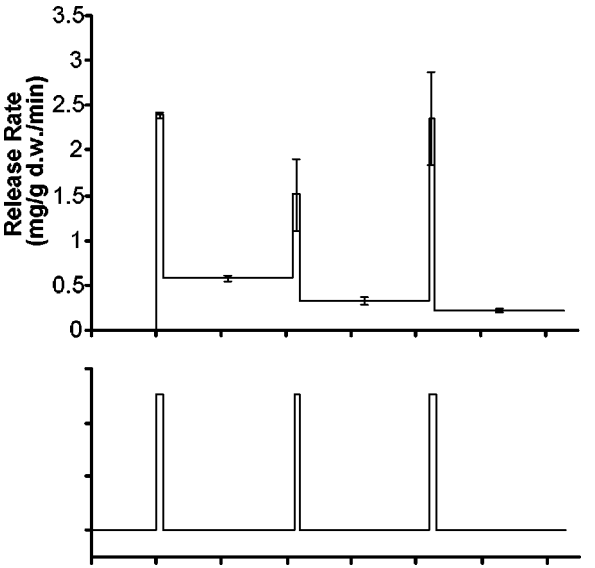

Figure 4 Release of insulin from NIPAAm-coAAm hydrogels with nanoshells embedded in their structure can be modulated by exposure to near-infrared light (832 nm, 1.5 W/cm^2). The top panel is the release rate of insulin from the nanocomposite hydrogel materials versus time, whereas the bottom panel indicates the pattern of laser illumination.

Figure 5, using wavelengths and laser powers that result in no observable damage in the absence of nanoshells. A benefit of this approach over molecular-based photodynamic therapy is that the gold nanoshells are nontoxic and highly biocompatible, only causing cellular damage when activated by light. Furthermore, tissue ablation only occurs when temperatures are raised above approximately 55°C. The temperature achieved in a tissue depends on both the nanoshell density and the intensity of light at the appropriate wavelength. Thus, unlike photodynamic therapy, concerns regarding side effects upon exposure to normal daylight in the days and weeks after an in vivo treatment should be minimal.

SILVER PLASMON RESONANT PARTICLES FOR BIOASSAY APPLICATIONS

Silver plasmon resonant particles have been used as reporter labels in microarray-based DNA hybridization studies (14) and sandwich immunoassays (15). Silver plasmon resonant particles consist of a gold nanoparticle core onto which a silver shell is grown. Particles of this type in the size range of 40–100 nm scatter light very strongly (14), allowing them to act as diffraction-limited point sources that can be observed using a standard dark-field microscope with white light illumination.

In the bioassay applications that have been developed, bioconjugates are prepared with antibodies against either a target antigen for an immunoassay or against biotin for subsequent attachment of biotinylated DNA (14, 15). In both the immunoassay and the hybridization assay, the results are determined by counting the number of particles bound to the substrate via microscopy. In the DNA hybridization assay, the sensitivity obtained was approximately 60 times greater than what is typically achieved using conventional fluorescent labels.

ACKNOWLEDGMENTS

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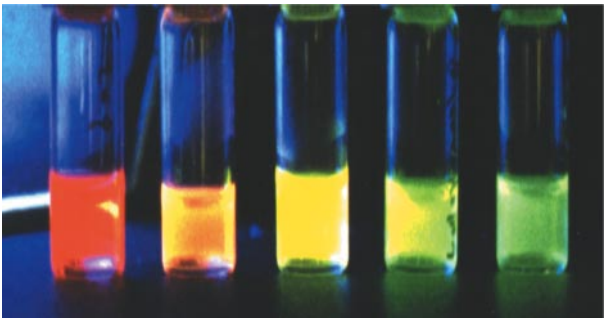

Figure 1 Semiconductor nanocrystals, also referred to as quantum dots, are highly light absorbing, luminescent nanoparticles whose absorbance onset and emission maximum shift to higher energy with decreasing particle size due to quantum confinement effects. These vials contain CdSe nanocrystals. Upon illumination with a single light source, different colors of emission are observed, depending on nanocrystal size. This may allow one to simultaneously probe for multiple analytes. Figure courtesy of Dr. Colvin.

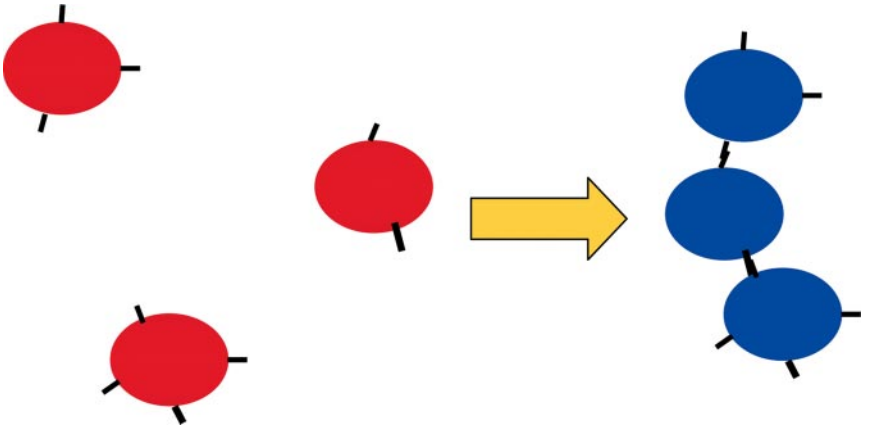

Figure 2 When gold nanoparticles come into close proximity, plasmon-plasmon interactions cause dramatic changes in optical properties. Using appropriately conjugated nanoparticles, this behavior can be exploited for DNA hybridization assays and immunoassays.

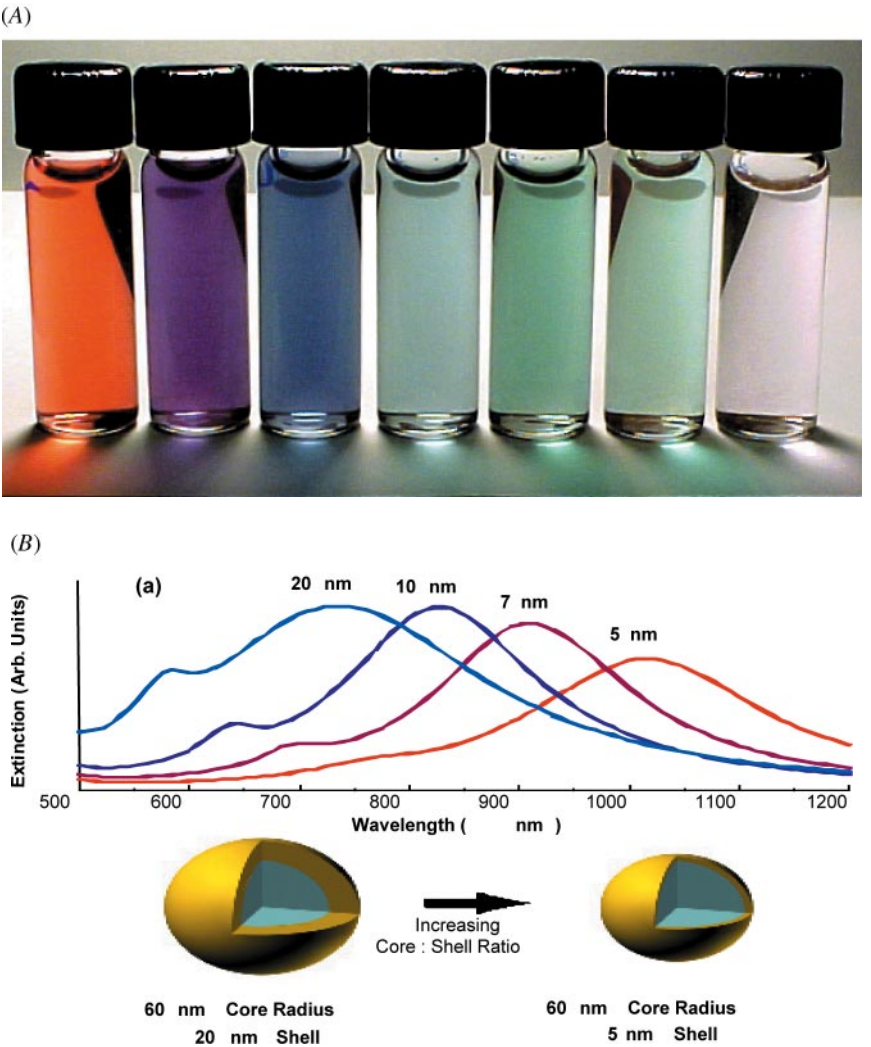

Figure 3 Gold nanoshells consist of a dielectric core nanoparticle surrounded by a thin metal shell. By varying the relative dimensions of the core and shell constituents, one can design particles to either absorb or scatter light over the visible and much of the infrared regions of the electromagnetic spectrum. (*A*) These vials contain suspensions of either gold colloid (*far left* with its characteristic red color) or gold nanoshells with varying core:shell dimensions. (*B*) The optical properties of nanoshells are predicted by Mie scattering theory. For a core of a given size, forming thinner shells pushes the optical resonance to longer wavelengths.

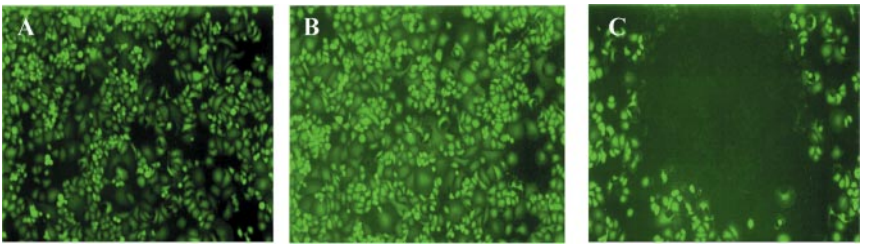

Figure 5 Breast carcinoma cells were exposed to either nanoshells (*A*), near-infrared light (*B*), or the combination of nanoshells and near-infrared light (*C*). As demonstrated by staining with the fluorescent viability marker calcein AM, the carcinoma cells in the circular region corresponding to the laser spot were completely destroyed, whereas neither the nanoshells nor the light treatment alone compromised viability.

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