Nanodiagnostics: A New Frontier for Clinical Laboratory Medicine

HASSAN M.E. AZZAZY,1* MAI M.H. MANSOUR,1 and STEVEN C. KAZMIERCZAK2

Background: The use of nanotechnologies for diagnostic applications shows great promise to meet the rigorous demands of the clinical laboratory for sensitivity and cost-effectiveness. New nanodiagnostics tools include quantum dots (QDs), gold nanoparticles, and cantilevers. QDs, which are the most promising nanostructures for diagnostic applications, are semiconductor nanocrystals characterized by high photostability, single-wavelength excitation, and size-tunable emission. QDs and magnetic nanoparticles can be used for barcoding of specific analytes. Gold and magnetic nanoparticles are key components of the bio-barcode assay, which has been proposed as a future alternative to the PCR.

Methods: We examined articles published over the past 10 years investigating the use of QDs, gold nanoparticles, cantilevers, and other nanotechnologies in promising diagnostic applications.

Results: Several nanodiagnostic assays have been developed, including a QD-based assay capable of detecting biotinylated prostate-specific antigen (PSA) at 0.38 ng/L, a bio-barcode assay capable of detecting 30 amol/L PSA in a 10-μL sample, and another able to detect 50 molecules of the Alzheimer marker amyloid β-derived diffusible ligand in 10 μL of cerebrospinal fluid.

Conclusions: Nanodiagnostics promise increased sensitivity, multiplexing capabilities, and reduced cost for many diagnostic applications as well as intracellular imaging. Further work is needed to fully optimize these diagnostic nanotechnologies for clinical laboratory set-uping and to address the potential health and environmental risks related to QDs.

© 2006 American Association for Clinical Chemistry

The pursuit of methods able to detect increasingly smaller amounts of biomolecules can be followed back to the mid-1970s (1–3). However, many of the advances and much of the research in the field of nanotechnology, particularly in the development of nanoparticles and optical detection methods, have taken place only during the last decade. Nanotechnology has been defined as the use of materials, devices, or systems on the nanometer (one billionth of a meter) scale (4). Nanodiagnostics, defined as the use of nanotechnology for clinical diagnostic purposes (4, 5), was developed to meet the demands of clinical diagnostics for increased sensitivity and earlier detection of disease. The increased demand for sensitivity requires that a diagnostically significant interaction occurs between analyte molecules and signal-generating particles, thus enabling detection of a single analyte molecule. Nanotechnology has enabled one-to-one interaction between analytes and signal-generating particles such as quantum dots (QDs; 3–8 nm) in the size range of proteins (1–20 nm) and other important biomolecules: i.e., the nano range (3–5).

Literature Survey

We searched the literature in the PubMed database (with the limits being articles in English and with abstracts) using the key words: nanodiagnostics OR nanotechnology and clinical diagnostics OR nanotechnology and diagnostics OR quantum dots OR quantum dots and diagnostics OR gold nanoparticles and diagnostics OR nanocantilevers OR bio-barcode assay. Our search strategy also involved going through the most recent reviews and backtracking the references used for the main nanotechnologies described. In addition, we used the reviews

1 Department of Chemistry and Science & Technology Research Center, the American University in Cairo, Cairo, Egypt.
2 Department of Pathology, Oregon Health & Science University, Portland, OR.
* Address correspondence to this author at: Department of Chemistry, Science Building 310, The American University in Cairo, 113 Kasr El-Abdin Street, Cairo 11511, Egypt. Fax 2-02-79357605; e-mail haszazy@aucegypt.edu.
Received January 9, 2006; accepted April 27, 2006.
Previously published online at DOI: 10.1373/clinchem.2006.066654

3 Nonstandard abbreviations: QD, quantum dot; PSA, prostate-specific antigen; Pgp, P-glycoprotein; BCA, bio-barcode assay; and ADDL, amyloid β-derived diffusible ligand.
to identify additional key words to search for more specific research articles. We reviewed the titles and abstracts of the articles generated by the search, and obtained the full texts of those relevant to the use of nanotechnology in clinical diagnostics. Recent reviews covering the application of nanotechnologies in diagnostics and key articles describing the properties of QDs, gold particles, and cantilevers were included. In addition, research articles were included based on the use of a nanotechnology-based assay for the detection of a new biomarker, or on the use of a nanotechnology-based assay for detection of established biomarkers with enhanced sensitivity.

**Diagnostic Nanotechnologies**

Nanoparticles possess certain size-dependent properties, particularly with respect to optical and magnetic parameters, that can be manipulated to achieve a detectable signal (1). The primary event in most nanoparticle-based assays is the binding of a nanoparticle label or probe to the target biomolecule that will produce a measurable signal characteristic of the target biomolecules. A variety of probes have been used for this purpose, including QDs, nanoshells, and metal nanoparticles (3, 6–10). A probe that is to function in a biological system must be water-soluble and stable with minimal interaction with the surrounding environment. For fluorescence readout, ideally the probe should have a high fluorescence quantum yield and minimal photobleaching rates to generate a detectable signal (11). At present, the most promising nanotechnologies for clinical diagnosis include QDs, cantilevers, and gold nanoparticles (Table 1). The characteristics of additional diagnostic nanotechnologies are summarized in Table 2.

**QUANTUM DOTS**

QDs are the most used and promising nanostructures for diagnostic applications. QDs are semiconductor nanocrystals, characterized by strong light absorbance, that can be used as fluorescent labels for biomolecules. A typical QD has a diameter of 2–8 nm (3–5) and is usually composed of a core consisting of a semiconductor material enclosed in a shell of another semiconductor material with a larger spectral band-gap (11, 12). Examples of different types of QDs and their corresponding emission wavelengths are presented in Table 3.

**Preparation of QDs.** There are several strategies for synthesizing nanocrystals with various properties depending on the required applications (13). Wang et al. (14) have developed a convenient strategy for synthesis of QDs based on general phase transfer and a separation mechanism that occurs at the liquid, solid, and solution interfaces during synthesis. A schematic diagram of the general structure of a QD is shown in Fig. 1.

QDs can be conjugated to antibodies, an oligonucleotide or aptamer, or coated with streptavidin. This helps direct the QD toward the target analyte. In addition, QDs can be used as nonspecific fluorescent labels (13).

QDs are neither water-soluble nor biocompatible, but many strategies are available to make biocompatible QDs. These include silanization or surface coating with water-soluble ligands (exchange with thiol-containing molecules or organic phosphines, dendrons, or peptides). Other methods include encapsulation with block-copolymer micelles, phospholipid micelles, polymer beads (nano or micro) or shells, or amphiphilic polysaccharides (3, 13, 15). To facilitate conjugation with avidin to detect biotinylated targets, QDs have been modified with dihydroxylic acid (3). Synthesis based on encapsulation of the nanocrystals within an amphiphilic polymer has been used to prepare CdSe/ZnS QDs, which are among the most commonly used QDs (10). Coating of the nanocrystals with mercaptoacetic acid has also been used to solubilize CdSe/ZnS QDs (12).

**Operation and detection of QDs.** When a QD absorbs a photon with energy higher than the band-gap energy of the composing semiconductor, an exciton, or electron-

---

### Table 1. Comparison of QDs, cantilevers, and gold nanoparticles.

<table>
<thead>
<tr>
<th>Feature</th>
<th>QDs</th>
<th>Cantilevers</th>
<th>Gold nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>Semiconductor nanocrystals typically composed of a core semiconductor enclosed in a shell of another semiconductor with a larger spectral band-gap; a third silica shell can be added for water solubility</td>
<td>Micro-machined silicon cantilevers similar to those used in atomic force microscopy</td>
<td>Gold particles in the nanometer size domain; gold nanoshells consist of concentric sphere nanoparticles with a dielectric core (typically gold sulfide or silica) surrounded with a thin gold shell</td>
</tr>
<tr>
<td>Applications</td>
<td>Multiplexed diagnostics; immunoassays; immunohistochemical assays; neurotransmitter detection; cellular imaging</td>
<td>Protein and DNA detection and quantification</td>
<td>Immunoassays; detection of infectious agents by DNA hybridization</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Risk of leakage of toxic core semiconductor materials into host system or into environment on disposal</td>
<td>No particular toxicity concerns</td>
<td>No particular toxicity concerns</td>
</tr>
<tr>
<td>References</td>
<td>(4, 11, 12, 19, 24)</td>
<td>(5, 20, 39)</td>
<td>(3–5, 20, 40–43)</td>
</tr>
</tbody>
</table>
hole pair, is created. As a result, a broadband absorption spectrum occurs because of the increased probability of absorption at shorter wavelengths. The return of the exciton, characterized by a long lifetime \(10\,\text{ns}\), to a lower energy state leads to emission of a photon with a narrow symmetric energy band \((13)\). QDs are reported to emit with lifetimes of \(5\)–\(40\,\text{ns}\), whereas conventional organic dyes emit in the \(0.5\)–\(2\,\text{ns}\) range \((16)\). This produces a strong, stable fluorescence signal. When the size of the nanocrystal or QD is smaller than the Bohr exciton radius, the exciton binding energy decreases, allowing for broadband emission. This property makes QDs suitable for a wide range of applications, including bioimaging and sensing.

### Table 2. Examples of diagnostic nanotechnologies.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Description</th>
<th>Advantages</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic nanoparticles and immunoassays</td>
<td>Label choice is made so that its interaction with the analyte gives a magnetic signal to be used instead of an optical one (magnetic nanoparticles); detection of label is done by magnetometer</td>
<td>High sensitivity attributable to detection of subtle modifications in magnetic character; ability to detect circulating cancer cells and microorganisms</td>
<td>((4, 5))</td>
</tr>
<tr>
<td>Nano barcodes</td>
<td>Sequential electrochemical deposition of metal ions to give submicrometer metallic barcodes whose differential reflectivity can lead to identification of the unique striping patterns by light microscopy</td>
<td>Used for multiplexed protein assays and single-nucleotide variation (SNP) mapping; does not interfere with use of fluorescence labeling</td>
<td>((4, 44))</td>
</tr>
<tr>
<td>Nanowires</td>
<td>Similar to other nanoparticles, e.g., gold and QDs, but are characterized by having different shapes, thus allowing for different interactions with different entities and more unique signals</td>
<td>Can be associated with almost any chemical or biological recognition system; allow real-time detection; analyte-independent; can be conjugated with almost any biomolecular recognition entity suitable for the use; in vivo diagnostics</td>
<td>((1, 4, 5, 20, 45))</td>
</tr>
<tr>
<td>Nanopores</td>
<td>Pores of definite size in the nanoscale range on membranes between 2 solutions; on application of current, the pores allow only nucleic acids or charged biomolecules of particular sizes to pass; these are then directly translated into electric signals on passage through the pore</td>
<td>Labelless immunoassays for flow systems; sensitive enough to detect single-base substitutions in DNA strands of the length 30 nucleotides</td>
<td>((4, 5, 20))</td>
</tr>
<tr>
<td>Nanocapacitors</td>
<td>Capacitors with electrodes spacing in the nano order, with single-stranded DNA probes; target hybridization produces a measurable change in capacitance</td>
<td>When used in arrays, nanocapacitors can allow for labelless simultaneous detection of nucleic acids</td>
<td>((20))</td>
</tr>
<tr>
<td>Nanotubes</td>
<td>Arrays of nanotubes, e.g., carbon or boron nitride, are used as electrodes with single-stranded DNA probes attached to their open end; hybridization of target DNA gives an electrochemical signal; they can also be adapted for analytes other than DNA, e.g., by attaching enzyme to detect substrate analyte</td>
<td>Detection sensitivity in the attomole range and reduction of signal-to-noise ratio; method also minimizes the need for amplification of target DNA</td>
<td>((20))</td>
</tr>
<tr>
<td>Resonance light scattering (RLS)</td>
<td>Technique uses incident collared light, which is scattered by metallic nanoparticles (gold or silver) of uniform size; this produces a highly intense fluorescence signal</td>
<td>Does not require enhancement of signal data; (1) million-fold increase in sensitivity over current methods based on fluorescence signals</td>
<td>((4, 5))</td>
</tr>
<tr>
<td>Ferrofluid magnetic nanoparticles</td>
<td>Consist of a polymeric layer of a biological substrate with a magnetic core; a type of molecule with affinity for the target analyte is coated on the outer layer; the ferrofluid particles bound to analyte are separated magnetically</td>
<td>Behave in liquid samples as solutions, not as a suspension, thus allowing close interaction with analyte; this allows development of specialized ferrofluids with high sensitivity</td>
<td>((5))</td>
</tr>
<tr>
<td>PEBBLE nanosensors</td>
<td>Probes encapsulated by biologically localized embedding (PEBBLE) nanosensors give sensor molecules ranging from 20 to 200 nm by entrapping sensor molecules in a chemically inert matrix via a microemulsion polymerization process</td>
<td>Real-time inter- and intracellular imaging of ions and molecules and insensitivity to proteins interference</td>
<td>((4))</td>
</tr>
</tbody>
</table>

### Table 3. Examples of different types of QDs and corresponding emission wavelengths.

<table>
<thead>
<tr>
<th>QD</th>
<th>Emission wavelengths</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdSe/ZnS</td>
<td>(550)–(630) nm</td>
<td>((3))</td>
</tr>
<tr>
<td>CdSe core</td>
<td>(525)–(655) nm</td>
<td>((16))</td>
</tr>
<tr>
<td>InP</td>
<td>Near-infrared</td>
<td>((3))</td>
</tr>
<tr>
<td>InAs</td>
<td>Near-infrared</td>
<td>((3))</td>
</tr>
<tr>
<td>PbS core</td>
<td>(850) and (950) nm</td>
<td>((46))</td>
</tr>
<tr>
<td>PbSe core</td>
<td>(1100, 1310, 1550, \text{ and } 1900) nm</td>
<td>((46))</td>
</tr>
</tbody>
</table>
which is defined as the natural separation distance between the positive and negative charges in the excited state of a material, the energy levels of the photon are quantized. Thus, a direct relationship exists between the QD size and the values of the quantized energy levels. This effect is called the quantum confinement effect and gives QDs their unique name (13).

Methods for tracking and detecting QDs are numerous and include fluorometry and several types of microscopy, such as fluorescence, confocal, total internal reflection, wide-field epifluorescence, atomic force, and multiphoton microscopy (3, 11–13, 15, 17). The choice of monitoring or detection technique depends on the type of application, such as in vitro diagnostics or imaging, for which QDs are used.

Features and advantages of QDs. The use of multiple QD labels enables optical barcoding of targets (5). This is made possible by choosing QD labels that have narrow emission spectra that could be spectrally isolated from one another and are excitable at the same wavelength (11). This multicolor optical label could be used as a specific barcode for a particular analyte because one light wavelength could be directed at the sample that would give a clear, distinct, and characteristic set of signals. It is now possible to achieve this by embedding QDs of different sizes into polymeric microbeads (5, 11, 18, 19). Theoretical, the use of just 10 intensity levels and 6 colors could enable coding for over $1 \times 10^6$ nucleic acids or protein sequences (18). A schematic diagram of a multiplex QD assay is shown in Fig. 2. The optical barcoding system using QDs embedded in polymeric microbeads has an accuracy of 99.9% because of the high reproducibility of the beads (1, 18, 19).

The advantages of QD over conventional organic dyes and fluorophores is their optical tunability, resistance to photobleaching, excitation of various QDs by a single wavelength of light (for multiplexing), narrow emission band, and exceptional stability of optical properties after conjugation to a biomolecule (3, 11, 12, 20). Currently, up to 40 000 assays can be run simultaneously with the current capabilities of the QD technology (5, 19). QDs very appealing for diagnostic applications because they do not need lasers for excitation and because the instrumentation needed for detection is simple, e.g., fluorometers and fluorescence microscopes (4).

The use of QD labels for DNA detection would help overcome 2 of the most important problems encountered when using organic dyes for DNA labeling; cleavage of DNA molecules as a result of photobleaching and subsequent formation of free radicals, and disturbing DNA–protein interactions. QDs circumvent these problems and also allow 2-color determination of the orientation of a single DNA molecule. This has been achieved by the use of DNA labeled with biotin and/or digoxigen at both ends of linear DNA molecules (17). Labeled DNA molecules are combed on glass surfaces and detected by use of streptavidin- or antibody-conjugated QDs and fluorescence microscopy (17).

Despite the high potential of QDs, there are some possible technical difficulties in their application, as well as considerable safety concerns. Semiconductor nanocrystals or QDs may be subject to reduced luminescence activity because of their relatively large surface areas (11).

Early problems with use of QDs included reproducibility during manufacturing and quenching in solution (from molecular collisions). Most of these problems have been overcome, but others may arise as new QDs are developed. Questions have also been raised regarding the
ability of QDs to reach targets within cellular compartments or multicomponent molecular complexes (20). This problem will impact the sensitivity of the technique. An considerable technical problem is also posed by an inherent property of CdSe/ZnS QDs; they do not emit in the near-infrared region. As a result, they cannot be used for whole blood analysis, however, they can be used with serum and other body fluids (5).

**Assays.** Examples of assays that use QDs with different recognition moieties are given in Table 4. QDs are at the core of a fluoroimmunoassay introduced by Härma et al. (21) for the detection of prostate-specific antigen (PSA). Their assay used 107-nm QDs consisting of β-diketones entrapping europium molecules (>30 000 molecules) and coated with streptavidin. The assay had a detection limit of 0.38 ng/L for biotinylated PSA and used a time-resolved fluorometer for signal detection. PSA detection was achieved in both solid and liquid phases, and visualization of individual PSA molecules was also possible with use of a fluorescence microscope (21).

QDs have also recently been used to optically detect acetylcholine (22). Water-soluble CdSe/ZnS QDs, the surfaces of which were modified with tetrahydroxyl ether derivatives of p-sulfonatocalix[4]arene, were used for this first successful detection of a neurotransmitter by use of QDs (22).

QDs have also been conjugated to antibodies and used to target the membrane protein P-glycoprotein (Pgp) in HeLa cells that had been transiently transfected by a plasmid encoding for Pgp. The conjugation process was done in 2 different ways. The first method involved biotinylation of the Pgp primary antibody and allowing it to bind to avidin-coated QDs. The second method used an engineered adaptor protein with affinity for the Fc region of the Pgp antibody and electrostatically attracted to the charged QDs. Both approaches provided specific binding of QDs to the transfected cells and gave a clear fluorescent signal (13).

Peptides have also been conjugated to QDs, as exemplified by the work of Akerman et al. (23). These investigators conjugated 3 different peptides to QDs to target lung endothelial cells, brain endothelial cells, and breast carcinoma cells, both in vitro and in vivo. Microinjection of QDs conjugated to suitable peptides can also target cellular organelles such as the nucleus or mitochondria (13).

Another promising area for application of QDs is the detection of cancer. An assay has been developed for detection of the receptor Her2 (hairy-related 2) on SK-BR-3 breast cancer cells (24). The assay used an humanized anti-Her2 antibody that bound to the Her2 marker and a biotinylated goat anti-human IgG secondary antibody. Streptavidin-coated QDs detected the marker Her2 via the biotinylated secondary antibody (13, 24). This combination was more successful than use of either QD–streptavidin conjugates (very weak signal) or QD–IgG alone (24). Similarly, QD–streptavidin conjugates have been used for the successful detection of intracellular targets, including microtubules in mouse 3T3 fibroblasts, nuclear antigens, and F-actin filaments. As reported by Wu et al. (24), the specificity of the QDs and their emission spectra enabled the detection of 2 cellular targets with a single excitation wavelength. These investigators used QD–IgG (emission maximum at 555 nm) and QD–streptavidin (emission maximum at 630 nm) to detect Her2 and nuclear antigens in SK-BR-3 cancer cells, respectively.

**Imaging applications.** QDs have applications in cellular imaging. Antibody-coupled QDs injected into the tail veins of mice were successful in detecting prostate cancer xenografts. The spectral image of the mice clearly showed the location of the target-bound QDs (13). A more generalized imaging application was described by Larson et al. (15), who injected water-soluble QDs into mice to image skin and adipose tissues. Despite the fact that both of these tissues have high light-scattering properties, the vasculatures containing QDs at ~1 μmol/L were clearly visible by multiphoton microscopy (13, 15). Another potential use for QDs is angiography. The high photostability of QDs is a primary reason for their high potential in imaging applications. Fluorescence correlation spectroscopy indicated stability of over 9 months for water-soluble QDs (15). A recently described genotyping method using QDs is reported to detect zeptomoles of targets (25).

**GOLD NANO PARTICLES**

Gold nanoparticles and gold nanoshells provide great sensitivity for the detection of DNA and proteins (1, 3, 5, 20). They can be used to label DNA or protein molecules (including antibodies), which can then bind to their respective targets.

Surface plasmon resonance is an optical technique that measures the refractive index of very thin layers of

---

**Table 4. Selected QD-based assays.**

<table>
<thead>
<tr>
<th>Recognition moiety conjugated to QD</th>
<th>Target analyte</th>
<th>Comments</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptavidin</td>
<td>Biotinylated PSA</td>
<td>Detection limit = 0.38 ng/L</td>
<td>(21)</td>
</tr>
<tr>
<td>Oligonucleotide probes</td>
<td>DNA target</td>
<td>Successful (60%)</td>
<td>(17)</td>
</tr>
<tr>
<td>p-Sulfonatocalix[4]arene</td>
<td>Acetylcholine</td>
<td>Successful</td>
<td>(22)</td>
</tr>
<tr>
<td>Streptavidin</td>
<td>Her2 breast cancer marker</td>
<td>Successful</td>
<td>(13, 24)</td>
</tr>
<tr>
<td>Primary antibodies</td>
<td>Pgp</td>
<td>Successful</td>
<td>(13)</td>
</tr>
</tbody>
</table>
material adsorbed on a metal. It offers real-time in situ analysis of dynamic surface events and is capable of defining rates of adsorption and desorption for surface interactions (26). Plasmon–plasmon resonance, resulting from the interaction of locally adjacent gold nanoparticle labels that have bound to a target, produces changes in optical properties that can be used for detection. It is known that the characteristic red color of gold colloid changes to a bluish-purple color on colloid aggregation because of this effect (3).

Silver can be involved in the visualization process, with Raman spectroscopy as a favored detection method (27, 28). Gold nanoparticles can be coated with silver shells; silver-coated gold particles (40–100 nm in size) have strong light-scattering properties and can easily be detected by standard dark-field microscopy with white light illumination (3, 29). The technique takes the detection limit for oligonucleotides down to ~10 fmol; 50-fold lower than conventional fluorophore-based methods (1, 3, 5, 20). Gold nanoshells could allow direct, rapid, and economically feasible analysis of whole blood samples. These nanoshells consist of concentric spherical nanoparticles with a dielectric core, usually consisting of gold sulfide or silica, surrounded by a thin gold shell. Variations in the relative thicknesses of the core and outer shell allow the optical resonance of gold to go into the mid-infrared region. Further manipulation of the surface properties can be used to expand the absorption wavelength range to the near-infrared, just above the absorption of hemoglobin and below that of water (3, 6–10). This feature helps avoid interference from hemoglobin and allows direct analysis of whole blood. An additional advantage of nanoshells is their exceptional biocompatibility, as their behavior is almost identical to that of gold colloids. These properties of nanoshells, along with the tunability (change with nanoparticle size) of their optical properties, could allow for development of immunoassays capable of simultaneous analysis of multiple antigens (3, 10).

**Assays**

**PSA assay.** The bio-barcode assay (BCA) has been used for the detection of proteins such as PSA, which is an important marker for prostate and breast cancers (30). The BCA assay uses 2 different probes. The first is a magnetic microparticle conjugated to a PSA monoclonal antibody, and the second probe is a gold nanoparticle to which a PSA polyclonal antibody and “barcode” oligonucleotides are attached. In this assay, PSA becomes sandwiched between the 2 antibodies conjugated to the 2 probes. The antigen-containing complex is separated magnetically from the rest of the mixture via the magnetic microparticle. The magnetic field draws the unbound magnetic microparticles to the walls of the container, leaving behind only those particles that were involved in the detection. Washing of the separated complex then dehybridizes the barcode oligonucleotides from the nanoparticle. The free oligonucleotides act as reporters for the target of interest and enable the detection of PSA. The barcode is detected by conventional DNA detection methods or by sensitive silver amplification (27). The sensitivity is in the attomolar range, and some assays have detected PSA at concentrations of 30 amol/L in a 10-μL sample (30). This is a rapid assay, and the sensitivity may also be increased by manipulating the equilibrium of the reaction by changing the concentration of the magnetic microparticle probe. In addition, the conjugation steps involved are relatively easy to achieve compared with directly attaching DNA to antibodies. The multiplexing potential is limited only by the number of antibodies that can be directed against different antigen analytes (30). However, this type of assay may be too sensitive for practical clinical use. Further signal amplification by PCR can enhance the detection limit down to ~3 amol/L (20), but this would significantly increase the time needed to perform the assay.

**Alzheimer BCA.** BCA has also been used to successfully detect a biomarker for Alzheimer disease (31–33). In a study by Georganopoulou et al. (29), the BCA was used to determine the concentration of the biomarker, amyloid β-derived diffusible ligands (ADDLs), in cerebrospinal fluid. These investigators used ADDL oligomer-specific monoclonal and polyclonal antibodies. The assay was able to detect as few as 50 ADDL molecules in 10-μL aliquots of cerebrospinal fluid. Signal was detected by a scanometric method of light scattering using silver-amplified gold particles. Initially, this biomarker was difficult to detect because of its low concentrations outside the brain, where ADDLs are most prevalent. The assay, which is one million-fold more sensitive than the current ELISA technique, could be developed for the measurement of ADDLs in blood, thus providing an early diagnostic tool for Alzheimer disease (4, 29, 31).

**BCA as a possible alternative for PCR.** BCA uses 2 sets of oligonucleotides for DNA detection: one set is bound to magnetic microparticles, and the other set is bound to gold nanoparticles. Both sets of oligonucleotides bind to the complementary target DNA sequence in the sample, forming a sandwich complex. Thousands of identical DNA strands attached to the gold nanoparticles act as a unique barcode. After hybridization with the target, the barcode DNA is dehybridized from the gold nanoparticle, thus generating the signal. The magnetic particle is used to remove the target DNA sandwich from the medium. Barcodes can then be detected by silver amplification and a scanometric method. The method allows detection of target DNA at concentrations as low as 500 amol/L and is thus a strong challenger to PCR (30, 34). Unlike QDs, gold nanoparticles do not compromise environmental safety. Also unlike PCR, the target DNA is not amplified; therefore, the risk of contamination is nearly eliminated.
CANTILEVERS
Cantilevers, small beams similar to those used in atomic force microscopy, function by use of nanomechanical deflections. The micromachined silicon cantilevers are used to monitor molecular events such as DNA hybridization. For DNA detection, the cantilever surface holds a particular DNA sequence capable of binding to a specific target (4, 5). The cantilever scans the sample, and when the target DNA sequence is found, hybridization occurs with the cantilever single-stranded DNA. This places mechanical stress on the beam, which subsequently deflects. The binding event upsets the balance, with the resulting nanodeflection proportional to the amount of DNA hybridized; an optical signal is then generated. This technique can be used as a microarray, allowing for multiple analyses. In addition, cantilever arrays can detect molecular targets without the targets being labeled. However, this homogeneous method needs further development to solve the problem of nonspecific binding (5, 20). Early work with cantilever-based assays used cantilevers with micrometer dimensions (5, 20). New fabrication methods have enabled production and characterization of cantilevers with nanometer dimensions (35, 36).

Assays. Cantilevers have also been used for the detection of PSA. A single-reaction test was successful in detecting PSA among other proteins (5). Cantilevers may also be useful for the detection of microorganisms such as Salmonella enterica (4). This pathogen was successfully detected in situ by use of a silicon nitride cantilever. As few as 25 organisms should suffice for successful detection, as indicated by scanning electron micrographs. Cantilever arrays may also be used for the detection of cardiac troponins, which are well-established biomarkers of myocardial damage. In addition, this assay technique could be used for breath analysis to detect acetone and dimethylamine (4).

Why Nanodiagnostics?
The potential of nanodiagnostics arises from the fact that most biological molecules and cell organelles fall within the nanometer scale. For example, the typical protein has a size of 5 nm. The nanoparticles used for detection can be synthesized to be within this same size domain (1, 3). Current diagnostic methods measure or determine a particular property as an average of the individual contributions of an ensemble of units or particles. Thus, such methods do not convey any information about the individual members in a heterogeneous population or about the lifespans of the various members within the population (2). The fact that most nanotechnology-based assays are amenable to automation and do not require a separation step definitely adds to their appeal for clinical scientists. What the field of nanodiagnostics has to offer to improve clinical diagnostics is mainly increased sensitivity and faster detection. As nanotechnology evolves to include automation and economies of scale, the cost of this diagnostic testing process could become comparable to or lower than those of other testing modalities (2, 5, 12, 21).

The Price of the QD Promise
The most eminent problem regarding QDs is toxicity. The fact that QDs have basic components that are highly toxic to humans, e.g., cadmium, raises serious safety issues. Many of the studies mentioned previously used...
QDs with concentrations just high enough to achieve optimal labeling, and the duration of use ranged from several hours to several days. The authors of these studies found no adverse effects on cellular functioning or development during the study. However, at higher QD concentrations (>5 × 10³ QDs/cell), Xenopus embryo development was affected (13, 37). Toxicity findings from studies using QDs are summarized in Table 5. For in vivo studies, the question that remains is whether the surface coating, assuming one is used, of the QD will be sufficiently robust to guard against leakage of the core metals and semiconductors into the biological system being studied?

The same factors that lead to questions concerning the safety of QDs and other nanostructures for use in humans lead to questions of environmental safety. Specifically, can QDs be stored and disposed of without leakage of the toxic metals used? In addition, if an organism is exposed to the toxic agents of certain nanostructures, how can we be assured that the toxic agents will not accumulate within the ecosystem, even if the organism itself did not experience the adverse consequences of the exposure?

Conclusions

The promise of increased sensitivity and speed and reduced cost and labor makes nanodiagnostics an appealing alternative to current diagnostic techniques. The potential diagnostic uses of QDs are numerous, with the most promising applications being in the areas of tumor detection, tissue imaging, intracellular imaging, immunohistochemistry, infectious agent detection, multiplexed diagnostics, and fluoroimmunoassays. QDs also have considerable potential for in vivo imaging, but there are concerns over their toxicity, both to patients and the environment. The technologies are numerous and the applications are constantly increasing, with QDs, gold nanoparticles, and BCAs in the lead. The numerous types of nanoparticles differ in shape and properties, which could be made use of for specific diagnostic applications. PCR could soon lose its lead position as the gold standard for DNA detection to BCA, which offers increased sensitivity and safety. Cantilevers may also challenge PCR, but to a much lesser extent than BCA. In time, nanodiagnostics may become very cost-effective, as is currently the case with some magnetic nanoparticles. This should allow better clinical diagnostic services, particularly in economically deprived regions. These technologies can also be applied to point-of-care testing and lab-on-a-chip technologies (5, 38). Whether nanodiagnostics will replace current diagnostic methods remains to be seen. Many aspects of these nanodiagnostic techniques need to be evaluated further, especially the safety issues. However, the advantages that these new technologies offer are too good to dismiss.

References


