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Applications of Nanobiotechnology in Clinical Diagnostics

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Background: **Nanobiotechnologies are being applied to molecular diagnostics and several technologies are in development.**
Methods: **This review describes nanobiotechnologies that are already incorporated in molecular diagnostics or have potential applications in clinical diagnosis. Selected promising technologies from published literature as well as some technologies that are in commercial development but have not been reported are included.**
Results: **Nanotechnologies enable diagnosis at the single-cell and molecule levels, and some can be incorporated in current molecular diagnostic methods, such as biochips. Nanoparticles, such as gold nanoparticles and quantum dots, are the most widely used, but various other nanotechnological devices for manipulation at the nanoscale as well as nanobiosensors are also promising for potential clinical applications.**
Conclusions: **Nanotechnologies will extend the limits of current molecular diagnostics and enable point-of-care diagnostics, integration of diagnostics with therapeutics, and development of personalized medicine. Although the potential diagnostic applications are unlimited, the most important current applications are foreseen in the areas of biomarker discovery, cancer diagnosis, and detection of infectious microorganisms. Safety studies are needed for in vivo use. Because of its close interrelationships with other technologies, nanobiotechnology in clinical diagnosis will play an important role in the development of nanomedicine in the future.**

Nanomolecular diagnostics, the use of nanobiotechnology in molecular diagnostics *(1)*, and nanobiotechnology, the use of various nanotechnologies and their applications in life sciences *(2)* offer new options for clinical diagnostic procedures. No recognized classification system for nanodiagnostics is currently in use, but a proposed system with the main categories of technologies is shown in Table 1. Further descriptions of some of these categories are provided in subsequent sections of this review. Nanoparticle biolabels are listed in Table 2 and are described in detail elsewhere *(3)*. Molecular diagnostics is an essential part of the development of personalized medicine, which features point-of-care performance of diagnostic procedures. This report focuses on the application of these technologies in the clinical laboratory setting. Interrelationships of nanotechnology and molecular diagnostics and their role in nanomedicine as well as personalized medicine are shown schematically in Fig. 1.

Nanoscale probes are suitable for detailed analysis of receptors, pores, and other components of living cells that are on a nanoscale. Thus nanotechnology can be used to improve PCR as well as provide non-PCR methods for rapid diagnostics. Advantages of applying nanotechnology to molecular diagnostics are that only small amounts of sample material are needed and that diagnostic tests that use nanoscale particles as tags or labels are faster and more sensitive *(4)*. Nanoparticles can also be used to combine diagnostics with therapeutics.

Nanotechnology-Based Biochips and Microarrays

Nanotechnology on a chip is a new paradigm for total chemical analysis systems *(5)*. The ability to make chemical and biological information easier and less costly to obtain will impact molecular diagnostics and healthcare. Some examples of devices that incorporate nanotechnology-based biochips and microarrays are nanofluidic arrays and protein nanobiochips. These devices can be adapted for point-of-care use.

One of the more promising uses of nanofluidic devices is isolation and analysis of individual biomolecules, such as DNA. This capability could lead to new detection schemes for cancer. One such device entails the construction of silicon nanowires on a substrate, or chip, using standard photolithographic and etching techniques, followed by a chemical oxidation step that converts the

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Table 1. Classification of categories of nanodiagnostic technologies.

Nanoscale visualization, e.g., atomic force microscopy, scanning probe microscopy
Nanoparticle biolabels
Nanotechnology-based biochips/microarrays
Nanoparticle-based nucleic acid diagnostics
Nanoproteomic-based diagnostics
Biobarcode assays
Nanopore technology
DNA nanomachines for molecular diagnostics
Nanoparticle-based immunoassays
Nanobiosensors
Combinations of multiple diagnostic technologies

nanowires into hollow nanotubes *(6)*. With this process, the investigators can reliably create nanotubes with diameters as small as 10 nm, although devices used for biomolecule isolation contain nanotubes with diameters of 50 nm. Trapping DNA molecules requires a device consisting of a silicon nanotube connecting 2 parallel microfluidic channels. Electrodes provide a source of current used to drive DNA into the nanotubes. Each time a single DNA molecule moves into the nanotube, the electrical current suddenly changes. The current returns to its baseline value when the DNA molecule exits the nanotube. Nanofluidic technology is expected to have broad applications in systems biology, personalized medicine, pathogen detection, drug development, and clinical research.

Protein microarrays for the study of protein function are not widely used, in part because of the challenges in producing proteins to spot on the arrays. Protein microarrays can be generated by printing complementary DNAs onto glass slides and then translating target proteins with mammalian reticulocyte lysate *(7)*. Epitope tags fused to the proteins allow them to be immobilized in situ. This procedure obviates the need to purify proteins, avoids protein stability problems during storage, and captures sufficient protein for functional studies. This technology has been used to map pairwise interactions among 29 human DNA replication initiation proteins and to recapitulate the regulation of Cdt1 binding to select replication proteins and map the geminin-binding domain.

Table 2. Nanoparticle biolabels.

Quantum dots as labels
Silver nanoparticle labels
Silica nanoparticles for labeling antibodies
SERS nanotags
DNA nanotags
Fluorescent lanthanide nanorods
Perfluorocarbon nanoparticles
Organic nanoparticles as biolabels

Nanotechnology-Based Cytogenetics

Cytogenetics has been used mainly to describe the chromosome structure and identify abnormalities related to disease. The localization of specific gene probes by fluorescent in situ hybridization (FISH)[2] combined with conventional fluorescence microscopy has reached its limit. Molecular cytogenetics in now enhanced by use of nanobiotechnology, e.g., atomic force microscopy and quantum dot (QD) FISH.

Both atomic force microscopy and scanning near-field optical microscopy have been used to obtain local information from G-bands and chromosomal probes. The final resolution allows a more precise localization compared with standard techniques, and the extraction of very small amounts of chromosomal DNA by the scanning probe is possible. This method is also focused on the combination of biochemical and nanomanipulation techniques, which enable both nanodissection and nanoextraction of chromosomal DNA.

The photostability and narrow emission spectra of nonorganic QD fluorophores make them desirable candidates for the use of FISH to study the expression of specific mRNA transcripts. A method for direct QD labeling of modified oligonucleotide probes using streptavidin and biotin interactions increased sensitivity of multiple-label FISH *(8)*. This technique also gives excellent histological results for FISH combined with immunohistochemistry.

QD's broad absorption spectra allowed different colored probes specific for distinct subnuclear genetic sequences to be simultaneously excited with a single excitation wavelength and imaged free of chromatic aberrations in a single exposure. A rapid method for the direct multicolor imaging of multiple subnuclear genetic sequences uses novel QD-based FISH. A Texas red dye gamma-satellite probe produces fluorescent foci at the periphery of interphase nucleus and labels every centromere in metaphase chromosomes *(9)*.

Application of Nanoparticles for Tracking Stem Cells

A superparamagnetic iron oxide (SPIO) nanoparticle is emerging as an ideal probe for noninvasive cell tracking. However, its low intracellular labeling efficiency has limited its use and stimulated interest in the development of new labeling strategies.

The use of 200-nm perfluorocarbon nanoparticles to label endothelial progenitor cells taken from human umbilical cord blood enables in vivo progenitor cell detection by MRI *(10)*. The MRI scanner can be tuned to the specific frequency of the fluorine compound in the nanoparticles, and only the nanoparticle-containing cells are visible in

[2] Nonstandard abbreviations: FISH, fluorescent in situ hybridization; QD, quantum dot; SPIO, superparamagnetic iron oxide; RSV respiratory syncytial virus; GMA, glycidyl methacrylate; SPR, surface plasmon resonance; SERS, surface enhanced Raman scattering.

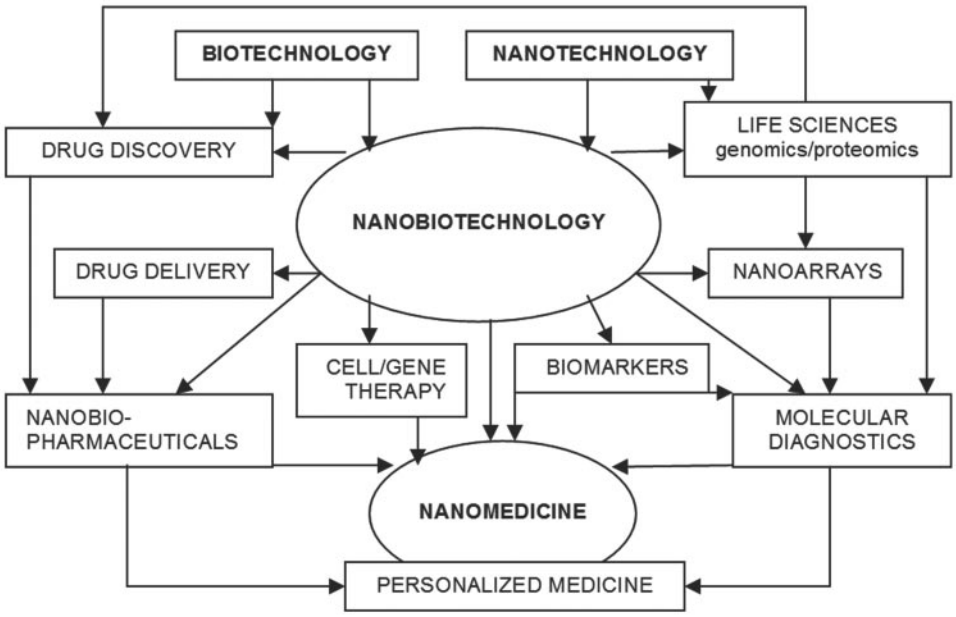

Fig. 1. This scheme shows the interrelationship of various technologies that contribute to clinical nanodiagnostics.

These technologies also contribute to development of nanomedicine under the concept of personalized medicine.

the scan. This method eliminates any background signals, which often interfere with medical imaging. Moreover, the lack of interference enables measurement of very low amounts of the labeled cells and estimation of their number on the basis of the brightness of the image. Because several perfluorocarbon compounds are available, different types of cells could be labeled with different compounds, injected, and then detected separately by tuning the MRI scanner to the individual frequency of each cell type. This technology offers significant advantages over other cell-labeling technologies in development. Laboratory tests showed that the cells retained their usual surface markers and that they were still functional after the labeling process. The labeled cells were shown to migrate to and incorporate into blood vessels forming around tumors in mice. These methods could soon enable researchers and physicians to use unique signatures from the ingested nanoparticle beacons to directly track cells used in medical treatments. Such tracking ability could prove useful for monitoring tumors and diagnosing as well as treating cardiovascular problems.

Nanoscale Single-Cell or Molecule Identification

Nanotechnology has facilitated the development of methods for detection of single cells or a few molecules. Nanolaser scanning confocal spectroscopy, with the capability of single-cell resolution, can be used to identify previously unknown properties of certain cancer cells that distinguishes them from closely related nonpathogenic cells *(11)*. Nanoproteomics, the application of nanobiotechnology to proteomics, can enable detection of a single molecule of protein *(2)*. Biobarcode assays enable detection in body fluids of miniscule amounts of proteins that cannot be detected by conventional methods *(12)*. A 2-dimensional method for mass spectrometry in solution is based on the interaction between a nanometer-scale pore and analytes *(13)*. An applied electric current is used to force charged molecules (such as single-stranded DNA) one at a time into the nanopore, which is only 1.5 nm at its smallest point. As the molecules pass through the channel, the current flow is reduced in proportion to the size of each individual chain, allowing its mass to be easily derived. This single-molecule analysis technique could prove useful for the real-time characterization of biomarkers.

Application of Nanoparticles for Discovery of Biomarkers

Currently available molecular diagnostic technologies have been used to detect biomarkers of various diseases. Nanotechnology has refined the detection of biomarkers. Some biomarkers also form the basis of innovative molecular diagnostic tests. The physicochemical characteristics and high surface areas of nanoparticles make them ideal candidates for developing biomarker-harvesting platforms. Given the variety of nanoparticle technologies that are available, it is feasible to tailor nanoparticle surfaces to selectively bind a subset of biomarkers and sequester them for later study using high-sensitivity proteomic tests *(14)*. Biomarker harvesting is an underutilized application of nanoparticle technology and is likely to undergo substantial growth. Functional polymer-coated nanoparticles can be used for quick detection of biomarkers and DNA separation.

Nanoparticles for Molecular Diagnostics

Several nanoparticles have been used for diagnostics. Of these, the most frequently used are gold nanoparticles, QDs, and magnetic nanoparticles.

Gold Nanoparticles for Diagnostics

Small pieces of DNA can be attached to gold particles no larger than 13 nm in diameter. The gold nanoparticles assemble onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously.

The current nonoptimized detection limit of this method is 20 fmol/L. Gold nanoparticles are particularly good labels for sensors because a variety of analytical techniques can be used to detect them.

QDs

QDs are inorganic fluorophores that offer significant advantages over conventionally used fluorescent markers. They have high sensitivity, broad excitation spectra, stable fluorescence with simple excitation, and no need for lasers. Their red/infrared colors enable whole blood assays. QDs have a wide range of applications for molecular diagnostics and genotyping. QDs also enable multiplexed diagnostics and integration of diagnostics with therapeutics.

The most important potential applications of QDs are for cancer diagnosis. Luminescent and stable QD bioconjugates enable visualization of cancer cells in living animals. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals. QDs have been coated with a polyacrylate cap and covalently linked to antibodies for immunofluorescent labeling of breast cancer marker Her2. Carbohydrate-encapsulated QDs with detectable luminescent properties are useful for imaging of cancer.

Another application of QDs is for viral diagnosis. Rapid and sensitive diagnosis of respiratory syncytial virus (RSV) is important for infection control and development of antiviral drugs. Antibody-conjugated nanoparticles rapidly and sensitively detect RSV and estimate relative levels of surface protein expression *(15)*. A major development is the use of dual-color QDs or fluorescence energy transfer nanobeads that can be simultaneously excited with a single light source. A QD system can detect the presence of particles of the RSV in a matter of hours. It is also more sensitive, allowing detection of the virus earlier in the course of an infection *(16)*. When an RSV virus infects lung cells, it leaves part of its coat containing F and G proteins on the cell's surface. QDs have been linked to antibodies keyed to structures unique to the RSV coat. As a result, when QDs come in contact with either viral particles or infected cells they stick to their surface. In addition, colocalization of these viral proteins was shown using confocal microscopy.

Magnetic Nanoparticles

Iron nanoparticles, 15–20 nm in size and having saturation magnetization, have been synthesized and embedded in copolymer beads of styrene and glycidyl methacrylate (GMA), which were coated with polyGMA by seed polymerization *(17)*. The resulting Fe/St-GMA/GMA beads had diameters of 100–200 nm. Coating with polyGMA changed the zeta potential of the beads from −93.7 to −54.8 mV, as measured by an electrophoresis method. As revealed by gel electrophoresis, this process facilitates nonspecific protein adsorption suppression, which is a requisite for nanoparticles to be applied to carriers for bioscreening.

Nanoparticles are used as labeling molecules for bioscreening. Superparamagnetic nanoparticles are useful for cell-tracking cells and for calcium sensing. Ferrofluids (Immunicon's CellTracks™ Technology) consist of a magnetic core surrounded by a polymeric layer coated with antibodies for capturing cells. A family of calcium indicators for MRI is formed by combining a powerful SPIO nanoparticle-based contrast mechanism with the versatile calcium-sensing protein calmodulin and its targets *(18)*.

Superparamagnetic nanoparticles measuring 2–3 nm have been used in conjunction with MRI to reveal small and otherwise undetectable lymph-node metastases. Ultrasmall SPIO enhances MRI for imaging cerebral ischemic lesions. A dextran-coated iron oxide nanoparticle enhances MRI visualization of intracranial tumors for more than 24 h.

Safety Issues of Nanoparticles for Diagnostics

Potential toxic effects are a concern with in vivo use of nanoparticles but not with in vitro diagnostics, which forms the major portion of laboratory diagnostics. There are environmental concerns about the release of nanoparticles during manufacturing of nanoparticles and the environmental effects. These are being studied along with naturally present nanoparticles in the atmosphere.

There are still many unanswered questions about the fate of nanoparticles introduced into the living body. Because of the huge diversity of materials used and the wide range in size of nanoparticles, these effects will vary considerably. QDs made with fluorescent labels of calcium selenide or zinc sulfide to increase stability may release potentially toxic cadmium and zinc ions into cells. Capping QDs with ZnO effectively prevents Cd^{2+} formation on exposure to air but not to ultraviolet radiation, and the search for better coating materials is ongoing. A high-throughput gene expression test determined that specially coated QD fluorescent nanoprobes affect only 0.2% of the human genome, dispelling the concern that the mere presence of these potentially toxic sentinels disrupts cell function *(19)*.

It is conceivable that particular sizes of some materials may have a bearing on toxic effects. A number of studies have been done, but at this stage, no conclusions can be drawn about the safety of nanoparticles. Concern centers around nanoparticles smaller than 20 nm in diameter, which can penetrate the cells. One limitation for the approval of in vivo nanomaterials for human diagnostics would be that demonstration of safety of nanoparticles would be required.

Nanobiosensors

Nanobiosensors are nanosensors used for detection of chemical or biological materials. Nanomaterials are exquisitely sensitive chemical and biological sensors *(20)*. A classification of nanobiosensors is shown in Table 3.

These sensors can be electronically gated to respond to the binding of a single molecule. Prototype sensors have demonstrated detection of nucleic acids, proteins, and ions. These sensors can operate in the liquid or gas phase, opening up an enormous variety of downstream applications. The detection schemes use inexpensive low-voltage measurement schemes and detect binding events directly, so there is no need for costly, complicated, and time-consuming labeling chemistries such as fluorescent dyes or the use of bulky and expensive optical detection systems. As a result, these sensors are inexpensive to manufacture and are portable. It may even be possible to develop implantable detection and monitoring devices on the basis of these detectors.

Cantilever Biosensors

Cantilevers transform a reaction into a mechanical motion on the nanometer scale, approximately 10 nm, which can be measured directly by deflecting a light beam from the cantilever surface. Cantilever technology provides an alternative to PCR and complements current DNA and protein microarray methods. There is no need to label or copy the target molecules. The advantages of cantilevers are that they provide fast, label-free recognition of specific DNA sequences for single-nucleotide polymorphisms, oncogenes, and genotyping. Nanocantilevers could be crucial in designing a new class of ultrasmall sensors for detecting viruses, bacteria, and other pathogens *(21)*. Finally, a real-time cantilever biosensor can provide continuous monitoring of clinical parameters in personalized medicine.

Nanosensors Based on Surface-Enhanced Raman Scattering

Surface plasmon resonance (SPR) technology is the best-known example of optical biosensors. Optical-detectable tags can be formed by surface enhanced Raman scattering (SERS) of active molecules at the glass-metal interface. Each type of tag exploits the Raman spectrum of a different small molecule. SERS bands are 1/50 the width of fluorescent bands and enable a greater degree of multiplexing than current fluorescence-based quantification tags. The spectral intensity of SERS-based tags is linearly proportional to the number of particles, allowing these tags to be used for multiplexed analyte quantification. Because SERS-based tags are coated with glass, attachment to biomolecules is straightforward, and the tags can be detected with low-cost instrumentation. The particles can be interrogated in the near-infrared range, enabling detection in blood and other tissues. Another advantage of these particles is that they are stable and are resistant to photodegradation. Nanoplex Biotags (Oxonica) enable measurement of up to 20 biomarkers in a single test without interference from biological matrices such as whole blood.

A variety of sensors, metallic nanostructured probes, metallic nanoshells and half-shells, and nanoarrays for SERS sensing have been developed at the Oak Ridge National Laboratory. The SERS technology can directly detect chemical agents and biological species (e.g., spores, biomarkers of pathogenic agents). A DNA-based technique based on surface-enhanced Raman gene (SERGen) probes can also be used to detect gene targets via hybridization to DNA sequences complementary to these probes. Advanced instrumental systems designed for spectral measurements and for multiarray imaging as well as for field monitoring (RAMiTS technology) have been constructed. Plasmonics and SERS nanoprobes are useful for biological sensing.

Table 3. Nanobiosensors.

Electronic nanobiosensors
Electrochemical nanobiosensors
Ion channel switch biosensor technology
Nanowire biosensors
Cantilevers as biosensors
Carbon nanotube biosensors
FRET-based DNA nanosensor
Optical biosensors using laser, nanoshell, SPR, SERS, mRNA
PEBBLE (Probes Encapsulated by Biologically Localized Embedding)
Quartz nanobalance DNA sensor
Viral nanosensor

Viral Nanobiosensors

Virus particles are biological nanoparticles. Herpes simplex virus and adenovirus can be used to trigger the assembly of magnetic nanobeads as nanosensors for clinically relevant viruses *(22)*. It is possible to detect as few as 5 viral particles in a 10-mL serum sample. This system is more sensitive than ELISA-based methods and is an improvement over PCR-based detection because it is cheaper and faster and has fewer artifacts.

Future Issues in the Development of Nanobiosensors

New biosensors and biosensor arrays are being developed using new materials, nanomaterials, and microfabricated materials and new methods of patterning. Biosensor components will use nanofabrication technologies. Nanosized devices can be produced by use of nanotubes, fullerenes (buckyballs), and silica and its derivatives. Some of the challenges will be development of real-time noninvasive technologies that can be applied to detection and quantification of biological fluids without the need for multiple calibrations using clinical samples.

It would be desirable to develop multiple integrated biosensor systems that use doped oxides, polymers, enzymes, or other components to give the system the required specificity. Such integrated sensor systems

would include all of the sensor components, software, plumbing, and reagents along with sample processing. There is also a need for reliable fluid handling systems for "dirty" fluids and for relatively small quantities of fluids (nanoliter to attoliter quantities). These should be low cost, disposable, reliable, and easy to use as part of an integrated sensor system. Sensing in picoliter to attoliter volumes might create new problems in the development of microreactors for sensing and novel phenomena in very small channels.

Clinical Applications of Nanodiagnostics

Some of the clinical applications of nanodiagnostics are mentioned along with technologies. This report briefly describes a few examples of the use of nanodiagnostics for diagnosis of cancer, infections, and neurological disorders. More detailed descriptions can be found in a handbook on nanomedicine *(23)*.

Applications of Nanodiagnostics in Management of Cancer

Nanoparticles can be designed for dual-mode imaging of cancer. The best characteristics of QDs and magnetic iron oxide nanoparticles can be combined to create a single nanoparticle probe that can yield clinically useful images of both tumors and the molecules involved in cancer *(24)*. Silica nanoparticles, approximately 30 nm in size, are impregnated with rhodamine, a bright fluorescent dye, and 9-nm diameter water-soluble iron oxide nanoparticles. The resulting combination of nanoparticles is approximately 45 nm in diameter and performs better in both MRI and fluorescent imaging tests than any of the individual components. An antibody that binds to polysialic acid molecules found on the surface of lung tumors is attached to these nanoparticles, which are quickly taken up by cultured tumor cells and can be visualized with fluorescence microscopy.

Bioconjugated QDs, collections of differently sized nanoparticles embedded in tiny polymer beads, provide a new class of biological labels for evaluating biomarkers on intact cells and tissue specimens. In particular, the use of multicolor QD probes in immunohistochemistry is considered one of the most important and clinically relevant applications. The medical use of QD-based immunohistochemistry has been limited by the lack of specific instructions and protocols for clinicians. Preliminary results and detailed protocols for QD-antibody conjugation, tissue specimen preparation, multicolor QD staining, image processing, and biomarker quantification have been published *(25)*. The results demonstrate that bioconjugated QDs can be used for multiplexed profiling of biomarkers, and ultimately for correlation with disease progression and response to therapy. These applications will increase the clinician's ability to predict the likely outcomes of drug therapy in a personalized approach to disease management. Bioinformatics and systems biology are used to link each patient's molecule profile with disease diagnosis and treatment decisions. The usefulness of these protocols was demonstrated by the simultaneous identification of multiple biomarkers in prostate cancer tissue. In general, QD bioconjugation is completed within 1 day, and multiplexed molecular profiling takes 1–3 days depending on the number of biomarkers and QD probes used.

Gold nanoparticles conjugated to anti–epidermal growth factor receptor monoclonal antibodies specifically and homogeneously bind to the surface of cancer cells with 600% greater affinity than to noncancerous cells *(26)*. This specific and homogeneous binding is found to give a relatively sharper SPR absorption band with a red-shifted maximum compared with that observed when added to the noncancerous cells. Efficient conversion of strongly absorbed light by plasmonic gold nanoparticles to heat energy and their easy bioconjugation suggest their use as selective photothermal agents in molecular cancer cell targeting *(27)*. Thus, gold nanoparticles can link diagnosis to therapeutics by noninvasively detecting the cancer and then destroying it.

Application of Nanodiagnostics in Infectious Diseases

The rapid and sensitive detection of pathogenic bacteria at the point of care is extremely important. Limitations of most of the conventional diagnostic methods are lack of ultrasensitivity and delay in getting results. A bioconjugated nanoparticle-based bioassay for in situ pathogen quantification can detect a single bacterium within 20-min *(28)*. Detection of single-molecule hybridization has been achieved by a hybridization-detection method using multicolor oligonucleotide-functionalized QDs as nanoprobes *(29)*. In the presence of various target sequences, combinatorial self-assembly of the nanoprobes via independent hybridization reactions leads to the generation of discernible sequence-specific spectral codings. This method can be used for genetic analysis of anthrax pathogenicity by simultaneous detection of multiple relevant sequences.

A spectroscopic assay based on SERS using silver nanorods, which significantly amplify the signal, has been developed for rapid detection of trace levels of viruses with a high degree of sensitivity and specificity *(30)*. The technique measures the change in frequency of a near-infrared laser as it scatters viral DNA or RNA. That change in frequency is as distinct as a fingerprint. This novel SERS assay can detect spectral differences between viruses, viral strains, and viruses with gene deletions in biological media. The method provides rapid diagnostics ($\leq$60 s) for detection and characterization of viruses generating reproducible spectra without viral manipulation. This method is also inexpensive and easily reproducible.

Applications of Nanodiagnostics in Neurological Disorders

Nanoparticle contrast agents are in development to enhance MRI. A new MRI contrast agent using manganese oxide nanoparticles to visualize the anatomic structures of mouse brain produces images that are as clear as those obtained by histological examination *(31)*. The new con-

trast agent will enable better research and diagnosis of neurological disorders such as Alzheimer disease, Parkinson disease, and stroke. Furthermore, antibodies can be attached to the manganese oxide nanoparticles, which recognize and specifically bind to receptors on the surface of breast cancer cells in mouse brains with breast cancer metastases. The tumors were clearly highlighted by the antibody-coupled contrast agent. The same principle should allow other disease-related changes or physiological systems to be visualized by using the appropriate antibodies.

Future Prospects

Within the next decade, measurement devices based on nanotechnology, which can make thousands of measurements very rapidly and very inexpensively, will become available. Future trends in diagnostics will continue in miniaturization of biochip technology to the nanoscale range. The most common clinical diagnostic application will be blood protein analysis. Blood in systemic circulation reflects the state of health or disease of most organs. Therefore, detection of blood molecular fingerprints will provide a sensitive assessment of health and disease.

Molecular electronics and nanoscale chemical sensors will enable the construction of microscopic sensors capable of detecting patterns of chemicals in a fluid. Information from a large number of such devices flowing passively in the bloodstream allows estimates of the properties of tiny chemical sources in a macroscopic tissue volume. Estimates of plausible device capabilities have been used to evaluate their performance for typical chemicals released into the blood by tissues in response to localized injury or infection *(32)*. These indicate that the devices can readily enable differentiation of a single cell–sized chemical source from the background chemical concentration in vivo, providing high-resolution sensing in both time and space. With currently used methods for blood analysis, such a chemical source would be difficult to distinguish from background when diluted throughout the blood volume and withdrawn as a blood sample.

The trend will be to build the diagnostic devices from the bottom up, starting with the smallest building blocks. Whether interest and application of nanomechanical detection will hold in the long range remains to be seen. Another trend is to move away from fluorescent labeling as miniaturization reduces the signal intensity, but there have been some improvements making fluorescent labeling methods viable with nanoparticles. Nanobiotechnology will facilitate the development of non-PCR diagnostic technologies. As a non-PCR method for preimplantation genetic diagnosis, microarrays are used in comparative genomic hybridization, where they can replace the metaphase spread to which the mixture of test and comparative DNA is hybridized. As a further refinement, nanotechnology can potentially be applied for analysis of a single cell for preimplantation genetic diagnosis.

In the near future, the use of nanodiagnostics could reduce waiting time for test results. For example, patients with sexually transmitted diseases could provide urine samples when they first arrive at the outpatient clinic or physician practice; the results could then be ready by the time they see the physician. Patients could then be given the prescription immediately, reducing the length of time that the patient has to wait for results, thus decreasing patient anxiety, improving compliance, and making the whole process less costly.

In the next decade nanobiotechnology will play important roles not only in diagnosis but also in linking diagnosis with treatment and development of personalized medicine. Because of the integration and interrelationships of several technologies involved in nanodiagnostics, those who conduct these tests or devise new tests will be taking a more active part in decision-making in the future healthcare systems.

Another important area of application will be cancer diagnostics. Molecular diagnosis of cancer, including genetic profiling, will likely be widely used by the year 2015. By the time a cancer is detected by currently available methods, it is often too late for cure. Nanorobotics may be applied in the future for early detection as well as treatment of cancer. Nanodevices for this purpose are now beyond the realm of science fiction, in the feasibility stage. A nanodevice for combined diagnosis and therapeutics could be implanted as a prophylactic measure in individuals who do not exhibit any obvious manifestations of cancer, and cancer surveillance could be conducted by external remote monitoring. Such monitoring would circulate freely and could detect cancer at the earliest stages and deliver appropriate therapeutic intervention. These monitoring devices should be biodegradable, and safety must be established before implantation. Such a surveillance system would be the ultimate in preventive personalized management of cancer. Early detection would increase the chances of cure. Such a device would have advantages over detection of biomarkers in specimens of body fluids, because such examinations can be performed only periodically and would be less accurate than analyses conducted continuously in vivo *(33)*.

In conclusion, nanotechnologies promise to extend the limits of current molecular diagnostics and enable point-of-care diagnosis, integration of diagnostics with therapeutics, and development of personalized medicine. The most important clinical applications of currently available nanotechnology are in the areas of biomarker discovery, cancer diagnosis, and detection of infectious microorganisms. Nanomedicine promises to play an important role in the future development of diagnostic and therapeutic methods.

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