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REVIEW ARTICLE

Recent applications of nanodevices

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ABSTRACT

Although the "war on cancer and other disease like AIDS" is now in its fourth decade and despite much progress has been made in categorizing the environmental causes and cellular and molecular biological basis for this dreaded disease, we still do not have a precise understanding of the differences between a diseased cell and its normal counterpart. If we do not understand such disease, we cannot control and eliminate it. The completion of the human genome sequence and its subsequent improvements in the sequence data are important steps to fully comprehend disease cell biology. Nanotechnology, a new, novel focus of research evolved from the convergence and coalescence of many diverse scientific disciplines and as a general term for the creation, manipulation, and application of structures in the nanometer size range. In this review article, nanomedicine aspects of nanotechnology will be stressed and will cover areas such as drug delivery systems and new drug therapies as they relate to such disease. One of the ultimate goals of nanomedicine is to create medically useful nanodevices that can function inside the body. It is envisioned that nanodevices will be hybrids of biologic molecules and synthetic polymers that can enter cells and the organelles to interact directly with DNA and proteins. Additionally, nanomedicine will have an impact on the key challenges in cancer therapy: localized drug delivery and specific targeting. Among the newly developed nodevices such as quantum dots, magnetic nanoparticles, gold nanoparticles are the most promising applications for various cancer and other disease treatments.

Key words: Nanodevices, Nanomedicine, Drug Targeting.

INTRODUCTION:

Many diseases, including cancer, oriented from genomic mutations and alterations to normal cellular functioning and metabolic pathways at molecular level(1,2). Accurate and sensitive diagnosis has been constrained by the lack of biosensors and molecular probes capable of rapidly recognizing the features of these diseases. The ability of nanomaterials and nanopatterned devices to directly interact with biologically significant molecules, and to convert those interactions into directly electromagnetic signals, has enabled a new generation of early-stage diagnostic techniques. The greater the detail with which the molecular components of a specific disease can be determined the more specifically the therapeutic regime can be tailored to the individual (3). Many products have emerged for laboratory clinical and diagnostic uses including separation technologies for blood into its components, the fractionation of complex biofluidic mixtures into, its protein and nucleic acid digest sub-populations, DNA amplification strategies via PCR-on-chip, precise fluidic dispensation technologies for automated high throughput analyses, multiplexed analyte sensors for point-of-care diagnostics, and many more. Despite the enormous potential of nanotechnology as it relates to diagnosis, many important concerns must still be addressed including the toxicological effects of the *in vivo* use of the relevant nanomaterials, the identification of appropriate target molecules and biomarkers to be screened for, the proper protocols for sample preparation, and the complete interpretation of diagnostic results collected from both animal models and human trials. The ability to address these concerns will ultimately determine nanotechnology diagnostic techniques can used into the clinic, but as initial preclinical efforts are quite promising and the array of possible applications are quite broad. Currently, conventional radiological diagnostic techniques focus on detecting the physical manifestations of disease. Given the high correlation between survival rate and early detection(4), it is highly advantageous to identify abnormal cellular function before significant physiological modifications become apparent. Mammography is the process of using low energy X-rays to examine the human breast for characteristic masses or microcalcifications

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(5). It is a widely used technique because of its relatively low cost and operational simplicity allowing for population-wide screening. Regular mammographic screening can detect between 80 and 90% of breast cancers in asymptomatic individuals, resulting in reduced breast cancer mortality(4).However, for women with dense breasts, the sensitivity of mammography is low (45.8–55%)(6). Finally, this imaging-based technology requires a critical mass of localized accumulated tumor cells for effective neoplasm identification and is not capable of detecting tumors with masses or densities below this critical dimension. The diagnosis of breast cancer can be also accomplished by directly analyzing tumor cells through techniques such as the cytomorphology of exfoliated cells (7).Direct visualization of tumor cells does not lend itself to regular population-wide screening of asymptomatic women, however, because of the subjective nature of tumor cell identification and the labor-intensiveness of the process. Several studies have recognized that diagnostic biomarkers isolated from blood, such as circulating proteins and nucleic acids, may offer the greatest potential for the reliable and earliest possible screening of diseases (8-11).The nanotechnologies will greatly enhance the throughput and sensitivity of the identification and screening of potential biomarkers. For example, the breakthroughs in using inorganic nanoparticles for contrast enhance in imaging biomarkers provide a robust framework for biomedical application (12-16). The marriage of biology and micro- and nanofabrication has revolutionized biosensing by allowing for the integration of biological recognition elements into devices that will significantly impact the commercial availability of detection and diagnostic technologies at the genome and proteome levels. This in turn should dramatically decrease the time between disease onset and the initiation of tailored medical intervention and thus greatly increase the likelihood of positive clinical results.

In this review, we will discuss disease diagnosis through molecular recognition in addition to several of the most promising and advanced nanotechnologies for achieving the goal of the earliest possible detection of abnormal cellular function. The nanomaterials used in diagnostics, including the DNA-based nanobarcode, quantum dots (QDs), and gold nanoparticles, magnetic nanoparticles, predominantly as contrast agents for magnetic resonance imaging (MRI). Finally, we will conclude with a discussion of future trends, including the movement toward theranostics whereby the functional flexibility of many nanotechnologies allows for the coupling of both the personalized diagnostic and therapeutic modalities within the same construct.

Nanomaterials and their applications:

Nanomaterials are defined as materials comprised of basic components, which have at least one dimension in the nanoscale (<100 nm). This confined dimensionality yields a host of unique properties not present in the bulk material, which are being investigated for an array of clinical applications (17). These are materials whose basic building blocks have all three dimensions in the nanoscale. These nanoscale structures represent powerful diagnostic tools as they can be surface functionalized with a range of specific targeting agents and then systemically circulated *in vivo* to locate and monitor specific biological targets. They can also be used for *in vitro* assays and experiments to increase the sensitivity of a particular assay or label important subcellular or molecular features of cells. Nanomaterials are typically fabricated using a bottom-up approach in which molecular components self-assemble into more complex structures.

The first we discuss the use of magnetic iron oxide nanoparticles as MRI contrast agents and then investigate gold nanoparticles that can absorb light through the excitation of a surface plasmon resonance (SPR), a phenomenon with wide ranging applicability. The next topic will be a discussion on bio-barcodes, an assay amplification strategy that incorporates both gold and magnetic nanoparticles. This will be followed by a look at aptamers, a targeting modality which, when conjugated to the surface of gold and magnetic nanomaterials, can be used for both targeting as well as for assay amplification. The use of QDs in a variety of labeling strategies based on the ability to tune their optical emission spectra to almost any visible wavelength will follow next. Finally, this section will conclude with a treatment of multiplex dendrimers whereby carbon lattices are used to encapsulate contrast agents allowing for very tight control over their nanoscale structure.

Magnetic Nanoparticles:

Since the early 1980s, superparamagnetic nanoparticles and their derivatives have been developed and commercialized to enable the purification, separation, and detection of important components within biological samples (18,19). More recently, the use of this class of nanomaterials has been extended *in vivo* to drug delivery and to the development of molecular contrast agents for MRI(21).MRI is a diagnostic imaging technique, which uses a strong external magnetic field to align the nuclear magnetization of hydrogen atoms incorporated on water or fat molecules within the body and then uses

radiofrequency (RF) waves to excite these aligned magnetizations out of equilibrium(21). The excited hydrogen nuclei then relax back into their aligned equilibrium positions with two characteristic relaxation times, the longitudinal relaxation time T1 and the transverse relaxation time T2, resulting in the emission of the excitation energy absorbed from the RF waves (21). Stronger external magnetic field strength leads to shorter relaxation times and thus better image contrast and resolution. An MRI with an external magnetic field of 7 Tesla is capable of a minimum resolution of 50–100 microns (22). By imaging techniques weighted toward either T1 or T2, different tissue contrasts can be realized. Furthermore, the relaxation times are also highly dependent on the chemical composition of the tissue yielding enhanced soft tissue contrast. This ability to better image soft tissue and the use of nonionizing radiation in the imaging process endow MRI with many important advantages as compared with other imaging modalities such as computed tomography (CT). Colloidal superparamagnetic iron oxide (SPIO) have been investigated as MRI contrast agents because of their ability to reduce T2 proton relaxation times of specific tissues. The tissue is targeted through the surface functionalization of the colloidal nanoparticles with recognition elements, such as antibodies or ligands, leading to an improvement of the MRI resolution down to the level of single cells and allowing for the specific recognition of the molecular components of important cellular features and processes (23).

The SPIO nanoparticles are synthesized using a variety of techniques, the easiest and most common being an aqueous co-precipitation process initiated by mixing an iron salt with polymer surfactants under alkaline conditions (23). The precise pH value in the solution and the amount and structure of the surfactant-coating materials play the predominant role in tuning the nanoparticle properties (24,25). High-temperature decomposition of organometallic precursors has also been used to improve nanoparticle size control. This technique is capable of producing uniform spherical Fe_3O_4 nanoparticles with a size variation of <2 nm and diameters ranging from 4 to 20 nm(26).

Once the magnetic nanoparticles have been synthesized, they are coated with various types of chemical modifiers. These modifiers can include polymers to prevent nanoparticle aggregation and functional ligands, organic dyes, permeation enhancers, or antibodies to imbue them with greater biological functionality. SPIO nanoparticles can be coated with poly(ethylene glycol) (PEG) to avoid nanoparticle uptake by macrophages allowing for extended blood circulation time *in vivo*(27,28). CTX-targeted iron oxide nanoparticles have been demonstrated to specifically accumulate in 9L glioma flank xenografts *in vivo*, resulting in more thorough contrast enhancement of these tumors in comparison with non-targeted control nanoparticles. streptavidin-conjugated SPIO nanoparticles has used for magnetic resonance molecular imaging of Her-2/neu receptors expressed by breast cancer cells *in vitro*. The receptors were tagged with biotinylated monoclonal antibodies, which allowed for the binding of streptavidin-conjugated nanoparticles. The contrast level of the resulting image was proportional to the level of expression of Her-2/neu receptors.

Quantum Dots

QDs consist of a semiconductor core encapsulated by another semiconductor shell with a typical diameter of 2–10 nm. Because of their tunable nanoscale dimensions, high photostability, broad absorption spectra, and narrow emission bands, QDs have been used as florescent labels to optically image a host of biological structures and processes, ranging from DNA, small organelles, and tumors to cell–cell interactions and cell signaling processes (29-31). The quantum confinement effect allows for careful control of the emission properties of QDs by varying their size and material composition (32). The fact that multiple QDs may be excited by a single excitation wavelength, thanks to their aforementioned broad absorption spectra, allows for them to facilitate multiplexed diagnosis(33).The impressive multiplexing capability of QD tags in live animals, comparing with the detection sensitivity and spectral features of encoded fluorescent proteins (green fluorescent protein, GFP)(13). Although QDs themselves are insoluble in water, their active surface can be conjugated by a layer of functionalized silica or any number of linkers, including mercaptoacetic acid, dihydrolipoic acid, or modified polyacrylic acid, thus rendering them biocompatible(34).In addition, QDs can be adapted for specific target recognition by coating them with antibodies, streptavidin, or oligonucleotides.

A multiplex immunoassay has been developed (35) for the simultaneous and sensitive detection of cholera toxin, ricin, shiga-like toxin 1, and staphylococcal enterotoxin B using the relevant antibodies conjugated to QDs with different sizes, which lie in different colors. In addition, a multiplex diagnostic system using a microfluidic chip and antigen-coated QDs embedded in polystyrene microbeads was developed (36). The integrated device is able to detect antibodies against hepatitis B virus, hepatitis C virus, and HIV in serum samples with

sensitivity at the level of near picomolar. The microfluidic system enables the approach to be more automatic, accurate, and efficient, rendering it 50 times more sensitive than the currently available methodology using the same antigen and antibodies.

Gold Nanoparticles:

Gold nanoparticles represent another promising diagnostic technology as their optical properties can easily be tuned and their surfaces functionalized using a variety of well-characterized chemical moieties (thiols, disulfides, amines)(37). These nanoscale constructs can be fabricated either as solid gold spherical nanoparticles with diameters in the range of 0.8–250 nm, asthin gold shells surrounding a dielectric core (i.e., silica) or as high aspect ratio nanorods(37).Au nanoparticles, unlike their QD counter parts, do not emit light but absorb and scatter it in a process called SPR(37). SPR is a phenomenon whereby a coherent oscillation of electrons at the surface of a gold nanoparticle (or thin film) is excited by incident electromagnetic radiation of a particular frequency causing the incident radiation to be either absorbed, scattered, or both(38). This oscillation, or plasmon, can only be sustained in materials (including the noble metals gold, silver, and copper) that possess a dielectric constant that is complex-valued with a negative real part and a slightly positive imaginary part (37,38) The size and shape of the Au nanoparticles determine their optical resonance and can be tuned to achieve absorption and scattering at electromagnetic wavelengths from visible light to the mid infrared(39,40). The resonance frequency of the excited plasmon is also highly sensitive to the local refractive index of the solution in which the nanoparticles are dissolved, but only at small distances (nanometers) because of the exponential decay of the evanescent field normal to the surface (38,40).This property can be used to determine both the presence and binding kinetics of molecular targets adsorbed to the particle surface.

When used for *in vitro* assays, the binding of Au nanoparticle-labeled recognition elements to their respective targets leads to aggregation of the nanoparticles resulting in a color change in the optical signal as compared with that for the unbound monodispersed gold nanoparticle solution(37). An immunoassay has developed which is capable of quickly detecting (within 10–30 min) subnanogram/mL quantities of various analytes using gold nanoshells optimized for the near-infrared, including rabbit IgG in different media (41). For *in vivo* imaging, nanoparticles optimized for a subset of the near-infrared (650–900 nm) are frequently used, as the body and biological tissue are highly transmissive in this wavelength range (37).

Aptamer-Conjugated Nanoparticles:

The *in vitro* detection of specific mRNA and DNA sequences originating *in vivo* from circulating cancer cells and micrometastases has proven to be a very useful diagnostic technique, because their extracellular instability leads to low extracellular lifetimes and prevents significant circulation (42). Thus, there is a high correlation between the presence of these specific sequences and the presence of diseased cells in biological samples. Some of the most widely used techniques for detecting these DNA and mRNA sequences are based on the use of the polymerase chain reaction (PCR) to amplify these biomarkers in blood and tissue samples by six to eight orders of magnitude (42,43). These PCR-based techniques have been demonstrated to yield a detection limit of one diseased cell in 1–10 million normal cells (42). This extreme sensitivity can also increase the number of false positives, however, because of the combination of the small probability that non-target sequences will initiate the amplification process and the small number of total molecules necessary to achieve significant amplification. Thus, the clinical effectiveness of this technique is limited (42,44,45). Fluorescent and magnetic nanoparticles surface functionalized with a variety of biological recognition elements, such as aptamers and antibodies, have been used to screen for specific cells, including circulating cancer cells, in buffer, blood, and fetal bovine serum, and to extract those cells from the samples using magnetic fields(46).Aptamers are receptors constituted of short DNA or RNA sequences, which have been selected *in vitro* from a large library of random sequences to bind a host of biological components in a manner similar to antibodies(47,48).The apatamer selection process, referred to as systematic evolution of ligands by exponential enrichment (SELEX), was first reported in 1990(49,50). The unique features of aptamers, making them superior to antibodies in clinical use, include the ability to produce them from repeatable chemical synthesis, as opposed to antibodies which must be biologically synthesized in an animal model, and their ability to fold into complex 3D structures with distinct molecular binding motifs. Gold nanoparticles functionalized with thiolated aptamers (about 80 aptamers per particle) were used for the optical transduction of aptamer–protein interaction (51). The binding of aptamers to a thrombin protein target resulted in aggregation of the gold nanoparticles. Removal of the aggregates from solution with the use of a centrifuge precipitation technique led to a decrease in the solution's

plasmon absorbance as compared with a sample with an equal concentration of unbound dispersed gold nanoparticles.

Nanoparticle-Based Bio-Bar Codes:

Effective clinical biomarker screening requires three crucial capabilities to be effective, the ability to look for several targets simultaneously, the ability to detect small concentrations of proteins in samples containing a complex mixture of biological constituents, and the ability to operate using minimal sample sizes. A variety of multiplex assays have already been developed, such as microarrays(52,53) and microsphere-based flow cytometry,(54)capable of high throughput screening for a wide range of different molecular species simultaneously. Greater assay sensitivity has also been achieved through either directly amplifying the quantity of low abundance targets, such as the replication of DNA and RNA sequences using PCR, for use with a technique such as microarray analysis, or by developing more sensitive detection methodologies, several of which are described in this review. Finally, microfluidic systems are being developed to achieve a lot of these techniques, such as flow cytometry, with greatly reduced sample volumes (55).

One of the more promising recent developments with the potential for multiplexed, sensitive, low volume biomarker analysis is the bio-barcode (56). They used an aluminum oxide film as a stencil to create metallic bars, nanometers in diameter and microns in length, with alternating submicron bands of metals with varying optical reflectance. These bars were then coated with a targeting element that bound to their targets by affinity capture. This system allowed for multiplexed analysis, because the array of metallic bands could be arbitrarily controlled, and was therefore not limited by the range of available fluorophores. Furthermore, a limited amount of sample was required as the analysis could be performed directly in the sample with no further processing. In 2003, Chad Mirkins group at Northwestern University developed a different barcode system based on the use of magnetic microparticles and gold nanoparticles(57). The magnetic microparticle is functionalized with one recognition element, such as aptamers or antibodies, and the gold nanoparticle is conjugated by second similar recognition element as well as large numbers of specific double-stranded DNA sequences. The two particles then sandwich and extract a specific target using a magnetic field. Although all of the magnetic microparticles are captured, only gold nanoparticles that have bonded to the target are captured. The double-stranded DNA immobilized on the gold nanoparticle surface is then dehybridized to release one of the strands into solution, either chemically (dithiothreitol)(58) or thermally(59-61) and then experimentally detected using chip-based DNA techniques with or without PCR. The bio-barcode assay. This bio-barcode technique not only incorporates multiplexing and small sample volumes, but it is also capable of significant signal amplification, given the high ratio of DNA barcodes to target recognition elements. The sensitivity of this methodology exceeds that of ELISA by up to 10^6, yielding the possibility for the use of low abundance biomarkers in diagnosis whose concentrations where considered too low to be useful with previous technologies. The bio-barcode assay has been demonstrated to successfully detect free PSA at concentrations in the range of 3aM to 300 fM.

Multiplex Dendrimers:

Dendrimers, artificial macromolecules presenting a tree-like structure, comprise tunable nanostructures that may be synthesized with tight regulation over their physical characteristics, including size, shape, interior void space, and surface chemistry. Their medical use in targeted diagnostic imaging has been widely investigated through extensive*in vitro* studies (62, 63). Dendrimers provide an excellent platform for the attachment and presentation of cell-specific targeting groups, the modification of solubility, the reduction of immunological interactions, and the labeling of biological constructs for imaging applications, i.e. MRI. Currently Gd(III) ions are the most popular contrast agents for clinical MRI. However, their high toxicity to serum proteins limited Gd(III) ions' potential in the clinical use. Although some alternative contrast agents have been developed, such as a complex of diethylenetriaminepenta-acetic acid (DTPA), these LMW species normally diffuse from blood vessels and are excreted very quickly. Several HMW compounds were synthesized by binding Gd complexes to albumin, dextran, and polylysine, but their application has yet obtained the desirable level because of their low relaxivities and slow clearance. The low generation dendrimers, with low toxicity and favorable biocompatibility, is able to improve upon all of these drawbacks. The first reports of *in vivo* diagnostic imaging using dendrimers as a contrast agent for MRI can be traced back to Lauterbur et al. in the early 1990(64). In this study, chelated gadolinium groups were attached to the PAMAM dendrimer surface to facilitate their blood pool properties and dramatically enhanced MRI contrast. The efficacy of dendrimer-based size-mediated targeting for the *in vivo* imaging of primary tumors was described in 2003 (65). Discrete dendrimer sizes obtained through

sequential synthetic generations were applied in designing organo-specific diagnostic imaging modalities to identify size-dependent mammalian excretion routes (66). Recently, the conjugation of antibodies to PAMAM dendrimers bearing the fluorescein imaging tag has been explored further increasing the usefulness of this synthetic architecture in diagnostic applications.

Conclusion and future trends:

A major challenge in diagnosis for the 21st century is to be able to detect disease biomarkers non-invasively at an early stage of disease progression, and to determine the exact relationship between the abundance of these biomarkers and the extent of their corresponding clinical pathologies. In breast cancer, for instance, one goal of molecular imaging is to be able to accurately determine when a tumor mass has reached a population size of approximately 100–1000 cells, in contrast to the current techniques such as mammography, which require more than a million cells for accurate clinical diagnosis. The ability of nanotechnology to interact with matter at the molecular scale provides not only the possibility to ascertain the molecular constituents of a disease, but also the way in which these constituents affect the totality of biological function. The capacity to incorporate an array of structural and chemical functionalities onto the same micro-and nanoscale architecture should also enable more accurate, sensitive, and precise screening of diseases, which present with more significant pathological heterogeneity. This same flexibility should also enable a new generation of clinical constructs, referred to as theranostics, which combine both diagnostic and therapeutic elements. The nanodevices described herein, including nanoporous chips, nanowire biosensors, SETs, and micro- and nanocantilevers, are able to detect extremely low concentrations of target proteins as well as DNA and RNA sequences of interest, many of them label-free, in real-time, and at-point-of-care. Researchers are also making progress in using free standing nanoparticles to spot disease biomarkers both *in vitro* and *in vivo*. The application of gold nanoparticles in bio-barcode assays can help detect protein and DNA signatures for a number of diseases, including cancer, at concentration limits in the attomolar range. Many of these nanoparticles have the potential to be incorporated with more traditional imaging and diagnostic techniques to improve contrast and resolution or can be used in conjunction with the aforementioned nanodevices to further enhance their diagnostic and screening sensitivity and flexibility. The development of nanodevices and nanoparticles for use in diagnostic applications requires expertise from a diverse collection of pure and applied disciplines, from biology, chemistry, and physics, to mechanical, electrical, chemical, and biomedical engineering. Cross-discipline cooperation will be essential in the design, fabrication, characterization, and implementation of future nanotechnological diagnostic platforms capable of the direct observation, analysis, and manipulation of the molecular signatures of disease, from a single molecule to an entire proteomic library.

The concept of personalized medicine entails the incorporation of novel biotechnologies into the diagnosis and treatment of individual patients such that their unique genetic and phenotypic characteristics may be factored into the choice and administration of a particular therapy. Diagnosed breast cancer is currently screened for a number of key factors that vary from patient to patient, including but not limited to the presence of alterations in the breast cancer susceptibility genes BRCA1 and BRCA2 (5–10% of reported cases), the overexpression of the growth promoting hormone HER2/neu (15–30% of cases), and whether the tumor is estrogen and progesterone receptor positive.The therapeutic regime is then highly dependent on which of these factors are present and at what overall levels of expression. Given that tumor progression in most forms of cancer is not yet fully understood, there will undoubtedly emerge a host of other important biomarkers which must be identified and monitored as well. How patients respond to treatment also depends on individual differences in radiation and drug absorption, drug metabolizability, and drug elimination from the body. The nanotechnologies detailed in this review should make significant contributions toward more accurate, precise, sensitive, and timely diagnosis of disease and subsequent screening for the important molecular factors unique to the individual. They should also, through improvements to*in vitro* and *in vivo* assay throughput, speed, cost, and flexibility, allow for more detailed real-time systematic monitoring of the therapeutic process on the level of the patient and thus a more fully optimized therapeutic regimen.

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