Annual Review of Analytical Chemistry

Polyvalent Nanoobjects for Precision Diagnostics

David Omstead,[1] Jenna Sjoerdsma,[1] and Basar Bilgicer[1,2,3,4]

[1]Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, Indiana 46556, USA; email: bbilgicer@nd.edu

[2]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA

[3]Advanced Diagnostics and Therapeutics Initiative, University of Notre Dame, Notre Dame, Indiana 46556, USA

[4]Mike and Josie Harper Cancer Research Institute, University of Notre Dame, South Bend, Indiana 46617, USA

Annu. Rev. Anal. Chem. 2019. 12:11.1–11.20

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

https://doi.org/10.1146/annurev-anchem-061318-114938

Keywords

multivalency, nanoparticle, nanotechnology, theranostics, detection, biosensor

Abstract

As our ability to synthesize and modify nanoobjects has improved, efforts to explore nanotechnology for diagnostic purposes have gained momentum. The variety of nanoobjects, especially those with polyvalent properties, displays a wide range of practical and unique properties well suited for applications in various diagnostics. This review briefly covers the broad scope of multivalent nanoobjects and their use in diagnostics, ranging from ex vivo assays and biosensors to in vivo imaging. The nanoobjects discussed here include silica nanoparticles, gold nanoparticles, quantum dots, carbon dots, fullerenes, polymers, dendrimers, liposomes, nanowires, and nanotubes. In this review, we describe recent reports of novel applications of these various nanoobjects, particularly as polyvalent entities designed for diagnostics.

INTRODUCTION

Nanoobjects have been utilized by humanity since fourth-century Rome, but their usage in medicine has only come into fruition recently. Faraday first described the creation of gold nanoparticles in 1857, but to this point such techniques were only used for their optical effects in crafting and pottery. With the current availability of numerous techniques and methods for generating a wide variety of nanoobjects, the field of nanotechnology has bloomed not just in medicine but also in electronics and physics. Especially in medicine, an extensive variety of nanoobjects with their many different chemical and physical properties has expanded the reach of previously impossible techniques. The size range and ease of surface modifiability of nanoobjects have made them preferred platforms for taking advantage of polyvalent designs in particular. While the focus of nanoobjects in medicine was originally solely on the therapeutic side, it has since expanded into new and unique diagnostic methods that could potentially greatly aid in detecting and locating many diseases and ailments.

In this review, we summarize each main type of nanoobject used in diagnostics. The nanoobjects covered include silica nanoparticles, gold nanoparticles, quantum dots, carbon dots, fullerenes, polymers, dendrimers, liposomes, nanowires, and nanotubes. After a brief introduction to each of these nanoobjects, we review their most common historical usages in diagnostics, along with novel recent usages that may prove to be quite promising in the future. Special focus is given to systems that take advantage of nanoobjects' useful base as a platform for polyvalent interactions.

SILICA NANOPARTICLES

Silica has recently gained popularity as a choice material used in imaging agents because it is considered intrinsically nontoxic, and silica coatings provide additional protection to the carried molecules or particles via photobleaching resistance and shelter from enzymatic degradation (1). The photophysically inert characteristics of silica mean that it is transparent to visible light and is not involved in dye-quenching electron-transfer processes (1). Silica particles synthetized in diameters of less than 10 nm have been reported for uses in imaging (2). Silica-based and silica-coated nanoparticles have been studied since the early 1990s, and in the early 2000s, researchers at Cornell University discovered that silica nanoparticles labeled with radioactive iodine could be tracked in vivo with positron emission tomography (PET) (2). A second version of this "Cornell dot" uses fluorescent imaging and is currently undergoing clinical trials (2). Silica nanoparticles continue to be combined with various fluorescent molecules to produce targeted imaging agents (3, 4).

Wu et al. (3) describe using a base of mesoporous silica nanoparticles (MSNs) with pH-sensitive fluorophores attached to track changes to lysosomal pH. They tagged the MSNs with fluorescein isothiocyanate to track their transport into the lysosomes. The pH-sensitive fluorescence of the MSNs was produced by attachment of rhodamine lactams (R6G-lactams), which are nonfluorescent under standard physiological conditions but fluoresce red under acidic conditions due to the opened lactam. The R6G-doped MSNs fluoresced 250-fold brighter at lysosomal pH (pH 4.0) than at cytosolic pH (pH 6.5) (3).

Zhou et al. (4) coated clusters of gold nanoparticles (nanoclusters, or AuNCs) with silica to prevent nonspecific absorption due to AuNC aggregation and to improve the ease of surface functionalization (4). As discussed in the next section, gold nanoparticles can serve as both fluorescent imaging and improved CT imaging agents. Zhou et al. designed the silica-AuNCs to target the folate receptor, an endocytosis-mediator protein on the surface of many human cancer cells (**Figure 1**). To this end, folic acid was conjugated to the silica-coated AuNCs. The

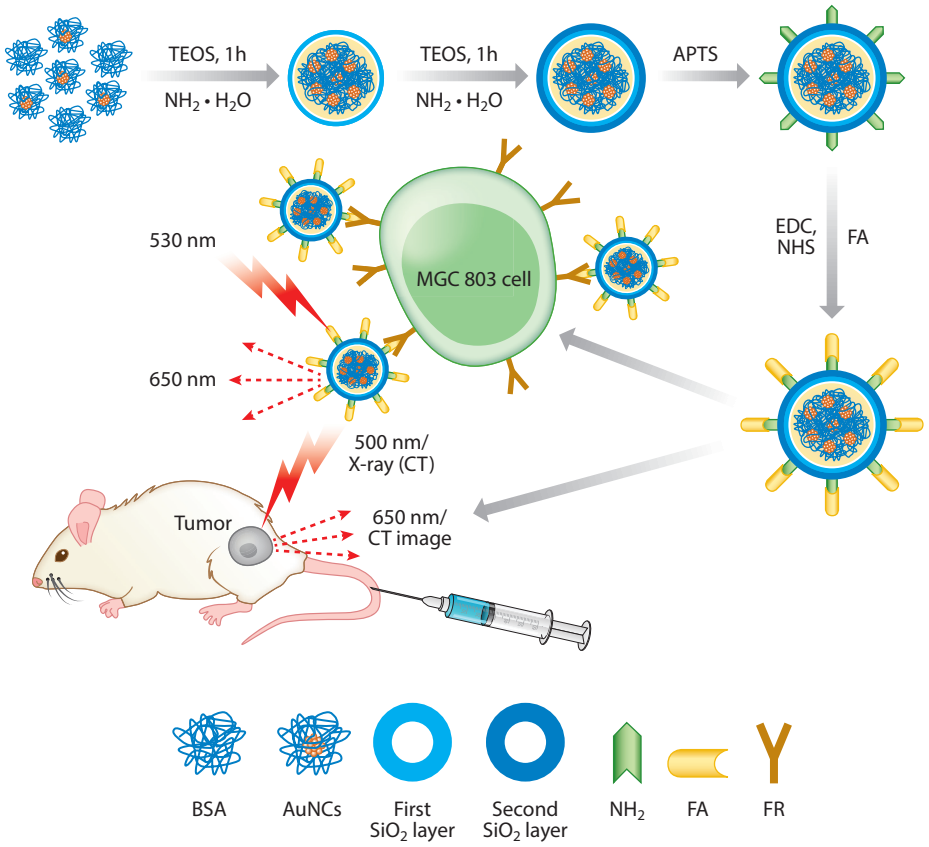

Figure 1

Schematic illustrating the synthesis of silica (SiO_2)-coated gold nanoparticle clusters (AuNCs) and their use for targeted imaging of tumors in vivo. Bovine serum albumin (BSA)-stabilized AuNCs were coated by two layers of SiO_2. The SiO_2-AuNC surfaces were functionalized with folic acid (FA) to target the folate receptor (FR) present on MGC-803 gastric cancer cells. After injection of the folate targeting AuNCs in tumor-bearing mice, the tumors were detected using both fluorescent (excitation 530 nm, emission 650 nm) and CT imaging techniques. Figure adapted from Reference 4. Reproduced with permission from BioMed Central Ltd. under the Creative Commons Attribution License.

silica-AuNCs were shown to target tumors and provide fluorescent and CT imaging in mouse models (4). Silica appears to be a promising material for use in nanoparticle diagnostics, either as a base or in combination with other nanoparticles.

GOLD NANOPARTICLES

Although gold nanoparticles have been used, however unknowingly, for their ornamental properties over many millennia, it is only in the last five decades that their uses in the biomedical fields have been explored. Since that time, gold nanoparticles have been studied extensively as diagnostic tools owing to their optical properties and tunability. Gold nanoparticles can be made in a wide range of diameters (2–250 nm) while achieving precisely made uniform particles with good synthetic reproducibility (5). Surface modifications, typically formed through Au-S bonds, are very stable and can be used to conjugate a variety of molecules (6). The optical properties

of gold nanoparticles make them useful for various types of imaging, including localized surface plasmon resonance (LSPR), fluorescence resonance energy transfer (FRET), surface-enhanced Raman scattering (SERS) (6), and photoacoustic imaging (PAI) (7). In solution, gold nanoparticles also show a strong, visible-light plasmon resonance absorption band, resulting in vividly colored samples (5). As such, they are well suited for colorimetric assays and are used in commercial lateral-flow immunoassays such as home pregnancy tests (8).

Imaging

Gold nanoparticles provide a modifiable surface that yields a polyvalent presentation for a broad range of targeting ligands. When combined with imaging-functional molecules, gold nanoparticles are typically exploited for applications in enhanced imaging. In early examples of such applications, fluorescent contrast agents were combined with the gold nanoparticles. Perrault & Chan (9) designed gold nanoparticle–fluorescent agent conjugates that assembled in vivo at a tumor site. After passively targeting the tumor, the biotinylated gold nanoparticles served as anchors for the fluorescent contrast agent (streptavidin-Alexa fluor 568) to actively target (9). This resulted in faster tumor accumulation and improved pharmacokinetics over purely passive targeting. However, the biotin-streptavidin binding system can result in nonspecific binding of the fluorescent agent throughout the body. Recently, researchers began combining other molecules with gold nanoparticles, such as FePt cubes that were sandwiched by two of the gold nanoparticles to make "dumbbell"-type hybrid nanoparticles (7). These Au-FePt-gold nanoparticles could be used as cancer diagnostics with both magnetic resonance imaging (MRI) and PAI techniques, resulting in highly sensitive, deeply penetrating imaging (7). The hybrid nanoparticles also represent a trend toward theranostics, a combination of diagnostics and therapeutics in a single particle or system. The Au-FePt-gold nanoparticles provide therapeutic activity through a pH-controlled release of iron ions at the tumor site to catalyze the synthesis of cytotoxic agents and by acting as a target and heat sink for photothermal therapy, whereas the results of these therapies can be tracked with MRI and PAI (7). Hybrid nanoparticles, such as these theranostics, provide a multidiagnostic imaging platform and a unique opportunity to track tumor therapy progress in real time.

Immunoassays

Another researched use of gold nanoparticles is in the development of gold nanoparticle–plated assays for detecting a target molecule or cell in a manner similar to that of a standard enzyme-linked immunosorbent assay (ELISA). Gold nanoparticles functionalized with a binding domain are chemically plated in a monolayer, with a second set of gold nanoparticles in solution optionally used to enhance detection via sandwich binding to the desired molecule or cell. This technique has been used to perform relatively standard ELISAs for detecting the cancer biomarker carcinoembryonic antigen (10), but its most interesting uses have been in the form of gold nanoparticle sandwich assays that allow for quantification via voltammetric methods by using a silver coating (11). This method has allowed for precise measurement of targeted cells over a wide range from 15 to 1×10^6 cells/mL (12). While most of these techniques have made use of an antibody to achieve the desired molecular or cellular targeting, small-molecule targeting systems have also been developed. Of note is the use of a streptavidin-biotin-annexin V binding complex for the capture and immobilization of apoptotic cells. Via the application of a gold nanoparticle sandwich along with silver coating, apoptotic cells are detectable not just via voltammetric measurements but also with simple light microscopy (13). These systems show various advantages over basic ELISAs and other immunoassays through their sensitivity, wide range, and ability to take advantage of

polyvalency for cellular detection. Nevertheless, other techniques such as flow cytometry can often be used to collect similar cellular data in a simpler manner, narrowing the range of applications for this technique.

Aggregation

Gold nanoparticles can also be engineered to aggregate in response to the presence of an analyte molecule. This aggregation can be measured using dynamic light scattering (DLS), colorimetric systems, or other methods to determine the presence and concentration of the analyte molecule, allowing a rapid and easy method for detection (6). DNA-targeted techniques are a staple of the aggregation-mediated gold nanoparticle systems, wherein two DNA probes for different sections of a targeted DNA strand are used to functionalize and cause aggregation of gold nanoparticles when the targeted DNA is present. This aggregation leads to change in coloration easily detectable even by the naked eye and sensitive to the level of a single mismatch (5). This system can also be modified to be used to detect ions such as magnesium and pyrophosphate (14). Recently, this gold nanoparticle sandwich–DNA technique has also been utilized to develop a method to quickly quantify the concentration of a targeted DNA strand without the use of time-intensive techniques such as Southern and Northern blotting. According to a report by Marczak et al. (15), this quantification was accomplished through the use of a gel membrane biochip platform that takes advantage of the electrochemical properties of gold nanoparticles. The nanoparticles are concentrated electrically against a membrane along with an impure DNA sample, and then by reversing the electrical field, the aggregated nanoparticle-DNA-nanoparticle dimers are separated from the unbound monomers via gel electrophoreses. The dimers can then easily be quantified with the entire process taking only approximately 20 min. DNA-labeled gold nanoparticles have also been recently used for the detection of thrombin by Li et al. (16), with DNA hybridization between particles initiated by cobinding to the targeted thrombin, leading to concentration-dependent aggregation.

Alongside successful sensing applications of DNA-functionalized gold nanoparticles, antibody functionalization has also been incorporated in gold nanoparticle–based biosensing applications. The most critical requirement for successful implementation of antibodies in any biosensing application is ensuring the proper orientation of the antibodies on the surfaces to which they are conjugated. This is done to ascertain that the antigen-binding sites on the Fab domains are exposed and not hindered to retain their antigen-binding functionality. Standard methods of antibody conjugation, such as ionic interaction, thiol-reduction, and amine coupling (via EDC-NHS), are unable to guarantee this proper orientation. Nevertheless, a method to achieve oriented antibody conjugation was developed in our laboratory and described by Mustafaoglu et al. (17), in which the conserved nucleotide-binding site (NBS) was utilized for oriented immobilization. The NBS is a little-known pocket that exists on the Fab domain of all antibody molecules regardless of isotype. A thiol functionality was site specifically introduced by using an NBS ligand that binds to this site with moderate affinity, followed by permanent covalent bond formation using photochemistry. Next, the Au-S reaction was utilized to immobilize the functionalized antibody on gold nanoparticles. Antibody molecules conjugated in this manner retained their binding capability and achieved antigen concentration–dependent aggregation as measured by DLS (**Figure 2**) (17). Because the NBS conjugation method protects the structural integrity and the antigen-binding activity of antibody molecules during gold nanoparticle conjugation, it allows the system to maintain many of the advantages that high-affinity antibodies possess. The aggregation occurs quite rapidly, within less than 10 min, and the method has a markedly high sensitivity, achieving a level of detection for the prostate-specific antigen of 55 pM. Most importantly, the technique can be performed directly using patient serum without any need for purification of blood proteins and other components.

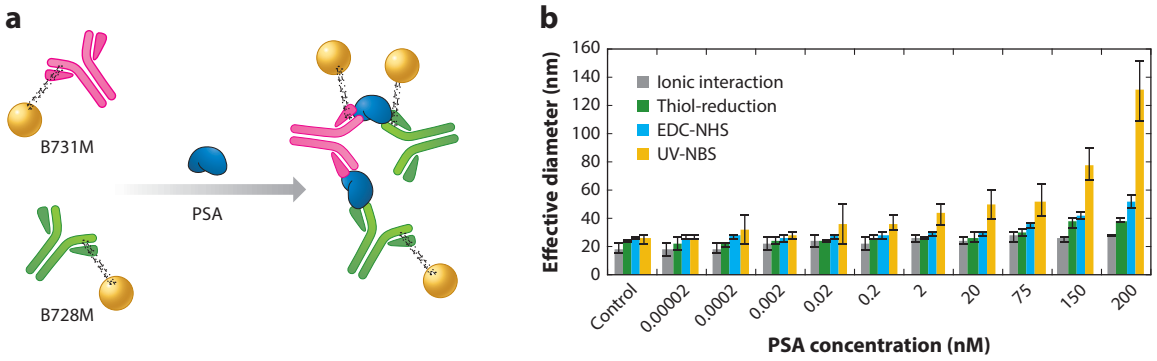

Figure 2

Detection of prostate-specific antigen (PSA) via dynamic light scattering of antibody-conjugated gold nanoparticles using site-specific conjugation. (*a*) Schematic showing antibody-conjugated gold nanoparticle clustering via antibody binding to PSA. Two sets of gold nanoparticles are used where each set has been conjugated with a different monoclonal antibody to target two different epitopes on the antigen. (*b*) Comparison of detection efficiency for antibody-functionalized gold nanoparticles prepared using various methods. Gold nanoparticles prepared using the novel ultraviolet–nucleotide binding site (UV-NBS) method for site-specific antibody conjugation displayed greater sensitivity and a lower limit of detection compared to standard antibody conjugation methods. Figure adapted with permission from Reference 17. Copyright 2017, Royal Society of Chemistry.

Owing to the ease and simplicity of this method, it could potentially be a powerful tool for the detection and diagnosis of many diseases, even in remote geographic areas.

QUANTUM DOTS

Quantum dots (QDs) are semiconductor nanoparticles with unique fluorescent properties, such as broad excitation and narrow emission spectra, an extended excited state, and good photostability (i.e., no bleaching) (18–19). The emission wavelengths of QDs can be tuned precisely by changing size (approximately 1–10-nm diameters) and composition (18–20). QDs have been explored as imaging agents since 1998; more recently, diverse methods of combining QDs with other materials have allowed for multimodal composite nanoparticles with enhanced imaging capabilities (18). Magnetic QDs, which are useful for combining MRI with fluorescent imaging, are most commonly made with iron oxides or sometimes gadolinium (18, 21).

Imaging

The high photostability and tunable fluorescence of QDs make them ideal for imaging. Active targeting elements may be added to allow imaging of a wide variety of sites ranging from tumors to the nervous system. Tsuboi et al. (22) targeted breast cancer tumors with anti-Her2 antibodies conjugated to QDs. First, the His-tagged immunoglobulin-binding (B1) domain of protein G is conjugated to the QD surface as an adapter protein. Then, the protein G B1 domain forces the oriented binding of antibody (Ab) molecules on the QD, as B1 binds only to the antibody F_C region. In vivo studies showed that targeted Ab-QD conjugates accumulated preferentially in the breast tumor, whereas nontargeted Ab-QD conjugates accumulated in the liver (22). A similar study with peptide-targeted QDs showed their usefulness as fluorescent probes for pancreatic cancer. Du et al. (21) developed a system for imaging the nervous system, particularly spinal cord injuries, by combining magnetic QDs with an axon tracer. A lipid coating on the QDs allows for the varied loading of paramagnetic gadolinium and axon tracer BDA (biotinylated dextran amine)

onto the QDs (23). Initial testing showed low cytotoxicity and strong magnetic resonance and fluorescence imaging capabilities for this system.

Ex Vivo Assays

The fluorescence of QDs can also be exploited in ex vivo assays for detecting targets varying from malaria to neurotoxins. Kim et al. (24) reported an immunoassay using magnetic beads and QDs to capture and detect malaria marker histidine-rich protein 2 (HRP2) at a limit of detection of 0.1 ng/mL, tenfold lower than commercially available alternatives. A lipid coating was applied to the QDs, with 10% of the lipids having a terminal amine to which a protein G was conjugated. Anti-HRP2 antibodies were then conjugated to the protein G to detect malaria (24). The system was optimized to be used with an automated droplet assay device for use in resource-scarce settings.

Wang et al. (19) developed a FRET-based combination of QDs and peptide-conjugated "dark quenchers," a type of nonfluorescent dye, to detect the deadly neurotoxin botulinum (**Figure 3*a*,*b***). The two most common serotypes of botulinum, A and B, were detected with a QD-quencher pair, each with a different emission wavelength (QD size) and quencher-conjugated peptide (19). In the absence of botulinum, the peptide-quencher conjugates bound to the QDs polyvalently and suppressed FRET signals. When botulinum was present, it attacked the enzymatic cleavage site on each peptide, severing the peptide that acts as the linker between the QD and the quencher. Consequently, as the peptide was cleaved, quenchers were no longer attached the to the QDs, diminishing the FRET effect and relieving the QD to fluoresce. Wang et al. (19) reported limits of detection of 0.2 ng/mL and 2 ng/mL for serotypes A and B, respectively, which are less sensitive than the current gold standard, but their method delivers results significantly faster, on the order of hours instead of days.

CARBON DOTS

Carbon dots are a class of nanoparticles that are mostly of interest as a potential replacement for the toxic, metal-based QDs (25). They were discovered serendipitously in 2004 during the purification of single-walled carbon nanotubes created via arc discharge (26). The researchers noticed that during gel electrophoresis, a fast-moving band was highly luminescent, which was later found to contain luminescent carbon dots. While there are a wide variety of methods to create carbon dots, they all result in the creation of a small (<10 nm) graphene oxide nanoparticles that contain many carboxylic acid groups ideal for both high water solubility and functionalization with various other molecules and particles. Carbon dots display size- and excitation-dependent photoluminescent behavior with a purely organic construct, allowing them to lack the dangerous side effects associated with comparable QDs and other metal-based nanoparticles (27).

Recently developed carbon dot techniques have focused on the diagnostic potential of a modifiable and fluorescent nanoparticle. Several groups have focused on the usage of carbon dots in FRET systems. Hamd-Ghadareh et al. (28) developed a system for detecting the tumor biomarker CA125 by utilizing carbon dots alongside a gold nanoparticle–antibody quenching complex. This system was able to achieve a detection limit of 400 ovarian cancer cells/mL and showed selective imaging of CA125-positive cells. Other FRET-based carbon dot systems have also been created for the detection and measurement of small molecules such as acetone and mercury in human fluids. These small molecule–detecting platforms utilize interaction with the small molecule itself as a quenching device, as opposed to the previous use of a gold nanoparticle complex (29–30).

In an alternative method of detection, Lee et al. (31) developed a simple assay that utilizes carbon dots to identify a certain DNA sequence—a notable bacterial gene—using a simple assay

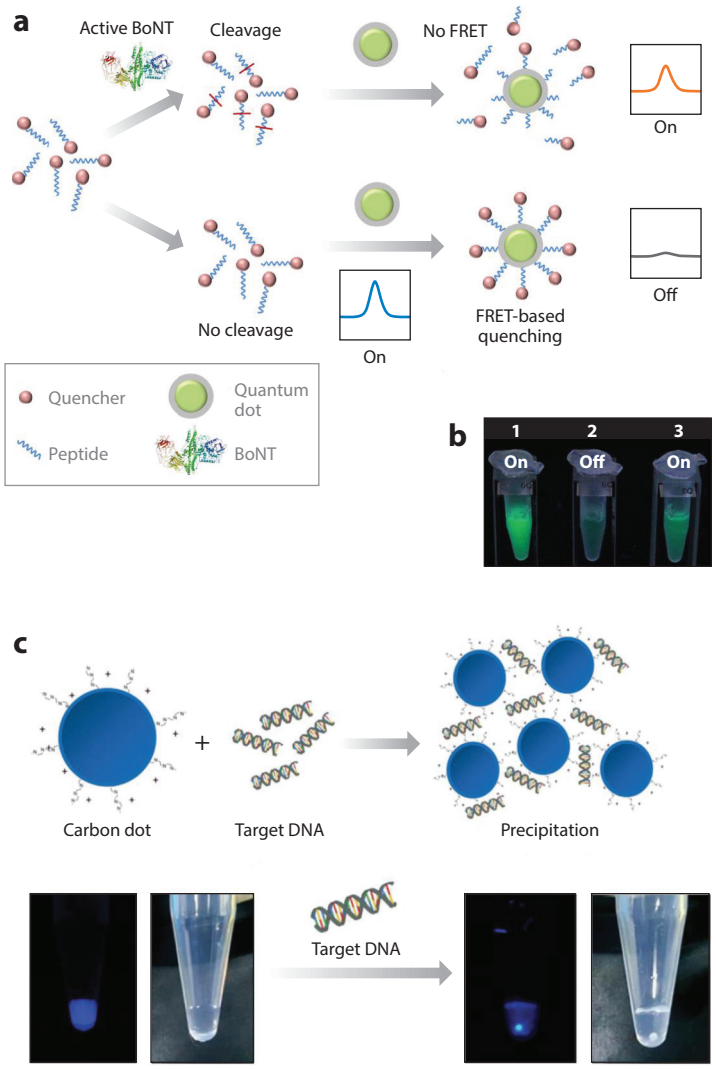

Figure 3

Fluorescent detection of neurotoxins and bacterial DNA via quantum dots and carbon dots, respectively. (*a*) In the absence of BoNT, peptide-quencher conjugates bind to quantum dots and quench fluorescence. When active BoNT is present, it cleaves the peptides and prevents them from linking the quenchers to the quantum dot, eliminating the quenching effect and turning the fluorescence back on. Panel adapted with permission from Reference 19. Copyright 2017, American Chemical Society. (*b*) Sequence of events during detection: (1) prior to the addition of quenchers fluorescence is on; (2) fluorescence turns off after quencher-conjugates are added; and (3) fluorescence turns back on or is unquenched if BoNT is present. Panel adapted with permission from Reference 31. Copyright 2017, John Wiley & Sons. (*c*) Carbon dots, functionalized with many copies of a complementary DNA sequence, bind to and cluster with target DNA, resulting in the precipitation of the large carbon dot-DNA aggregates. Abbreviations: BoNT, botulinum neurotoxin; FRET, fluorescence resonance energy transfer.

(**Figure 3*c***). The carbon dots are functionalized with many copies of a complementary DNA sequence for the target gene, so that binding with the gene results in precipitation of the carbon dot–DNA aggregate. This precipitate is typically detectable by ultraviolet (UV) illumination or even by the naked eye, giving it valuable potential for field application for a quick and easy way to identify bacterial strains. Perhaps the most interesting recent use of carbon dots is the use of their fluorescence as an internal light source for binding via photopolymerization to a larger targeting shell. Demir et al. (32) have developed a system for detecting the cancer biomarker glucuronic acid by utilizing the fluorescence of the carbon dots both to polymerize them to a molecularly imprinted polymer shell and to detect the targeted cancerous cells. Although much work has been done to develop diagnostic methods utilizing carbon dots for in vitro techniques, there are still great possibilities for their use in vivo, especially considering their potentially less toxic and more biofriendly nature compared to that of QDs.

FULLERENES

Fullerenes are one of the more recently discovered nanoobjects; the first was the C60 Buckyball discovered in 1985 at Rice University. They are similar in structure to graphite but form hollow shapes such as spheres or tubes. Because they exhibit five stages of oxidation/reduction, they can act as either an electrophile or a nucleophile, which combined with their structure, allows a wide range of probable structural and chemical modifications for use in diagnostic applications (33). Classically, fullerenes are often used in combination with a biosensor such as an enzyme or antibody working as a mediator between the sensor and an electrode, allowing conversion of a biochemical action to an electrical signal for measurement (**Figure 4**) (34). This section focuses on the recent applications of spherical fullerene nanoparticles in diagnostics, whereas nanotube fullerenes are examined in a later subsection.

One recent use of fullerenes as an electron mediator for diagnostics is the creation of a nanoprobe for the detection of *Mycobacterium tuberculosis* MPT64 antigen. This nanoprobe was created using C60 fullerenes wrapped in coils of polyaniline and decorated with gold nanoparticles and an anti-MPT64 aptamer. This complex was then used in a sandwich-binding assay with a surface-bound aptamer attached to a gold electrode. This binding enabled electron transfer from

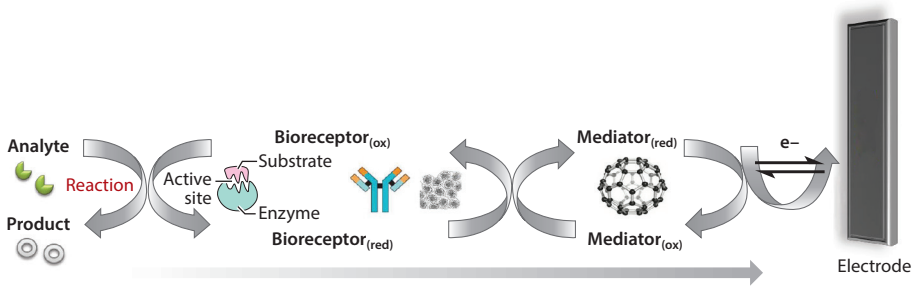

Figure 4

The role of the fullerene as an electron transfer mediator between a bioreceptor and an electrode in diagnostics. The fullerene's reductive and oxidative potential allows it to accept electrons from a conjugated bioreceptor such as an enzyme or antibody and transfer those electrons to an electrode for detection, converting a chemical reaction to an electrical signal for easy measurement. Adapted with permission from Reference 34. Copyright 2015, Elsevier. Abbreviations: Ox, oxidized; Red, reduced.

the polyaniline to the electrode, allowing presence of the tuberculosis antigen to be measured electronically (35).

In addition to this more classical usage of fullerenes as electron mediators, their use as contrast agents for use in MRI has also been explored. Water-soluble C60 derivatives have been used as scaffolds for the design of gadolinium-containing nanoparticles that prove to be highly sensitive contrast agents in vivo (36). Targeted functionalized fullerenes have also been used in the detection of cancer. One such method was the creation of a complex consisting of a C60 fullerene with many iron oxide nanoparticles chemically deposited onto it; this was coated with polyethylene glycol (PEG) and functionalized through conjugation of the tumor-targeting molecule folic acid to the ends of the PEG. These nanoparticles were shown to be a powerful MRI contrast agent and additionally showed potential for usage in synergistic photodynamic and radiofrequency thermal therapy (37). The electrical aspects of fullerenes combined with ease of chemical functionalization will facilitate their continuous development in a wide range of applications in diagnostics and potentially even as therapeutics.

POLYMERS

Polymers—macromolecules composed of many repeating units of the same or different molecules—may be branched or linear, natural or synthetic. Polymers may be used to form vesicular or modular nanoparticles that contain nanoparticles of different materials and molecules for detection, or they may be colloidal nanoparticles with surface functionalizations. Polymeric nanoparticles can vary between 10 nm and 1,000 nm in diameter. Polymers such as PEG have been used for decades to coat nanoparticles used for therapeutics or imaging to prevent aggregation and extend circulation time in vivo (38). Polymers have also been used since the early 2000s in films and coatings in microfluidics for detection in many fields ranging from environmental science to biology and biomedicine (39).

Oxidative Stress

Oxidative stress, which is an increase in oxidants such as hydrogen peroxide (H_2O_2), occurs in inflammatory conditions, including cancer, vascular diseases, and neuronal degeneration, and is believed to be a useful diagnostic marker for such diseases (40–41). In early work, Kim et al. (40) used PEG hydrogel nanospheres to encapsulate horseradish peroxidase (HRP, an enzyme typically used in ELISAs for signal amplification) as optical sensors for H_2O_2 production. Oh et al. (41) developed a platform of polyacrylonitrile nanoparticles with boronate surface modifications to allow for polyvalent presentation of donor-bridge-acceptor fluorescent systems on the nanoparticles (**Figure 5**). In response to oxidative stress, the fluorescent intensity of the boronate-modified polyacrylonitrile nanoparticles decreases. In vitro, the system allows for selective ratiometric detection of H_2O_2, over other reactive oxygen species, with a reported limit of detection of 100 pM.

Theranostics

Recent research has turned to nanoparticles that allow for theranostics. Liu et al. (42) engineered a polymer vesicle comprising block copolymers (polyglutamic acid and polycaprolactone) with tumor-targeting groups (folic acid) and MRI contrast agents (gadolinium, Gd^{3+}) attached on either end. The vesicles encapsulated cancer therapeutics (i.e., doxorubicin) for targeted delivery.

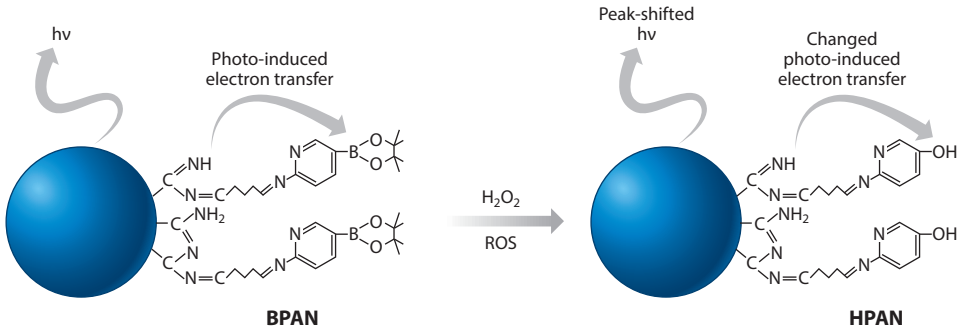

Figure 5

Schematic describing the boronate-functionalized polyacrylonitrile (BPAN) nanoparticles. Due to donor-bridge-acceptor fluorescent systems on the BPANs' surfaces, their fluorescent intensity decreases in response to oxidative stress, allowing for the ratiometric detection of reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2), through the conversion of the BPAN nanoparticles to H_2O_2-treated PAN (HPAN) nanoparticles. Adapted with permission from Reference 41. Copyright 2012, American Chemical Society.

Liu and colleagues found an eightfold enhancement in the performance of the gadolinium as a contrast agent.

Polymer nanoparticles can also be used as imaging materials on their own, with incorporated physical properties including significant extinction coefficients, bright fluorescence, resilient photostability, and biocompatibility. Guo et al. (43) developed thiophene-benzo[*c*][1,2,5]thiadiazole (TBT) polymer vesicles with three imaging functionalities, namely fluorescence, photoacoustic, and thermal imaging. The TBT vesicles also have two therapeutic functionalities: photodynamic and photothermal. The TBT possesses these functionalities because it is a donor–acceptor polymer composed of alternating electron donor and acceptor subunits. A charge flows along the polymer chains and jumps between neighboring polymer chains as the electrons are passed from donors to acceptors (44). To improve water solubility, the TBTs are encapsulated by DSPE-mPEG2000 (2-distearoyl-*sn*-glycero-3- phosphoethanolamine-*N*-[methoxy-(polyethyleneglycol)-2000]) to improve water solubility and biocompatibility (43). The TBT vesicles could be improved by adding targeting elements, as they currently rely on passive targeting by accumulating in tumors via the enhanced permeability and retention effect.

DENDRIMERS

Discovered in 1978, dendrimers are formed as multiple layers, called generations, of highly branched polymers around a core (45). The spherical shape of dendrimers arises from the multiple generations, with dendrimer size varying between 1 nm to 10 nm, depending on generation number (45–46). The number of termini also increases with each generation, along with some heterogeneity. These many termini yield exceptional polyvalent functionalization opportunities, including uses ranging from cosmetics to therapeutics to imaging and detection (46). Several dendrimer-based diagnostics are already on the market, including a polyamidoamine dendrimer assay for cardiac biomarkers (Stratus CS from Siemens), an anthrax detector (Alert Ticket from US Army laboratories), and a polylysine dendrimer with gadolinium for use as an MRI contrast agent (Gadomer 17 from invivoContrast) (47).

The range of diagnostic possibilities for dendrimers continues to expand, with recent research applying them to detection of allergies, viruses, and cancer. Soler et al. (48) developed a

label-free method of detecting amoxicillin-binding IgEs in amoxicillin-allergic patients by using polyamido-based dendrimers surface functionalized onto gold nanodisks. The IgEs bind to amoxicilloyl groups bound to peripheral termini. The allergen–IgE bindings are detected with a nanoplasmonic biosensor via surface plasmon resonance (SPR) with a detection limit of 0.6 ng/mL (0.25 kU/L).

Dendrimers have also been used to enhance established diagnostics like immunoassays and microfluidics. Sun (49) used a system of AgCl nanospheres surface functionalized with dendrimers in place of the detection antibody in a sandwich-type immunoassay to identify enterovirus 71 (EV71). EV71 is an RNA virus implicated in pediatric epidemics of serious neurological diseases and is the main cause of hand, foot, and mouth disease. Sun's system allowed for the detection of EV71 to a limit of 0.058 ng/mL. To improve a microfluidic capture system, Yeh et al. (50) added dendrimers to increase the degree of movement of the antibodies used to capture circulating tumor cells (CTCs) and circulating tumor microemboli (CTMs) in an antibody-based, ex vivo system. The polyamidoamine dendrimers bind the antibodies to the supported lipid bilayer surface while allowing them to move in three dimensions. The incorporation of dendrimers in the biosensor design resulted in 1.6× and 2.3× enhancements to the capture of CTCs and CTMs, respectively, compared to antibodies bound directly to the supported lipid bilayer.

LIPOSOMES

Although largely studied for their uses in drug delivery, liposomes have also been researched for possible uses in diagnostics. The most common diagnostic method explored with liposomes has been their use in bioimaging. The nanosized liposomes passively target the tumor via the enhanced permeability and retention effect, which enables their use for cancer imaging in vivo or even combined with imaging and therapy. Many early studies have already been reviewed in detail (51–52), including those that apply theranostic approaches such as combined MRI and temperature-triggered drug release (53), and combined nuclear imaging and internal radiotherapy (54). More recently, combined liposome-QDs have been studied as a way to deliver a chemotherapeutic within the aqueous core of the liposome to tumor cells, while the QDs provide fluorescent imaging capabilities (55).

Another novel application for liposomes in diagnostics is their use as a synthetic allergen platform, named nanoallergens (56). Although recent research has suggested that only a few certain distinct epitopes on an allergen protein are critical in provoking the allergic response, current diagnostic methods cannot identify epitope-specific information. The nanoallergen platform that has been developed in our laboratory is utilized to multivalently present each individual allergenic epitope from an allergen protein (56–57) (**Figure 6**). This allows the identification of the immunodominant allergen epitopes that largely carry the responsibility for the initiation of the significant allergenic responses using patient sera ex vivo. In this manner, a more personalized approach to allergy diagnosis is offered, and immunodominant epitopes critical to the allergenic response for a specific patient can be identified (**Figure 6*b***). Moreover, this technique could aid the development of personalized allergen inhibition for those with severe allergies to common food items such as peanuts and potentially lifesaving medicines such as antibiotics and chemotherapeutics (57–58).

NANOWIRES AND NANOTUBES

Although silicon nanowires and carbon nanotubes are quite different chemically, they have similar geometric structures and electrical properties; therefore, they have many similar uses in diagnostic applications. Both are primarily used to convert a chemical interaction into an electrical signal for

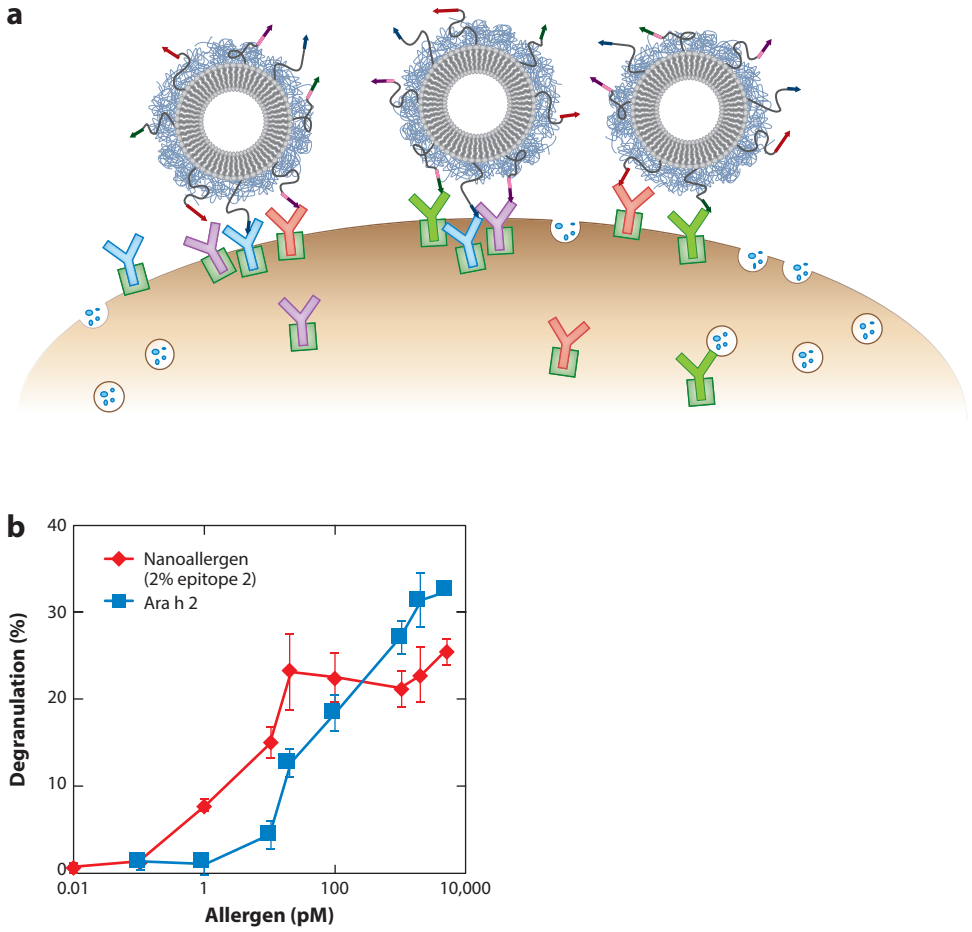

Figure 6

Liposome-based nanoallergens induce degranulation responses with intensities comparable to a natural allergen (Ara h 2). (*a*) Graphic representation of nanoallergen with multivalent presentation of epitopes (represented by different colors) allowing for cross-linking of polyclonal IgEs and triggering a signal transduction that results in degranulation. (*b*) The most immunogenic epitope of Ara h 2 (epitope 2) was presented on nanoallergens at 2% loading and was able to induce degranulation with a comparable intensity to that of the natural allergen Ara h 2. Figure adapted with permission from Reference 57 and Nature Publishing Group under the Creative Commons Attribution 4.0 International License (**http://creativecommons.org/licenses/by/4.0**).

measurement, with the polyvalent nature of their functionalized chemical forms allowing sensitive detection over a range of sensitivity. Other uses for nanowires and nanotubes do exist, and new iterations of their classic usage as well as novel unique uses for both are discussed below.

Nanowires

As stated above, the primary use of silicon nanowires in diagnostics is in the transformation of a chemical interaction event to an electrical signal. Many different applications and methods of accomplishing this have been implemented for a variety of different targets. These nanowire arrays

are all produced via standard silicon wafer fabrication techniques, allowing extremely precise patterning down to the nanometer size. The nanowires are then functionalized with multiple copies of a moiety that has affinity for the desired target molecule. Binding and capture of the target molecule on its surface lead to a change in the resistance of the wire, which is measurable via the current flowing through the wire (59). This principle forms the basis of most nanowire diagnostic techniques.

One of the earliest applications has been in the ultrasensitive detection of certain sequences of DNA. Improvements in fabrication and design have allowed techniques to offer detection limits down to 1 fM (60). These silicon nanowire arrays have recently been used to detect the presence of dengue virus and even miRNA associated with breast cancer from blood plasma at very low levels (61–62). Similar techniques have also been developed for the detection of small-molecule targets such as cardiac troponin T and cardiac troponin I, both markers for acute myocardial infarction, and human thyroid stimulating hormone, a useful screening tool for the diagnosis of thyroid disease. Both of these techniques demonstrate detection limits in the subpicomolar range, showing much promise as an easy and reusable early detection technique that could potentially also be translated into field use (63–65).

A more recent and more novel use of silicon nanowires simply comes from arranging them in a different geometry, perpendicular to their base instead of parallel to it. These nanowires are termed nanoneedles, and reports have described novel applications in minimally invasive manipulation of the intracellular environment (66). These nanoneedles can be functionalized to detect localized enzymatic activity within regions of an individual cell. One study utilized this specific application to detect localized cathepsin B activity through the use of fluorophore bound to the nanoneedles with a cathepsin B–sensitive bond (67) (**Figure 7**). An array of these needles was then interfaced with cells, and areas with high cathepsin B activity, such as lysosomes of healthy cells or the cytosol of cancer cells, displayed high levels of fluorescence as the fluorophore was cleaved from the nanoneedles (67). This and other techniques could hold a wide range of interesting applications for nanoneedles in diagnostics in the future.

Nanotubes

Similar to nanowires, carbon nanotubes are usually used in diagnostics by converting a binding event into an electrical signal. Nanotubes are highly modifiable with different binding moieties, but they require the use of additional elements to create the commonly used field effect transistors that form the bulk of their use in diagnostics (68–69). The addition of a gold element can greatly amplify the signal from a carbon nanotube–field-effect transistor. One such technique utilizes gold nanoparticles in a sandwich-binding assay with the targeted ligand to enhance the signal and sensitivity of the detector (70). A similar technique adds a layer of gold between the nanotubes and the binding sites to form a metal semiconductor field effect transistor, in this case, allowing the device to detect levels of amyloid-beta (an early Alzheimer's biomarker) down to 1 pg/mL in human serum (71). One unique biosensor for utilizing nanotubes takes advantage of their geometry by having the nanotubes not be fixed in place, but instead added as the top slice of a sandwich binding. An AC field is then applied so that their length bridges two gold electrodes, allowing a current to flow (72) (**Figure 8**). This method also utilizes hydrodynamic shear to further increase selectivity and can achieve detection limits for biotin-streptavidin down to 100 aM (72).

In addition to their use as biosensors, carbon nanotubes have also been used for imaging and treatment of cancer. A recent study utilized nanotubes as a package for delivering gadolinium-carbon QDs as well as doxorubicin for combined tumor imaging and treatment via chemotherapy and photothermal therapy (73). This combination was then coated with PEG, and folic acid was

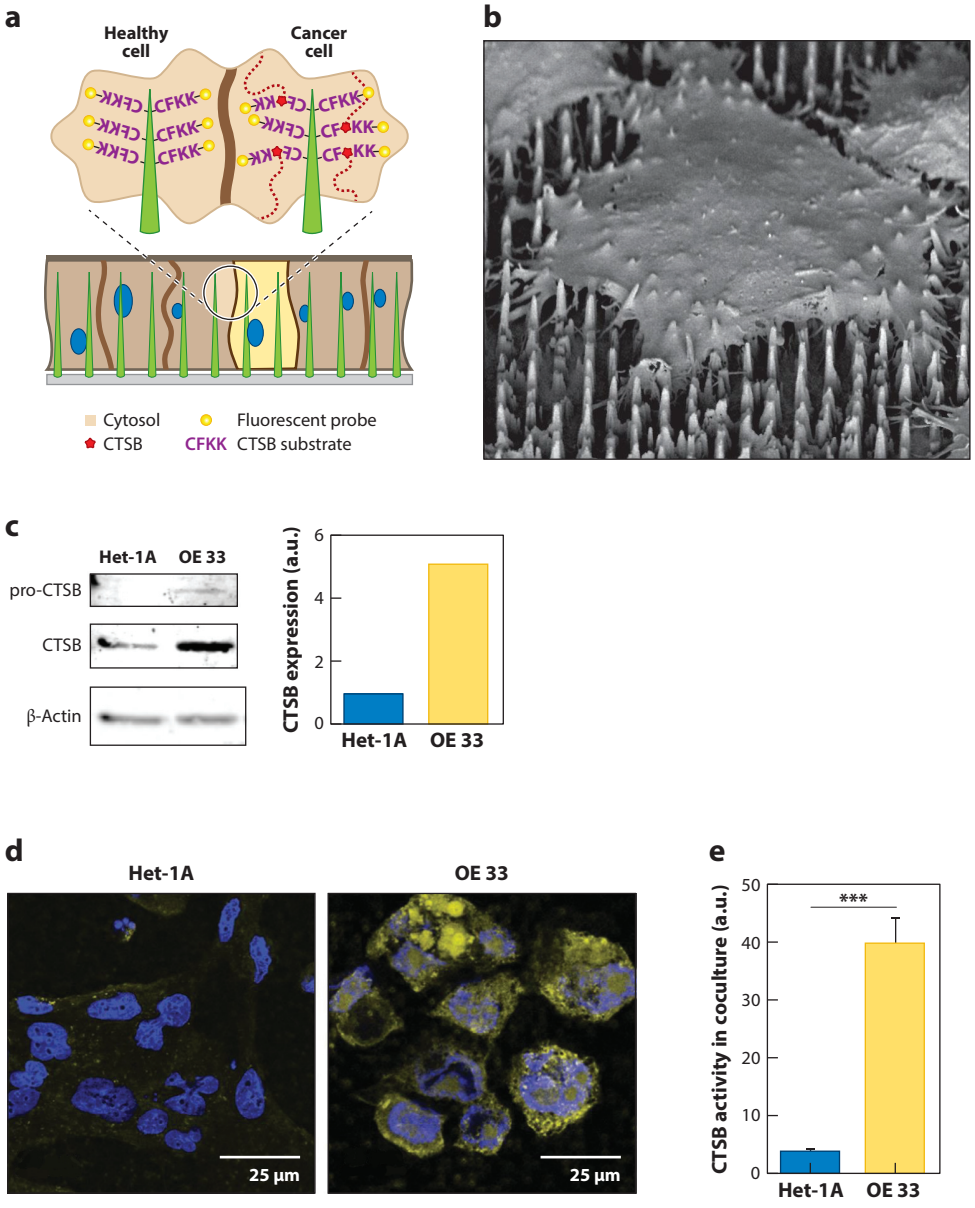

Figure 7

Local cytosolic enzymatic activity mapping using silicon nanoneedles. (*a*) Schematic of nanoneedle (*green*) function when interacting with the cell. In the presence of active cathepsin B (CTSB), the fluorescent probe is cleaved from the nanoneedle, resulting in the probe's release from the nanoneedle into the intracellular space. (*b*) Scanning electron microscope image of a cell interfaced with a nanoneedle array. (*c*) Image of the relevant Western blot bands and quantification of the Western blot displaying the expression of CTSB in HET-1A and OE 33 cell lines. (*d*) Confocal microscopy images of representative HET-1A and OE 33 cells after application of nanoneedles for 15 min. The cleaved fluorescent probe is displayed in yellow, whereas nuclei are stained blue. (*e*) Quantification of area-normalized fluorescence for HET-1A and OE 33 cells grown in coculture after application of nanoneedles. Figure adapted with permission from Reference 67. Reproduced by permission of John Wiley & Sons under the Creative Commons Attribution License.

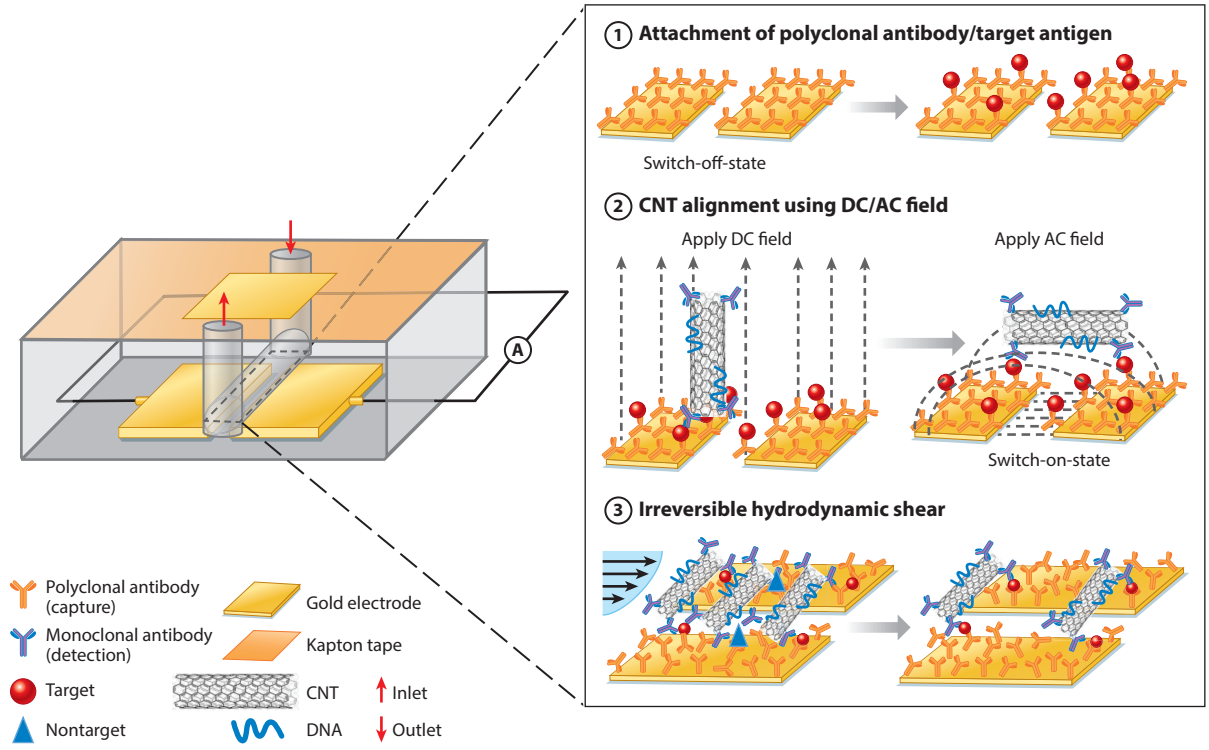

Figure 8

Schematic for a carbon nanotube (CNT) switch sensor for ultrasensitive protein detection. Step 1: Sample with target is incubated with gold electrode–bound polyclonal antibodies. Step 2: Monoclonal antibody–conjugated and DNA-wrapped (to allow DC electrophoresis and AC dielectrophoresis) CNTs are introduced. These bind to the target and self-assemble under a DC field before being switched on through the application of an AC field, bridging the gap between the gold electrodes by binding targets on each side. Step 3: Hydrodynamic shear irreversibly removes nonspecifically bound CNTs and can be tuned to the binding strength of the desired antibody–antigen interaction. Binding can then be measured through the use of an ammeter (A). Adapted with permission from Reference 72. Copyright 2017, Elsevier.

conjugated to the nanotubes to provide tumor targeting. This combined nanopackage allowed tumor imaging in vivo via MRI, fluorescence imaging, and near-infrared imaging. Additionally, the synergistic chemo- and photothermal therapy reportedly showed significant efficacy in an in vivo tumor xenograph model (73). Considering their applications in biosensing, imaging, and therapeutic treatments, carbon nanotubes hold a wide array of promising techniques for future study.

CONCLUSION

The range of applications of nanotechnology in diagnostics has grown a great deal in the last twenty years. Several new nanotechnology-based scientific reports that describe a variety of engineering and design feats and discoveries are published every week across many disciplines, including biomedicine. As the medical field keeps shifting toward more individualized and specialized treatments, improved and faster diagnostic methods are needed to arm doctors with the accurate and reliable information they need to decide on the most suitable treatment regimen. Nanoobjects provide a powerful tool with which to fill that role. The wide range of nanoobjects, particularly

polyvalent and highly functionalizable ones, enable engineering of more complex and diverse diagnostics than ever before. In this review, we have discussed both established and some more recent, developing applications of nanoobjects in diagnostics. We anticipate that some of these ideas will be translated from academia to industry and will help researchers achieve significant accomplishments in both the clinic and the field.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Arap W, Pasqualini R, Montalti M, Petrizza L, Prodi L, et al. 2013. Luminescent silica nanoparticles for cancer diagnosis. *Curr. Med. Chem.* 20:2195–211
2. Harianja DH. 2015. *Cornell C' dots, the cancer hunters*. Cornell Res., Cornell Univ. Ithaca, NY. **https://research.cornell.edu/news-features/cornell-c-dots-cancer-hunters**
3. Wu SQ, Li Z, Han JH, Han SF. 2011. Dual colored mesoporous silica nanoparticles with pH activable rhodamine-lactam for ratiometric sensing of lysosomal acidity. *Chem. Commun.* 47:11276–78
4. Zhou ZJ, Zhang CL, Qian QR, Ma JB, Huang P, et al. 2013. Folic acid-conjugated silica capped gold nanoclusters for targeted fluorescence/X-ray computed tomography imaging. *J. Nanobiotechnol.* 11:12
5. Xie XJ, Xu W, Liu XG. 2012. Improving colorimetric assays through protein enzyme-assisted gold nanoparticle amplification. *Acc. Chem. Res.* 45:1511–20
6. Zhou W, Gao X, Liu DB, Chen XY. 2015. Gold nanoparticles for in vitro diagnostics. *Chem. Rev.* 115:10575–636
7. Zhang RP, Cheng K, Antaris AL, Ma XW, Yang M, et al. 2016. Hybrid anisotropic nanostructures for dual-modal cancer imaging and image-guided chemo-thermo therapies. *Biomaterials* 103:265–77
8. Millipore Sigma. 2018. *Gold nanoparticles: properties and applications*. Millipore Sigma, Burlington, MA. **https://www.sigmaaldrich.com/technical-documents/articles/materials-science/nanomaterials/gold-nanoparticles.html**
9. Perrault SD, Chan WCW. 2010. In vivo assembly of nanoparticle components to improve targeted cancer imaging. *PNAS* 107:11194–99
10. Zhou F, Yuan L, Wang HW, Li D, Chen H. 2011. Gold nanoparticle layer: a promising platform for ultra-sensitive cancer detection. *Langmuir* 27:2155–58
11. Zhu Y, Chandra P, Shim YB. 2013. Ultrasensitive and selective electrochemical diagnosis of breast cancer based on a hydrazine–Au nanoparticle–aptamer bioconjugate. *Anal. Chem.* 85:1058–64
12. Mira TA, Yoon JH, Gurudatt NG, Won MS, Shim YB. 2015. Ultrasensitive cytosensing based on an aptamer modified nanobiosensor with a bioconjugate: detection of human non-small-cell lung cancer cells. *Biosens. Bioelectron.* 74:594–600
13. Pan YL, Shan WQ, Fang HT, Guo ML, Nie Z, et al. 2014. Sensitive and visible detection of apoptotic cells on Annexin-V modified substrate using aminophenylboronic acid modified gold nanoparticles (APSA-GNPs) labeling. *Biosens. Bioelectron.* 52:62–68
14. Wang HB, Xu W, Zhang H, Li DW, Yang ZQ, et al. 2011. EcoRI-modified gold nanoparticles for dual-mode colorimetric detection of magnesium and pyrophosphate ions. *Small* 7:1987–92
15. Marczak S, Senapati S, Slouka Z, Chang HC. 2016. Induced nanoparticle aggregation for short nucleic acid quantification by depletion isotachophoresis. *Biosens. Bioelectron.* 86:840–48
16. Li L, Liang Y, Zhao Y, Chen ZB. 2018. Target binding and DNA hybridization-induced gold nanoparticle aggregation for colorimetric detection of thrombin. *Sens. Actuators B* 262:733–38
17. Mustafaoglu N, Kiziltepe T, Bilgicer B. 2017. Site-specific conjugation of an antibody on a gold nanoparticle surface for one-step diagnosis of prostate specific antigen with dynamic light scattering. *Nanoscale* 9:8684–94

18. Mahajan KD, Fan QR, Dorcena J, Ruan G, Winter JO. 2013. Magnetic quantum dots in biotechnology—synthesis and applications. *Biotechnol. J.* 8:1424–34
19. Wang Y, Fry HC, Skinner GE, Schill KM, Duncan TV. 2017. Detection and quantification of biologically active botulinum neurotoxin serotypes A and B using a Förster resonance energy transfer-based quantum dot nanobiosensor. *ACS Appl. Mater. Interfaces* 9:31446–57
20. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, et al. 2005. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* 307:538–44
21. Du Y, Rajamanickam K, Stumpf TR, Qin YB, McCulloch H, et al. 2018. Paramagnetic quantum dots as multimodal probes for potential applications in nervous system imaging. *J. Inorg. Organomet. Polym. Mater.* 28:711–20
22. Tsuboi S, Sasaki A, Sakata T, Yasuda H, Jin T. 2017. Immunoglobulin binding (B1) domain mediated antibody conjugation to quantum dots for *in vitro* and *in vivo* molecular imaging. *Chem. Commun.* 53:9450–53
23. Li SL, Yang J, Lei XF, Zhang JN, Yang HL, et al. 2016. Peptide-conjugated quantum dots act as the target marker for human pancreatic carcinoma cells. *Cell. Physiol. Biochem.* 38:1121–28
24. Kim C, Hoffmann G, Searson PC. 2017. Integrated magnetic bead quantum dot immunoassay for malaria detection. *ACS Sens.* 2:766–72
25. Yang ST, Wang X, Wang HF, Lu FS, Luo PJG, et al. 2009. Carbon dots as nontoxic and high-performance fluorescence imaging agents. *J. Phys. Chem. C* 113:18110–14
26. Xu XY, Ray R, Gu YL, Ploehn HJ, Gearheart L, et al. 2004. Electrophoretic analysis and purification of fluorescent single-walled carbon nanotube fragments. *J. Am. Chem. Soc.* 126:12736–37
27. Baker SN, Baker GA. 2010. Luminescent carbon nanodots: emergent nanolights. *Angew. Chem. Int. Ed.* 49:6726–44
28. Hamd-Ghadareh S, Salimi A, Fathi F, Bahrami S. 2017. An amplified comparative fluorescence resonance energy transfer immunosensing of CA125 tumor marker and ovarian cancer cells using green and economic carbon dots for bio-applications in labeling, imaging and sensing. *Biosens. Bioelectron.* 96:308–16
29. Das P, Ganguly S, Mondal S, Bose M, Das AK, et al. 2018. Heteroatom doped photoluminescent carbon dots for sensitive detection of acetone in human fluids. *Sens. Actuators B* 266:583–93
30. Liang XC, Chen SQ, Gao JM, Zhang HY, Wang Y, et al. 2018. A versatile fluorimetric chemosensor for mercury (II) assay based on carbon nanodots. *Sens. Actuators B* 265:293–301
31. Lee HN, Ryu JS, Shin C, Chung HJ. 2017. A carbon-dot-based fluorescent nanosensor for simple visualization of bacterial nucleic acids. *Macromol. Biosci.* 17:1700086
32. Demir B, Lemberger MM, Panagiotopoulou M, Rangel PXM, Timur S, et al. 2018. Tracking hyaluronan: molecularly imprinted polymer coated carbon dots for cancer cell targeting and imaging. *ACS Appl. Mater. Interfaces* 10:3305–13
33. Biju V. 2014. Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. *Chem. Soc. Rev.* 43:744–64
34. Afreen S, Muthoosamy K, Manickam S, Hashim U. 2015. Functionalized fullerene (C_{60}) as a potential nanomediator in the fabrication of highly sensitive biosensors. *Biosens. Bioelectron.* 63:354–64
35. Bai LJ, Chen YH, Bai Y, Chen YJ, Zhou J, Huang AL. 2017. Fullerene-doped polyaniline as new redox nanoprobe and catalyst in electrochemical aptasensor for ultrasensitive detection of *Mycobacterium tuberculosis* MPT64 antigen in human serum. *Biomaterials* 133:11–19
36. Wang LR, Zhu XL, Tang XY, Wu CQ, Zhou ZJ, et al. 2015. A multiple gadolinium complex decorated fullerene as a highly sensitive T_1 contrast agent. *Chem. Commun.* 51:4390–93
37. Shi JJ, Wang L, Gao J, Liu Y, Zhang J, et al. 2014. A fullerene-based multi-functional nanoplatform for cancer theranostic applications. *Biomaterials* 35:5771–84
38. Thakor AS, Jokerst JV, Ghanouni P, Campbell JL, Mittra E, Gambhir SS. 2016. Clinically approved nanoparticle imaging agents. *J. Nuclear Med.* 57:1833–37
39. Ruiz JAR, Sanjuán AM, Vallejos S, García FC, García JM. 2018. Smart polymers in micro and nano sensory devices. *Chemosensors* 6:12
40. Kim SH, Kim B, Yadavalli VK, Pishko MV. 2005. Encapsulation of enzymes within polymer spheres to create optical nanosensors for oxidative stress. *Anal. Chem.* 77:6828–33

41. Oh WK, Jeong YS, Kim S, Jang J. 2012. Fluorescent polymer nanoparticle for selective sensing of intracellular hydrogen peroxide. *ACS Nano* 6:8516–24
42. Liu QM, Chen S, Chen J, Du JZ. 2015. An asymmetrical polymer vesicle strategy for significantly improving T_1 MRI sensitivity and cancer-targeted drug delivery. *Macromolecules* 48:739–49
43. Guo L, Niu GG, Zheng XL, Ge JC, Liu WM, et al. 2017. Single near-infrared emissive polymer nanoparticles as versatile phototheranostics. *Adv. Sci.* 4:1700085
44. Marszalek T, Li MM, Pisula W. 2016. Design directed self-assembly of donor-acceptor polymers. *Chem. Commun.* 52:10938–47
45. Abbasi E, Aval SF, Akbarzadeh A, Milani M, Nasrabadi HT, et al. 2014. Dendrimers: synthesis, applications, and properties. *Nanoscale Res. Lett.* 9:247
46. Abd-El-Aziz AS, Agatemor C. 2018. Emerging opportunities in the biomedical applications of dendrimers. *J. Inorg. Organomet. Polym. Mater.* 28:369–82
47. Sharma A, Kakkar A. 2015. Designing dendrimer and miktoarm polymer based multi-tasking nanocarriers for efficient medical therapy. *Molecules* 20:16987–7015
48. Soler M, Mesa-Antunez P, Estevez MC, Ruiz-Sanchez AJ, Otte MA, et al. 2015. Highly sensitive dendrimer-based nanoplasmonic biosensor for drug allergy diagnosis. *Biosens. Bioelectron.* 66:115–23
49. Sun AL. 2018. A potentiometric immunosensor for enterovirus 71 based on bis-MPA-COOH dendrimer-doped AgCl nanospheres with a silver ion-selective electrode. *Analyst* 143:487–92
50. Yeh PY, Chen YR, Wang CF, Chang YC. 2018. Promoting multivalent antibody-antigen interactions by tethering antibody molecules on a PEGylated dendrimer-supported lipid bilayer. *Biomacromolecules* 19:426–37
51. Langer R. 1998. Drug delivery and targeting. *Nature* 392:5–10
52. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. 2007. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* 2:751–60
53. Langereis S, Keupp J, van Velthoven JLJ, de Roos IHC, Burdinski D, et al. 2009. A temperature-sensitive liposomal ^{1}H CEST and ^{19}F contrast agent for MR image-guided drug delivery. *J. Am. Chem. Soc.* 131:1380–81
54. Ting G, Chang CH, Wang HE, Lee TW. 2010. Nanotargeted radionuclides for cancer nuclear imaging and internal radiotherapy. *J. Biomed. Biotechnol.* 2010:953537
55. Seleci M, Seleci DA, Scheper T, Stahl F. 2017. Theranostic liposome-nanoparticle hybrids for drug delivery and bioimaging. *Int. J. Mol. Sci.* 18:1415
56. Deak PE, Vrabel MR, Pizzuti VJ, Kiziltepe T, Bilgicer B. 2016. Nanoallergens: a multivalent platform for studying and evaluating potency of allergen epitopes in cellular degranulation. *Exp. Biol. Med.* 241:996–1006
57. Deak PE, Vrabel MR, Kiziltepe T, Bilgicer B. 2017. Determination of crucial immunogenic epitopes in major peanut allergy protein, Ara h2, via novel nanoallergen platform. *Sci. Rep.* 7:3981
58. Handlogten MW, Serezani AP, Sinn AL, Pollok KE, Kaplan MH, Bilgicer B. 2014. A heterobivalent ligand inhibits mast cell degranulation via selective inhibition of allergen–IgE interactions in vivo. *J. Immunol.* 192:2035–41
59. Gao ZQ, Agarwal A, Trigg AD, Singh N, Fang C, et al. 2007. Silicon nanowire arrays for label-free detection of DNA. *Anal. Chem.* 79:3291–97
60. Dorvel BR, Reddy B, Go J, Guevara CD, Salm E, et al. 2012. Silicon nanowires with high-k hafnium oxide dielectrics for sensitive detection of small nucleic acid oligomers. *ACS Nano* 6:6150–64
61. Nuzaihan MNM, Hashim U, Arshad MKM, Kasjoo SR, Rahman SFA, et al. 2016. Electrical detection of dengue virus (DENV) DNA oligomer using silicon nanowire biosensor with novel molecular gate control. *Biosens. Bioelectron.* 83:106–14
62. Ivanov YD, Pleshakova TO, Malsagova KA, Kozlov AF, Kaysheva AL, et al. 2018. Detection of marker miRNAs in plasma using SOI-NW biosensor. *Sens. Actuators B* 261:566–71
63. Chua JH, Chee RE, Agarwal A, Wong SM, Zhang GJ. 2009. Label-free electrical detection of cardiac biomarker with complementary metal-oxide semiconductor-compatible silicon nanowire sensor arrays. *Anal. Chem.* 81:6266–71

64. Kim K, Park C, Kwon D, Kim D, Meyyappan M, et al. 2016. Silicon nanowire biosensors for detection of cardiac troponin I (cTnI) with high sensitivity. *Biosens. Bioelectron.* 77:695–701
65. Lu N, Dai PF, Gao AR, Valiaho J, Kallio P, et al. 2014. Label-free and rapid electrical detection of hTSH with CMOS-compatible silicon nanowire transistor arrays. *ACS Appl. Mater. Interfaces* 6:20378–84
66. Yum K, Wang N, Yu MF. 2010. Nanoneedle: a multifunctional tool for biological studies in living cells. *Nanoscale* 2:363–72
67. Chiappini C, Campagnolo P, Almeida CS, Abbassi-Ghadi N, Chow LW, et al. 2015. Mapping local cytosolic enzymatic activity in human esophageal mucosa with porous silicon nanoneedles. *Adv. Mater.* 27:5147–52
68. Battigelli A, Ménard-Moyon C, Da Ros T, Prato M, Bianco A. 2013. Endowing carbon nanotubes with biological and biomedical properties by chemical modifications. *Adv. Drug Deliv. Rev.* 65:1899–920
69. Ly SY, Cho NS. 2009. Diagnosis of human hepatitis B virus in non-treated blood by the bovine IgG DNA-linked carbon nanotube biosensor. *J. Clin. Virol.* 44:43–47
70. Kim JP, Lee BY, Lee J, Hong S, Sim SJ. 2009. Enhancement of sensitivity and specificity by surface modification of carbon nanotubes in diagnosis of prostate cancer based on carbon nanotube field effect transistors. *Biosens. Bioelectron.* 24:3372–78
71. Oh J, Yoo G, Chang YW, Kim HJ, Jose J, et al. 2013. A carbon nanotube metal semiconductor field effect transistor-based biosensor for detection of amyloid-beta in human serum. *Biosens. Bioelectron.* 50:345–50
72. Li DY, Wang CM, Sun GC, Senapati S, Chang HC. 2017. A shear-enhanced CNT-assembly nanosensor platform for ultra-sensitive and selective protein detection. *Biosens. Bioelectron.* 97:143–49
73. Zhang M, Wang WT, Cui YJ, Zhou NL, Shen J. 2018. Magnetofluorescent carbon quantum dot decorated multiwalled carbon nanotubes for dual-modal targeted imaging in chemo-photothermal synergistic therapy. *ACS Biomater. Sci. Eng.* 4:151–62