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OVERVIEW

Application of nanotechnology in biosensors for enhancing pathogen detection

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Rapid detection and identification of pathogenic microorganisms is fundamental to minimizing the spread of infectious disease, and informing clinicians on patient treatment strategies. This need has led to the development of enhanced biosensors that utilize state of the art nanomaterials and nanotechnology, and represent the next generation of diagnostics. A primer on nanoscale biorecognition elements such as, nucleic acids, antibodies, and their synthetic analogs (molecular imprinted polymers), will be presented first. Next the application of various nanotechnologies for biosensor transduction will be discussed, along with the inherent nanoscale phenomenon that leads to their improved performance and capabilities in biosensor systems. A future outlook on characterization and quality assurance, nanotoxicity, and nanomaterial integration into lab-on-a-chip systems will provide the closing thoughts.

This article is categorized under:
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1 | INTRODUCTION

The need for rapid detection and characterization of pathogenic microorganisms has led to the evolution of the biosensor. A biosensor consists of two components: a molecular recognition element and a transducer. The recognition element or bioreceptor is a molecule that recognizes and specifically interacts with a target analyte. The transducer converts the interaction into a measurable signal for quantification. But despite significant advancements in diagnostics, there still exists a need for faster, portable, and more sensitive diagnostic methods. Nanoscale materials, materials with at least one dimension on the order of 1–100 nm, have drawn great interest for enhancing diagnostic systems because of their size-dependent optical, electrical, physical, and chemical properties (Hodak, Henglein, & Hartland, 2000). At the same time nanoscale systems are much more prone to the adverse effects of nonspecific adsorption when compared to macroscale systems, making their incorporation into diagnostics that process a variety of complex sample mediums an important topic to consider. This review will cover numerous approaches for successful integration of nanomaterials into biosensors, as well as highlight how this integration is propelling the diagnostics field forward.

2 | BIORECEPTORS

In a biosensor, or diagnostic device, the bioreceptor is meant to interact with the specific analyte of interest so that its quantity can be measured among a matrix of other biological components. The analyte of interest can be proteins, nucleic acids,

and so forth, depending on the mechanisms of the underlying pathogen. The most common form of bioreceptor interactions are: antibody/antigen, nucleic acids, enzymes/ligands, cellular, or synthetic bioreceptors, that is, molecular imprinted polymers (Vo-Dinh & Cullum, 2000). Bioreceptors have nanoscale dimensions and acquire selectivity, an important diagnostic requirement, from nanoscale interactions.

2.1 | Antibodies

The antibody structure is composed of two identical copies of both heavy and light polypeptide chains held together by disulfide and noncovalent bonds to form a Y-shaped molecule about 10 nm in size (Reth, 2013). The heavy and light chains consist of constant and variable regions (sequences of 110 amino acids) (Lipman, Jackson, Trudel, & Weis-Garcia, 2005). Each region contributes differently to the functionality of the antibody. The constant region is responsible for interactions the with various effector molecules, which mediate the immune response, while the variable region is responsible for antigen binding. Figure 1a shows an illustration of the antibody structure. Specificity is the degree to which an antibody can discriminate between different antigens and is influenced by the structure of the antibody in its variable region, as well as the binding affinity (Frank, 2002). Binding affinity, or binding strength, is influenced by the conformation and may be altered by any number of factors, including association with other proteins, temperature, pH, and salt concentration (Lipman et al., 2005).

The concept of using antibodies for antigen identification is known as an immunoassay and it has been employed in various biosensor formats starting as early as 1959, when Yalow and Berson described the first immunoassay for insulin. As the technology took off so did the demand for antibodies. At first, antibodies were harvested from immunized animals (polyclonal antibodies) a technique that struggled to meet the demand of expanding use in diagnostics. Then in 1975, Kohler and Milstein reported the discovery of monoclonal antibody technology that allowed the production of a unique antibody in large quantities. More recently, recombinant antibodies—monoclonal antibodies with genetically fused antigen binding domains, have emerged as more cost-effective alternatives to traditional polyclonal and monoclonal antibodies (Emanuel et al., 2000). These advantages have led to a number of applications for pathogen detection that incorporate recombinant antibodies (Bugli et al., 2004; de Greeff, van Alphen, & Smith, 2000; Deng, Nesbit, & Morrow, 2003; Desogus et al., 2003; Saldarelli et al., 2005).

There are two basic formats for immunoassays: direct and indirect. With direct immunoassays (Figure 1b) sample with an unknown concentration of antigen is bound to a solid support. Antibody with attached reporter (dye, nanoparticle, enzyme, etc.) is added and then binds to the exposed epitope of immobilized antigen. The signal from the reporter will directly correlate to the quantity of the antigen present such that unknown concentrations can be quantified using a standards curve. With indirect immunoassays (Figure 1c) the steps are similar to a direct immunoassay with one additional step. Secondary antibody with a reporter molecule is added to bind with the antibody attached to the antigen. In this way, multiple secondary antibodies are able to attach to one antigen, amplifying the signal and providing enhanced sensitivity.

Immunoassay technology continues to be developed leading to new and improved applications for pathogen detection. Platforms are employing refined nanomaterials and combining methodologies in molecular biology to push sensitivity limits, which for certain pathogens has drastic implications on clinical outcomes (Caliendo et al., 2013; Peeling, Smith, & Bossuyt, 2010). One recent approach has been to employ polymerase chain reaction (PCR) for exponential amplification of DNA that is connected to an antibody, for signal enhancement. This is known as Immuno-Polymerase Chain Reaction (IPCR) and has been explored in numerous formats for detection of bacterial and viral pathogens where up to 10^9 fold increase in sensitivity has been reported compared to ELISA based immunoassays (Chang, Li, & Wang, 2016). Incorporation of nanoparticle-oligonucleotide

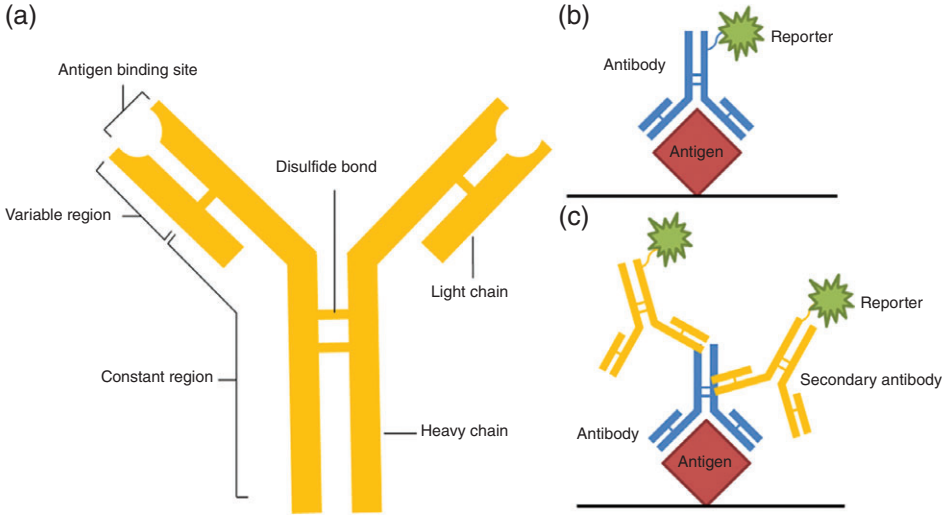

FIGURE 1 (a) Immunoglobulin G (IgG) antibody structure. (b) Direct immunoassay format. (c) Indirect immunoassay format

complexes for signal enhancement has also been demonstrated in the "bar code" immunoassay (Nam, Thaxton, & Mirkin, 2003). The "bar code" assay detected prostate specific antigen (PSA) down to 30 aM concentration, a sensitivity boost of 10^6 over traditional immunoassay formats (Nam et al., 2003).

2.2 | Nucleic acids

Nucleic acids are another example of a nanoscale material used for biorecognition in biosensors. Oligonucleotides are short single polymer chains about 10 nm in length (Matthews, 1997), consisting of 13–40 nucleotides. Nucleotides are composed of three subunit molecules: a nitrogenous base (Adenine, Thymine (Uracil for RNA), Guanine, Cytosine) a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. Oligonucleotides will hybridize with a complementary sequence of nucleic acids by reversible hydrogen bonding to form a stable duplex or triplex. During hybridization, higher affinity does not correlate to higher specificity due to the relatively small energetic penalty of a single mismatch (Demidov & Frank-Kamenetskii, 2004). The amount of base pair mismatch tolerated during hybridization under particular conditions is known as stringency, with higher stringency corresponding to higher specificity of the interaction (Edberg, 1985). Each unique duplex formed has a specific melting temperature dependent on physiochemical parameters such as base pair composition (Marmur & Doty, 1962), and salt concentration (Schildkraut, 1965). The capability of temperature controlled association and disassociation of the nucleic acid polymer strands forms the basis for nucleic acid hybridization probes, as well as nucleic acid amplifications reactions (e.g., PCR), which has over the last several decades been successfully applied to many diagnostics platforms. Moreover, due to sequence specific melting temperatures, melt temperature analysis can be used as a way to validate amplification products (Wittwer, Reed, Gundry, Vandersteen, & Pryor, 2003), reducing false positive signals.

The synthesis of specific oligonucleotides for hybridization with target nucleic acid sequences for quantification is known as a nucleic acid test (NAT). The target nucleic acid sequence can be DNA or RNA depending on the pathogen of interest, while the oligonucleotide probes can be based on DNA, RNA, and peptide nucleic acids (PNA). DNA is the basis for most NATs because of its stability, and its ability to be isolated relatively simply from a variety of biological matrices (O'Connor & Glynn, 2010). RNA has also been used for biosensor probes (Ferapontova, Olsen, & Gothelf, 2008; Ke et al., 2011), but is generally less stable than DNA based probes because of its susceptibility to ribonuclease digestion and chemical cleavage (Ferapontova et al., 2008). More recently, PNA, a synthetic analog to DNA/RNA with a backbone composed of repeating *N*-(2-aminoethyl)-glycine units linked by amide bonds (Nielsen & Egholm, 1999), has also been employed for pathogen detection (Kuhn et al., 2002; Mateo-Marti, Briones, Pradier, & Martin-Gago, 2007; Metaferia et al., 2013; Ozkan et al., 2002; Steichen, Decrem, Godfroid, & Buess-Herman, 2007; X. Su, Teh, Lieu, & Gao, 2007; Uno, Tabata, & Kawai, 2007; J. Wang et al., 1996; C. Zhao et al., 2014). PNA recognition layers impart remarkable sequence specificity and offers greater flexibility in reaction conditions over DNA/RNA based probes (Brandt & Hoheisel, 2004; J. Wang et al., 1996).

There are two common formats for nucleic acid based biosensors: direct detection and amplification based detection. In direct detection assays, oligo probes are immobilized onto a surface which is meant to hybridize with target nucleic acid sequences present in sample. Much like immunoassays, most NATs require a reporter molecule. Reporter labels act as transducers which convert a biochemical reaction into a measurable signal that can be correlated with the amount of target present. Optical and electrochemical are the two most common formats for oligo label detection. Optical labels offers high sensitivity, and the ability to detect multiple probes by emission spectra design (M. Han, Gao, Su, & Nie, 2001), but require transparent sample matrices. On the other hand, electrochemical based labels can be used for nontransparent samples, such as blood and urine (Katz & Willner, 2004). Currently, there is a growing interest in developing label-free methods using electrochemical detection (Das, Sumana, Nagarajan, & Malhotra, 2010; Fang, Jiao, Li, Qu, & Jiang, 2008; Oliveira et al., 2015; Souza et al., 2011) to simplify operation and eliminate the downfalls of complex probe synthesis (Oliveira et al., 2015). Label-free detection schemes can rely on electrodes to probe the intrinsic electrochemical properties of DNA (e.g., oxidation of purine bases), or detect changes in the interfacial properties after hybridization (Oliveira et al., 2015). DNA microarray technology, arrays of thousands of oligo probes deposited on a substrate in 10–100 μm reactions zones, has evolved as a particularly successful platform for gene expression and pathogen diagnosis because of the high degree of multiplexing it offers (Conejero-Goldberg et al., 2005; Gardner, Jaing, McLoughlin, & Slezak, 2010; Palacios et al., 2007; Quan et al., 2007; D. Wang et al., 2002; J. Q. Zhao et al., 2015).

In amplification based NATs, a small amount of target DNA or RNA can be amplified into billions of copies for more sensitive detection of small target analyte. This method is known as PCR, a technique first discovered in 1985 by Kary Mullis (Mullis et al., 1986). PCR is an enzyme driven reaction whereby oligonucleotide primers hybridize specifically in certain locations on a DNA sequence in free solution. These locations mark the starting and finishing locations where a thermostable DNA polymerase enzyme will rebuild the DNA sequence in between using free nucleic acids. Through multiple cycles of heating and cooling in steps corresponding to DNA denaturation, primer hybridization, and primer extension, a small amount

of target DNA is exponentially amplified in less than 2 hr. In this way PCR is significantly faster than traditional techniques like virus culturing and plating which can take 2–14 days for diagnosis (Baer & Kehn-Hall, 2014).

Since its introduction PCR has undergone some important evolutions to improve utility and versatility. One approach has been to develop an amplification technique that does not require thermocycling (Notomi et al., 2000; Piepenburg, Williams, Stemple, & Armes, 2006; Vincent, Xu, & Kong, 2004; Walker et al., 1992) so as to make it more practical for diagnostics in resource limited settings. Multiplexed PCR is the simultaneous amplification of multiple targets in one assay to provide higher diagnostic output per test. Multiplex PCR has led to the ability to perform comprehensive diagnostic panels, and screen for antibiotic resistance genes (Caliendo et al., 2013). Additionally, the invention of reverse transcriptase PCR (RT-PCR) has provided the important capability of detecting RNA based pathogens such as, HIV and Hepatitis C. Moreover, RT-PCR allows for the discrimination of viable cells and nonviable cells (Yaron & Matthews, 2002) as well as being able to detect gene expression (Derveaux, Vandesompele, & Hellemans, 2010).

2.3 | Nanoscale imprinted polymers

Nano molecular imprinted polymers (nMIPS) are polymer materials that are fabricated to mimic the structure of naturally occurring biorecognition elements, such as antibodies, aptamers, and nucleic acids. There are several advantages to using synthetic mimics to natural biorecognition elements, namely stability at high pH and temperature, as well as being less expensive to synthesize (Poma, Turner, & Piletsky, 2010). The basic concept for imprinted polymers is that a target molecule (template) is introduced to a monomer solution that interacts through complementary functional groups (e.g., hydrogen bonding, reversible covalent bonds, electrostatic interactions, and van der Waals forces; Scorrano, Mergola, Del Sole, & Vasapollo, 2011). The monomer-target mixture is then polymerized producing an imprint with cavities and binding sites that are complementary in shape and chemistry to the original target (Schauperl & Lewis, 2015) (Figure 2a). Target is removed by a solvent leaving behind an open imprint specific to the target.

Functional monomer-target interactions during synthesis have a significant effect on specificity and binding (Scorrano et al., 2011). These interactions can either be covalent, or noncovalent in nature. To improve target specificity, more than one functional group can be included in the prepolymer. Common functional monomers used for molecular imprinting include acids (i.e., acrylic acid, methacrylic acid, vinylbenzoic acid, 2-acrylamido-2-methylpropane sulphonic acid), and bases (vinylpyridine, vinylimidazole). Temperature during synthesis also influences specificity. Several studies have confirmed this by demonstrating that photo-initiated polymerization at low temperatures is preferable over thermal-initiated polymerization for improved stability and binding capacity (Vasapollo et al., 2011).

Conventional MIPs are commonly manufactured by polymerizing bulk monoliths and grinding into smaller microparticles (Yan & Row, 2006). However, this method is time and material consuming and yields particles with damaged and heterogeneous binding sites reducing overall effectiveness as a biorecognition element. Prepolymer emulsions can be

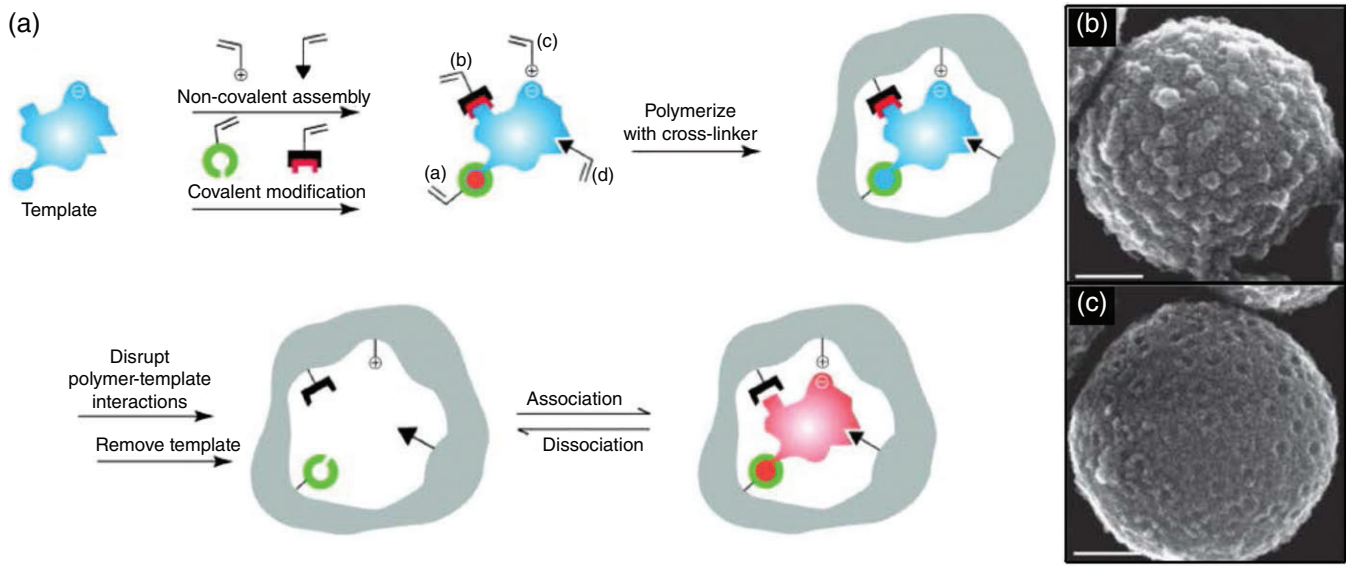

FIGURE 2 (a) Molecular imprinting process adopted from reference (Poma et al., 2010) with permission from Elsevier. (b) Scanning electron micrograph (SEM) image of nanoparticles with virus attached for the construction of the recognition layer (Reprinted with permission from Cumbo, Lorber, Corvini, Meier, and Shahgaldian (2013). Copyright 2013 Nature) (c) SEM image of nMIPS after virus template is removed (Reprinted with permission from Cumbo et al. (2013). Copyright 2012 Nature)

crosslinked such that MIP micro- and nanospheres are formed (T. Zhou, Zhang, Kamra, Bulow, & Ye, 2015), offering better control of size and binding site quality in comparison with ground particles. Alternatively, thin films of imprinted polymers can be fabricated on top of bulk substrates (X. Gu et al., 2015; Karthik, Margulis, Ren, Zare, & Leung, 2015) where the lack of mechanical breakup offers better binding site homogeneity. Homogeneity was further improved with the discovery of atom transfer radical polymerization (ATRP) a technique that uses transition metal complexes to control polymer chain growth. However, ATRP is constrained to thin film growth with only certain monomers (L. Chen, Wang, Lu, Wu, & Li, 2016). In general, the thickness of the imprinted layer must be comparable with the hydrodynamic radius of the protein to avoid fully encapsulating the target in the polymer matrix, which would prevent its removal (Menger et al., 2016).

More recently, a process has been developed to graft imprinted polymer films onto premade nanoparticle substrates, providing enhancement of surface area and improved homogeneity of binding sites. In this way nMIP layers have been constructed on to nanoparticle substrates for sensitive and selective detection of viruses (Karthik et al., 2015) and bacteria (Gültekin et al., 2009). For example, the Shahgaldian group demonstrated picomolar sensitivity for turnip yellow mosaic virus (TYMV) using a TYMV imprinted layer grown onto a silica nanoparticle. Scanning electron micrographs were taken during each fabrication step showing the layer growth with virus template (Figure 2b), as well as the imprinted layer after the virus template is removed (Figure 2c) (Cumbo et al., 2013). Moreover, nanoparticles can be incorporated directly into the layer to enhance localized transduction phenomena for Surface Plasmon Resonance (SPR), and Surface Enhanced Raman Spectroscopy (SERS). For example, Riskin, Tel-Vered, Frasconi, Yavo, and Willner (2010) embedded gold nanoparticles into a molecular imprinted film to improve the SPR detection sensitivity of l-phenylalanine 10× over a nanoparticle-free layer. While nMIP based recognition is still inferior to antibody and nucleotide based recognition in terms of selectivity and sensitivity, ongoing research in nMIPs has lessened that performance gap considerably.

3 | NANOPARTICLE TRANSDUCERS

Biosensors also require reporter labels to convert the biorecognition–target interaction into a signal for analysis. For diagnostics to be effective it is imperative to detect low concentrations of the pathogen to provide early warning before the pathogen has overwhelmed the patient's immune system. Thus, reporters must not only indicate the recognition event, but also provide a mechanism of amplifying that indication to an appropriate level such that very low concentrations are not masked by background noise. Nonetheless, when nanoparticles encounter biological media (i.e., blood, mucus, etc.) the biorecognition elements that coat their surface may undergo a change in surface charge. This in turn may lead to loss in specificity and increased nanoparticle aggregation (X.-Q. Zhang et al., 2012) rendering them useless as a diagnostic tool. This is compounded by their lack of ability to be immobilized for washing operations that mitigate nonspecific absorption. Thus, careful nanoparticle functionalization strategies along with control over sample matrix are integral to any clinical diagnostic that utilizes nanoparticles.

3.1 | Quantum dots

Quantum dots (QDs) are nanometer-scale inorganic semiconductor crystals composed of groups II–VI or III–V elements with a diameter typically ranging from 2 to 6 nm. In this size range, quantum confinement allows for highly discretized band structures that result in emission wavelength shifts corresponding to the size of the nanocrystal (Figure 3). Some of the advantageous properties of QDs are size-tunable emissions from visible to near infrared range (NIR), high quantum yield,

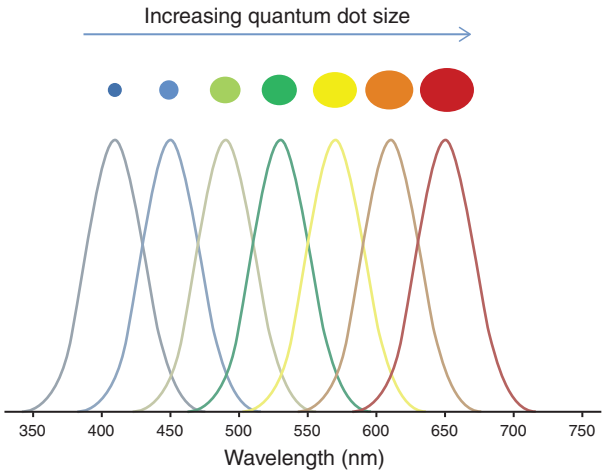

FIGURE 3 Size dependent emission spectra of quantum dots (Reprinted with permission from Vasudevan, Gaddam, Trinchi, and Cole (2015). Copyright 2015 Elsevier)

long fluorescent lifetimes, narrow emission spectra, and a high resistance to photobleaching. These unique properties have warranted their use in biosensing applications.

One of the most useful features of QDs is their synthesis can be tailored to specific requirements for the core, shell, and coating. QDs are mostly synthesized in nonpolar organic solvents such as trioctylphosphine oxide and hexadecylamine. An inorganic capping layer of zinc sulfide (ZnS) can be added to improve quantum yield by as much as 50%, and protect the core from oxidation (Dabbousi et al., 1997). QDs are coated with organic molecules and macromolecules to provide aqueous solubility and opportunities for bioconjugation. These coatings can be broadly classified as ligand-based (mercaptopropionic acid and dihydrolipoic acid) or polymer-based (polyethylene glycol) (Algar, Tavares, & Krull, 2010).

The unique and highly desirable luminescent properties of QDs have led to their increased adoption as reporter labels in biosensing applications. In general, multiple QD labels can be excited by a single light source and emit light with minimal spectral overlapping. This allows for multi-target assays without the added cost of filtering excitation light. While the characteristic narrow emission spectra of differentially-sized QDs can be leveraged for distinct target labels, it can also be used combinatorially, varying color and intensity levels for higher order multiplexing (Goldman et al., 2004; M. Han et al., 2001). This combinatorial approach, termed "QD barcoding," has demonstrated significant enhancement in Hepatitis B Virus, Hepatitis C, and HIV detection (Kim, Biondi, Feld, & Chan, 2016; Klostranec et al., 2007). Similarly, H. Xu et al. (2003) also demonstrated a 2-color/3-intensity barcoding assay for SNP genotyping analysis. QDs are also excellent donor molecules in Förster resonance energy transfer (FRET) detection compared to molecular fluorophores because of their large stokes shifts. FRET is an energy transfer mechanisms extremely sensitive to small scale changes in proximity (1–10 nm) of the acceptor and donor molecules, making it useful in characterizing biological interactions. Recently, Qiu and Hildebrandt (2015) demonstrated a QD-FRET assay that could quantify three different miRNA targets from clinical samples down to 0.3 pM. Furthermore, Noor and Krull (2014) took advantage of QD-FRET probes sensitivity and developed an assay with a single UV-lamp excitation source and an iPad camera for detection of nucleotide targets down to 450 fM. This demonstration shows the potential to implement highly sensitive QD-FRET probes in low cost POC diagnostics in the future.

3.2 | Nobel metal nanoparticles

Nobel metal nanoparticles are widely used in biosensing applications because of their ability to be synthesized in many sizes and compositions to suit a variety of functional requirements. In general, metal nanoparticles consist of a core containing a few hundred to thousands of atoms depending on the radius (J. Zhou, Ralston, Sedev, & Beattie, 2009). Nanoparticles can have a metal core consisting of one element, or be composed with a shell of a different metal (i.e., gold-silver core-shell nanoparticles). These bimetallic nanoparticles have the advantage of taking properties from both metals to enhance their optical and electronic properties over monometallic nanoparticles (Lu, Burkey, Halaciuga, & Goia, 2013). At sizes smaller than 3 nm noble metal nanoparticles are called nanoclusters (L.-Y. Chen, Wang, Yuan, & Chang, 2015). Unlike nanoparticles, nanoclusters do not display SPR absorption in the visible region, but possess fluorescence emission in the near-infrared to visible region (J. Zheng, Zhou, Yu, & Liu, 2012). The wavelength of emission can be tuned by controlling the size of the cluster, making nanoclusters very useful fluorescent biosensor labels.

There are a variety of methods for the fabrication of metal nanoparticles using physical means (i.e., laser ablation, microwave, arc discharge, etc.) and chemical means (i.e., chemical reduction, photochemical, micro emulsions, etc.) (Sreeprasad & Pradeep, 2013). Chemical reduction is the most common because of its simplicity in tailoring the nanoparticles to various sizes, with different functional groups. The Turkevich method (Turkevich, Stevenson, & Hillier, 1951) in particular has been used to synthesize colloidal gold nanoparticles 10–20 nm in size using sodium citrate as a reducing agent. Frens (1973) modified this method showing that control over nanoparticle size could be achieved by varying the trisodium citrate to gold ratio. A thorough review of the many synthesis routes for noble metal nanoparticles can be found here (Sreeprasad & Pradeep, 2013).

Gold and silver nanoparticles have been studied extensively for use with local surface plasmon resonance (LSPR). LSPR is the phenomenon where collective oscillations of free electrons in the nanoparticle, called plasmon, vibrate at a resonance frequency with the incident radiation. This appears as an absorption peak in the visible spectra (Nguyen, Park, Kang, & Kim, 2015). Absorption is influenced by the size of the nanoparticle, while peak position is influenced by the local refractive index surrounding the particle, which changes with the adsorption of biomolecules (Vincenzo, Roberto, Marco, Onofrio, & Maria Antonia, 2017). The ability to control the optical properties via particle size, shape, and surface functionalization (Tonga, Saha, & Rotello, 2014), has prompted significant exploration of nanoparticles for highly selective LSPR sensors for clinical diagnostics (W. Zhou, Gao, Liu, & Chen, 2015). Recently, Au/Ag composite nanoparticles have detected Human IgG down to 0.15 μg/mL concentration (J. Wang et al., 2011). Au has the advantage of chemical stability and biocompatibility, while Ag produces a sharper resonance peak than just bare Au nanoparticles (Mitsushio, Miyashita, & Higo, 2006). Additionally, Au nanoparticles also make excellent colorimetric probes and have frequently been incorporated into lateral

flow assays (LFA; Guo, Xu, Ferhan, Chen, & Kim, 2013; Kato & Oishi, 2014; Veigas et al., 2012). In this regard, AuNP-LFAs have been demonstrated for low cost detection of human ferritin down to 10 ng/mL (Mao, Du, Meng, & Song, 2015). Au nanoclusters (AuNCs), while comparable to QDs in terms of their size dependent emissions, strong photoluminescence, and photostability, show superior biocompatibility. In the past few years there has been considerable effort directed in the development of highly sensitive AuNC fluorescent probes for use in biosensors. For example, AuNCs were employed for selective detection of liver disease biomarker GSH S-transferase (C. T. Chen, Chen, Liu, Chang, & Chen, 2009). AuNCs have also been used to enhance the detection capabilities of other materials as well. For instance, AuNC embedded boron nitride nanocomposite provided fluorescent detection of interleukin-6 (IL-6), a pro-inflammatory cytokine, down to 30 pg/mL concentration in a sandwich assay format.

3.3 | Lanthanide nanoparticles

Lanthanide luminescent nanoparticles are composed of ions from elements located in the sixth period and IIIB group in the periodic table. The key feature of lanthanide luminescence is that lanthanide ions have exceedingly long-lived luminescence (μs to ms range), as opposed to conventional dyes that luminesce on the nanosecond scale. This feature coupled with time resolved fluorometery (TRF) gives lanthanide based labels the unique ability to be probed after the extinction of luminescence from background noise, leading to improved ability to resolve analyte signal at lower concentrations where background noise would normally suppress that signal. Lanthanide based probes also exhibit excellent photostability, large Stokes shift (>150 nm), and sharp-band emissions (<10 nm full width at half-maximum) (Y. Chen & Lu, 2007).

In the past decade, a variety of approaches for the synthesis of lanthanide luminescent NPs have been developed allowing for controlled morphologies, sizes, and optical properties. The most popular of these routes is thermal decomposition and hydrothermal/solvothermal because of their effectiveness in preparing high-quality target nanoparticles (Dong et al., 2015). Lanthanide doped nanoparticles require a chelating agent for binding the lanthanide ion to a functional group on the biomolecule. Common chelators are polyaminocarboxylates, β-diketonates, and carboxylates (Dong et al., 2015). Antennas, or highly π-conjugated aromatic or heteroaromatic systems, are also incorporated into the lanthanide nanoparticle complex as a means to efficiently transfer excitation energy to the lanthanide ions (Dong et al., 2015).

The enhanced sensitivity of lanthanide materials makes them popular alternatives to conventional fluorescent dyes for use in diagnostics. Furthermore, their long-lived luminescence allows them to be probed after the extinction of luminescence from other biological components in the sample matrix, an important feature for diagnostics that test unpurified clinical samples. As an alternative to simply tagging biorecognition elements with lanthanide chelates, there has been a shift to doping nanoparticle carriers with lanthanide materials and coating the NP carriers with biorecognition elements to form a complex (Hagan & Zuchner, 2011). For example, a streptavidin coated 107-nm polystyrene bead was doped with 30,000 Eu(III)–2-thenoyltrifluoroacetons and used in a PSA assay (Harma et al., 2000). The assay achieved zM (10^{-21} mol) sensitivity with a linear range spanning four orders of magnitude, a 100-fold increase over using just Eu-labeled streptavidin. Similarly, the Hewlett group has used polystyrene-core Eu-doped nanoparticles for detection of HIV-1 (Tang et al., 2010), anthrax (Tang et al., 2009), and Influenza A and B (J. Liu, Zhao, Petrochenko, Zheng, & Hewlett, 2016) down to single pg/mL concentrations. Nevertheless, polystyrene has its limitations as a lanthanide carrier due to generally large particle sizes, swelling, and leakage of the encapsulated molecules through surface defects (F. Wang, Tan, Zhang, Fan, & Wang, 2006). Consequently, silica, is being used as an alternative carrier because of improved aqueous solubility, and silane coupling chemistry (F. Wang et al., 2006). For instance, Chunduri et al. (2017) engineered streptavidin coated Europium doped silica nanoparticles to significantly increase HIV-1 detection sensitivity down to 0.02 pg/mL, a 1,000-fold enhancement over a conventional colorimetric assay.

4 | LABEL FREE TRANSDUCTION

Nanomaterials have also been use in label free transduction schemes such as, nanowires, nanotubes, nanocantilevers, and nanopores as a simpler alternative to relying on attachment with reporter labels for signal transduction. In label-free techniques the nanoscale size of the transduction substrate affords the same signal amplification mechanisms as do the nanoscale reporters, but generally reduces operational and material requirements. Not to mention, a platform that can multiplex, or detect for multiple targets in one sample, is imperative to fully characterize the pathogenic variant and guide the course of therapy. Reporter labels are inherently limited in multiplexing capability by spectral overlap of emission signals, or for nonoptical transduction, lack of ability to individually probe reporters in solution. The potential to improve usability and add multiplexing functionality has been the driving factor in development of new label free transducers for diagnostic devices.

4.1 | Nanowires

Silicon based nanomaterials have been popular because of their semiconducting and mechanical properties, flexible surface modification, and compatibility with conventional silicon processing techniques. Silicon nanowires (SiNWs) are high aspect ratio wires (Figure 4a) that typically have a diameter of <100 nm, and have been employed recently in highly sensitive microfluidic detection platforms for nucleic acids (W. Y. Chen et al., 2013; Gao et al., 2012; Hahm & Lieber, 2004; G.-J. Zhang, Luo, Huang, Tay, & Lim, 2010; G. J. Zhang, Zhang, et al., 2010) and proteins (Chua, Chee, Agarwal, Wong, & Zhang, 2009; Cui, Wei, Park, & Lieber, 2001; G. J. Zhang, Huang, Ang, Liu, & Desai, 2011; G. J. Zhang, Luo, et al., 2011). The original concept for a nanowire sensor was proposed by Lieber's group in 2001 (Cui et al., 2001), who demonstrated SiNWs incorporated into a field effect transistor (FET). A SiNW FET sensor has three electrodes: source, drain, and gate electrode. The nanowire acts as the semiconductor channel between the source and drain electrodes, while the gate electrode modulates the channel conductance. This basic operating principle is that charged biomolecules will bind to the surface of the wire and alter its electric field. This binding event results in a change in conductivity depending on the nanowire dopant (p-type or n-type) and the charge of the captured molecule (Figure 4b,c). For instance, a FET sensor will detect a decrease in conductivity when a negatively charged biomolecule binds to the surface of an n-type SiNW. The FETs response to changes in surface charge is dependent on the Debye screening length, so it is important to maintain a low ionic strength in the buffer to maximize sensitivity. Nanoscale physics lead to unique sensor properties like quantum confinement (Niquet et al., 2006) in nanowires with diameters <2.2 nm (Mohammad, 2014). At sufficiently small diameters, SiNWs are sensitive to very small changes in charge because a single binding event can lead to depletion or accumulation of carriers throughout a much larger percentage of the conducting channel cross-section. Reduction of diameter also leads to increased surface area available for target capture. Nanowire doping also influences biosensor sensitivity. For instance, it has been shown that lightly doped nanowires show greater sensitivity than highly doped or undoped ones (Nair & Alam, 2007). In generally, SiNWs have a dynamic range extending from tens of fM to pM and a detection limit of 10^{-14} M (Bunimovich et al., 2006).

There are two approaches to SiNW synthesis: bottom-up and top-down. Bottom-up processes involve wire growth on a silicon wafer either metal-catalyzed assisted or metal-catalyzed free. Vapor liquid solid (VLS) growth was first described by Wagner and Ellis (1964) and later explored extensively for SiNW fabrication by the Lieber group (Cui et al., 2001; Y. Wu et al., 2004). One key advantage of VLS is that the size of the AuNC defines the size of the nanowire as opposed to lithographic patterning, which has inherent limitations at the sub-10 nm scale. Nevertheless, the technology suffers from lack of

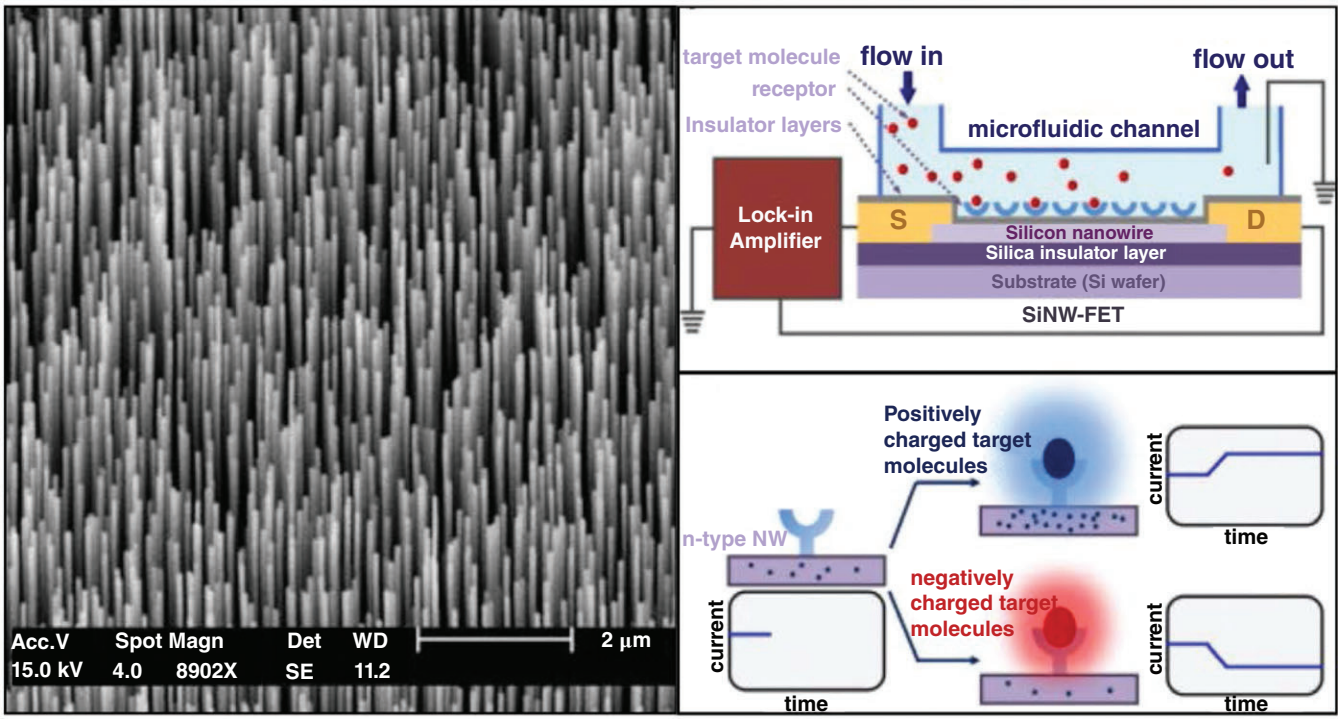

FIGURE 4 (a) SEM image of the nitrogen-doped ZnO nanowire array (Reprinted with sion from Yuan et al. (2008).Copyright 2008 Nature Publishing Group). (b) Schematic of an SiNW-FET integrated into a microfluidic device for biosensing (Reprinted with permission from M.-Y. Shen, Li, and Li (2014). Copyright 2014 Elsevier). (c) When positively charged targets bind on an n-type SiNW-FET, holes are accumulated in the SiNW leading to an increase in the electrical conductance. Conversely, negatively charged targets cause a depletion of charge carriers stored reducing the conductance of SiNW (Reprinted with permission from M.-Y. Shen et al. (2014). Copyright 2014 Elsevier)

control of wire orientation, size uniformity, and limited throughput (G. J. Zhang & Ning, 2012). Metal assisted etching is another common bottom-up approach where metal nanoparticles are deposited on top of a Si substrate causing it to oxidize, which is subsequently etched by HF etchant. M. L. Zhang et al. (2008) found that different morphologies of SiNWs arrays could be obtained using this technique by manipulating parameters including temperature, deposition time, etchant concentration, surface orientation, and doping level. On the other hand, in top-down approaches bulk silicon is scaled down to the preferred size and shape using lithographic means. Several examples of top-down approaches are e-beam lithography (Z. Li et al., 2004; Stern et al., 2007), lithographically patterned nanowire electrodeposition (LPNE; Tyagi et al., 2009; Xiang et al., 2008), nanoimprint lithography (Vu et al., 2010), and superlattice nanowire pattern transfer (SNAP; Bunimovich et al., 2006). In general, top-down approaches have the advantages of being amenable to conventional microfabrication manufacturing and provide easy integration with other system level complementary metal oxide semiconductor (CMOS) technology (C.-W. Huang et al., 2013).

SiNW surface functionalization is also necessary so that a molecular recognition element can be attached to the nanowire for specific target capture. There are two strategies generally used for attachment; electrostatic absorption, and covalent bonding. Electrostatic absorption employs electrostatic attraction to absorb ionic species onto oppositely charged absorbents and has been successfully applied for capturing DNA with negatively charged oligo probes linked to an amine-terminated layer on the nanowire (Bunimovich et al., 2006). In covalent bonding, silane chemistry is used to introduce amino terminal groups on the SiNW surface that react with aldehyde, carboxylic acid, and epoxy groups present on proteins and other biomolecules (Mu et al., 2015). For a more in-depth overview of different SiNW functionalization strategies please see (de Smet, Ullien, Mescher, & Sudholter, 2011).

The most common format for SiNW diagnostics is electrochemical detection in which a SiNW monitors the change in the electrical properties of a solution where a reaction between a recognition molecule and target analyte takes place. SiNW show many advantages over conventional electrochemical sensors because of the high surface area to volume ratios, large pore structures, and enhanced electrocatalytic activity when dopants are added (S. Su et al., 2013). Su et al. reported that electron transfer was greatly enhanced when Au nanoparticles were incorporated into the nanowire structure. Several other groups have also showed the sensitivity enhancements of nanoparticle dopants in SiNW electrochemical sensors (D. H. Kwon et al., 2011; K. Yang, Wang, Zou, & Zhang, 2006). Likewise, modifying SiNWs with AgNPs or AuNPs has been reported as a way of increasing sensitivity in SERS detection platforms as well (X. M. Han, Wang, Ou, & Zhang, 2012; He et al., 2011; Peng et al., 2010; T.-T. Xu et al., 2011). For instance, He et al. (2011) demonstrated an AgNP-SiNW with a SERS enhancement factor of ~10^{10} that could detect DNA down to a concentration of 1 fM. This enhancement factor represents a 100-fold improvement over Ag-free SiNWs. Interestingly, the Maiolo group combined both SERs and electrochemical detection in one platform to increase analysis efficiency and reduce detection of nonspecific interactions (Convertino, Mussi, & Maiolo, 2016).

4.2 | Nanotubes

Carbon nanotubes (CNTs), first discovered by Iijima in 1991, are hollow cylindrical tubes made up of carbon atoms. CNTs take two main forms: single-walled CNTs (SWCNTs; Figure 5a) and multi-walled carbon nanotubes (MWCNTs; Figure 5a, b). SWCNTs are seamless one dimensional cylindrical tubes rolled from a single layer of graphene (Iijima & Ichihashi, 1993), while MWCNTs are multiple concentric tubes of graphene encircling one another (Iijima, 1991). CNT diameters can range from single to hundreds of nanometers (Iijima & Ichihashi, 1993) with up to centimeter scale lengths (L. X. Zheng et al., 2004). The physical and chemical properties of CNTs, such as high aspect ratio, high conductivity, high mechanical strength, and biocompatibility make them excellent electrode materials for use in biosensors. CNTs offer many advantages over bulk carbon, such as a large edge plane/basal plane ratio that affords CNT-based sensors higher sensitivity and faster kinetics (Jacobs, Peairs, & Venton, 2010). Moreover, SWCNTS can act as either semi-conducting, or metallic in nature depending on the chirality of the structure, while MWCNTS will exhibit metallic behavior if only a single layer within is metallic (Jacobs et al., 2010). The combination of these characteristics makes CNT a unique nanomaterial well suited for sensitive molecular detection.

CNTs are generally fabricated by three techniques: laser ablation, electric arc discharge, and chemical vapor deposition (CVD). In the laser ablation technique, a high power laser (typically Nd, YAG, or CO_2) is used to vaporize a graphite target at high temperature in an inert atmosphere (Purohit, Purohit, Rana, Rana, & Patel, 2014). Both MWNTs and SWNTs can be produced in this way, but SWCTS require metal nanoparticle catalysts (Yudasaka et al., 1999). CNT quality and size depend on several factors such as the laser power, amount and type of catalysts, operating temperature, and gas flow rate (Vashist, Zheng, Al-Rubeaan, Luong, & Sheu, 2011). In arc discharge, current is applied through two electrodes to generate an arc that vaporizes carbon precursors on one of the electrodes. The vaporized carbon aggregates into CNTs as the gas cools. An extensive review of the parameters that affect size, quality, and yield of CNTs produced by arc discharge can be found here

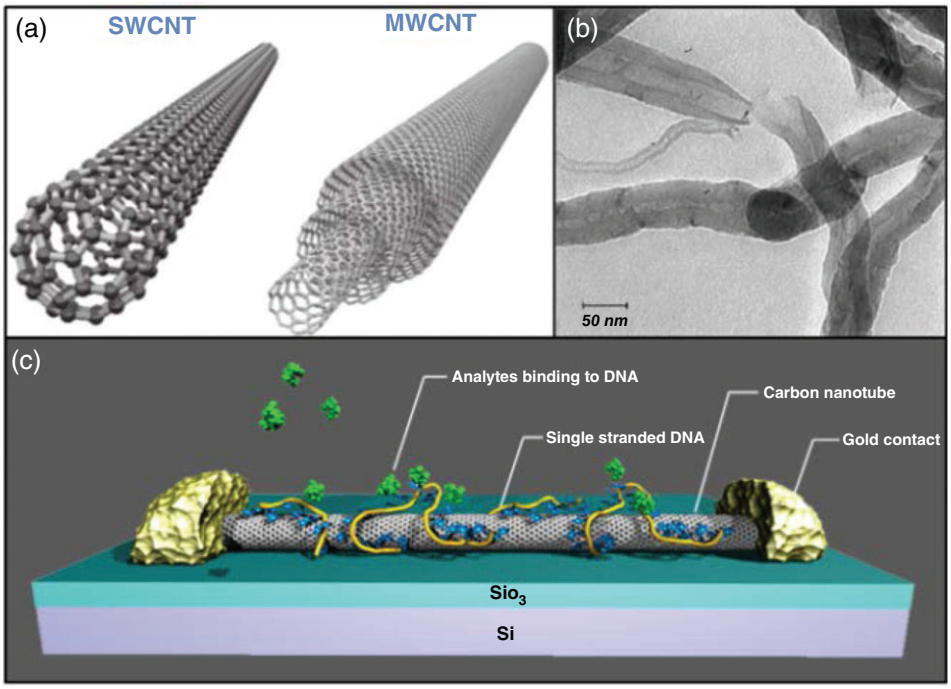

FIGURE 5 (a) Single-walled carbon nanotube (SWCNT) and multiwalled carbon nanotube (MWCNT) (Reprinted with permission from Gajewicz et al. (2012). Copyright 2012 Elsevier). (b) TEM images of MWCNTs (Reprinted with permission from Abbasi, Zebarjad, Baghban, and Youssefi (2015). Copyright 2015 Taylor & Francis). (c) A graphic of a DNA functionalized CNT field effect transistor (Reprinted with permission from Johnson (2017). Copyright 2017 Charlie Johnson)

(Arora & Sharma, 2014), but in general the technique is costly and requires the removal of contaminants (Vashist et al., 2011). CVD is the most common method for CNT synthesis due to its simplicity and low cost. CVD involves the decomposition of carbon containing gases at high temperature as it passes over metal catalysts such as Fe, Co, or Ni. While CVD can achieve the high yield required for mass manufacturing, the CNTs produced by CVD have more defects than those produced by arc-discharge or laser ablation (Koziol, Boskovic, & Yahya, 2010). One of the major challenges with these techniques is the control over CNT placement because synthesis often yields a random tangled assortment. Recent interest has thus been placed on the development of techniques that offer better control over CNT alignment (X. M. H. Huang et al., 2005; Nagahara, Amlani, Lewenstein, & Tsui, 2002), but it remains to be seen if it can reach the scale and throughput required for commercial manufacturing.

After synthesis CNTs can be functionalized using covalent or noncovalent means for the attachment of biorecognition elements. The most common functionalization strategy is to treat the CNTs with acids to expose oxides on the surface. These carboxylates can be linked to the amino groups on nucleotides or proteins using a carbodimide procedure. Additionally, the presence of carboxyl groups leads to a reduction of van der Waals interactions between the CNTs, which enhances nanotube dispersibility and solubility in aqueous and organic solutions (Balasubramanian & Burghard, 2005). Yet, covalent functionalization results in a change in carbon hybridization (sp2→sp3), leading to a potential loss in the electron-transport properties critical to biosensor sensitivity (Y. L. Zhao & Stoddart, 2009). Therefore, noncovalent functionalization strategies, such as use of aromatic compounds (i.e., pyrene, porphyin, and derivatives), surfactants, and hydrophobic interactions can be employed to preserve the CNTs favorable electrical properties. Oxidized CNTs will aggregate in the presence of salts due to charge screening effects, and thus have limited applicability when using biological media. However, attachment of hydrophilic polymers such as PEG can improve stability (Z. Liu, Sun, Nakayama-Ratchford, & Dai, 2007). Further details into CNT functionalization strategies are covered extensively in Balasubramanian and Burghard (2005) and Y. L. Zhao and Stoddart (2009).

Carbon nanotubes are often integrated into FETs (Figure 5c) and used as electrochemical sensors for DNA, proteins, cells, and other pathogen biomarkers. Similar to SiNWs, CNTs can enhance the electrochemical response detected when a biorecognition element and target react due to the increased surface area, and excellent electrocatalytic activity contributed by exposed graphite edge planes (Nugent, Santhanam, Rubio, & Ajayan, 2001). Thus, highly sensitive and real time electrochemical CNT (EC-CNT) sensors have been used for the detection of DNA hybridization. Tam, Van Hieu, Chien, Le, and Anh Tuan (2009) used probe DNA bound covalently to a MWCNT to detect influenza type A virus down to 0.5 nM. EC-CNT sensors have also demonstrated the detection of C-reactive protein, a biomarker associated with inflammatory response, down to concentrations as low as 0.5 ng/mL (Buch & Rishpon, 2008). Likewise, a CNT-FET with PNA probe was used to detect Hepatitis C virus down to 0.5 pM (Dastagir et al., 2007). In a novel approach Ishikawa, Stauffer, Caron, and Zhou (2009) deposited metal clusters onto CNTs to enhance sensitivity by 2000-fold for the detection of *Aureococcus anophagefferens* and *BT3* algae. Similarly, TiO_2/CNT composite electrodes were reported (Q. Shen et al., 2008) showing a significant

increase in detection sensitivity of K562/ADM and K562/BDW cancer biomarker cells. An excellent review covering a myriad of CNT applications for pathogen detection can be found here (Vashist et al., 2011).

4.3 | Nanocantilevers

Cantilever-based sensing was first used for diagnostics in 2000 where it was demonstrated that DNA coated cantilevers could distinguish targets with a single base mismatch for single-nucleotide polymorphism (SNP) detection (Fritz et al., 2000). Nanocantilevers are flexible beams typically constructed of silicon, silicon nitride, or quartz that are clamped on one side (Figure 6a). Beams are functionalized with biorecognition elements to absorb target analytes if they are present in sample. The analyte adds mass to the beam which affects the beams conformational or resonant properties (S. Xu, 2012). There is a significant interest in achieving nanoscale dimensions because the overall effect on the beam caused by the added mass will be more pronounced during measurements, resulting in better sensitivity. The nanoscale also provides an increased surface-to-volume ratio which enhances the target capture efficiency.

Advances in nanofabrication techniques have led to the ability to construct nanoscale cantilever beams. Fabrication approaches generally involve the deposition of cantilever material by CVD then nanoscale patterning by electron beam lithography (Guillon, Saya, Mazenq, Perisanu, et al., 2011), laser lithography (Davis et al., 2003), or UV lithography (Guillon, Saya, Mazenq, Nicu, et al., 2011). Following patterning, excess material is removed via dry or wet etch to release the cantilever beam. It has also been shown possible to lithographically pattern and etch using an Al mask without affecting cantilever process flows. This allows for simple incorporation of CMOS components onto the substrate during manufacturing (Davis et al., 2003).

Nanocantilever functionalization shares many aspects with the functionalization strategies of biosensors in general, and an extensive review on the subject can be found here (Nicu & Leichle, 2008). That said, there are some unique requirements of the nanocantilevers systems based on their fragility, and susceptibility to stiction effects (Tamayo, Kosaka, Ruz, San Paulo, & Calleja, 2013). Silicon based nanocantilevers can be functionalized for biorecognition attachment using a salinization reaction (see Section 4.1 for details on this process). Gold is also used for functionalization of the cantilever surfaces, particularly in systems that measure bending due to surface stresses between cantilever sides. In this particular format, asymmetric functionalization (one side only) is required so that binding does not occur on the native cantilever side and alter surface stress measurements. A gold layer is deposited on one side using thermal evaporation or sputtering deposition, and then

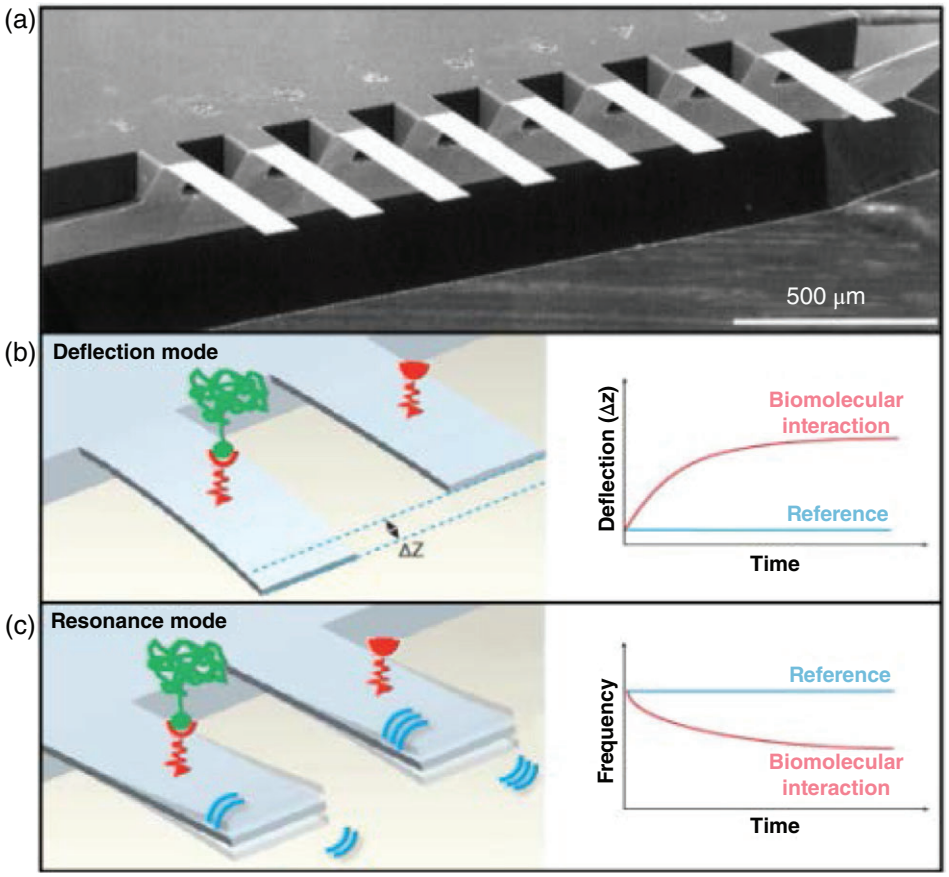

FIGURE 6 (a) Cantilever array based artificial nose (Reprinted with permission from Baller et al. (2000). Copyright 2000 Elsevier). Two modes of cantilever-based biomolecule detection: (b) deflection mode and, (c) resonance mode (Reprinted with permission from Hwang, Lee, Kim, Lee, and Kim (2009). Copyright 2009 Annual Reviews)

a self-assembled monolayer is formed on the surface via derivatized thiol groups (Tamayo et al., 2013). These groups can be used to link nucleic acid probes (Cagliani, Kosaka, Tamayo, & Davis, 2012; Hansen et al., 2001; McKendry et al., 2002; Ramos et al., 2009; J. Zhang et al., 2006) and antibodies (Alvarez et al., 2003; Arntz et al., 2003; Yue et al., 2008) to cantilever surfaces for target capture.

Nanocantilever diagnostic formats can be broken down by the mode of cantilever excitation; static (deflection based) (Figure 6b), or dynamic (resonance based) (Figure 6c), as well as detection means; optical, or electrical. In all formats, a reference beam with no biorecognition is used for signal comparison against an active beam (Figure 6b,c). Static based devices consist of a cantilever beam clamped at one end and when a target analyte binds to that beam the surface stress is changed. The change causes the beam to deflect up or down proportional to the amount of target bound. The deflection can be measured optically with sub-angstrom resolution (Boisen, Dohn, Keller, Schmid, & Tenje, 2011) by reflecting a laser off the cantilever surface and back to a position sensitive photodiode (X. L. Feng, He, Yang, & Roukes, 2007). Deflection can also be measured with a change in resistance of a piezoelectric material incorporated into the beam. The latter reduces the instrumentation required and lessens the overall footprint of the system. Static based devices have a higher limit of detection compared to dynamic formats, however static formats can function in a variety of sample mediums (Bellan, Wu, & Langer, 2011) offering greater potential as a ubiquitous sensing modality.

In dynamic excitation, the cantilever is actuated and the added mass of captured target will produce a shift in the cantilevers resonant frequency. The smaller the cantilever the easier it is to resolve the added mass of captured target. Dynamic devices have a lower limit of detection compared to static formats where masses on the order of zeptogram scale have been detected (X. L. Feng et al., 2007). Yet, generally dynamic actuation is constrained by the sample buffer. Aqueous buffers, common in biosensing, produce poor performance due to dampening. To overcome this challenge, J. Lee, Shen, Payer, Burg, and Manalis (2010) showed a unique nanochannel suspended resonator that could achieve attogram sensitivity in aqueous solutions. A number of sensors utilizing the piezoelectric effect for conversion of cantilever deflection into direct electrical signal for detection of proteins have been demonstrated (H.-S. Kwon et al., 2007; J. H. Lee et al., 2005; J. H. Lee, Kim, & Yoon, 2004). One advantage of electrical based detection is its multiplexability. For instance, it has been demonstrated that more than 1,000 cantilevers can be monitored simultaneously (Lutwyche et al., 2000). This is an integral factor for high-throughput diagnostics systems for antibiotic resistance screening. Additionally, electrical based detection formats are easier to miniaturize through integration, an important requirement in next generation portable diagnostics.

4.4 | Mesoporous membranes

The mesoporous membrane was first discovered by researchers at Mobil Oil Corporation in 1992 (Kresge, Leonowicz, Roth, Vartuli, & Beck, 1992), and has since been implemented in many applications for water purification, separation science, and biosensors. Porous elements for molecular detection can be constructed of a variety of materials and categorized by their different pore morphologies: microporous (<2 nm), mesoporous (2–50 nm), and macroporous (>50 nm) (Dai & Ju, 2012). Pore design is application dependent and must be determined using the target size and diffusion considerations to prevent membrane clogging. Typically for biosensing applications the ideal pore size is in the mesoporous regime because of its large surface areas, high pore volumes, and well-ordered pore distributions that reduce the aggregation of proteins (S. Wu, Wu, Liu, & Ju, 2008). Pores can be cylindrical, conical, slit-like, or irregular in shape, and be arranged in well-ordered pores with vertical alignment, or in a random network of tortuous pores (Adiga, Jin, Curtiss, Monteiro-Riviere, & Narayan, 2009). Aside from pore size and distribution, membrane properties such as conductivity, wettability, and surface chemistry are also important considerations in biosensor design. Very high surface-to-volume ratios and small diffusion lengths make mesoporous membranes very rapid and sensitive label free transduction elements. Additionally, the uniform nanosized framework structures also bring quantum effects (Scott, Wirnsberger, & Stucky, 2001), which provide the sensor with excellent performance in electrically and optically based transduction.

The fabrication process for mesoporous structures depends on the membrane material. Nonoxide materials are generally fabricated using a templating processing where either of two templates are used: supramolecular aggregates of amphiphilic species (soft templating), or preformed mesoporous solid structures (hard templating) (Shi, Wan, & Zhao, 2011). In soft templating (Figure 7a), the mesopore morphology is driven by the thermodynamics of the surfactant-inorganic precursor interaction. Electrostatic and steric interactions also play a role in pore morphology, while temperature, ionic strength, pH, and concentration control the long range pore organization (Firouzi et al., 1995). In hard templating (Figure 7b) a mesoporous sacrificial mold is first constructed as a template for a replica mold. A review of the many materials and synthesis pathways that have been demonstrated for hard templating can be found here (D. Gu & Schuth, 2014; Tiemann, 2008; Wan & Zhao, 2007; H. F. Yang & Zhao, 2005). The replica mold has its own unique pore morphology (reverse of the template) leading to greater porosity. This helps with the integration of membranes into microsystems where back pressure is an important

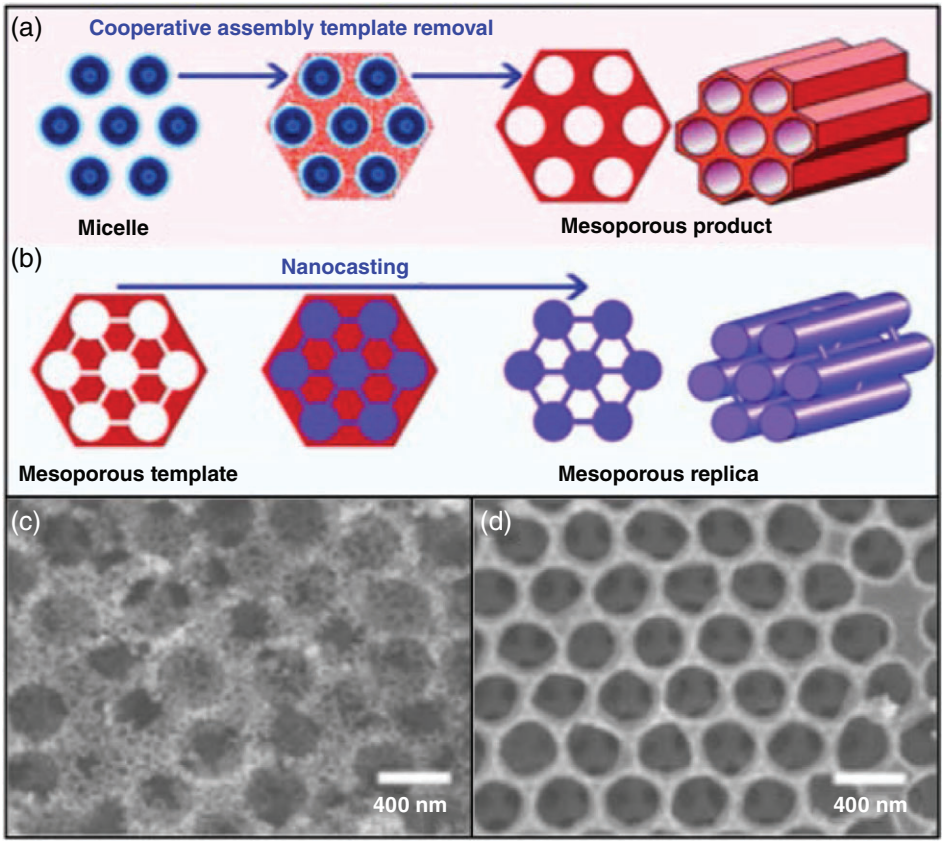

FIGURE 7 (a) Synthesis route for soft-templating, (b) Synthesis route for hard-templating (Reprinted with permission from Shi et al. (2011). Copyright 2011 The Royal Society of Chemistry). (c, d) FE-SEM images of the porous membranes prepared using an organized hard template. The particle sizes of silica colloids used as templates were (c) 40–50 nm, (D) 10–20 nm (Reprinted with permission from Woo, Dokko, Sasajima, Takei, and Kanamura (2006). Copyright 2006 The Royal Society of Chemistry)

consideration. A combination of hard template elements such as nanospheres, nanorods, and so forth, of different size can create replicas with a range of pore morphologies that optimize surface area and porosity (Figure 7c,d).

Functionalization of the membrane for attachment of biorecognition elements depends on the membrane material. For silicate based membranes silane chemistry is commonly used (Tan et al., 2011; Ye, Shi, Chan, Zhang, & Yang, 2014). Details of this functionalization protocol can be found in Section 4.1. Glutaraldehyde, a crosslinker that links amine groups, has also been used for linking both antibodies and DNA to polymer and aluminum based membranes (Jain et al., 2012; Rai, Deng, & Toh, 2012). For a more comprehensive review of functionalization protocols for a variety of materials used in mesoporous membranes please refer to (H. F. Yang & Zhao, 2005).

Mesoporous membranes for biosensing generally take two forms based on transduction formats: optical and electrochemical. Optical properties such as index of refraction can be tailored by parameters such as degree of ordering of the pores, or the variation in the pore diameter (Xifre-Perez, Ferre-Borull, Pallares, & Marsal Lluis, 2015), and be exploited during light probing for highly sensitive analyte detection. Reflectance interference (RI), is a method of light probing that measures the change in refractive index in the presence of an analyte. This detection technique has been applied to single layers of a single pore size, or multiple layers with varying pore diameters (Kumeria et al., 2014). Enhanced RI has been employed with anodic aluminum oxide (AAO) membranes for sensitive detection of circulating tumor cells (Kurneria, Kurkuri, Diener, Parkinson, & Losic, 2012). Additionally, Dronov, Jane, Shapter, Hodges, and Voelcker (2011) used a platinum coated AAO membrane to detect human IgG proteins. Pan and Rothberg (2003) use AAO membranes for the detection of DNA down to picomolar concentrations. Long range order of pore diameter and interpore distances make AAO membranes excellent substrates for SERS base detection. For instance, Choi, Choi, Hong, Kang, and Lee (2010) showed enhancement factors of 10^7 by careful control of membrane thickness.

Mesoporous membranes also serve as excellent substrates for electrochemical detection because of their unique highly ordered pore structure with high surface-to-volume ratios. AAO and silicon based substrates are common materials for electrochemical sensing because of their flexibility in pore design. Pore diameters can be fabricated to specific target dimensions such that when target is captured in the channel via biorecognition there is a significant reduction in ionic mobility within the channel due to steric effects (S. J. Li et al., 2010). The change in ionic conductance can be measured to quantify captured targets. This approach has been applied successfully for label free detection of DNA (S. J. Li et al., 2010; Rai, Hapuarachchi, et al., 2012; Senapati et al., 2014; X. Wang & Smirnov, 2009) and cells (Cheng, Lau, Chow, & Toh, 2011). DNA translocation time through nanopores is another parameter that can be used to identify specific nucleotides passing through the sensor. For example, Iqbal, Akin, and Bashir (2007) demonstrated that measuring translocation time could resolve SNPs. Yet, due to

the stochastic nature of DNA motion resulting in fluctuations in translocation kinetics, coupled with nonspecific interactions with the pore wall, it is possible to have a broad distribution of dwell times and different electrical signatures for identical length and sequences of DNA (Haque, Li, Wu, Liang, & Guo, 2013). Overcoming such challenges has been the focus of recent efforts (Y. Feng, Zhang, Ying, Wang, & Du, 2015) to develop a diagnostic system that can both universally quantify and sequence pathogens rapidly and inexpensively.

5 | FUTURE OUTLOOK

As nanoscale materials begin to be adopted for the next generation of diagnostics, their practicality depends heavily on quality assurance. Indeed, the same nanoscale phenomenon that serves to enhance sensitivity can also be detrimental to performance if nanomaterial size and surface properties are not consistent across devices. The complexity regarding nanosystems is often ignored for more idealized model systems in discovery literature (Figure 8) (Richman & Hutchison, 2009). The key to nanosystem QA, and often considered a commercialization bottleneck, is well-defined and well-characterized material standards (i.e., mass, surface area, conductivity, optical absorbance, toxicity, reactivity, catalytic activity, symmetry) (Richman & Hutchison, 2009). While this is relatively straightforward for bulk material, it is far more challenging with nanomaterials considering the different shapes (i.e., tubes, rods, spheres, planes, porous, etc.), forms (i.e., suspensions, powders, films, and single crystals), and nonhomogenous elemental compositions (i.e., core, functional groups, dopants, biorecognition elements, etc.). Thus, a combination of different characterization techniques is required to capture the complete picture. For example, in the case of Au nanoparticles, nuclear magnetic resonance can be used to identify surface functionalization and impurities; UV–Vis spectroscopy can provide size estimates; and transmission electron microscopy and energy-dispersive X-ray spectroscopy can inform on core size, and elemental composition (Richman & Hutchison, 2009). A downside to the combinatorial approach is slow throughput, and the capital cost associated with purchasing multiple characterization tools. Future adoption of nanomaterials for commercial diagnostics will rely heavily on the development of QA protocols formed with rigorous material characterization and standardization.

As nanomaterials are increasingly integrated into diagnostic systems, their toxicity is an important consideration. Due to their small size, nanomaterials are able to cross biological membranes and access cells, tissue, and organs whereby these materials can cause increased oxidative stress, inflammatory response, and even cell death (G. Y. Chen, Roy, Yang, & Prasad, 2016). Nanomaterials can gain access to body by ingestion, inhalation, and even skin contact (Oberdörster et al., 2005). The use of nanomaterials in diagnostics, and the disposal of these diagnostics represent a significant risk to public health and the environment if proper measures are not taken to reduce toxicity and exposure risks. The high surface energy of nanoparticles is one of the primary causes of toxicity (G. Y. Chen, Roy, et al., 2016). Hydrophobic nanoparticles can be encapsulated in hydrophilic-hydrophobic block copolymers that self-assemble in water as polymeric micelles, improving stability and reducing aggregation (Miyata, Christie, & Kataoka, 2011). Also, thiolated-PEG and mercaptoacetic acid have been used to improve nanoparticle stability in aqueous solutions and blood samples. It has also been reported that coating CdSe QDs with a ZnS shell can reduce the toxicity resulting from generation of free radicals (Cd^{2+} and Cd^{3+} ions) (Derfus, Chan, & Bhatia, 2004).

Capable diagnostics are needed most in developing countries where health infrastructure cannot keep pace with epidemics like HIV and tuberculosis. For the next generation of diagnostics to be utilized in these areas they must be sensitive,

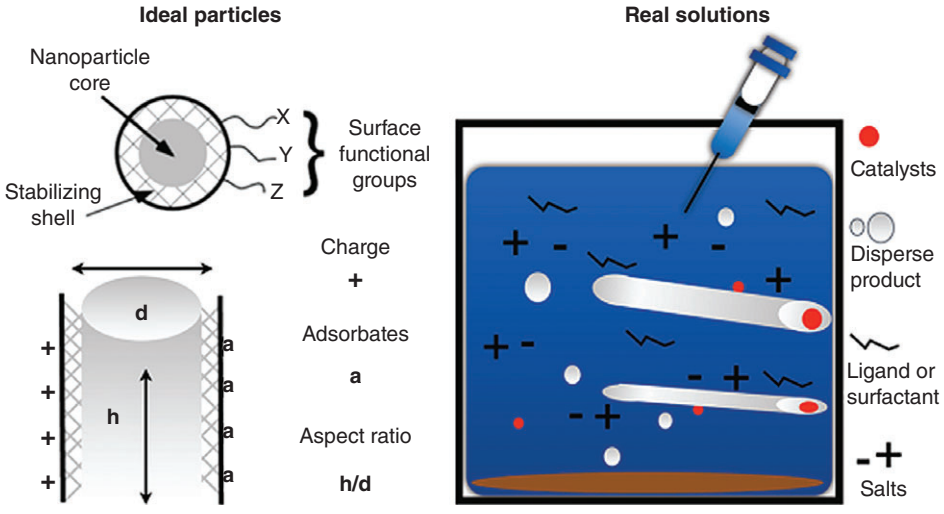

FIGURE 8 (Left) Ideal portrayal of complex nanosystems composed of core, shells, ligands, functional groups surface charge, surface adsorbates, and structural geometry. (Right) Real materials include far more species: side products, decomposition products, extra ligands, reactants, catalysts, and salts. (Reprinted with permission from Richman and Hutchison (2009). Copyright 2009 American Chemical Society)

inexpensive, and rapid. Lab-on-a-chip (LOC) technology, or the miniaturization and automation of lab operations on integrated low cost platforms, has been explored together with nanomaterials for this purpose. The small footprint of microfluidic systems allows for modularity and integration that can serve to enhance sensitivity of nanomaterials even further (Yoo & Lee, 2016). For instance, Kao et al. (2011) demonstrated a diagnostic with a rapid PCR sample-enrichment module that improved sensitivity to 20–30 fg/μL for H1N1 and Influenza A. Similarly, Stern et al. (2010) developed a microfluidic chip that could simultaneously capture multiple biomarkers from blood samples, wash, and release them into purified buffer for detection by a nanowire sensor. In doing so the complexity of detection in blood was reduced, enabling detection of two model cancer antigens from a 10-mL sample of whole blood in less than 20 min. J. Liu et al. (2016) reported the development of a simple and low cost microfluidic Europium nanoparticle immunoassay with comparable sensitivity to commercial plate-based ELISA assays for the detection of Influenza A. More fundamentally, LOC technology reduces reaction volumes to the μL to pL range, which significantly improves mass transport, and thus assay time, compared to macroscale devices. For example, QDs were employed in a polydimethylsiloxane device that combined the rapid binding kinetics of microfluidics with the sensitivity of QDs to provide a platform for hepatitis B detection with relatively small reagent consumption, and short assay times (H. Zhang, Xu, Li, & Yang, 2010). A more comprehensive summary of LOC devices for pathogen detection can be reviewed in (Foudeh, Fatanat Didar, Veres, & Tabrizian, 2012). Taking advantage of the synergy between nanomaterials and LOC technology today will enable faster and more capable diagnostics for tomorrow's healthcare challenges.

6 | CONCLUSION

Nanomaterials are integral in the development of the next generation of diagnostic systems. Nanoscale phenomenon inherent to these materials, such as high surface area-volume ratios and efficient electron transfer has been harnessed to provide significant enhancement to sensitivity in a variety of biosensor applications. Nanomaterials and nanotechnology are also providing new capabilities like label free transduction and highly scalable multiplexing that was not achievable in previous macroscale systems. Furthermore, nanomaterial integration with lab-on-a-chip systems is enabling very sensitive detection in more automated and portable formats.

While there are many advantages to developing nanomaterials for future diagnostics, there are still several challenges that must be addressed to ensure the successful adoption of these diagnostics in clinical settings. The toxicity of nanomaterials must be studied and properly mitigated in device workflows, especially considering their increased use in point of care (POC) settings. Nanomaterials and nanosystems are also fundamentally more susceptible to defects and environmental conditions than their macroscale counterparts. This fact coupled with the rigorous quality standards required for diagnostic accuracy makes establishing appropriate quality assurance guidelines critical. Overall, these challenges are not insurmountable and the enhancements gained are well worth the directed efforts.

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The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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