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BIOSENSORS FOR EARLY DISEASE DIAGNOSIS

Ahmet E. Topal, Alper Devrim Ozkan, Aykutlu Dana, Ayse B. Tekinay, and Mustafa O. Guler

Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, Turkey

10.1 INTRODUCTION

Early diagnosis is an important aspect of modern medicine and significantly improves the prognosis of a wide range of life-threatening disorders (Ping et al., 1997; Wang, 2006). However, early diagnosis is often difficult or impossible without an appropriate means of detecting small differences in the patient's physiology. As such, a great diversity of both general and specialized methods has been developed to identify the presence of genetic disorders, cancers, viral or bacterial infections, and other diseases prior to the point they present their pathological effects (Saravolatz et al., 2003; Wulfkuhle et al., 2003). These methods principally involve either medical imaging, in which the patient's tissues are checked *in situ* with or without the assistance of a dye or contrast agent (see Chapter 9); histopathology, in which a sample of the patient's tissues is removed by biopsy for visual inspection, usually with the help of specific cell and tissue stains; or a variety of diagnostic assays, in which the patient's bodily fluids (or tissue samples) are biochemically analyzed for disease-specific *biomarkers*.

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While powerful, current medical diagnostic techniques nonetheless suffer from clinical limitations. Histopathology is the gold standard for the diagnosis of many diseases but also requires an expert practitioner and introduces an element of subjectivity to disease diagnosis (Uckermann et al., 2014). Likewise, medical imaging is commonly used in disease diagnosis but cannot easily detect early-stage disorders, which often affect tissues on scales too small to be resolved by the imaging technique. In contrast, biochemical assays combine low detection limits with objective criteria of quantification and are therefore more suitable for the diagnosis of hard-to-detect diseases. Standard biochemical assays are now employed for the diagnosis of tuberculosis, HIV, hepatitis, *Escherichia coli* enteritis, and many other pathological conditions.

Nonetheless, these assays frequently require a laboratory and a human practitioner, which may not be readily available in rural areas where infectious diseases are common (Peeling and Mabey, 2010). In addition, complex diseases often require testing of multiple biomarkers in tandem, and an automated system would be of considerable advantage for the parallel testing (or "multiplexing") of a broad range of molecules. The amount of analytes and buffer solutions used in these assays can also be reduced if the protocols of these assays could be replicated on a small device, allowing the development of cheaper diagnostic methods. The benefits of such fast, repeatable "autoassay" devices are therefore obvious, and numerous attempts have been made to perform each step of a diagnostic assay on a small device that ultimately produces an output that can either be confirmed by the naked eye or quantified by a spectrometer or similar, commonly available equipment. These devices are typically referred to as *biosensors*.

A biosensor is a compact analytical device that is capable of selectively identifying biological signals, such as proteins, nucleic acids, small molecules, or secondary metabolites, which are collectively called *analytes*. The presence of a specific biological moiety can either be detected directly ("label-free") or through the assistance of a *label*. Label-bearing biosensors usually have a *recognition element*, which specifically interacts with the target analyte, and a *signal transducer element*, which transforms that interaction into an optical, electrochemical, or mechanical signal. Recognition elements are called *bioreceptors* if they consist of biological materials with recognition capability, such as antibodies and complementary DNA or RNA sequences (due to their specificity, most biosensors employ bioreceptors as their recognition element). Transducers, in turn, convert the analyte/recognition element interaction into a measurable signal and are composed of one or more *interface elements*, which are device elements such as thin films and field-effect transistor (FET) devices or nanomaterials

such as nanoparticles and nanowire arrays. The output of the biosensor is either confirmed visually (especially in the case of colorimetric biosensors) or quantified by a readout system (Medley et al., 2008).

The first biosensor, designed for the detection of glucose, was introduced in 1962 by Clark and Lyons (Rapp et al., 2010). Also called an "enzyme electrode," it was a biosensor of the amperometric type. Since then, the number and diversity of biosensors have grown enormously, and the design of biosensors has become an important field in medical diagnostics. As such, this chapter will focus on biosensor types, their detection limits, analysis times, and the diseases they are suitable for detecting. In addition, as nanomaterials are an effective means of producing small-scale diagnostic devices, nanostructures have been commonly employed in biosensor design. Consequently, a section will be devoted to the types of nanomaterials currently under use in biosensor design.

10.2 BIOSENSOR ELEMENTS

Biosensors can be classified according to their recognition element (e.g., enzymes, antibodies, nucleic acids), output type (e.g., optical, electrical, mechanical), detection principle (e.g., surface plasmon resonance (SPR) based, surface-enhanced Raman spectroscopy (SERS) based, quartz crystal microbalance (QCM) based), or intended use (*in vivo* or *ex vivo*). These factors all play vital roles in determining the sensitivity and selectivity of a biosensor and will be considered separately.

10.2.1 Recognition Elements

Recognition elements are almost uniformly biological, since enzymes, antibodies, and complementary nucleic acid sequences display specificities unparalleled by almost any nonbiological material (molecular imprinting, however, is also capable of creating highly specific binding sites for biological analytes and can be employed in sensor design). Biological sensing materials used for analyte recognition include enzymes, antibodies, and DNA or RNA constructs, which may either be used in purified form or expressed on the surface of bacteria or viruses (live-cell detection) (Van Dorst et al., 2010).

Enzyme-based biosensors typically detect the breakdown products of an enzymatic reaction between the analyte and the enzyme (i.e., catalytic recognition) (Iles and Kallichurn, 2012). They are often employed for the detection of small molecules, as antibody- and nucleic acid-based methods are either unfeasible (if the analyte isn't recognized as an antigen) or unnecessary (if easy-to-produce enzymes are able to yield accurate measurements) for these

materials. As enzyme targets are often found in both healthy and diseased tissues, diagnosis often relies on the concentration, rather than the presence, of the analyte. Enzymes have been used in the design of biosensors for glucose and other sugars, nerve gas agents, heavy metals, urea, ascorbic acid, acetylcholine, malate, and other small molecules (Mulchandani et al., 1999; Tsai and Doong, 2005; Wang, 2001). In addition to acting as the recognition element, enzymes can also be used as a means of visualizing the analyte–recognition element, such as by the use of HRP-tagged secondary antibodies.

Antibodies are often considered the gold standard for the detection of proteins and are commonly used in protein-detecting biosensors. Antibodies may either be monoclonal or polyclonal; monoclonal antibodies target a specific recognition site, while polyclonal antibodies bind to different recognition sites on the same antigen. As such, monoclonal antibodies are generally more specific, although they are costlier to produce and the recognition specificity of even polyclonal antibodies is considerable. Antibodies are also selective enough to quantify very small differences in the concentration of a specific protein, which is of considerable advantage in situations where small changes in expression patterns are indicative of early-stage disease. In addition to antibodies, binding peptides with similarly high affinities (down to the picomolar range) to specific analytes can be used as detector elements (Sidhu et al., 2000). Antibody-based biosensors have been designed for the detection of a wide range of proteins, varying from cancer markers to viral antigens and bacterial cell membrane components (El-Sayed et al., 2005; Pathirana et al., 2000; Torrance et al., 2006).

The most prominent technique for antibody-based detection is the enzyme-linked immunosorbent assay (ELISA). Digital ELISA is capable of detecting analyte concentrations at as low as the femtomolar scale, for example, prostate-specific antigen (PSA) could be detected at 14 fg/ml (0.4 fM) from patients who had undergone radical prostatectomy (Rissin et al., 2010). However, ELISA is relatively costly as a routine diagnostic technique and, in some cases, not sensitive enough for use in diagnosis, especially for the detection of early biomarkers for cancer (Tothill, 2009). As such, one main focus in biosensor development is on surpassing the detection limit of conventional ELISA (Park et al., 2009).

While antibodies can be raised against specific nucleic acid sequences, a complementary DNA or RNA strand can also be used for nucleic acid detection. These can be used not only for the detection of bacterial or viral nucleic acids but also to rapidly measure gene expression at the RNA level or to detect mutations in genomic DNA (Dell'Atti et al., 2006). However, DNA and RNA may face stability issues when exposed to serum or other biological media; as such, nucleic acids with alternative backbones, such as peptide

nucleic acids (PNAs), have been developed for use as recognition elements with increased stability (Ray and Norden, 2000). In addition, oligonucleotide aptamers are promising recognition agents that, much like antibodies, can be raised against specific protein targets (Du et al., 2013). Aptamer production does not require a cell line or animal to serve as a source, which is an advantage compared to antibodies (Iliuk et al., 2011). Nucleic acid-based biosensor probes have been reported for the detection of mutations, human and animal viruses, and heavy metals, as well as for use in gene expression studies.

Recognition elements are typically of biological origin, as it is difficult to match the efficiency of detection mechanisms that have been continuously improved by natural selection since the emergence of the earliest immune systems. However, polymers can also be etched to create highly specific binding sites using a technique called molecular imprinting, which has been employed in the detection of amino acids, sugars, antibiotics, and simple organic molecules (Kriz et al., 1997). Electronic noses, devices that contain no biological elements and instead rely on the differential binding of gas or solute molecules to the device surface, have also been used in the development of breath test biosensors for diabetes, pneumonia, fungal toxins, and blood in urine (Di Natale et al., 1999; Hanson and Thaler, 2005; Logrieco et al., 2005; Ping et al., 1997).

10.2.2 Output Type and Detection Techniques

The signal created by the binding of the analyte to the recognition element is transformed into a detectable form by a *transducer*. The transducer may detect either the binding event itself or, in the case of an enzymatic reaction, the products that are formed in the aftermath of catalytic activity. Alternatively, the recognition element itself might yield a detectable signal, such as a change in absorption properties, after binding to the analyte. No matter the case, the resulting output must be measured and quantified for the biosensor to function. This output may be optical, electrical, electrochemical, gravimetric/piezoelectric, mechanical, or magnetic and may be amplified and processed to increase signal quality prior to diagnostic assessment. Secondary equipment, such as spectrometers, is commonly used in data evaluation in this manner.

The output signal and the method used for its detection are important factors in determining the sensitivity of the biosensor. A table of biosensor output types, detection techniques, and the associated sensitivity and analysis time comparisons is provided in Table 10.1. It is worth noting that most biosensors provide optical, electrical/electrochemical, or mechanical output and other detection methods are relatively rare.

TABLE 10.1 Output Types, Recognition Elements, Sensitivities, and Analysis Times for Recent Biosensors

	Technology, Technique or Phenomenon	Nanomaterial, Bioreceptor, or Component	Analyte	Sensitivity	Analysis Duration	References
Electrical	Amperometric	Fructosyl amino acid oxidase immobilized onto zinc oxide nanoparticle–polypyrrole film	Hemoglobin A1c	50 µM	5–25 s current response time	Chawla and Pundir (2012)
		Bionanocomposite film consisting of glucose oxidase/Pt/functional graphene sheets/chitosan (GOD/Pt/FGS/chitosan)	Glucose	0.6 µM	~200 s	Wu et al. (2009)
	Capacitance	Citrate-capped gold nanoparticles (AuNPs) on a screen-printed electrode	Cardiac troponin I	0.2 ng/ml	~25 min	Bhalla et al. (2012)
	Field-effect transistor (FET) biosensors	Thiamine-immobilized, glutaraldehyde-modified SiO_2 gate on p-type Si	Prion proteins	2 nM	>1 h	Wustoni et al. (2014)
		Borrelia burgdorferi (Lyme) flagellar antibodies on SWNT FET	Lyme disease antigen	1 ng/ml	20 min	Lerner et al. (2013)
		Silicon nanowires (SiNWs)	Prostate-specific antigen (PSA)	10^{-6} ng/ml	Real time	Luo and Davis (2013)
Electrochemical	Electrochemical impedance spectroscopy (EIS)	Antihuman alpha-synuclein-based detection; device consists of PEG-thiol monolayer on gold electrode	Alpha-synuclein	55 + 3 pM	A few minutes	Bryan et al. (2012)

Amperometric	Fructosyl amino acid oxidase (FAO) immobilized on core–shell magnetic Fe–Si bionanoparticles (with chitosan); device uses modified gold electrode	Glycosylated hemoglobin	0.1 mM	<4 s	Chawla and Pundir (2011)
	Porous redox-active Cu_2O–SiO_2 nanoparticles (NPs)	Ferritin	0.4 ng/ml	30 min	Yang et al. (2009)
Squarewave voltammetry	CdSe QDs functionalized with streptavidin- (SA) labeled DNA; detection based on endonuclease activity; AuNPs used for signal amplification	*Mycobacterium* DNA	8.7×10^{-15} M	10 s for pH = 2	Zhang et al. (2015)
Cyclic and differential pulse voltammetry	Chitosan-modified glassy carbon electrode (GCE)	SCWL-diseased plant ssDNA	4.709 ng/μl	>10 min	Wongkaew and Poosittisak (2014)
	SiNW/AuNP-modified indium tin oxide (ITO)	DNA related to dengue virus	3.5 ng/ml	Within seconds	Rashid et al. (2014)
Electrochemiluminescence (ECL)	Biotin-anti-cTnI-luminol-AuNPs and SA-AuNPs	Troponin I	0.06 ng/ml	A few minutes	(Li et al., 2013a)
	Gold electrode labeled with hairpin DNA incorporating a ruthenium complex	Target ssDNA	9×10^{-11} M	2–4 s	Bertoncello and Forster (2009)
Electrochemical immunosensor	Antitestosterone antibody on hybrid gold NP/CNT–Teflon composite electrodes	Testosterone in human serum	85 ± 6 pg/ml		Serafín et al. (2011)
	Interdigitated electrodes (IDE) with nanoislands; device functions through protein immobilization to a parylene-A surface	Hepatitis B virus surface antigen (HBsAg)	<100 pg/ml		Jung et al. (2014)

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TABLE 10.1 (*Continued*)

	Technology, Technique or Phenomenon	Nanomaterial, Bioreceptor, or Component	Analyte	Sensitivity	Analysis Duration	References
	Electrochemically enhanced LSPR	Nanoscale Lycurgus cup arrays	BSA	13 µg/ml		Zhang et al. (2014)
Optical	Labeled SPR	ssDNA aptamer-functionalized gold chip	DNA	75 nM (1.58 µg/ml) RBP4		Lee et al. (2008)
		Oligoethylene glycol linker conjugated testosterone covalently immobilized to gold; detection enhanced using a secondary antibody with an attached AuNP	Testosterone	3.7 pg/ml with standard in running buffer 15.4 pg/ml in a stripped human saliva matrix	Real-time in 13 min	Mitchell and Lowe (2009)
	Fluorescence	Hepatitis B virus (HBV) capsid-derived chimeric NPs on nickel nanohairs; detection achieved by antibody–antigen binding	Troponin I	0.1 nM		Park et al. (2009)
		Graphene oxide (GO) array deposited on amino-modified glass; antibody–DNA–AuNP complexes used for detection	Rotavirus	10^3–10^5 pfu/ml		Jung et al. (2010)
		PDMS microchannels with gold thin-film sensing layers coated with anti-CTX-II antibodies covalently conjugated to fluoro-microbeads	C-telopeptide fragments of type II collagen (CTX-II) serum (sCTX-II; homodimers) and urine (uCTX-II; monomers or variant monomers)	50 ng/mmol	<70 min	Park et al. (2014)

Transmission spectroscopy	Fluorescent Qdot-antinitrotyrosine conjugate	Nitrated ceruloplasmin, a significant biomarker for cardiovascular disease	1 ng/ml	10 min	Li et al. (2010)
	Plasmonic gold nanorods	Single nucleotide polymorphism (SNP) in DNA	310 nm/RIU, 10 nM		Dodson et al. (2015)
	100 nm Au thin film with plasmonic nanoholes	Vesicular stomatitis virus (VSV), pseudotyped Ebola (PT-Ebola), and Vaccinia virus	10^6 PFU/ml	>90 min	Yanik et al. (2010)
SERS	Detection of antibody conjugated to gold nanostar or spheres bound to malachite green isothiocyanate (MGITC) Raman signals is enhanced by a silica sandwich nanostructure	Human immunoglobulin G (human IgG), VEGF	7 fg/ml	10 s	Li et al. (2013b)
	Iodide-modified AgNPs in fluid	Lysozyme, avidin, bovine serum albumin (BSA), cytochrome c, and hemoglobin	3 µg/ml	Within minutes	Xu et al. (2014)
Colorimetric, naked eye	AuNP-based plasmonic ELISA	PSA HIV-1 capsid antigen p24	1×10^{-18} g/ml		de la Rica and Stevens (2012)

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TABLE 10.1 (*Continued*)

	Technology, Technique or Phenomenon	Nanomaterial, Bioreceptor, or Component	Analyte	Sensitivity	Analysis Duration	References
		Water-soluble cationic conjugated polymers (CCP)	DNA mutation	fM, 2% mutant DNAs from total DNA	A few hours	Song et al. (2012a)
		Mutation-specific single-base extension (SBE) primers	PIK3CA mutations for breast cancer			
		Trinder's reaction-based enzymatic assay	Glucose	1.3 ng/ml		Duk Han et al. (2014)
		Competitive enzyme-linked immunosorbent assay	Urinary collagen type II C-telopeptide fragments (uCTX-II)			
		PDMS microchannel bonded with polydopamine coating and/or G4 PAMAM dendrimer				
		Cell-SELEX aptamers and gold colloid NPs	Cancer cells including T-cells, leukemia, and lymphoma	90 cells	>30 min	Medley et al. (2008)
Mechanical	Suspended microchannel resonator (SMR)	U-shaped microchannels or microcantilevers with anti-ALCAM monoclonal IgGs coated on silicon	Activated leukocyte cell adhesion molecule (ALCAM)	300 pM, 10 ng/ml	1 min	Arlett et al., (2011), von Muhlen et al. (2010)

Atomic force microscopy (AFM)	Functionalization with 3-aminopropyltriethoxysilane (APTES) followed by another functionalization step with a layer of very small peptides, which have a high affinity to the Fc region of the antibody	Antibody–antigen complexes	10 ng/ml		Ierardi et al. (2013)
Surface acoustic wave (SAW) biosensor	MUC1 aptamer-modified gold interdigital transducers for leaky SAW on lithium tantalite ($LiTaO_3$) surface	MCF-7 human breast cancer cells	32 cells/ml	Real time	Chang et al. (2014)
Quartz crystal microbalance (QCM) biosensor (acoustic-based biosensor)	ssDNA cross-linked polymeric hydrogel immobilized on the gold surface	Avian influenza virus (AIV) H5N1	0.0128 HAU (HA unit)	30 min	Wang and Li (2013)
	Biotin-labeled probe DNA immobilized on gold surface after immobilizing avidin on carboxyl chip prior to biotin	Fish pathogenic viral hemorrhagic septicemia (VHS) virus	About 0.1 nM of real-target viral RNA	~25 min	Hong et al. (2010)
Microcantilever biosensor	Biotinylated polyclonal antibody-functionalized silicon microcantilever arrays	Interleukin-6, interferon-γ, and alpha-fetoprotein	<0.1 pg/ml	~1 h	Joo et al. (2012)
	Multifunctional Fe_3O_4 at SiO_2 core TiO_2 shell magnetic–photocatalytic NPs				
	FhuA-containing proteoliposomes immobilized on the chemically activated gold-coated surface	Bacterial virus T5	3 pM T5 virus	>20 min	Braun et al. (2009)

(*Continued*)

TABLE 10.1 (*Continued*)

	Technology, Technique or Phenomenon	Nanomaterial, Bioreceptor, or Component	Analyte	Sensitivity	Analysis Duration	References
Lab-on-a-chip (combined approaches)	Chemiluminometric ELISA on a chip	HRP-labeled biotinylated anti-HRP antibody biotin–SA capture on plastic plates	Troponin I	0.027 ng/ml	30 s	Cho et al. (2009)
Magnetic biosensors		Core–shell Fe_3O_4 at poly (dopamine) magnetic nanoparticles (MNPs)	H_2O_2	182 nM	10 s	Martín et al. (2014)
	Superconducting quantum interference devices (SQUIDs)	Antibody-coated magnetic particles	*Listeria monocytogenes* bacteria	5×10^4 NPs for a substrate-based assay and 1.1×10^5 bacteria in a 20 ml sample volume	<0.1 s	Grossman et al. (2004)
		Dextran-coated iron oxide MNPs attached to antibodies	Amyloid ß 40 and 42	10 pg/ml	Real time	Chiu et al. (2011)
		Freely floating SA-functionalized MNPs with biotinylated monoclonal antibodies	PSA	0.7 nM or 100 ng/ml in a 2 µl sample volume	Real time	Oisjöen et al. (2010)
		Antibody-coated magnetic particles	*L. monocytogenes* bacteria	$(5.6 \pm 1.1) \times 10^6$ bacterial cells in sample volume of 20 µl	<0.1 s	Grossman et al. (2004)

Whole cell	Microbial cell-based biosensor	Bacterium *Sphingomonas* sp. on the inner epidermis of onion bulb scale	p-Nitrophenol (PNP)	4–80 µM of methyl parathion	>5 min	Michelini and Roda (2012)
	Cell-based biosensor	TurboRFP-based DNA damage reporter cell line in NIH-3T3 cells that fluoresce in response to stress	Genotoxic stress caused by a wide variety of agents, from chemical genotoxic agents to UV-C radiation	>80%	After 12 h of exposure to UV-C	Fendyur et al. (2015)
Microfluidics	Paper-based chemiluminescence biosensor	Paper-based, µPCAD, glucose oxidase (GOx), urate oxidase (UOx) enzymes	Glucose and uric acid	0.14 mmol/l for glucose and 0.52 mmol/l for uric acid	40–50 s	Yu et al. (2011)
	Electrophoretic microfluidic immunoassay with magnetic-beads detection	Biotinylated magnetic beads combined with anti-SA-IgG antibody array	SA	20 aM or 2×10^{-17} M	Within 2–3 min	Morozov et al. (2007)
Other types	Nanopore-based detection	Bionanopore phi29 DNA-packaging motor	Colon cancer antigen EpCAM antibody	1 nM at single-molecule level	Real time	Wang et al. (2013)

10.2.3 Optical Biosensors

Optical biosensors yield outputs that can be confirmed by the naked eye, by changes in absorption, fluorescence peaks, or refractive index or by using other optical or spectroscopic techniques such as fluorescence, FTIR, and Raman spectroscopies. The widespread availability of optical detection equipment makes optical biosensor techniques attractive for the development of low-cost lab-on-a-chip devices for use in areas where rapid diagnosis of potential disease is essential and more sophisticated diagnostic methods are not readily available, such as during pathogen outbreaks in rural regions.

Colorimetric biosensors change color when exposed to their target analyte, and quantification is performed through changes in absorption at a specific wavelength. Despite their simplicity, these biosensors may have considerable sensitivity and selectivities: An absorption wavelength shift-based biosensor for vesicular stomatitis virus (VSV), pseudotyped Ebola (PT-Ebola), and vaccinia virus was able to reach a sensitivity figure of merit (FOM) of 40, a detection limit of below 10^5 PFU/ml virus and a resolution of 0.05 nm (Yanik et al., 2010). Likewise, HIV RNA molecules could be colorimetrically detected using PNAs bearing different numbers of cyclopentane chemical groups, with sufficient sensitivity to detect 20–30,000 copies/ml plasma of this virus (Zhao et al., 2014a).

Changes in chemical composition can also be detected through spectroscopic methods, such as Raman or FTIR spectroscopy. These methods quantify the absorbance, reflectance, or fluorescence of a material following exposure to light at a specific wavelength or range of wavelengths and yield chemical information in the form of molecular interactions, ionic and covalent bonds, and vibrational and rotational motions. They are useful for label-free biosensing efforts, as IR absorption and Raman scattering can be used to detect conformational changes of proteins and structural variations between materials and molecules, allowing the detailed analysis of chemical bonds without an intermediary reporter or dye. Alzheimer's disease, for example, could be detected by quantifying amyloid β (Aβ) peptide titers using an attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy biosensor (Kleiren et al., 2010).

Raman signals can be enhanced through a method called SERS, which utilizes the fact that the Raman signal becomes much more prominent (up to a factor of 10^{14}, although typical values are in the 10^6–10^8 range) when the analyte molecules are situated between gold and silver surfaces set apart by distances around 20–30 μm (Kneipp et al., 1997). The interstructural distances required for SERS are usually created using nanoparticles

or nanopatterned surfaces, and gold and silver are often the materials of choice because their plasmon resonances (and, therefore, Raman enhancements) are in the near-infrared and visible spectral ranges, respectively. SERS-active substrates for protein detection may also be designed to display a tunable resonance in the infrared range in order to produce a signal enhancement effect around the spectral positions of amide bands; or the samples may be labeled by a Raman-active dye for easier detection (Han et al., 2009). A detection level of 7 fg/ml could be achieved in a cancer biomarker detection study from plasma using SERS with 3D hierarchical plasmonic nanoarchitectures, including Au nanospheres and Au stars, as well as with a Raman dye (malachite green isothiocyanate, MGITC) and silica nanoparticles (Li et al., 2013b).

Surface plasmons can be used for the enhancement of other spectroscopic detection methods, often using noble metal nanoparticles or metallic thin films. SPR reflectivity measurements in particular are useful for the detection of molecular interactions and may reach very high sensitivities. SPR-based sensing systems measure the coupling of light with the plasmons (electron cloud oscillations) present at the surface of a nanoscale thin film of gold or silver. Recognition agents for SPR sensing and imaging are generally antibodies. Picogram per milliliter-level sensitivities can be obtained using SPR; for example, testosterone detection limits in one SPR-based system was found to be 3.7 pg/ml in standard running buffer and 15.4 pg/ml in a human saliva matrix (Mitchell and Lowe, 2009). Biological assays, nonetheless, may prove superior in detection capacity: In another study, the detection capacity of SPR for retinol binding protein 4 (RBP4, a useful marker for type 2 diabetes) was found to be greater than ELISA but lower than Western blot (Lee et al., 2008).

A variant technique of SPR, called localized SPR (LSPR), uses metal nanoparticles (usually gold or silver) of specific sizes and geometries to further improve the detection limits of this technique (Swierczewska et al., 2012). LSPR nanostructures may be employed for biomolecule detection through the measurement of refractive index changes and colorimetric SPR imaging, and it has been shown in a theoretical study that the detection capacity of LSPR-based methods can exceed that of systems based on traditional Kretschmann geometry systems in terms of wavelength shift sensitivity outperforming the detection system with Kretschmann geometry (Kaya et al., 2014). LSPR-based detection of colon cancer was also achieved using Au nanorods, and the width- and length-dependent changes in the plasmonic and photonic properties of nanorod antennae were investigated to identify optimal geometries for nanorod arrays for use in cancer biosensors (Dodson et al., 2015).

10.2.4 Electrical and Electrochemical Biosensors

Like optical signals, electrical signals are generally easy to detect and quantify. Electrical and electrochemical biosensors consequently see common use in both research and commercial purposes. Electrical biosensors typically use an electrode as their transducer, and the recognition element (usually an enzyme) is immobilized onto this electrode. The enzyme–ligand interaction creates a change in the electrical properties of the electrode (this may be electrical potential, resistivity, impedance, conductivity, or capacitance, as well as the current running between the recognition element-bound electrode and a reference electrode), which is measured and quantified to determine the concentration of the analyte. Cyclic voltammetry, chronoamperometry, chronopotentiometry, impedance spectroscopy, and various FET-based methods are some common tools that are used in the measurement of the output signal. More sensitive measurements may be performed with the use of nanomaterials such as nanowires, nanotubes, and nanoparticles.

As one of the most popular biosensor types, amperometric biosensors quantify changes in current, which is usually linear with the change in analyte concentration within the detection range (Wang, 1999). Amperometric biosensors typically display relatively modest detection capabilities; for example, a biosensor based on a multiwalled carbon nanotube (CNT)–gold nanoparticle composite yielded a limit of detection of 0.01 mM for uric acid (Chauhan and Pundir, 2011). Likewise, a detection range of 0.001–5 mM was obtained for glucose detection using an early biosensor based on Prussian blue-functionalized electrodes (Wu et al., 2012). In addition to gold, nanoparticles of other metals, such as zinc and platinum, can also be used in biosensor design (Chawla and Pundir, 2012; Wu et al., 2009).

The transducer electrode can be modified to produce amperometric sensors with much higher sensitivities. Sotiropoulou and Chaniotakis have reported dichlorvos detection limits at the picomolar level using a biosensor with a nanoporous carbon electrode, which enhances enzyme–ligand interactions by adsorbing the enzyme on its surface (Sotiropoulou and Chaniotakis, 2005). Salimi et al. also reported picomolar-level detection capacity using a modified electrode; their system used a glassy carbon electrode modified by guanine and nickel oxide nanoparticles with the help of cyclic voltammetry (Salimi et al., 2008). Likewise, an electrical detection-based FET biosensor was used to detect prion proteins through the use of thiamine molecules immobilized on a glutaraldehyde-modified SiO_2 gate surface on p-Si (Wustoni et al., 2014). Another biosensor design, using an antibody-immobilized single-walled carbon nanotube (SWNT)

FET, was hypothesized to be able to detect the Lyme disease antigen at concentrations up to 1 ng/ml in buffer (Lerner et al., 2013).

Potentiometric biosensors measure changes in the electrical potential of the electrode and are less common than amperometric biosensors. Nonetheless, potentiometry-based biosensors using modified electrodes have been described for biomaterials such as urea and polyglycerides, with working ranges between 10^{-2} and 30 mM (Lakard et al., 2004; Saurina et al., 1998). Due to the relative rarity of this detection type, high-sensitivity studies involving potentiometric biosensors are lacking, although a combined SPR–potentiometric analysis method for nerve gas detection at nanomolar-to-picomolar detection ranges was reported (Taranekar et al., 2006). Biosensors that utilize changes in conductivity, capacitance, admittance, and impedance have also been recorded in the literature, although nonamperometric output types are somewhat uncommon (Berggren et al., 2001; Gerard et al., 2002; Varshney and Li, 2007).

10.2.5 Mechanical Biosensors

The binding interaction between the recognition element and the analyte imposes an additional weight on the surface on which the former is immobilized. This change can be quantified using specialized devices and measurement methods, such as microcantilevers, QCM, microring resonators (MRR), suspended microchannel resonators (SMR), or atomic force microscopy (AFM). Many of these methods have exceptionally high sensitivities; QCM biosensors, for example, can detect femtomolar concentrations of DNA. However, they also have long analysis times and require uncommon and highly sensitive equipment for analysis (Arlett et al., 2011). As such, these techniques are generally suitable for research purposes rather than point-of-care diagnostics.

Mechanical biosensors can broadly be divided into two types, those that experience a surface deflection following analyte binding (surface stress mechanical biosensors) and those that change their oscillation frequency in the presence of the analyte (dynamic-mode mechanical biosensors) (Arlett et al., 2011). Cantilever biosensors can be of both types and have been used for sensing cancer biomarkers by using the change in cantilever frequency or deflection following antigen binding (Choi et al., 2010). Microcantilever array biosensors can be used with protein–ligand interactions to detect viruses at sensitivity levels down to subpicomolar concentrations (Braun et al., 2009). QCM biosensors can likewise reach picomolar sensitivities and have been developed for the detection of biomaterials such as human antibodies and bacterial toxins (Alfonta et al., 2001; Das et al., 2003; Yao et al., 2010).

Other types of mechanical biosensors include nanoelectromechanical systems (NEMS), such as nanomechanical resonators, which may possess single-molecule detection capacities. NEMS devices may use mass, elastic modulus, and surface stress changes as sensing parameters and can possess complex device designs that render them ideal for lab-on-a-chip applications that require more sophisticated assay conditions than the detection of a recognition element–analyte binding interaction. In addition, while not generally employed for the analysis of biosensor outputs, AFM is an exceptionally sensitive material characterization and manipulation tool that has seen extensive use in medical proteomics and potential diagnosis of cancer and infectious disease (Archakov and Ivanov, 2007). AFM is not an ideal technique for commercial biosensors; however, its subatomic detection capacity is effective for mechanically characterizing the recognition element–analyte interactions that drive biosensor development, and the similarity of AFM probes to mechanical biosensor cantilevers allows this technique to yield potentially valuable information about the design of novel mechanical biosensors (Baselt et al., 1998).

10.2.6 Other Biosensor Types

In addition to optical, electrical, and mechanical detection methods, changes in the magnetic field of an analyte-bound material can be quantified for biological detection. One example in the literature involves quantification of magnetic responsivity with a high-density sensor array that exhibits giant magnetoresistance (GMR), and the method was shown reach very high antigen–antibody binding sensitivities. A solute sensitivity of 20 zeptomoles, which is difficult to reach even with SPR and mechanical detection methods, was obtained using this strategy (Gaster et al., 2011).

Acoustic biosensors are another uncommon type and can be regarded as a subtype of mechanical biosensors. In these biosensors, changes in the properties of an acoustic wave are used to gain information about the binding interaction, usually by measuring the mass increase that results when the recognition element binds to the analyte. QCM and other types of acoustic resonators are employed in biosensors of this type and such devices have been shown to detect bacteria at sensitivities up to 0.4 cells/μl (Ferreira et al., 2009; Rocha-Gaso et al., 2009).

Entire cells can also be used for biosensor applications, especially for the detection of pollutants and other chemicals. In these biosensors, the physiological response of the cell is used as an indicator of analyte presence, and the complex sensory systems of cells are employed in place of advanced device design. The cells used may be bacteria, yeasts, and

fungi and are often genetically modified to yield an easily detectable signal in the presence of the analyte. These "one-cell biosensors" may be exposed to a water or soil sample to yield a bioluminescent or fluorescent response that is subsequently quantified in an assay-like process or the cells may be immobilized in an integrated system that truly qualifies as a biosensor (Belkin, 2003).

While analytes are typically presented in a solution, volatile organic compounds (VOCs) in gas form can also be quantified through mass spectroscopy methods for disease diagnosis. Gas chromatography–mass spectrometry (GC-MS), for example, was used to detect VOCs in human breath for the diagnosis of lung cancer (Dragonieri et al., 2009). Sensors for VOCs typically display sensitivities in the ppm range (Adiguzel and Kulah, 2014).

10.3 THE IMPACT OF NANOTECHNOLOGY AND NANOMATERIALS IN BIOSENSOR DESIGN

Although the analytes and recognition elements involved are often in the nanoscale, nanomaterials in the strict sense are not required for the design of biosensors. Nonetheless, the large total surface areas associated with nanomaterials, as well as the small amounts of analytes and buffers required for nanoscale device-based assays, render them attractive for biosensor applications. In addition, nanostructures may present ideal surface properties for analyte or recognition element immobilization or offer increased detection capacity through phenomena such as self-assembly or plasmon enhancement. Nanospheres, nanorods, nanowires, graphene- and CNT-based structures, quantum dots, magnetic nanoparticles, NEMS systems, and other nanoscale materials are therefore often used in biosensor design.

Nanospheres are the most common nanoscale biosensor components and may be functionalized with surface groups such as thiols to better bind to an enzyme, antibody, or other recognition molecules. In addition, they may be organized into nanostructure arrays to better present their enzyme or antibody load (Xu and Han, 2004). The total surface area presented by nanoparticles is larger relative to the bulk material of equal volume; as such, nanospheres and similar nanostructures can immobilize a greater amount of recognition molecule per volume compared to their macroscale counterparts (Li et al., 2008). In addition, the size, composition, or material properties of the nanospheres might allow enhanced detection capability, as is the case with magnetic nanoparticles: Cross-linked magnetic nanoparticles (CLIOs), manganese-doped magnetic nanoparticles, and core–shell structures such as elemental iron-ferrite nanoparticles are among the

magnetic materials used in biosensor applications (Haun et al., 2010). Quantum dots can also be used to improve the detection capacity of conventional recognition elements, and QD sensors that react to a broad range of factors, such as pH change, protein or nucleic acid cleavage, or DNA synthesis, have been reported in the literature (Suzuki et al., 2008). A quantum dot–aptamer conjugate was also shown to possess a detection limit in the attomolar scale, while conventional aptamer-based biosensors generally display nanomolar-level sensitivity (Hansen et al., 2006).

While nanospheres are commonly used for their ease of production, nanorods and other nonspherical nanoparticles may present advantages over the nanosphere morphology. Thiol groups, for example, prefer to bind to the tips of gold nanorods, and this effect can be used to produce self-assembled nanoparticle chains by attaching thiolated oligonucleotides to gold nanorods and allowing complementary sequences to link individual nanoparticles (Sepulveda et al., 2009). Nonspherical morphologies also alter the optical response of nanoparticles; both theoretical and experimental reports suggest that triangular silver nanoparticles enhance sensitivity in SPR-based biosensors (Haes and Van Duyne, 2002; Peng and Miller, 2011; Xu and Kall, 2002). Another study on a ZnO-based biosensor found that biotin-bound ZnO nanorods could detect streptavidin at concentrations lower than that previously reported for ZnO nanospheres (Kim et al., 2006).

Nanowires of silicon and noble metals can also be incorporated into sensor design. An Au nanowire waveguide, for example, was successfully used for plasmonic waveguide sensing in water and other liquids (Wang et al., 2014). Nanowires can also be organized into larger assemblies using techniques such as flow-assisted, Langmuir–Blodgett, bubble-blown, electric-field, smearing-transfer, roll-printing, and PDMS-transfer assembly processes (Chen et al., 2011). In addition to metal or metal oxide nanowires, single- or multiwalled CNTs can also be used for biosensor applications. In these biosensors, the carbon structure is used to modify the electrode of an amperometric biosensor and may be functionalized to carry the recognition molecule of interest. Different configurations can be used for CNT-based biosensors: CNTs may be coated onto the electrode or synthesized in aligned networks around it or the CNT itself may serve as the electrode. Submicromolar detection limits have been obtained in CNT-based biosensors for glucose monitoring (Wang, 2005).

Nanoporous silica and similar materials have also been used in the design of biosensors. Light reflected off a thin, porous layer of silicon creates interference patterns (Fabry–Perot fringes) that change following the binding of the target analyte to a recognition element immobilized on the surface of the silicon film. The extent of this change depends on the

change in the refractive index of the silicon layer and can be quantified to detect DNA, proteins, and other organic molecules at picomolar-to-femtomolar scales (Lin et al., 1997). Molecular imprinting can also be employed to create polymer matrices with "holes" that serve as recognition sites for specific biomolecules and may reach detection levels of 0.1 μM for small organic molecules (Yano and Karube, 1999). Lipid membranes can also be employed as biosensors, either serving as a platform to support an enzyme or antibody or acting as the recognition element itself; however, these sensors are more suited to membrane transport-related research than clinical diagnosis (Nikolelis and Krull, 1992; Reimhult and Kumar, 2008). Pore-bearing proteins that mimic the structure and selectivity of biological pore components can also be used in biosensor applications (Braha et al., 1997).

In addition to nanoparticle-, thin film-, and nanoporous matrix-based detection methods, the techniques used in biosensor design and quantification are often deeply rooted in nanotechnology. As such, advances in nanomaterial-based detection methods, such as QCM (the sensitivity of which depends on crystal thickness) and SERS, or nanofabrication methods, such as laser, AFM, or electron beam lithographies (which are used for the fabrication of cantilevers used in mechanical biosensors), will indirectly improve the sensitivity of current biosensors. However, while nanostructures offer several advantages over conventional biosensors, it must be kept in mind the *in vivo* use of nanoparticles in disease diagnosis is largely still in preclinical stages of development (Thakor and Gambhir, 2013). Further advances are no doubt necessary for the use of these devices in clinical settings.

10.4 EARLY DIAGNOSIS AND BIOSENSOR-BASED DISEASE DETECTION

The greatest potential of biosensors lies in their medical applications. While the detection of a broad spectrum of biomaterials is feasible using biosensors, the sheer incidence rates, physiological diversity, and difficulty of treatment that is characteristic of cancer, heart disease, and similar disorders ensure that a substantial amount of biosensor-related research is directed toward their diagnosis. Likewise, the detection of widespread foodborne pathogens such as *Salmonella*, *Klebsiella*, and *Staphylococcus* or viruses such as HIV and dengue virus is vital for the control of the associated diseases. As such, a list of diseases and target molecules that have been the subject of high-sensitivity biosensor development efforts is provided in the present section (Table 10.2).

TABLE 10.2 Target Molecules, Detection Methods, and Sensitivities of Biosensor-based Diagnostic Methods

Disease		Markers	Method	Sensitivity	Reference
Cancer	Prostate cancer	Prostate-specific antigen (PSA)	MEMS/NEMS Silicon nitride cantilever Optical deflection	0.2 ng/ml	Waggoner and Craighead (2007)
			Digital ELISA	14 fg/ml (0.4 fM).	Rissin et al. (2010)
	Hepatocellular carcinoma	Alpha-fetoprotein (AFP)	Resonant microcantilevers	Nanogram/ml	Liu et al. (2009)
			Immunosensor with single-walled CNT inside mesoporous silica	0.06 ng/ml	Lin et al. (2013)
	Ovarian	Human epididymis protein 4 (HE4)	Anti-HE4 antibody covalently attached to silver nanoparticles (synthesized through NSL technology) on glass substrate	4 pM	Yuan et al. (2012)
		Cancer antigen 125 (CA125) (most frequently tested)	Anti-CA125 was immobilized on gold surface through a self-assembled monolayer	0.05 U/ml with a capacitive biosensor system, 0.1 U/ml with SPR	Suwansa-ard et al. (2009)
			SPR biosensor and capacitive label-free immunosensor, separately		
	Breast	PIK3CA mutations	FRET-based visual detection and fluorescence measurement	fM, 2% mutant DNAs from total DNA	Song et al. (2012a)
	Lung	Urine, CD59 glycoprotein, transthyretin (TTR), GM2 activator protein (GM2AP), and Ig-free light chain			Arya and Bhansali (2011)
	Nonsmall cell	Fragile histidine triad (FHIT), mucin1 (MUC1), β-catenin (CTNNB1)			Altintas and Tothill (2013)

	Mutations in the EGFR gene	Circulating tumor cell detection using a microfluidic device	Median of 74 cells per milliliter (mean, 133; range, 5–771)	Collura et al. (2008)
Cervical cancer		Electrochemical impedance spectroscopy, impedimetric biosensor based on PEGylated arginine-functionalized magnetic nanoparticles	10 cells/ml	Chandra et al. (2011)
	Squamous cell carcinoma antigen (SCCa)	LSPR biosensor	0.125 pM	Zhao et al. (2014b)
Myocardial infarction	Troponin I, T			Steuer et al. (2009)
	Fatty acid binding protein			Kakoti and Goswami (2013)
Rheumatoid arthritis (RA)	Rheumatoid factor (RF), anticyclic citrullinated peptide antibody			Chandrashekara (2014)
	Activated CD8 cells			
	IL-6, IL-7, IL-10			
	Antimutated citrullinated vimentin (anti-MCV) antibody			
Systemic lupus erythematosus (SLE)	Autoantibodies against C1q	ELISA		Andrejevic et al. (2013), Vanhecke et al. (2012)
Osteoarthritis	C-telopeptide fragments of type II collagen (CTX-II)	Fluorescence-based biosensor	200 ng/mmol	Kim et al. (2013)
	Cartilage oligomeric matrix protein (COMP)	ELISA with fluoro-microbeads	0.8 ng/ml	Song et al. (2012b)

Cancer markers are some of the most common targets for biosensor design, as early detection is vital for successful treatment in many cancers. Early diagnosis of oral cancer, for example, increases survival rates from 50 to 80%, while pancreatic cancer, which is often diagnosed after the initial tumor has either spread locally or metastasized into another tissue, has a 5-year survival rate of about 5% (Pannala et al., 2009; Silverman, 1988). Diagnosis of cervical cancer, likewise, must be performed at an early stage or "the cancer metastasizes to the rest of the uterus, bladder, rectum and abdominal wall and eventually reaches pelvic lymph nodes, thereby invading other organs and leading to death" (Chandra et al., 2011). As such, biosensors using protein markers, DNA sequences, membrane glycans, and cancer-associated estrogen derivatives have been developed for the early detection of cancer, and the responses of cancer cells, such as drug resistance after taxane treatment in breast cancer cells, hydrogen peroxide production in human hepatoma cells, or formaldehyde presence in glioblastoma cells following treatment with a formaldehyde-releasing drug, have been observed using various biosensor designs (Bareket et al., 2010; Braunhut et al., 2005; Cavalieri et al., 2006; Feng et al., 2006; Myung et al., 2011; Rui et al., 2010; Zhang et al., 2010).

Advancements in nanoscience have also created the prospect of developing small biosensors that are implanted to the body of a prospective patient and trigger at the onset of disease state or other changes in physiological conditions. This type of *in vivo* diagnostic method is useful for the detection of diseases that are nonsymptomatic in their early stages and for the monitoring of chronic conditions, such as diabetes, that require regular treatment. In addition to their direct healthcare benefits, these biosensors may also serve as a means of collecting physiological data from a large set of patients, which would in turn enable the development of better treatment options. These sensors typically detect glucose and are designed for the management of diabetes; although biosensors that use fluorescein (introduced by the biosensor) or lactate for the detection of internal bleeding have also been developed (Kotanen et al., 2012; Mo and Smart, 2004; Ryou et al., 2011). Some glucose sensors of this type are approved for use by the FDA (Klonoff, 2007).

10.5 CONCLUSION AND FUTURE DIRECTIONS

Biosensor research combines the investigation of biochemical recognition processes, signal transduction systems, and output-specific detection methods, which makes it a highly multidisciplinary research field. As

such, the development of highly specific recognition elements and advancements in various signal detection techniques will indirectly result in the development of more accurate biosensors. Recent discoveries in gene regulation and biological signaling mechanisms have revealed the presence of various regulatory elements that are highly specific to their targets and may be used in biosensor applications. In addition, methods for the synthesis of proteins and nucleic acids incorporating nonstandard chemical groups have advanced greatly in the recent years; as such, combination systems incorporating signal detecting, amplifying, and transducing elements can now be produced with considerable ease.

As resolution limits are bypassed and more selective biomolecular recognition agents are discovered, the detection efficiency of future biosensors will no doubt continue to increase. Furthermore, advances in nanotechnology and material fabrication methods may both allow novel detection techniques that employ nanoscale phenomena to be used in biosensor design and decrease costs by lowering biosensor dimensions and the quantities of biological materials incorporated into the sensor structure. Consequently, it would appear that the multifaceted nature of biosensor design, as well as the advantages it offers in terms of assay sensitivity, selectivity, and detection time and costs, will continue attracting commercial and research efforts and may allow the early detection of diseases that currently are undetectable during periods for which their treatment would have been the most effective.

REFERENCES

Adiguzel, Y. and Kulah, H. (2014). Breath sensors for lung cancer diagnosis. Biosens. Bioelectron. *65*, 121–138.

Alfonta, L., Willner, I., Throckmorton, D., and Singh, A. (2001). Electrochemical and quartz crystal microbalance detection of the cholera toxin employing horseradish peroxidase and GM1-functionalized liposomes. Anal. Chem. *73*, 5287–5295.

Altintas, Z. and Tothill, I. (2013). Biomarkers and biosensors for the early diagnosis of lung cancer. Sens. Actuators B Chem. *188*, 988–998.

Andrejevic, S., Jeremic, I., Sefik-Bukilica, M., Nikolic, M., Stojimirovic, B., and Bonaci-Nikolic, B. (2013). Immunoserological parameters in SLE: High-avidity anti-dsDNA detected by ELISA are the most closely associated with the disease activity. Clin. Rheumatol. *32*, 1619–1626.

Archakov, A.I. and Ivanov, Y.D. (2007). Analytical nanobiotechnology for medicine diagnostics. Mol. Biosyst. *3*, 336–342.

Arlett, J., Myers, E., and Roukes, M. (2011). Comparative advantages of mechanical biosensors. Nat. Nanotechnol. *6*, 203–215.

Arya, S.K. and Bhansali, S. (2011). Lung cancer and its early detection using biomarker-based biosensors. Chem. Rev. *111*, 6783–6809.

Bareket, L., Rephaeli, A., Berkovitch, G., Nudelman, A., and Rishpon, J. (2010). Carbon nanotubes based electrochemical biosensor for detection of formaldehyde released from a cancer cell line treated with formaldehyde-releasing anticancer prodrugs. Bioelectrochemistry *77*, 94–99.

Baselt, D., Lee, G., Natesan, M., Metzger, S., Sheehan, P., and Colton, R. (1998). A biosensor based on magnetoresistance technology. Biosens. Bioelectron. *13*, 731–739.

Belkin, S. (2003). Microbial whole-cell sensing systems of environmental pollutants. Curr. Opin. Microbiol. *6*, 206–212.

Berggren, C., Bjarnason, B., and Johansson, G. (2001). Capacitive biosensors. Electroanalysis *13*, 173–180.

Bertoncello, P. and Forster, R.J. (2009). Nanostructured materials for electrochemiluminescence (ECL)-based detection methods: Recent advances and future perspectives. Biosens. Bioelectron. *24*, 3191–3200.

Bhalla, V., Carrara, S., Sharma, P., Nangia, Y., and Raman Suri, C. (2012). Gold nanoparticles mediated label-free capacitance detection of cardiac troponin I. Sens. Actuators B Chem. *161*, 761–768.

Braha, O., Walker, B., Cheley, S., Kasianowicz, J., Song, L., Gouaux, J., and Bayley, H. (1997). Designed protein pores as components for biosensors. Chem. Biol. *4*, 497–505.

Braun, T., Ghatkesar, M.K., Backmann, N., Grange, W., Boulanger, P., Letellier, L., Lang, H.-P., Bietsch, A., Gerber, C., and Hegner, M. (2009). Quantitative time-resolved measurement of membrane protein-ligand interactions using microcantilever array sensors. Nat. Nanotechnol. *4*, 179–185.

Braunhut, S., McIntosh, D., Vorotnikova, E., Zhou, T., and Marx, K. (2005). Detection of apoptosis and drug resistance of human breast cancer cells to taxane treatments using quartz crystal microbalance biosensor technology. Assay Drug Dev. Technol. *3*, 77–88.

Bryan, T., Luo, X., Forsgren, L., Morozova-Roche, L.A., and Davis, J.J. (2012). The robust electrochemical detection of a Parkinson's disease marker in whole blood sera. Chem. Sci. *3*, 3468.

Cavalieri, E., Chakravarti, D., Guttenplan, J., Hart, E., Ingle, J., Jankowiak, R., Muti, P., Rogan, E., Russo, J., Santen, R., et al. (2006). Catechol estrogen quinones as initiators of breast and other human cancers: Implications for biomarkers of susceptibility and cancer prevention. Biochim. Biophys. Acta *1766*, 63–78.

Chandra, S., Barola, N., and Bahadur, D. (2011). Impedimetric biosensor for early detection of cervical cancer. Chem. Commun. (Camb.) *47*, 11258–11260.

Chandrashekara, S. (2014). Current studies of biomarkers for the early diagnosis of rheumatoid arthritis. Curr. Biomark. Find. *4*, 107–119.

Chang, K., Pi, Y., Lu, W., Wang, F., Pan, F., Li, F., Jia, S., Shi, J., Deng, S., and Chen, M. (2014). Label-free and high-sensitive detection of human breast cancer cells by aptamer-based leaky surface acoustic wave biosensor array. Biosens. Bioelectron. *60*, 318–324.

Chauhan, N. and Pundir, C.S. (2011). An amperometric uric acid biosensor based on multiwalled carbon nanotube-gold nanoparticle composite. Anal. Biochem. *413*, 97–103.

Chawla, S. and Pundir, C.S. (2011). An electrochemical biosensor for fructosyl valine for glycosylated hemoglobin detection based on core-shell magnetic bionanoparticles modified gold electrode. Biosens. Bioelectron. *26*, 3438–3443.

Chawla, S. and Pundir, C.S. (2012). An amperometric hemoglobin A1c biosensor based on immobilization of fructosyl amino acid oxidase onto zinc oxide nanoparticles-polypyrrole film. Anal. Biochem. *430*, 156–162.

Chen, K.-I., Li, B.-R., and Chen, Y.-T. (2011). Silicon nanowire field-effect transistor-based biosensors for biomedical diagnosis and cellular recording investigation. Nano Today *6*, 131–154.

Chiu, M.J., Horng, H.E., Chieh, J.J., Liao, S.H., Chen, C.H., Shih, B.Y., Yang, C.C., Lee, C.L., Chen, T.F., Yang, S.Y., et al. (2011). Multi-channel SQUID-based ultra-high-sensitivity in-vitro detections for bio-markers of Alzheimer's disease via immunomagnetic reduction. IEEE Trans. Appl. Supercond. *21*, 477–480.

Cho, I.-H., Paek, E.-H., Kim, Y.-K., Kim, J.-H., and Paek, S.-H. (2009). Chemiluminometric enzyme-linked immunosorbent assays (ELISA)-on-a-chip biosensor based on cross-flow chromatography. Anal. Chim. Acta *632*, 247–255.

Choi, Y.-E., Kwak, J.-W., and Park, J.W. (2010). Nanotechnology for early cancer detection. Sensors (Basel) *10*, 428–455.

Collura, C.V., Inserra, E., Diederichs, S., Ph, D., Iafrate, A.J., Bell, D.W., Digumarthy, S., Muzikansky, A., Irimia, D., Settleman, J., et al. (2008). Detection of mutations in EGFR in circulating lung-cancer cells. N. Engl. J. Med. *359*, 366–377.

Das, K., Penelle, J., and Rotello, V. (2003). Selective picomolar detection of hexachlorobenzene in water using a quartz crystal microbalance coated with a molecularly imprinted polymer thin film. Langmuir *19*, 3921–3925.

De la Rica, R. and Stevens, M.M. (2012). Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nat. Nanotechnol. *7*, 821–824.

Dell'Atti, D., Tombelli, S., Minunni, M., and Mascini, M. (2006). Detection of clinically relevant point mutations by a novel piezoelectric biosensor. Biosens. Bioelectron. *21*, 1876–1879.

Di Natale, C., Mantini, A., Macagnano, A., Antuzzi, D., Paolesse, R., and D'Amico, A. (1999). Electronic nose analysis of urine samples containing blood. Physiol. Meas. *20*, 377–384.

Dodson, S.L., Cao, C., Zaribafzadeh, H., Li, S., and Xiong, Q. (2015). Engineering plasmonic nanorod arrays for colon cancer marker detection. Biosens. Bioelectron. *63*, 472–477.

Dragonieri, S., Annema, J.T., Schot, R., van der Schee, M.P.C., Spanevello, A., Carratú, P., Resta, O., Rabe, K.F., and Sterk, P.J. (2009). An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer *64*, 166–170.

Du, Y., Li, B., and Wang, E. (2013). "Fitting" makes "sensing" simple: Label-free detection strategies based on nucleic acid aptamers. Acc. Chem. Res. *46*, 203–213.

Duk Han, Y., Jin Chun, H., and Yoon, H.C. (2014). The transformation of common office supplies into a low-cost optical biosensing platform. Biosens. Bioelectron. *59*, 259–268.

El-Sayed, I., Huang, X., and El-Sayed, M. (2005). Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: Applications in oral cancer. Nano Lett. *5*, 829–834.

Fendyur, A., Varma, S., Lo, C.T., and Voldman, J. (2015). Cell-based biosensor to report DNA damage in micro- and nanosystems. Anal. Chem. *86*, 7598–7605.

Feng, K., Yang, Y., Wang, Z., Jiang, J., Shen, G., and Yu, R. (2006). A nanoporous CeO_2/chitosan composite film as the immobilization matrix for colorectal cancer DNA sequence-selective electrochemical biosensor. Talanta *70*, 561–565.

Ferreira, G., Da-Silva, A., and Tome, B. (2009). Acoustic wave biosensors: Physical models and biological applications of quartz crystal microbalance. Trends Biotechnol. *27*, 689–697.

Gaster, R.S., Xu, L., Han, S.-J., Wilson, R.J., Hall, D.A., Osterfeld, S.J., Yu, H., and Wang, S.X. (2011). Quantification of protein interactions and solution transport using high-density GMR sensor arrays. Nat. Nanotechnol. *6*, 314–320.

Gerard, M., Chaubey, A., and Malhotra, B. (2002). Application of conducting polymers to biosensors. Biosens. Bioelectron. *17*, 345–359.

Grossman, H.L., Myers, W.R., Vreeland, V.J., Bruehl, R., Alper, M.D., Bertozzi, C.R., and Clarke, J. (2004). Detection of bacteria in suspension by using a superconducting quantum interference device. Proc. Natl. Acad. Sci. U.S.A. *101*, 1–6.

Haes, A. and Van Duyne, R. (2002). A nanoscale optical blosensor: Sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles. J. Am. Chem. Soc. *124*, 10596–10604.

Han, X.X., Zhao, B., and Ozaki, Y. (2009). Surface-enhanced Raman scattering for protein detection. Anal. Bioanal. Chem. *394*, 1719–1727.

Hansen, J., Wang, J., Kawde, A., Xiang, Y., Gothelf, K., and Collins, G. (2006). Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor. J. Am. Chem. Soc. *128*, 2228–2229.

Hanson, C. and Thaler, E. (2005). Electronic nose prediction of a clinical pneumonia score: Biosensors and microbes. Anesthesiology *102*, 63–68.

Haun, J., Yoon, T., Lee, H., and Weissleder, R. (2010). Magnetic nanoparticle biosensors. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. *2*, 291–304.

Hong, S.-R., Jeong, H.-D., and Hong, S. (2010). QCM DNA biosensor for the diagnosis of a fish pathogenic virus VHSV. Talanta *82*, 899–903.

Ierardi, V., Ferrera, F., Millo, E., Damonte, G., Filaci, G., and Valbusa, U. (2013). Bioactive surfaces for antibody-antigen complex detection by atomic force microscopy. J. Phys. Conf. Ser. *439*, 012001.

Iles, R.K. and Kallichurn, H. (2012). What will be the future development of electrochemical biosensors for the detection and quantification of biomarkers? J Bioeng. Biomed. Sci. *2*, e110.

Iliuk, A.B., Hu, L., and Tao, W.A. (2011). Aptamer in bioanalytical applications. Anal. Chem. *83*, 4440–4452.

Joo, J., Kwon, D., Yim, C., and Jeon, S. (2012). Highly sensitive diagnostic assay for the detection of protein biomarkers using microresonators and multifunctional nanoparticles. ACS Nano *6*, 4375–4381.

Jung, J., Cheon, D., Liu, F., Lee, K., and Seo, T. (2010). A graphene oxide based immuno-biosensor for pathogen detection. Angew. Chem. Int. Ed. Engl. *49*, 5708–5711.

Jung, H.-W., Chang, Y.W., Lee, G., Cho, S., Kang, M.-J., and Pyun, J.-C. (2014). A capacitive biosensor based on an interdigitated electrode with nanoislands. Anal. Chim. Acta *844*, 27–34.

Kakoti, A. and Goswami, P. (2013). Heart type fatty acid binding protein: Structure, function and biosensing applications for early detection of myocardial infarction. Biosens. Bioelectron. *43*, 400–411.

Kaya, Y., Ayas, S., Topal, A.E., Guner, H., and Dana, A. (2014). Sensitivity comparison of localized plasmon resonance structures and prism coupler. Sens. Actuators B Chem. *191*, 516–521.

Kim, J., Park, W.I., Lee, C., and Yi, G. (2006). ZnO nanorod biosensor for highly sensitive detection of specific protein binding. J. Kor. Phys. Soc. *49*, 1635–1639.

Kim, S.J., Park, Y.M., Min, B.-H., Lee, D.-S., and Yoon, H.C. (2013). Optical immunosensor for quantifying C-telopeptide fragments of type II collagen as an osteoarthritis biomarker in urine. BioChip J. *7*, 399–407.

Kleiren, E., Ruysschaert, J., Goormaghtigh, E., and Raussens, V. (2010). Development of a quantitative and for Alzheimer' s disease : The effect of deuteration on the detection of the A β peptide. Spectroscopy *24*, 61–66.

Klonoff, D.C. (2007). The benefits of implanted glucose sensors. J. Diabetes Sci. Technol. *1*, 797–800.

Kneipp, K., Wang, Y., Kneipp, H., Perelman, L., Itzkan, I., Dasari, R., and Feld, M. (1997). Single molecule detection using surface-enhanced Raman scattering (SERS). Phys. Rev. Lett. *78*, 1667–1670.

Kotanen, C., Moussy, F., Carrara, S., and Guiseppi-Elie, A. (2012). Implantable enzyme amperometric biosensors. Biosens. Bioelectron. *35*, 14–26.

Kriz, D., Ramstrom, O., and Mosbach, K. (1997). Molecular imprinting—New possibilities for sensor technology. Anal. Chem. *69*, A345–A349.

Lakard, B., Herlem, G., Lakard, S., Antoniou, A., and Fahys, B. (2004). Urea potentiometric biosensor based on modified electrodes with urease immobilized on polyethylenimine films. Biosens. Bioelectron. *19*, 1641–1647.

Lee, S.J., Youn, B.-S., Park, J.W., Niazi, J.H., Kim, Y.S., and Gu, M.B. (2008). ssDNA aptamer-based surface plasmon resonance biosensor for the detection of retinol binding protein 4 for the early diagnosis of type 2 diabetes. Anal. Chem. *80*, 2867–2873.

Lerner, M.B., Dailey, J., Goldsmith, B.R., Brisson, D., and Johnson, A.T.C. (2013). Detecting Lyme disease using antibody-functionalized single-walled carbon nanotube transistors. Biosens. Bioelectron. *45*, 163–167.

Li, C., Liu, Y., Li, L., Du, Z., Xu, S., Zhang, M., Yin, X., and Wang, T. (2008). A novel amperometric biosensor based on NiO hollow nanospheres for biosensing glucose. Talanta *77*, 455–459.

Li, Z., Wang, Y., Wang, J., Tang, Z., Pounds, J.G., and Lin, Y. (2010). Rapid and sensitive detection of protein biomarker using a portable fluorescence biosensor based on quantum dots and a lateral flow test strip. Anal. Chem. *82*, 7008–7014.

Li, F., Yu, Y., Cui, H., Yang, D., and Bian, Z. (2013a). Label-free electrochemiluminescence immunosensor for cardiac troponin I using luminol functionalized gold nanoparticles as a sensing platform. Analyst *138*, 1844–1850.

Li, M., Cushing, S.K., Zhang, J., Suri, S., Evans, R., Petros, W.P., Gibson, L.F., Ma, D., Liu, Y., and Wu, N. (2013b). Three-dimensional hierarchical plasmonic nano-architecture enhanced surface-enhanced Raman scattering immunosensor for cancer biomarker detection in blood plasma. ACS Nano *7*, 4967–4976.

Lin, V., Motesharei, K., Dancil, K., Sailor, M., and Ghadiri, M. (1997). A porous silicon-based optical interferometric biosensor. Science *278*, 840–843.

Lin, J., Wei, Z., Zhang, H., and Shao, M. (2013). Sensitive immunosensor for the label-free determination of tumor marker based on carbon nanotubes/

mesoporous silica and graphene modified electrode. Biosens. Bioelectron. *41*, 342–347.

Liu, Y., Li, X., Zhang, Z., Zuo, G., Cheng, Z., and Yu, H. (2009). Nanogram per milliliter-level immunologic detection of alpha-fetoprotein with integrated rotating-resonance microcantilevers for early-stage diagnosis of hepatocellular carcinoma. Biomed. Microdevices *11*, 183–191.

Logrieco, A., Arrigan, D., Brengel-Pesce, K., Siciliano, P., and Tothill, I. (2005). DNA arrays, electronic noses and tongues, biosensors and receptors for rapid detection of toxigenic fungi and mycotoxins: A review. Food Addit. Contam. *22*, 335–344.

Luo, X. and Davis, J.J. (2013). Electrical biosensors and the label free detection of protein disease biomarkers. Chem. Soc. Rev. *42*, 5944–5962.

Martín, M., Salazar, P., Villalonga, R., Campuzano, S., Pingarrón, J.M., and González-Mora, J.L. (2014). Preparation of core–shell Fe_3O_4@poly(dopamine) magnetic nanoparticles for biosensor construction. J. Mater. Chem. B *2*, 739.

Medley, C.D., Smith, J.E., Tang, Z., Wu, Y., Bamrungsap, S., and Tan, W. (2008). Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells. Anal. Chem. *80*, 1067–1072.

Michelini, E. and Roda, A. (2012). Staying alive: New perspectives on cell immobilization for biosensing purposes. Anal. Bioanal. Chem. *402*, 1785–1797.

Mitchell, J.S. and Lowe, T.E. (2009). Ultrasensitive detection of testosterone using conjugate linker technology in a nanoparticle-enhanced surface plasmon resonance biosensor. Biosens. Bioelectron. *24*, 2177–2183.

Mo, J. and Smart, W. (2004). Lactate biosensors for continuous monitoring. Front. Biosci. *9*, 3384–3391.

Morozov, V.N., Groves, S., Turell, M.J., and Bailey, C. (2007). Three minutes-long electrophoretically assisted zeptomolar microfluidic immunoassay with magnetic-beads detection. J. Am. Chem. Soc. *129*, 12628–12629.

Mulchandani, A., Pan, S., and Chen, W. (1999). Fiber-optic enzyme biosensor for direct determination of organophosphate nerve agents. Biotechnol. Prog. *15*, 130–134.

Myung, S., Solanki, A., Kim, C., Park, J., Kim, K., and Lee, K. (2011). Graphene-encapsulated nanoparticle-based biosensor for the selective detection of cancer biomarkers. Adv. Mater. *23*, 2221–2225.

Nikolelis, D. and Krull, U. (1992). Establishment and control of artificial ion-conductive zones for lipid-membrane biosensor development. Anal. Chim. Acta *257*, 239–245.

Oisjöen, F., Schneiderman, J.F., Astalan, A.P., Kalabukhov, A., Johansson, C., and Winkler, D. (2010). A new approach for bioassays based on frequency- and time-domain measurements of magnetic nanoparticles. Biosens. Bioelectron. *25*, 1008–1013.

Pannala, R., Basu, A., Petersen, G., and Chari, S. (2009). New-onset diabetes: A potential clue to the early diagnosis of pancreatic cancer. Lancet Oncol. *10*, 88–95.

Park, J.-S., Cho, M.K., Lee, E.J., Ahn, K.-Y., Lee, K.E., Jung, J.H., Cho, Y., Han, S.-S., Kim, Y.K., and Lee, J. (2009). A highly sensitive and selective diagnostic assay based on virus nanoparticles. Nat. Nanotechnol. *4*, 259–264.

Park, Y.M., Kim, S.J., Lee, K.J., Yang, S.S., Min, B.-H., and Yoon, H.C. (2014). Detection of CTX-II in serum and urine to diagnose osteoarthritis by using a fluoro-microbeads guiding chip. Biosens. Bioelectron. *67*, 192–199.

Pathirana, S., Barbaree, J., Chin, B., Hartell, M., Neely, W., and Vodyanoy, V. (2000). Rapid and sensitive biosensor for Salmonella. Biosens. Bioelectron. *15*, 135–141.

Peeling, R. and Mabey, D. (2010). Point-of-care tests for diagnosing infections in the developing world. Clin. Microbiol. Infect. *16*, 1062–1069.

Peng, H. and Miller, B. (2011). Recent advancements in optical DNA biosensors: Exploiting the plasmonic effects of metal nanoparticles. Analyst *136*, 436–447.

Ping, W., Yi, T., Xie, H., and Shen, F. (1997). A novel method for diabetes diagnosis based on electronic nose. Biosens. Bioelectron. *12*, 1031–1036.

Rapp, B.E., Gruhl, F.J., and Länge, K. (2010). Biosensors with label-free detection designed for diagnostic applications. Anal. Bioanal. Chem. *398*, 2403–2412.

Rashid, J.I.A., Yusof, N.A., Abdullah, J., Hashim, U., and Hajian, R. (2014). The utilization of SiNWs/AuNPs-modified indium tin oxide (ITO) in fabrication of electrochemical DNA sensor. Mater. Sci. Eng. C *45*, 270–276.

Ray, A. and Norden, B. (2000). Peptide nucleic acid (PNA): Its medical and biotechnical applications and promise for the future. FASEB J. *14*, 1041–1060.

Reimhult, E. and Kumar, K. (2008). Membrane biosensor platforms using nano- and microporous supports. Trends Biotechnol. *26*, 82–89.

Rissin, D.M., Kan, C.W., Campbell, T.G., Howes, S.C., Fournier, D.R., Song, L., Piech, T., Patel, P.P., Chang, L., Rivnak, A.J., et al. (2010). Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat. Biotechnol. *28*, 595–599.

Rocha-Gaso, M., March-Iborra, C., Montoya-Baides, A., and Arnau-Vives, A. (2009). Surface generated acoustic wave biosensors for the detection of pathogens: A review. Sensors *9*, 5740–5769.

Rui, Q., Komori, K., Tian, Y., Liu, H., Luo, Y., and Sakai, Y. (2010). Electrochemical biosensor for the detection of H_2O_2 from living cancer cells based on ZnO nanosheets. Anal. Chim. Acta *70*, 57–62.

Ryou, M., Netniroski, A., Azagury, D., Shaikh, S., Ryan, M., Westervelt, R., and Thompson, C. (2011). An implantable wireless biosensor for the immediate detection of upper GI bleeding: A new fluorescein-based tool for diagnosis and surveillance. Gastrointest. Endosc. *74*, 189–194.

Salimi, A., Noorbakhash, A., Sharifi, E., and Semnani, A. (2008). Highly sensitive sensor for picomolar detection of insulin at physiological pH, using GC electrode modified with guanine and electrodeposited nickel oxide nanoparticles. Biosens. Bioelectron. *24*, 792–798.

Saravolatz, L., Manzor, O., VanderVelde, N., Pawlak, J., and Belian, B. (2003). Broad-range bacterial polymerase chain reaction for early detection of bacterial meningitis. Clin. Infect. Dis. *36*, 40–45.

Saurina, J., Hernandez-Cassou, S., Fabregas, E., and Alegret, S. (1998). Potentiometric biosensor for lysine analysis based on a chemically immobilized lysine oxidase membrane. Anal. Chim. Acta *371*, 49–56.

Sepulveda, B., Angelome, P., Lechuga, L., and Liz-Marzan, L. (2009). LSPR-based nanobiosensors. Nano Today *4*, 244–251.

Serafín, V., Eguílaz, M., Agüí, L., Yáñez-Sedeño, P., and Pingarrón, J.M. (2011). An electrochemical immunosensor for testosterone using gold nanoparticles—Carbon nanotubes composite electrodes. Electroanalysis *23*, 169–176.

Sidhu, S., Lowman, H., Cunningham, B., and Wells, J. (2000). Phage display for selection of novel binding peptides. In: Thorner, J., Emr, S.D., Abelson, J.N. (eds.), Methods in Enzymology, vol. *328*, Applications of chimeric genes and hybrid proteins, Pt. C, pp. Academic Press, San Diego, pp. 333–363.

Silverman, S. (1988). Early diagnosis of oral cancer. Cancer *62*, 1796–1799.

Song, J., Yang, Q., Lv, F., Liu, L., and Wang, S. (2012a). Visual detection of DNA mutation using multicolor fluorescent coding. ACS Appl. Mater. Interfaces *4*, 2885–2890.

Song, S.Y., Han, Y.D., Hong, S.Y., Kim, K., Yang, S.S., Min, B.-H., and Yoon, H.C. (2012b). Chip-based cartilage oligomeric matrix protein detection in serum and synovial fluid for osteoarthritis diagnosis. Anal. Biochem. *420*, 139–146.

Sotiropoulou, S. and Chaniotakis, N. (2005). Lowering the detection limit of the acetylcholinesterase biosensor using a nanoporous carbon matrix. Anal. Chim. Acta *530*, 199–204.

Reichlin, T., Hochholzer, W., Bassetti, S., Steuer, S., Stelzig, C., Hartwiger, S., Biedert, S., Schaub, N., Buerge, C., Potocki, M., Noveanu, M., Breidthardt, T., Twerenbold, R., Winkler, K., Bingisser, R., Mueller, C. (2009). Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. N. Engl. J. Med. *361*, 858–867.

Suwansa-ard, S., Kanatharana, P., Asawatreratanakul, P., Wongkittisuksa, B., Limsakul, C., and Thavarungkul, P. (2009). Comparison of surface plasmon resonance and capacitive immunosensors for cancer antigen 125 detection in human serum samples. Biosens. Bioelectron. *24*, 3436–3441.

Suzuki, M., Husimi, Y., Komatsu, H., Suzuki, K., and Douglas, K. (2008). Quantum dot FRET biosensors that respond to pH, to proteolytic or nucleolytic

cleavage, to DNA synthesis, or to a multiplexing combination. J. Am. Chem. Soc. *130*, 5720–5725.

Swierczewska, M., Liu, G., Lee, S., and Chen, X. (2012). High-sensitivity nanosensors for biomarker detection. Chem. Soc. Rev. *41*, 2641–2655.

Taranekar, P., Baba, A., Park, J., Fulghum, T., and Advincula, R. (2006). Dendrimer precursors for nanomolar and picomolar real-time surface plasmon resonance/potentiometric chemical nerve agent sensing using electrochemically cross-linked ultrathin films. Adv. Funct. Mater. *16*, 2000–2007.

Thakor, A.S. and Gambhir, S.S. (2013). Nanooncology: The future of cancer diagnosis and therapy. CA Cancer J. Clin. *63*, 395–418.

Torrance, L., Ziegler, A., Pittman, H., Paterson, M., Toth, R., and Eggleston, I. (2006). Oriented immobilisation of engineered single-chain antibodies to develop biosensors for virus detection. J. Virol. Methods *134*, 164–170.

Tothill, I.E. (2009). Biosensors for cancer markers diagnosis. Semin. Cell Dev. Biol. *20*, 55–62.

Tsai, H. and Doong, R. (2005). Simultaneous determination of pH, urea, acetylcholine and heavy metals using array-based enzymatic optical biosensor. Biosens. Bioelectron. *20*, 1796–1804.

Uckermann, O., Galli, R., Tamosaityte, S., Leipnitz, E., Geiger, K., Schackert, G., Koch, E., Steiner, G., and Kirsch, M. (2014). Label-free delineation of brain tumors by coherent anti-stokes Raman scattering microscopy in an orthotopic mouse model and human glioblastoma. PLoS One *9*, e107115.

Van Dorst, B., Mehta, J., Bekaert, K., Rouah-Martin, E., De Coen, W., Dubruel, P., Blust, R., and Robbens, J. (2010). Recent advances in recognition elements of food and environmental biosensors: A review. Biosens. Bioelectron. *26*, 1178–1194.

Vanhecke, D., Roumenina, L.T., Wan, H., Osthoff, M., Schaller, M., and Trendelenburg, M. (2012). Identification of a major linear C1q epitope allows detection of systemic lupus erythematosus anti-C1q antibodies by a specific peptide-based enzyme-linked immunosorbent assay. Arthritis Rheum. *64*, 3706–3714.

Varshney, M. and Li, Y. (2007). Interdigitated array microelectrode based impedance biosensor coupled with magnetic nanoparticle-antibody conjugates for detection of *Escherichia coli* O157: H7 in food samples. Biosens. Bioelectron. *22*, 2408–2414.

Von Muhlen, M.G., Brault, N.D., Knudsen, S.M., Jiang, S., and Manalis, S.R. (2010). Label-free biomarker sensing in undiluted serum with suspended microchannel resonators. Anal. Chem. *82*, 1905–1910.

Waggoner, P.S. and Craighead, H.G. (2007). Micro- and nanomechanical sensors for environmental, chemical, and biological detection. Lab Chip *7*, 1238–1255.

Wang, J. (1999). Amperometric biosensors for clinical and therapeutic drug monitoring: A review. J. Pharm. Biomed. Anal. *19*, 47–53.

Wang, J. (2001). Glucose biosensors: 40 years of advances and challenges. Electroanalysis *13*, 983–988.

Wang, J. (2005). Carbon-nanotube based electrochemical biosensors: A review. Electroanalysis *17*, 7–14.

Wang, J. (2006). Electrochemical biosensors: Towards point-of-care cancer diagnostics. Biosens. Bioelectron. *21*, 1887–1892.

Wang, R. and Li, Y. (2013). Hydrogel based QCM aptasensor for detection of avian influenza virus. Biosens. Bioelectron. *42*, 148–155.

Wang, S., Haque, F., Rychahou, P.G., Evers, B.M., and Guo, P. (2013). Engineered nanopore of Phi29 DNA-packaging motor for real-time detection of single colon cancer specific antibody in serum. ACS Nano *7*, 9814–9822.

Wang, Y., Guo, X., Tong, L., and Lou, J. (2014). Modeling of Au-nanowire waveguide for plasmonic sensing in liquids. J. Lightwave Technol. *32*, 3631–3636.

Wongkaew, P. and Poosittisak, S. (2014). Diagnosis of sugarcane white leaf disease using the highly sensitive DNA based voltammetric electrochemical determination. Am. J. Plant Sci., *5*, 2256–2268.

Wu, H., Wang, J., Kang, X., Wang, C., Wang, D., Liu, J., Aksay, I.A., and Lin, Y. (2009). Glucose biosensor based on immobilization of glucose oxidase in platinum nanoparticles/graphene/chitosan nanocomposite film. Talanta *80*, 403–406.

Wu, S., Liu, G., Li, P., Liu, H., and Xu, H. (2012). A high-sensitive and fast-fabricated glucose biosensor based on Prussian blue/topological insulator Bi_2Se_3 hybrid film. Biosens. Bioelectron. *38*, 289–294.

Wulfkuhle, J., Liotta, L., and Petricoin, E. (2003). Proteomic applications for the early detection of cancer. Nat. Rev. Cancer *3*, 267–275.

Wustoni, S., Hideshima, S., Kuroiwa, S., Nakanishi, T., Hashimoto, M., Mori, Y., and Osaka, T. (2014). Sensitive electrical detection of human prion proteins using field effect transistor biosensor with dual-ligand binding amplification. Biosens. Bioelectron. *67*, 256–262.

Xu, S. and Han, X. (2004). A novel method to construct a third-generation biosensor: Self-assembling gold nanoparticles on thiol-functionalized poly(styrene-*co*-acrylic acid) nanospheres. Biosens. Bioelectron. *19*, 1117–1120.

Xu, H. and Kall, M. (2002). Modeling the optical response of nanoparticle-based surface plasmon resonance sensors. Sens. Actuators B Chem. *87*, 244–249.

Xu, L.-J., Zong, C., Zheng, X.-S., Hu, P., Feng, J.-M., and Ren, B. (2014). Label-free detection of native proteins by surface-enhanced Raman spectroscopy using iodide-modified nanoparticles. Anal. Chem. *86*, 2238–2245.

Yang, X., Yuan, R., Chai, Y., Zhuo, Y., Hong, C., Liu, Z., and Su, H. (2009). Porous redox-active Cu_2O-SiO_2 nanostructured film: Preparation, characterization and application for a label-free amperometric ferritin immunosensor. Talanta *78*, 596–601.

Yanik, A.A., Huang, M., Kamohara, O., Artar, A., Geisbert, T.W., Connor, J.H., and Altug, H. (2010). An optofluidic nanoplasmonic biosensor for direct detection of live viruses from biological media. Nano Lett. *10*, 4962–4969.

Yano, K. and Karube, I. (1999). Molecularly imprinted polymers for biosensor applications. Trends Anal. Chem. *18*, 199–204.

Yao, C., Zhu, T., Qi, Y., Zhao, Y., Xia, H., and Fu, W. (2010). Development of a quartz crystal microbalance biosensor with aptamers as bio-recognition element. Sensors *10*, 5859–5871.

Yu, J., Ge, L., Huang, J., Wang, S., and Ge, S. (2011). Microfluidic paper-based chemiluminescence biosensor for simultaneous determination of glucose and uric acid. Lab Chip *11*, 1286–1291.

Yuan, J., Duan, R., Yang, H., Luo, X., Xi, M. (2012). Detection of serum human epididymis secretory protein 4 in patients with ovarian cancer using a label-free biosensor based on localized surface plasmon resonance. Int. J. Nanomed. *7*, 2921–2928.

Zhang, X., Teng, Y., Fu, Y., Xu, L., Zhang, S., He, B., Wang, C., and Zhang, W. (2010). Lectin-based biosensor strategy for electrochemical assay of glycan expression on living cancer cells. Anal. Chem. *82*, 9455–9460.

Zhang, D., Lu, Y., Jiang, J., Zhang, Q., Yao, Y., Wang, P., Chen, B., Cheng, Q., Liu, G.L., and Liu, Q. (2014). Nanoplasmonic biosensor: Coupling electrochemistry to localized surface plasmon resonance spectroscopy on nanocup arrays. Biosens. Bioelectron. *140*, 506–511.

Zhang, C., Lou, J., Tu, W., Bao, J., and Dai, Z. (2015). Ultrasensitive electrochemical biosensing for DNA using quantum dots combined with restriction endonuclease. Analyst *140*, 506–511.

Zhao, C., Hoppe, T., Setty, M.K.H.G., Murray, D., Chun, T.-W., Hewlett, I., and Appella, D.H. (2014a). Quantification of plasma HIV RNA using chemically engineered peptide nucleic acids. Nat. Commun. *5*, 5079.

Zhao, Q., Duan, R., Yuan, J., Quan, Y., Yang, H., and Xi, M. (2014b). A reusable localized surface plasmon resonance biosensor for quantitative detection of serum squamous cell carcinoma antigen in cervical cancer patients based on silver nanoparticles array. Int. J. Nanomed. *9*, 1097–1104.