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REVIEW

Affinity and enzyme-based biosensors: recent advances and emerging applications in cell analysis and point-of-care testing

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Abstract The applications of biosensors range from environmental testing and biowarfare agent detection to clinical testing and cell analysis. In recent years, biosensors have become increasingly prevalent in clinical testing and point-of-care testing. This is driven in part by the desire to decrease the cost of health care, to shift some of the analytical tests from centralized facilities to "frontline" physicians and nurses, and to obtain more precise information more quickly about the health status of a patient. This article gives an overview of recent advances in the field of biosensors, focusing on biosensors based on enzymes, aptamers, antibodies, and phages. In addition, this article attempts to describe efforts to apply these biosensors to clinical testing and cell analysis.

Keywords Biosensor · Enzyme · Aptamers · Phage · Point of care

Introduction

As the number of hazardous materials and pathogens in our environment and food increases, the development of highly selective and sensitive monitoring devices is ever more important. Despite significant progress, detection instruments are bulky, and require qualified personnel and large amounts of reagents. In this context, the field of biosensing strives to develop devices that are sensitive, specific, and yet miniature and simple to operate.

According to an IUPAC definition, a biosensor consists of biorecognition elements specific to the analyte of interest and a physiochemical transducer to relay the resultant signal from this biorecognition event [1]. Signal transduction strategies may be electrochemical [2–4], optical [5, 6], gravimetric [7, 8], or thermal [9]. Recognition elements may be readily integrated with electronics to create fast, accurate, and inexpensive sensing devices [10, 11]. Novel directions in biosensors often involve the design of improved biorecognition elements or the development of novel materials for improving signal transduction. Examples of such materials include carbon nanotubes (CNTs) and graphene in electrochemical systems and nanoplasmonic structures in optical systems.

Biosensors may be broadly classified on the basis of (1) the type of biorecognition elements (i.e., enzyme-, antibody-, DNA-, or RNA-based) and (2) the signal transduction method (optical, electrochemical, gravimetric, etc.) used for detection. Biosensors based on binding of specific analytes (e.g., antigens binding to antibodies) are an example of affinity biosensors. The best known example of an affinity biosensor is the enzyme-linked immunosorbent assay (ELISA). The affinity biosensors are designed to maximize association and minimize dissociation of target analytes; thus, these sensors become saturated and may not provide dynamic (kinetic) information about fluctuations in the level of the analyte over time. Enzyme-based biosensors on the other hand are based on the enzymatic turnover and may be used to record changes in analyte concentration over time. However, as discussed in

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this review, the emergence of new biorecognition elements such as aptamers is helping to blur the line between enzymatic and affinity biosensors. This review discusses several topics related to affinity and enzyme-based biosensors, including novel biorecognition elements, biosensor miniaturization, and novel biofunctional interfaces for biosensors. Because of the breadth of this research topic and the large number of published articles, this review will not be able to give due credit to all the excellent work done in this field.

Biorecognition elements for biosensors

Molecular recognition is a central event for most biosensors. Several articles [12–14] have given an overview of the various recognition approaches. The past few years have seen continued emphasis on enzyme and antibody-based biosensors and also the emergence of new recognition approaches, most notably those employing aptamers and phages. Presently, research into enzyme-based biosensing systems is being pursued in the areas of (1) enzyme immobilization, (2) integration of enzymes with specific nanostructures such as carbon nanotubes (CNTs) and graphene to enhance electron transfer capabilities, and (3) enzyme engineering to improve selectivity and immobilization. For affinity biosensing, some of the novel approaches are focused on (1) aptamer-based biosensors, where the most activity is in designing new aptamer structures and conjugation of aptamers with different physiochemical transducers, and (2) phages as a specific biorecognition structure that increases the specificity and improves the stability of the biosensor.

Enzyme-based biosensors

One of the challenges in making robust enzyme-based biosensors is ensuring that immobilized enzyme molecules remain functional over time. This section focuses on enzyme stabilization and immobilization strategies employing (1) CNTs, (2) sol-gel/hydrogel incorporation, and (3) immobilization of apoenzymes by cofactor coupling. We focus on glucose biosensors as these are most studied enzyme-based biosensors. However, we also highlight research making use of other enzymes, such as organophosphorus hydrolase (OPH) and horseradish peroxidase (HRP).

Carbon nanotubes

CNTs possess electrical conductivity and mechanical strength making them well suited to serve as a scaffold for enzyme immobilization and for relaying electrons to the electrode [15–18]. However, CNTs tend to aggregate into tangled networks when deposited on the surface. Utilization of CNTs in biosensing applications depends strongly on the ability to prevent bundling and disperse the CNTs homogeneously throughout the matrix without destroying their structural integrity [19, 20]. Dispersion of CNTs was demonstrated via association of biopolymers with CNT surfaces [21, 22].

A new approach was demonstrated by Yan et al. [23] using “dissolved” CNTs in a mixed solution of cyclodextrin and cyclodextrin prepolymer that works as a modifier to fabricate chemically tailored electrodes. An insoluble, conducting composite film of polycyclodextrin and CNTs was synthesized, and glucose oxidase (GOx) was immobilized on the film to fabricate an amperometric biosensor. It was shown that the composite biocompatible matrix of polycyclodextrin and CNTs maintains the bioactivity of the immobilized enzyme for 2 weeks [23]. The biosensor had a detection limit of 3.5 μM, with a linear range from 0.004 to 3.23 mM. In another effort, aimed at enhancing the amount of adsorbed enzyme, Nejadnik et al. [24] explored the use of CNT scaffolds for enzyme immobilization. It was hypothesized that the three-dimensional scaffolds can significantly increase the amount of enzyme adsorbed per unit area while preserving the catalytic activity of the adsorbed molecules. Nejadnik et al. found that improved sensitivity can be achieved by tailoring the thickness of scaffolds. The 65.5 nm scaffolds resulted in a sensitivity improvement of 390 % over the 44.5 nm scaffolds. Pedrosa et al. [25] demonstrated that single-walled CNTs used with a covalent immobilization strategy are very sensitive sensors with excellent long-term stability. OPH-functionalized single-walled and multiwalled CNT conjugates were exploited for direct amperometric detection of paraoxon, a model organophosphate. Single-walled CNTs onto which OPH had been covalently immobilized showed much higher activity than OPH-conjugated multiwalled CNTs. The detection limit was 0.01 μmol L^{-1}.

Utilizing layer-by-layer (LBL) assembly, Mantha et al. [11] demonstrated that a sandwich-like LBL structure provides a suitable microenvironment to retain the molecular activity of incorporated biopolymers. They used the electrostatic interaction of anionic/cationic biomolecular layers structured with multiwalled CNTs to build up the hybrid catalytic interfaces (Fig. 1). The electrodes generated by this approach were easy to fabricate and had excellent sensitivity and excellent electrochemical response.

Recent research efforts have shown graphene, a structural element of CNTs, to be an excellent matrix for biomolecule immobilization. The biocompatibility and excellent electrochemical properties of this material may prove beneficial in developing electrochemical enzyme-based biosensors. In a recent study, Liu et al. [26] demonstrated a highly efficient enzyme-based electrode using covalent attachment of

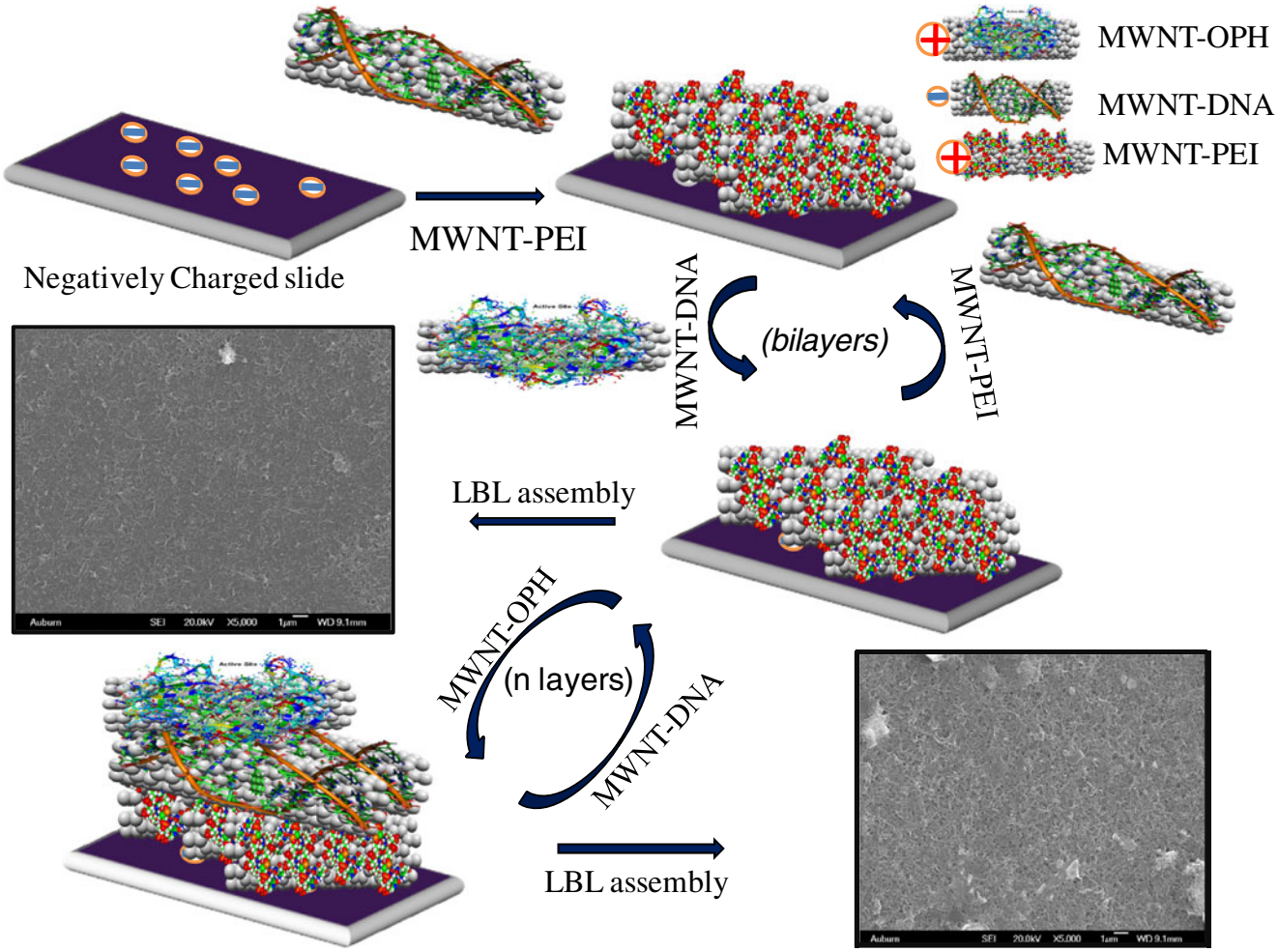

Fig. 1 *LBL* interface design. The initial layers of poly(ethylene imine)-functionalized multiwalled carbon nanotubes (*MWNT-PEI*) and DNA-functionalized multiwalled carbon nanotubes (*MWNT-DNA*) (four bilayers as shown in the scanning electron microscope image) provide support for subsequent layers of multiwalled carbon nanotubes functionalized with organophosphorus hydrolase (MWNT-OPH) and MWNT-DNA (nine bilayers are shown in the scanning electron microscope image). (From [11])

carboxy-functionalized graphene oxide sheets and GOx. The resulting biosensor exhibited a broad linear range up to 28 mM per square millimeter of glucose with a sensitivity of 8.045 mA cm^{-2} M^{-1}. Kang et al. [27] observed direct electron transfer in GOx immobilized on graphene–chitosan nanocomposite films. The immobilized enzyme exhibited a surface-confined, reversible two-proton and two-electron transfer reaction with an electron transfer rate constant of 2.83 s^{-1}. This nanocomposite sensor had glucose sensitivity of 37.93 μA mM^{-1} cm^{-2} and a detection limit of 0.02 mM.

Sol-gels and hydrogels for enzyme immobilization

Sol-gel/hydrogel chemistry has been used extensively to prepare metal oxide, silica, and organosiloxane materials for sensors, coatings, and catalysts [28]. Sol-gel technology may be used to prepare a three-dimensional network for the encapsulation of biomolecules [29] and for the creation of bio-inorganic conductive matrices for immobilization of enzymes. In this context, Ivnitski et al. [30] showed direct electron transfer by entrapping GOx in a silica/CNT nanocomposite prepared on a screen-printed carbon electrode. Ramanathan et al. [31] described a rapid method for enzyme immobilization directly on a waveguide surface by encapsulation in a silica matrix. OPH, an enzyme that catalytically hydrolyzes organophosphates, was used as a model to demonstrate the utility of lysozyme-mediated silica formation for enzyme stabilization. Silica-encapsulated OPH retained its catalytic activity for nearly 60 days, with a detection limit for paraoxon, a common organophosphate, of 35 μM. Jia et al. [32] have fabricated gold nanoparticles (AuNPs) containing a sol-gel network for HRP immobilization. The resulting biosensor exhibited a fast amperometric response to H_2O_2, with a detection limit of 2.0 μmol L^{-1}, and the linear range was 5.0 μmol L^{-1} to 10.0 mmol L^{-1}.

Yan et al. [33] developed a hydrogel-based microfabrication approach for interfacing functional enzymes with electrode arrays. Poly(ethylene glycol) hydrogel photopatterning was employed to integrate gold electrode arrays with the enzymes GOx and lactate oxidase. Subsequent work described the use of AuNPs-containing hydrogel microstructures in the development of electrochemical enzyme-based biosensors [34]. fabricate biosensors, AuNPs were conjugated with GOx or HRP molecules and were dispersed in the prepolymer solution of poly(ethylene glycol) diacrylate. Impedance measurements revealed that inclusion of AuNPs improved the conductivity of the poly(ethylene glycol) hydrogel by a factor of approximately 5. Importantly, the biosensors based on the AuNP–GOx complexes exhibited high sensitivity to glucose (100 $\mu A\ mM^{-1}\ cm^{-2}$) in the linear range from 0.1 to 10 mM.

Apoenzyme immobilization in biosensing

Apoenzymes are a class of enzymes that require a cofactor to function. This cofactor is not covalently linked to the enzyme, but may become very tightly bound. Taking advantage of this site-specific binding provides an opportunity to both immobilize and orient the target enzyme. The Willner group has been very active in research regarding the reconstitution of apoenzymes on cofactor-functionalized nanostructures associated with electrodes [35]. Their approach provides a means to both align enzymes on the conductive surface and to electrically contact redox enzymes with the electrodes. Work from their group includes a method to construct electrically contacted enzyme-based electrodes by using a phenylboronic acid ligand as a basis for the association of flavin adenine dinucleotide (FAD) or $NAD(P)^+$ cofactors [36]. The reconstitution of apo-GOx on a FAD cofactor linked to a pyrroloquinoline quinone phenylboronic acid monolayer yielded an enzyme monolayer (surface coverage 2.1×10^{-12} mol cm^{-2}) electrically coupled to the electrode exhibiting a turnover rate of 700 s^{-1}. This approach has also been successful for NAD^+ and $NADP^+$ monolayers and their respective apoenzymes. The Willner group has also used this strategy to couple apo-GOx to 1.4 nm gold nanocrystals functionalized with FAD. Integration of these constructs into a conductive film yields a bioelectrocatalytic system with exceptional electrical contact with the electrode support [37]. In this configuration, the electron-transfer ability of the reconstituted GOx exceeds that of the native enzyme.

Aptamer-based biosensors

Aptamers are small single-stranded DNA or RNA molecules (fewer than 100 bases) typically selected from a random oligonucleotide library to bind a target with high affinity and specificity. They offer a number of advantages over conventional antibodies. Aptamers are much smaller than antibodies and can easily be produced synthetically [38]. Furthermore, because of their oligonucleotide structure, aptamers can undergo multiple denaturation/regeneration cycles, whereas antibody-based biosensors are difficult to regenerate. Hypothetically, aptamers can be selected against any target analyte regardless of their antigenicity or toxicity [39, 40]. The simplicity of their chemical structure allows aptamers to be engineered that are capable of directly reporting analyte binding, thus obviating the need for the secondary labels typically required to detect antigen–antibody binding. Because of these advantages, many aptamer-based biosensors, sometimes called "aptasensors," have been developed [40]. A number of recent reviews have focused on the molecular recognition and sensing aspects of the aptamers [40–42], and these aspects will not be discussed in detail here. In this review, we focus on the strategies developed to transduce aptamer recognition events with an emphasis on optical and electrochemical methods.

Optical aptasensors

The first optical aptasensor based on fluorescently labeled aptamers was reported in 1996 [43]. Since then, a number of different optical aptasensors have been developed using a variety of fluorescence and colorimetric approaches. The design and characterization of these sensors have been discussed in previous reviews [44, 45], but fundamental developments will be discussed in this review for more understanding. One of the most widely used formats is based on aptamer beacons. Molecular beacons consist of a stem-loop-structured single-stranded oligonucleotide containing fluorophore and quencher moieties at either end [46]. When the beacon binds the target, the fluorophore and quencher are separated, resulting in a fluorescent signal [47, 48]. Yamamoto et al. [49] reported such an aptamer beacon for detection of TAT protein of human immunodeficiency virus (HIV). They developed an aptamer capable of detecting the presence of TAT protein at 100 nM concentration with high specificity. The same dequenching-based approach has also been applied without the use of stem-loop hairpin structures. In this implementation, a fluorophore-labeled aptamer is placed in a duplex structure with a complementary DNA sequence labeled with a quencher; the presence of a target forces the departure of the complementary strand from the aptamer, accompanied by an increase in fluorescence. Nutiu and Li [50] described a strategy for designing aptamer-based fluorescent reporters that

function by switching structures from the DNA/DNA duplex to the DNA/target complex. The duplex is formed between a fluorophore-labeled DNA aptamer and a small oligonucleotide modified with a quenching moiety (denoted QDNA).

In addition to dequenching, other fluorescence-based methods have been used as detection schemes. Yamana et al. [51] demonstrated that fluorophores specially selected to be sensitive to the local environment are also suitable for use in beacons. They made use of a bispyrene-labeled aptamer wherein the emission of the fluorophore shifts from that of the pyrene monomer to that of the excimer upon binding of the target.

More recent approaches have focused on improvements in both the design and the sophistication of aptamer beacons. Morse [52] reported an interesting aptamer-based tobramycin sensor. The aptamer design used resulted in improved affinity. Recent work in our laboratory [53] has focused on the design of aptamer beacons (Fig. 2A) for detection of inflammatory cytokines such as interferon gamma (IFN-γ). Variants of a DNA aptamer modified with biotin moieties and spacers were immobilized on avidin-coated surfaces and characterized by surface plasmon resonance (SPR). The SPR studies showed that immobilization of the aptamer via the 3′ end resulted in optimal binding with K_d of 3.44 nM. This aptamer variant was then used to construct a duplex structure by hybridizing a fluorophore-labeled aptamer with an antisense oligonucleotide strand carrying a quencher. SPR studies revealed that IFN-γ binding with an aptamer duplex occurred within 15 min of introduction of the analyte, suggesting rapid replacement of the quencher-carrying complementary strand by IFN-γ.

Another interesting optical aptasensor was constructed by Zhi et al. [54]. They used a colorimetric agent-caging hydrogel as a novel visual detection platform that relies on DNA base-pair recognition and aptamer–target interactions for simple and rapid target detection with the naked eye. As shown in Fig. 2B, they demonstrated the general design for a colorimetric visual detection platform based on an aptamer cross-linked hydrogel. Competitive binding of the target to the aptamer causes the reduction of cross-linking density and therefore induces gel dissolution. They used this simple system to detect less than 20 ng of cocaine with the naked eye within 10 min.

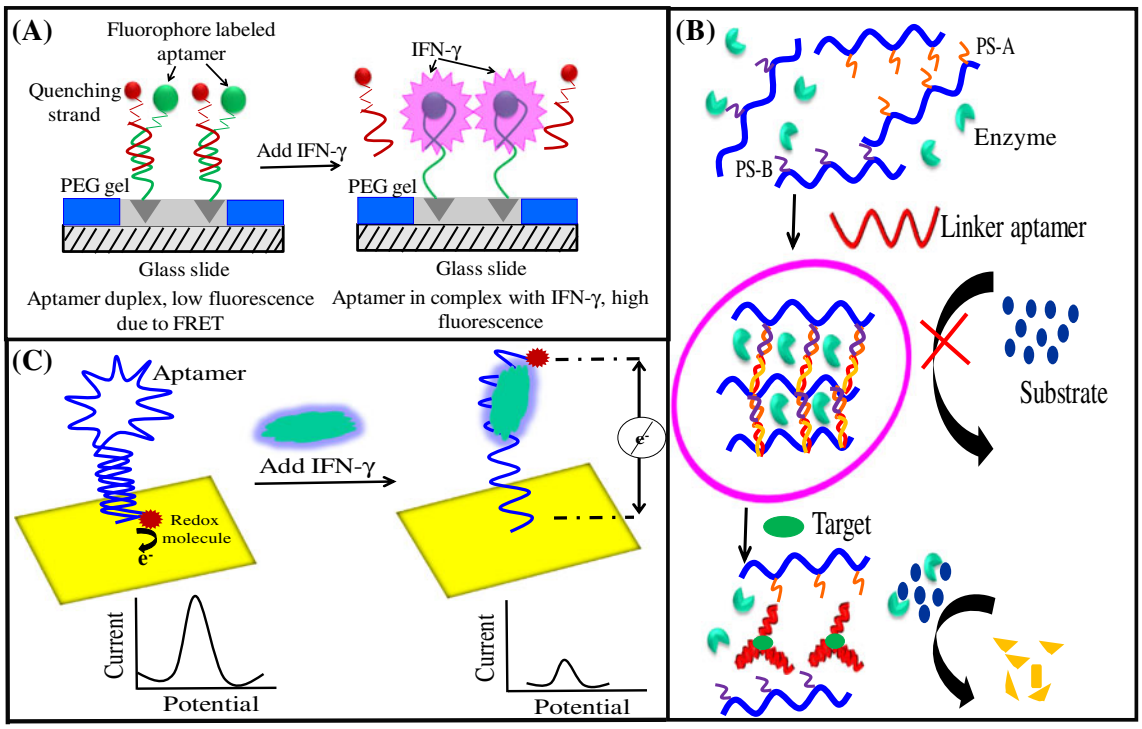

Fig. 2 **A** Aptamer beacons were immobilized inside microwells using avidin–biotin binding. A beacon molecule consisted of a fluorophore-labeled DNA aptamer and a quencher-labeled complementary strand. In duplex, fluorescence of an aptamer was quenched owing to the fluorescence resonance energy transfer (*FRET*) effect resulting from the proximity of the fluorophore-labeled aptamer to the acceptor-carrying complementary strand. Binding of interferon gamma (*IFN-γ*) disrupted the DNA duplex and resulted in a fluorescent signal. **B** Working principle of DNA cross-linked hydrogel for signal amplification and visual detection. **C** Aptamer-based electrochemical sensor for IFN-γ. The aptamer hairpin was thiolated at the 5′ end, allowing self-assembly on gold electrodes. The redox label was attached at the 3′ end of the hairpin and was close to the electrode surface. Upon addition of the IFN-γ aptamer, the hairpin changed confirmation and the redox label moved further away from the electrode, lowering the electron-transfer efficiency. *PEG* poly(ethylene glycol). (**A** From [53], **B** from [54], **C** from [68])

Electrochemical aptasensors

In comparison with the use of fluorescence, electrochemical transduction was implemented in aptamer-based biosensors considerably later, with the first report appearing in 2004 [55]. Although optical and electrochemical detection methods have a number of advantages and disadvantages, it may be argued that electrochemistry offers a less expensive means of reading the signal. If the electrochemical reporters and the electrolyte are chosen correctly, the electrical signal is likely to be stabler over time and may have less interferences compared with optical detection. Recent reviews have discussed fabrication of electrochemical aptasensors. Hianik and Wang [56] reviewed electrochemical aptasensors according to their transduction mode, including amperometric and impedimetric devices as well as a field effect transistor and a recently reported potentiometric aptasensor. Willner and Zayats [38] summarized recent accomplishments in developing electronic aptasensors, which include many of these methods as well as microgravimetric quartz crystal microbalance sensors. The review of Willner and Zayats further describes methods to develop amplified aptasensor devices and label-free aptasensors. In the current review, we discuss some of the recent work on electrochemical impedance spectroscopy (EIS)-based aptasensors.

A label-free aptamer-based sensor using EIS as a detection technique was reported by Xu et al. [57]. EIS is a technology typically used for studying biomolecular interactions [58]. Their approach was based on an aptamer for human IgE, containing a hairpin loop responsible for the target recognition, and three other nonspecific oligomers with some sequence changes, all immobilized on a gold electrode array via self-assembly and all of them containing a loop structure of the same size. The results indicated that only the targeted aptamer recognizes IgE, demonstrating the strong dependence of the affinity not only on the loop size but also on the sequence. Rodriguez et al. [59] reported another example of label-free impedance-based aptasensors. In this case, thiol-modified aptamers were self-assembled on a gold electrode and target binding was measured by an increase of electron transfer resistance in the presence of the $[Fe(CN)_6]^{3-/4-}$ redox couple. The increase in electron transfer resistance can be attributed to the repulsion between the negatively charged aptamer and redox couple. Similar protocols have also been developed for a number of other target analytes, including platelet-derived growth factor (PDGF) [60], interferon [61], and human IgE [62]. The reported detection limits ranged from 0.1 nM for thrombin to 100 fM for interferon.

In addition to EIS, binding-induced conformational switching has also been used in recent years in the development of a large number of electrochemical aptasensors. In particular, aptasensors based on "signal on," wherein the presence of the target increases the signal strength upon target binding, and "signal off," wherein the presence of the target reduces the signal strength upon target binding, have recently been studied [63, 64]. The Heeger and Plaxco groups have pioneered the use of these aptasensors. In their implementation an immobilized aptamer is either partially [65] or entirely [66] unfolded. Upon analyte binding, these aptamer probes fold and hold the redox labels closer to or farther from the electrode surface, respectively, thus increasing or decreasing the measured faradaic current. Different electrochemically active redox labels have been employed to relay the electrical signal resulting from aptamer–analyte binding. Of particular note is the work of Lubin and Plaxco [63], who modified electrodes with methylene blue (MB)-containing aptamers and demonstrated electrochemical detection (signal "off") of a range of analytes. Lai et al. [67] developed an aptasensor for the direct detection of PDGF in blood serum (signal "on"). Their approach employed alternating current voltammetry to monitor target-induced folding in an MB-modified, PDGF-binding aptamer. They detected the BB variant of PDGF at 1 nM directly in undiluted, unmodified blood serum and at 50 pM in serum diluted twofold with aqueous buffer. Our group described the development of an electrochemical DNA aptamer-based biosensor for detection of IFN-γ based on the "signal-off" sensing mechanism (Fig. 2c) [68]. Binding of IFN-γ caused the aptamer hairpin to unfold, pushing MB redox molecules away from the electrode and decreasing the electron-transfer efficiency. The change in redox current was quantified using square wave voltammetry and was highly sensitive to the IFN-γconcentration. The limit of detection for the optimized biosensor was 0.06 nM, with a linear response up to 10 nM.

A reagentless "signal-on" aptasensor for adenosine triphosphate (ATP) detection has also been described [69]. A ferrocene-labeled thiolated aptamer in its duplex form was self-assembled on a gold electrode surface. Upon binding of ATP, a stabilized rigid tertiary aptamer structure stabilizes and liberates the complementary DNA strand, which in turn brings the ferrocene moiety close to the electrode surface, thus generating an enhanced square wave voltammetry peak current.

Phages as biorecognition elements

Phages represent a promising new direction in the development of affinity biosensors. There are several reasons for using phages as biorecognition elements [70, 71]:

1. There are important analytes against which antibodies cannot be obtained easily [72].
2. Some applications, for example, environmental monitoring, may require extraction of samples from soil or groundwater that are soluble in organic solvents only. Such solvents may denature antibodies but not phages.

A phage, a threadlike virus, has a specific recognition peptide on its surface that binds to its host/target with high affinity and specificity [73]. Peptide-bearing phages can be selected from large phage libraries and can serve as receptors for detection of various bioanalytes. As opposed to synthesis of antibodies, which involves in vivo immunization of animals, phages can be easily synthesized by cheap generic fermentation processes. Besides this, they are more cost-effective, give high throughput, and have excellent stability at high temperatures (below 80 °C) and in harsh environmental conditions such as acidic or basic pH [74].

Phages can be immobilized via physical adsorption [75, 76], covalent binding [77, 78], affinity binding [79], and Langmuir–Blodgett deposition [80]. Cross-linking agents such as *N*-ethyl-*N*-(dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide [81] and glutaraldehyde [82] have been used for covalent immobilization of phages onto the desired sensor surfaces. The strong affinity between biotin and avidin/streptavidin has also been widely used by researchers for development of high-performance phage-based biosensors [79]. It has been found that sensors based on affinity (e.g., avidin–biotin) and covalent attachments of phages perform better than sensors based on physical adsorption [79, 83]. The sensing event (phage–target interaction) has been frequently investigated using several electroanalytical, optical, and mass-sensitive tools. Some of the recent work is discussed below

Electrochemical phage-based biosensors

Jia et al. [82] prepared a phage-modified light-addressable potentiometric sensor for label-free detection of cancer cells. The sensor surface was prepared by covalent immobilization of phage probes onto a silane-modified Si_3N_4 surface via glutaraldehyde. This sensor could detect human phosphatase of regenerating liver 3 in the concentration range from 0.04 to 400 nM, and mammary adenocarcinoma cells in concentrations from 0 to 105 mL. Other electroanalytical techniques such as EIS have also been employed for a specific label-free biosensor using phages covalently immobilized onto the sensor surface [81, 84]. Shabani et al. [81] showed detection of *Escherichia coli* by a sensor surface containing covalently immobilized T4 phages on screen-printed carbon electrode microarrays. An increase in electrolyte resistance and a decrease in charge-transfer resistance were observed owing to binding of bacteria of increasing concentration. This effect is contrary to the usually observed increase in charge-transfer resistance with increasing concentration of intact bacteria at the surface. Shabani et al. attributed this to onset of lysis (starts after 20 min of incubation), which involves the breakup of the bacterial cell, owing to which a large amount of ionic material is released and causes increased conductivity. Shabani et al. found a detection limit of 10^4 cfu mL^{-1}. Recently, Mejri et al. [85] utilized the effect of bacteria binding before and after the start of the lysis process and proposed a dual-signal-based detection of bacteria. The specific capture generates an initial increase in impedance, followed by an impedance decrease due to phage-induced lysis. Mejri et al. found a limit of detection of 10^4 cfu mL^{-1} with minimal interference from nontarget lactobacillus.

Optical phage-based biosensors

Balasubramanian et al. [86] reported SPR-based label-free detection of *Staphylococcus aureus* using lytic phages that showed a detection limit of 10^4 cfu mL^{-1}. However, most of the SPR-based phage biosensors [87, 88] have utilized physical adsorption for immobilization of phages on the sensor surface. This method takes a long time for complete attachment of the biorecognition elements. Also, leaching of phages from the surface does not allow complete regeneration of the sensor. In this context, covalent immobilization has aroused much interest as it may provide improved attachment of the phages to the sensor surface in less time and exhibits a wide dynamic range. Zhu et al. [77] developed a sensitive and inexpensive optofluidic ring resonator biosensor for biomolecule detection. They observed that less time is required for complete immobilization by the covalent binding method as compared with physical adsorption. They attributed this to the high surface-to-volume ratio in the circular optofluidic ring resonator's microfluidics channel, which promotes faster phage diffusion to the sensing surface from the bulk solution.

Mass-sensing-based phage-based biosensors

A piezoelectric biosensor based on a quartz crystal microbalance for detection of β-galactosidase from *E. coli* was developed by Nanduri et al. [89]. The sensor had a detection limit of a few nanomoles per liter over the range from 0.003 to 210 nM. Horikawa et al. [78] fabricated magnetoelastic phage biosensors based on differently functionalized gold surfaces. They found that surface functionalization has a large effect on the surface phage coverage. The appropriate surface can be utilized to pattern the phage probe layer to enhance the detection capabilities of magnetoelastic biosensors. Wan et al. [90] demonstrated detection of *Bacillus anthracis* spores using physical adsorption of phages on a magnetoelastic sensor surface.

Some of the recent biosensors based on aptamers, antibodies, and phages are listed in Table 1. Despite their high affinity and specificity, antibodies have limitations as

Table 1 Comparison of some of recent aptamer-, antibody-, and phage-based biosensors reported in the literature

Analyte	Biorecognition element	Transduction method	Linear range and detection limit	Reference
Tumor cells	Anti-human antibody	Cyclic voltammetry	DL: 5,714 cells mL^{-1}	[91]
	Mercapto-functionalized aptamer	Electrochemiluminescence	LR: 100–2,000 cells mL^{-1} DL: 78 cells mL^{-1}	[92]
	DNA aptamer	Micropillar-based microfluidic device	DL: 10 cells mL^{-1}	[93]
IFN-γ from T cells	Anti-CD4 antibody	Surface plasmon resonance	DL: 0.1 μg mL^{-1} (50 nM)	[94]
	Thiolated hairpin IFN-γ-binding aptamer	Square wave voltammetry	DL: 60 pM, IFN-γ production rate: 0.0079 pg $cell^{-1}$ h^{-1}	[95]
	IFN-γ aptamer beacon	Fluorescence resonance energy transfer	LR: 5-100 nM DL:5 nM	[53]
CRP	Anti-CRP antibody	Surface plasmon resonance	LR: 2–5 μg mL^{-1}	[96]
	RNA aptamer	Surface plasmon resonance	DL: 5 ng mL^{-1}	[97]
Mycobacterium tuberculosis in clinical samples	Fluorescein-labeled antibodies	Fluorescence-based	DL: 200 cfu mL^{-1}	[98]
	Phage D29	Quartz crystal microbalance	DL: 100 cfu mL^{-1}	[99]
Escherichia coli	Anti-*E. coli* antibody	Electrochemical impedance spectroscopy	DL: 4.36×10^{5} cfu mL^{-1}	[100]
	Lytic T4 phage	Cyclic voltammetry and electrochemical impedance spectroscopy	DL: 10^{3} cfu mL^{-1}	[101]

IFN interferon, *CRP* C-reactive protein, *LR* linear range, *DL* detection limit

biosensor probes that affect their applicability in the field. One of the major disadvantages of antibodies is their relative instability to environmental fluctuations, especially high temperature, compared with phage probes and aptamers. Phages and aptamers are attractive alternatives to antibodies in the fabrication of robust biosensing devices.

Applications of biosensors

Biosensors have numerous applications ranging from environmental sensing and biowarfare agent detection to tissue engineering. Because of our research interests, we will focus on recent development of biosensors for clinical diagnostics and cell analysis [102, 103]. Of the three types of biorecognition elements discussed in this review, enzyme-based biosensors are probably the most popular owing to their use in diabetes management. Aptamer-based sensing technologies are emerging and gaining ground and the number of applications is increasing [104]. Phage-based biosensors are just emerging but hold considerable promise in light of growing problems associated with antibiotic-resistant bacteria and food safety [105].

Biosensors and complex sample analysis

The ability to detect pathogenic molecules in complex samples such as blood and other bodily fluids is critical to clinical diagnosis and disease treatment. Blood contains numerous disease markers and is one the most commonly used bodily fluids in clinical diagnosis. Arguably the biggest success story to date in the field of biosensors is the development of glucose meters for blood analysis and diabetes management. An interesting new approach for glucose analysis in blood was reported recently by Xian et al. [106], who described a glucose biosensor based on a composite of AuNPs and conductive polyaniline nanofibers. This biosensor benefited from a dramatic increase in surface area, leading to a sensitive and selective glucose biosensor. The response of this biosensor was linear in the range from 1.0×10^{-6} to 8.0×10^{-4} M, with a detection limit of 5.0×10^{-7} M. Guo et al. [107] described an enzymatic glucose biosensor based on thin-walled graphitic nanocages with a well-developed graphitic structure and distinguished hollow interiors. The unique physicochemical properties of the graphitic nanocages, including well-developed graphitic structure, high surface area, and pronounced mesoporosity, resulted in a biosensor specific for and sensitive to glucose. Wu et al. [108] reported the attachment of GOx onto phosphorescent manganese-doped ZnS quantum dots via *N*-ethyl-*N*-(dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide chemistry for glucose sensing.. This sensor had a detection limit of 3 μM with two linear ranges: from 10 μM to 0.1 mM and from 0.1 to 1 mM. This biosensor was utilized to determine the glucose levels in real serum samples without the need for any complicated sample pretreatments.

In addition to enzyme-based detection of glucose, aptamers are being used to target analytes in complex media such as blood. Zuo et al. [109] reported a target-responsive

electrochemical aptamer switch for reagentless detection of standard and cellular ATP with high sensitivity and selectivity. The approach required neither exogenous reagents nor labeling of the target. The sensor functioned well when challenged with cell lysate, a complex, contaminant-ridden medium. In another study, Huang and Liu [110] combined flow cytometry with aptamer-functionalized magnetic microparticles for detection of adenosine in serum. In this implementation, the flow cytometer serves to isolate the magnetic microparticle sensors from the serum and detect the presence of the fluorescent signal. The antibiotic gentamicin has also been detected in serum using an RNA aptamer [111]. Stability issues necessitated the modification of the aptamer against resistance breakdown by nucleases. These modifications resulted in an unacceptable decrease in sensitivity and the researchers were eventually forced to filter the serum to achieve acceptable stability and sensitivity.

Recent advancements to improve the sensitivity of aptamers in small-molecule detection have seen a shift back toward the sandwich assay format originally used with antibody-based assays such as the enzyme-linked immunosorbent assay. The Plaxco group [112] recently reported detection of ATP from cell lysate and cocaine from serum using such an approach. By separating the redox reporter from the target-binding strand, they were able to increase the signal gain sixfold. In another sandwich-type assay study, cocaine was detected from banknotes left in simulated real-world environments with large amounts of contaminants [113]. This assay utilized aptamers and electrochemiluminescence. In this instance as well, the sandwich-type assay provided a higher signal and better sensitivity than a conventional aptamer-based electrochemiluminescence assay. The downside of the sandwich approach, however, is that the assayrequires the redox strand be present as a reagent.

Another advancement of note is the use of small molecules not as targets but as binding agents in biosensors. The Plaxco group [114] demonstrated the use of a nucleotide-based biosensor with a biotin moiety conjugated to one strand. Upon binding streptavidin, the electrical signal changes as it would in a typical aptamer biosensor. The sensor was insensitive to contaminants in a number of complex media, including serum, soil, and beer. Their work portends the possibility of adapting biosensor technologies to probe protein–small molecule interactions.

A number of phage-based biosensors reported recently were used for detection in real samples. Lakshmanan et al. [76] detected *Salmonella typhimurium* in fat-free milk using a magnetoelastic sensor immobilized with phages. Jia et al. [82] tested a phage-modified light-addressable potentiometric sensor for analysis of human phosphatase of regenerating liver 3 in blood samples.

Biosensors for cellular analysis

Biosensors for studying living systems, i.e., cells, tissue, organs, and organisms, have received a great deal of attention in the past few decades. Cell-based biosensors can monitor physiological changes in cells exposed to pathogens, pollutants, biomolecules, and drugs [115].

To obtain the information hidden inside the cell, some very refined procedures and sensing techniques are required that do not disrupt the cell membrane and maintain cell viability for a long time. Microfabrication and microfluidic systems show great potential is this area owing to their unique properties, such as controllable transport, immobilization, and facile manipulation of biological molecules and cells. With use of these techniques, various systems have been developed for analysis of intracellular parameters and to detect the presence of cell metabolites, even at the single-cell level [116, 117]. Electrochemical measurements using microelectrodes are powerful tools for spatiotemporal monitoring of electroactive chemicals, particularly when integrated into microfluidic systems. This allows many sample manipulations to be integrated into the microsystem and thus monitoring can be performed in a simple and automated way [118]. The electrochemical methods could serve as an excellent transducing system for invasive but nondestructive cell analysis. Moreover, in combination with optical probes and imaging techniques, electroanalytical methods show great potential for the development of multianalyte systems to monitor cellular dynamics. This section exemplifies biosensors for detection of various cell-secreted products.

Biosensors for cell-secreted products

The monitoring of cell-secreted products is of wide interest in the area of biomedical and health care as this process is an essential physiological function [119]. A variety of chemicals, including signal molecules such as hormones, neurotransmitters [120], trophic factors, and metabolic products, are released from cells upon stimulation. The responses of cells to physical or chemical cues have typically been measured in microfluidic devices [121] via optical [122] or electrochemical [123] means.

Detecting cellular release of neurotransmitters

Measurements of catecholamine and glutamate exocytosis have received tremendous interest in the past few decades. Disturbance of their controlled secretion may lead to many neurological disorders, such as Parkinson's disease [124, 125] and Alzheimer's disease [126, 127]. Glutamate plays

an important role in long-term changes in synaptic efficacy, forming the basis for learning and memory. Castillo et al. [128] described a glutamate sensor based on bienzyme (glutamate oxidase and HRP) redox hydrogel capable of detecting the release of this excitatory neurotransmitter from adherently growing cells upon stimulation. The sensor was able to operate at low working potential, which obviated the possibility of interference by easily oxidizable compounds present in complex biological samples. The sensor had a low detection limit of 0.5 μM glutamate, a response time of about 35 s, and a linear range of up to 60 μM. Another amperometric sensor based on bienzymes for direct monitoring of L-glutamate in a flow injection system was reported by Belay et al. [129]. The bienzyme electrodes were constructed by coating solid graphite rods with a premixed solution containing glutamate oxidase and HRP cross-linked with a redox polymer formed from poly(1-vinylimidazole). The sensor detected in the range from 0.3 and 250 μM glutamate concentration, with a sensitivity of 88.36 ±0.14 μA mM^{-1} cm^{-2}, a detection limit of 0.3 μM, and response time of less than 10 s.

Efforts were made toward electrochemical measurement of release of catecholamines from pheochromocytoma (PC12) cells [130]. Microfluidics was used to transport and trap a single cell while the stimulants were introduced from the microchannel and a carbon fiber microelectrode was positioned over the cell for amperometric measurements. Li et al. [131] used collagen coating in the microchannels to attach PC12 cells. They were able to measure exocytosis from a large population of cells with a micro molded carbon ink electrode. Huang et al. [132] developed a polydimethylsiloxane/glass microfluidic system for transport of a single PC12 cell to a microvial. Shi et al. [133] developed a novel microfluidic electrochemical sensor with a CNT-modified indium tin oxide microelectrode for release of dopamine from a single living rat PC12 cell. The sensitivity of the electrochemical sensor after CNT modification was more than that of the unmodified electrode by 2.5 to three orders of magnitude. Lin et al. [134] fabricated a microsensor by electrochemically depositing a film containing overoxidized polypyrrole and multiwalled CNTs onto a carbon fiber microelectrode. The sensor was utilized in in vivo microdialysis with electrochemical microsensing determination of dopamine in striatum of freely moving rats. The sensor exhibited linear detection in the concentration range from 5.0 nM to 10 μM, and the detection limit (at a signal-to-noise ratio of 3) was 0.5 nM.

Biosensors have been developed for monitoring D-serine, which is the predominant D-amino acid in the mammalian central nervous system and has been recently related to several neurological and psychiatric diseases. Pernot et al. [135] reported a microbiosensor based on cylindrical platinum microelectrodes, covered with a membrane of poly(*m*-phenylenediamine) and a layer of immobilized D-amino acid oxidase from the yeast *Rhodotorula gracilis*. By detecting the hydrogen peroxide produced by enzymatic degradation of D-serine, this microbiosensor exhibited a detection limit of 16 nM and a mean response time of 2 s.

Detection of reactive oxygen species secreted from cells

Inflammatory processes play a crucial role in a number of diseases, including diabetes [136], cancer [137], and tissue fibrosis [138]. Molecules secreted by immune cells during inflammation are critical in combating pathogens. Macrophages, immune cells residing in the tissue, are first to respond to invading pathogens. These cells produce inflammatory markers, including cytokines and reactive oxygen species (ROS). ROS include superoxide anion ($O_2^{\cdot-}$) hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$). H_2O_2 is one of the major indicators of oxidative stress and the stablest of ROS compounds. Our group [139] has developed electrochemical biosensors based on enzyme-containing hydrogel for detection of H_2O_2 secreted from macrophages. Enzyme-containing hydrogel was coated onto micropatterned gold electrodes and the hydrogel was cross-linked with UV exposure. The macrophages were seeded into ply (ethylene glycol) microwells and stimulated with phorbol 12-myristate 13-acetate. Polydimethylsiloxane was put on the micropatterned slides to make the electrochemical setup for amperometric measurements. Fluorescence microscopy was used to sense the H_2O_2 released from the immune cells [140]. The production of H_2O_2 after mitogenic stimulation of macrophages resulted in the appearance of fluorescence in the HRP-containing hydrogel microstructures, with the fluorescence intensity being a strong function of analyte concentration. Amatore et al. [141] studied release of ROS and reactive nitrogen species by electrochemistry within a microfluidic device. Macrophages were cultured in a detection chamber containing a three-electrode system and were stimulated by the microinjection of a calcium ionophore. Cheah et al. [142] developed a microfluidic device for heart tissue perfusion with real-time electrochemical monitoring of ROS release. Sun et al. [143] reported determination of ROS in a single erythrocyte by using a simple crossed-channel glass chip with integrated operational functions such as docking, lysing, and capillary electrophoretic separation with laser-induced-fluorescence detection.

Detection of other cell-secreted products

An optical approach for monitoring allergic response was demonstrated by Matsubara et al. [144]. A rat basophilic

leukemia cell line (RBL-2 H3), a tumor analog of rat mucosal mast cells, was used as a model to observe the allergic response upon antigenic stimulus. When exocytosis events occurred, the microfluidic system detected the fluorescent signal of quinacrine, which was released from RBL-2 H3 cells. The Kennedy group [145, 146] has developed microfluidic devices for high-throughput, automated, and online monitoring of insulin secretion from individual islets in parallel. This chip consists of 15 channel networks each capable of superfusing a single islet and mixing superfusate from each islet online with fluorescein isothiocyanate labeled insulin and anti-insulin antibody for a competitive immunoassay. The resulting continuous reaction streams were periodically injected onto parallel electrophoresis channels, where the mixtures were separated. The chip was used to demonstrate that free fatty acid induced lipotoxicity in islets eliminates pulsatile insulin secretion. Our group [147–149] has developed biosensors for the detection of molecules secreted or taken up by cells. We also developed antibody- or aptamer-based biosensors for detection of inflammatory cytokines released by activated immune cells [68, 94, 149, 150]. In addition to these optical and electrochemical methods, the application of SPR-based techniques could contribute extensively to cellular analysis. We also described a strategy combining antibody-based affinity cell separation and SPR for capturing human CD4 T cells and for label-free detection of cell-secreted IFN-γ.

Recent advancements in cell-secretion analysis have greatly improved the throughput of these biosensors. Work by the Heath group [151] (Fig. 3A) demonstrated a microfluidic platform designed for highly multiplexed (more than ten proteins), reliable, sample-efficient (approximately 1×10^4 cells), and quantitative measurements of secreted proteins from single cells. The platform was tested by assessment of multiple inflammatory cytokines from lipopolysaccharide-stimulated human macrophages before being applied to quantification of T-cell polyfunctional diversity from tumor antigen-specific cytotoxic T lymphocytes responding to a tumor and compared against the response of cells from healthy donor controls. Their results showed a large degree of heterogeneity in function between cells with similar surface markers, highlighting the importance of secretion analysis. In another study, Love et al. [152, 153] presented a soft lithographic method based on intaglio printing to generate microarrays comprising the secreted products of single cells. These engraved arrays enable a rapid (less than 12 h) and high-throughput (100,000 individual cells) system for identification, recovery, and clonal expansion of cells producing antigen-specific antibodies. This method shown in Fig. 3B can be adapted, in principle, to detect any secreted product in such a multiplexed manner.

Biosensors at the point of care

Bringing diagnostics to the point of care (POC) could allow some form of preliminary self-screening or sorting and even basic treatment by front-line nursing staff, therefore reducing the burden on practitioners and hospitals and allowing specialist care to be dedicated to those most in need [154, 155]. Microfluidics has been reviewed recently in this context [156], and applications in this field are increasing rapidly. Miniaturizing biosensor inside microfluidic devices has grown tremendously and rapidly, sustained by the promise it offers to revolutionize conventional laboratory handling, processing, and analytical techniques. One particularly interesting microfluidic device for POC applications is the integrated blood barcode chip [157]. This device has been developed to address the need for microchips that integrate on-chip plasma separations from microliter quantities of whole blood with rapid in situ measurements of multiple plasma proteins. The versatility of this barcode immunoassay has been demonstrated by detecting human chorionic gonadotropin from human serum over a 10^5 concentration range and by stratifying 22 cancer patients via multiple measurements of a dozen blood protein biomarkers for each patient.

Another important area where microfluidic-based biosensors is having an impact is the monitoring of HIV and acquired immune deficiency syndrome (AIDS). Advances in the field have enabled rapid, inexpensive, and reliable approaches for detecting and quantifying the HIV and AIDS status in patients. One way to evaluate the AIDS status in HIV-infected patients is to measure the absolute number of $CD4^+$ T lymphocytes in blood. Handheld, reliable, and low-cost CD4 counting devices for use in resource-scarce regions of the world are needed. As a result, considerable effort has been directed toward the development of miniature devices for simple and inexpensive leukocyte analysis [158–162]. The Toner group [163] has developed a simple CD4-counting microfluidic device that uses cell affinity chromatography operated under differential shear flow to specifically isolate $CD4^+$ T lymphocytes with high efficiency directly from 10 μL of unprocessed, unlabeled whole blood. CD4 counts are obtained under an optical microscope in a rapid, simple, and label-free fashion. Our group [164] has also developed methods for capturing and counting T cells using microfluidics in combination with printed microarrays of antibody spots. These methods capture cells with high purity (more than 94 %) and allow the quantitation of subset populations purely on the basis of the capture location. Our results were shown to be in good agreement with those from flow cytometry.

Building on these cell capture studies, our laboratory has developed methods for using antibody microarrays inside microfluidic devices for immunophenotyping of leukocytes

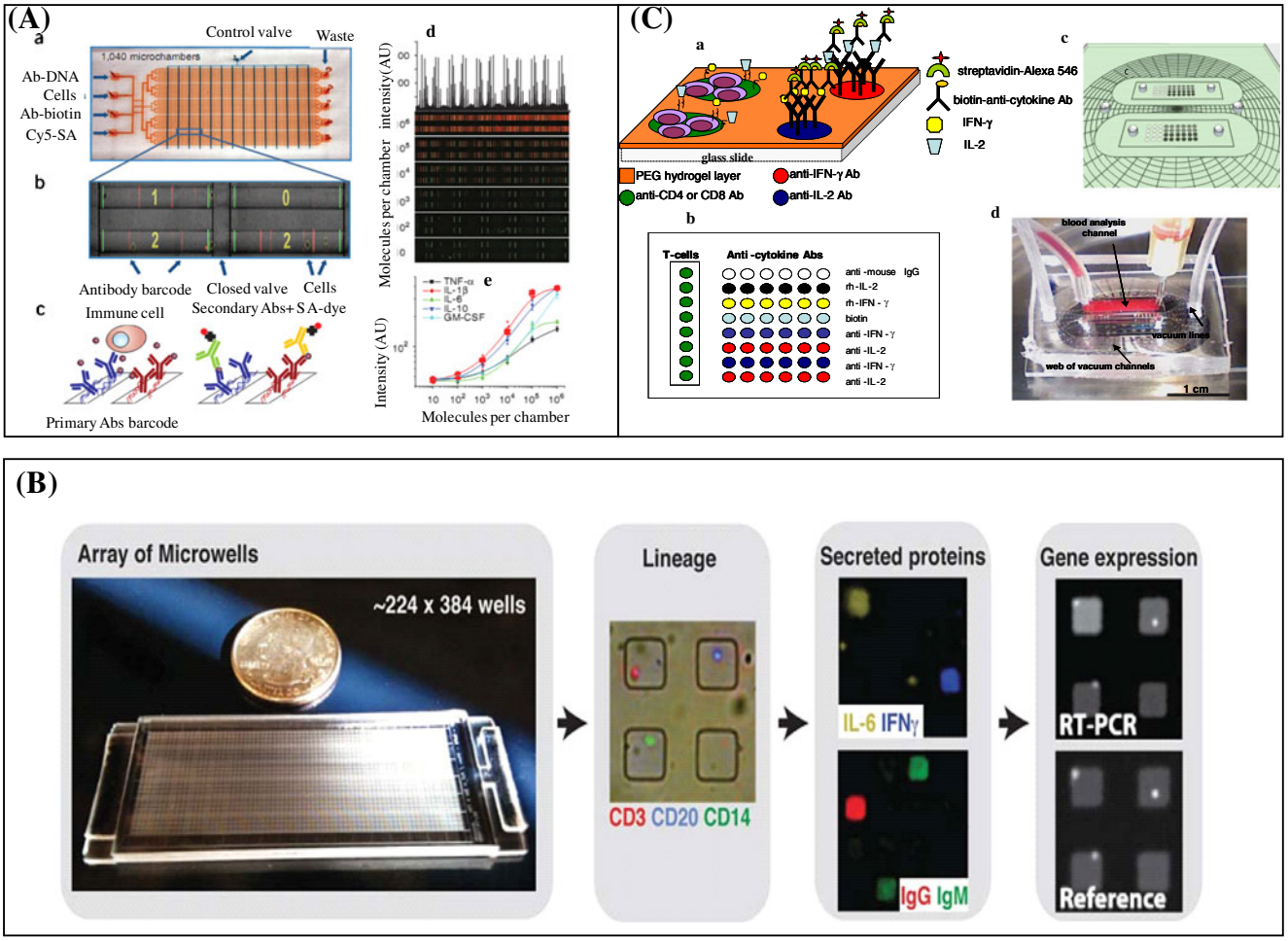

Fig. 3 **A** Design of the microfluidic device for single-cell analysis. *a* image of a microdevice in which flow channels are shown in *red* and the control channels are shown in *blue*, *b* an optical micrograph showing cells loaded and isolated within the microchambers, *c* the multiplex primary antibody barcode array used for capture of secreted proteins from single cells and then developed for the detection of those proteins, *d* scanned fluorescent images used for the antibody barcode calibration measurements using spiked recombinant proteins, *e* recombinant protein calibration curves for different cytokine measurements. **B** Example of one type of process for integrated single-cell analysis using modular single-cell bioanalytical operations. The elastomeric array of microwells (*left*) contains more than 85,000 individual wells to isolate cells deposited from a suspension. This platform allows in-well imaging to enumerate the number and types of cells (*lineage*), functional assays to detect secreted proteins by microengraving (*secreted proteins*), and gene expression by gene-specific reverse transcription and amplification (*gene expression*). These operations can be reconfigured to monitor dynamics or increase multiplexing of analysis. **C** *a* the conceptual design of microarrays for detection of T-cell-secreted cytokines. Printing of cell and cytokine-specific antibody spots side-by-side allowed T cells to be captured next to cytokine-sensing regions. T-cell-secreted cytokines were detected on the adjacent anti-cytokine antibody spots. *b* a map of the 8×20 microarray for capturing T cells and detecting T-cell-secreted cytokines. *c* design of a microfluidic platform employed for integration with antibody microarrays. *D* an image of a polydimethylsiloxane microdevice employed for T-cell capture and cytokine detection experiments with one reaction chamber filled with unlysed whole blood. *Ab* antibody. (**A** From [151], **B** from [153], **C** from [147])

from whole blood [147, 148]. We have successfully employed this approach to capture pure CD4 and CD8 T cells from blood and to monitor cytokine release from these cells, as shown in Fig. 3C.

The introduction of aptamers on a microchip has been expected to bring several advantages to POC applications, such as reduced reagent and sample consumption, simplicity, automated processing, faster separation, high throughput, and portability. The Tan group [130] immobilized aptamers in a microfluidic channel to capture rare cells to achieve a rapid assay without any pretreatment. Their device demonstrated both outstanding enrichment purity (97 %) and over 80 % capture efficiency. They were able to further extend the utility of their microfluidic device to simultaneously sort, enrich, and then detect multiple types of cancer cells. The Soper group [165] has also utilized an aptamer-immobilized microfluidic device to selectively capture low-abundance cancer cells. Such a combination of sensitive technology and selective aptamer binding resulted in a 90 % recovery rate of rare circulating prostate tumor cells

from a peripheral blood matrix. As another interesting development in aptamer-based microfluidics, Swensen et al. [166] introduced a microfluidic, electrochemical, aptamer-based sensor chip by integrating target-specific DNA. The system was applied to achieve continuous, real-time monitoring of cocaine in blood serum at the physiologically relevant concentration and with physiologically relevant time resolution.

Apart from aptamers, phage-based systems have recently emerged as promising tools for POC testing [155, 167]. The phage probes provide high throughput, adaptability to thet esting environment, and are cheaper than antibodies. Jia et al. [82] reported a novel phage-modified light-addressable potentiometric sensor system for cancer cell monitoring. In recent work, Mi et al. [99] fabricated a multichannel quartz crystal microbalance based biosensor for detection of *Mycobacterium tuberculosis* in clinical samples using phage D29, which infects *M. tuberculosis*. The sensor had a detection limit of 100 cfu mL^{-1}. Several other cell-specific peptide ligands identified from phage display libraries have shown binding with different tumor types [155].

Infectious agents also have an indirect effect on human health via agricultural and other related commodities [168]. Recent biological terrorism threats and outbreaks of microbial pathogens clearly emphasize the need for biosensors that can quickly and accurately identify infectious agents. Phages are novel innovative affinity-based recognition elements that are becoming increasingly important for food and environmental sensors because of their exceptional characteristics, such as their high affinity and specificity for their targets, their fast, cheap and animal-friendly production, their stability, and their high resistance against environmental stress. Phage probes are more amenable than antibodies to manipulation at the molecular level to improve their interaction with the analyte. A recent review by Dorst et al. [168] has discussed application of a phage biosensor for food and environmental monitoring.

Phage probes have also been used for detection of food- and water-borne pathogens in real samples such as water and fat-free milk [169]. Edgar et al. [170] used a biotin-tagged lytic T7 phage to form quantum dot complexes for detection of *E. coli* in river water. Thus, application of these biorecognition elements can lead to the fabrication of various POC devices.

Conclusions

This articles has presented an overview of recent advances in the development and application of affinity and enzyme-based biosensors. Some of recent applications of these biosensors in clinical sample testing and cell analysis have been highlighted. Moving forward, we envision biosensors increasing their foothold in the areas of cell analysis and clinical diagnostics. We are particularly excited about new sensing technologies allowing continuous monitoring of cell function in vitro and in vivo with high spatiotemporal resolution. We predict cell function analysis (the types and the levels of molecules produced by cells) will become more widespread in disease diagnosis and foresee novel biosensors playing an important role in this trend.

At the beginning of this article, we underscored the differences between enzyme-based and affinity biosensors and mentioned that the latter sensors cannot be regenerated and may not be used for long-term analyte monitoring. This statement holds true for antibody-based affinity biosensors, but the outlook is quite different for aptamer-based sensors. Because aptamers are chemically stable and may be easily regenerated by denaturation of the target analyte, we envision aptamer-based biosensors being used for continuous monitoring. Unlike the natural turnover of enzyme-based biosensors, aptasensors will be regenerated chemically and will likely be coupled with microfluidics to automate regeneration process.

In addition, the field of phage-based biosensors is likely to grow dynamically in the immediate future. This growth will be fueled by the increasing prevalence of hospital-acquired infection and antibiotic-resistant bacteria. Current technologies for bacterial detection are inexpensive but slow (based on cell growth) or rapid but expensive (PCR-based). Phages evolved to detect and invade bacteria and therefore provide a natural biorecognition element for bacterial detection. Because phages multiply/replicate within bacterial hosts, there is also a natural amplification effect to phage-based detection of bacteria. Finally, we envision that continued innovation in biosensors will come not only from the traditional biosensing disciplines of biorecognition engineering and transducer development but also from nanotechnology and microfluidics.

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