Electrical Chips for Biological Point-of-Care Detection

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Abstract

As the future of health care diagnostics moves toward more portable and personalized techniques, there is immense potential to harness the power of electrical signals for biological sensing and diagnostic applications at the point of care. Electrical biochips can be used to both manipulate and sense biological entities, as they can have several inherent advantages, including on-chip sample preparation, label-free detection, reduced cost and complexity, decreased sample volumes, increased portability, and large-scale multiplexing. The advantages of fully integrated electrical biochip platforms are particularly attractive for point-of-care systems. This review summarizes these electrical lab-on-a-chip technologies and highlights opportunities to accelerate the transition from academic publications to commercial success.

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1. INTRODUCTION

The field of diagnostics is a cornerstone of modern health care throughout both the developed and the developing world. As the need for simple and rapid medical care expands in the next few decades, it will become increasingly important to develop highly sensitive, specific, and portable methods for detection of relevant biological entities. Such biosensor schemes are critical not only to garner vital patient information in a timely fashion but also to accelerate progress toward a better understanding of the complex biological pathways that govern disease. Electrical biosensors offer a simple and inexpensive means to elucidate biological pathways, diagnose diseases, and save lives—particularly for point-of-care (POC) applications, where portability and cost are critical concerns. This review provides an introduction to basic concepts behind POC electrical biosensing and presents a critical overview of active research focused on emerging technologies to improve electrical sample preparation and biological detection. The major electrical biosensing strategies, including impedimetric, potentiometric, and amperometric detection, are evaluated and discussed. We intend to provide a set of critical guidelines that will enable a reader to select the optimal electrical biochip technology for a specific POC application.

1.1. Building an Integrated Point-of-Care System

A true POC device ideally inputs a raw sample from a patient and outputs useful information. To do so, several distinct components are necessary (**Figure 1**). A sample loading module is crucial to introduce the raw sample to the device. Sample preparation, in which lab steps such as manual centrifuging, pipetting, and mixing are implemented on chip using filters, microfluidic sorting, and concentration techniques, is a critical part of any POC device to transform the raw sample into a format that is compatible with sensor components. The resulting prepared fluid then interacts with the biological interface of the POC device, which takes the form of a biorecognition element,

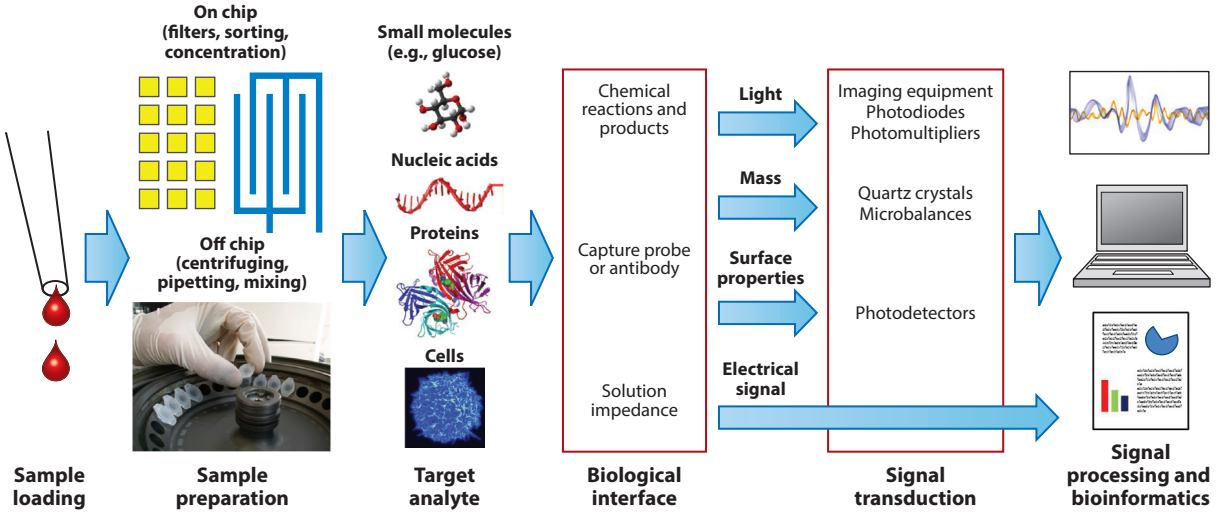


Figure 1

Main components of a point-of-care (POC) device. Electrical biosensors offer inherently attractive advantages, particularly for the sample preparation and signal transduction modules of POC devices.

membrane, or solution that is sensitive to the specific target analyte. This event induces a signal, which can be light, mass changes, surface property changes, or electrical signals. For quantitative measurements, this signal is then converted into an electrical output by the signal transduction component. Finally, for many applications with high volumes of data, a signal processing or bioinformatics step is needed to filter out the most relevant information.

Electrical biochips offer several inherently attractive means of improving the sample preparation and signal transduction components of a POC device. Several electrical techniques can be used on chip for sample preparation, including cell lysing, concentration, and flowing and mixing techniques. The use of electrical sensors can minimize or completely eliminate the need for a signal transduction component in a POC device, as the sensor itself produces an electrical signal that does not need to be converted. This direct measurement of an inherent property of the target analyte can fundamentally simplify the detection process, both by removing the need for optical readout equipment and by eliminating extra assay steps needed to transform the presence of the target analyte into a measurable signal. Both of these advantages directly translate into reduced complexity, increased portability, and decreased cost.

1.2. Charge: The Measurable in Electrical Biosensors

For optical biosensors, the output is almost always light intensity from a secondary reagent such as a fluorescent dye or a reaction by-product. The analogue in electrical biosensors is a biomolecule's charge, which is a primary component of a biomolecule. An electrical biochip is any technology that uses either (*a*) external applied electric fields to manipulate or sense biological molecules on the basis of the intrinsic electric field or charge of the biological molecules themselves or (*b*) a by-product to influence output characteristics on a chip-based platform. Eliminating the need for optical readout machinery can translate to important advantages for POC sensors. To fully benefit from these advantages and to engineer an electrical biosensor, one must understand the basis of a biomolecule's charge, how it changes under different conditions, and how it can be measured.

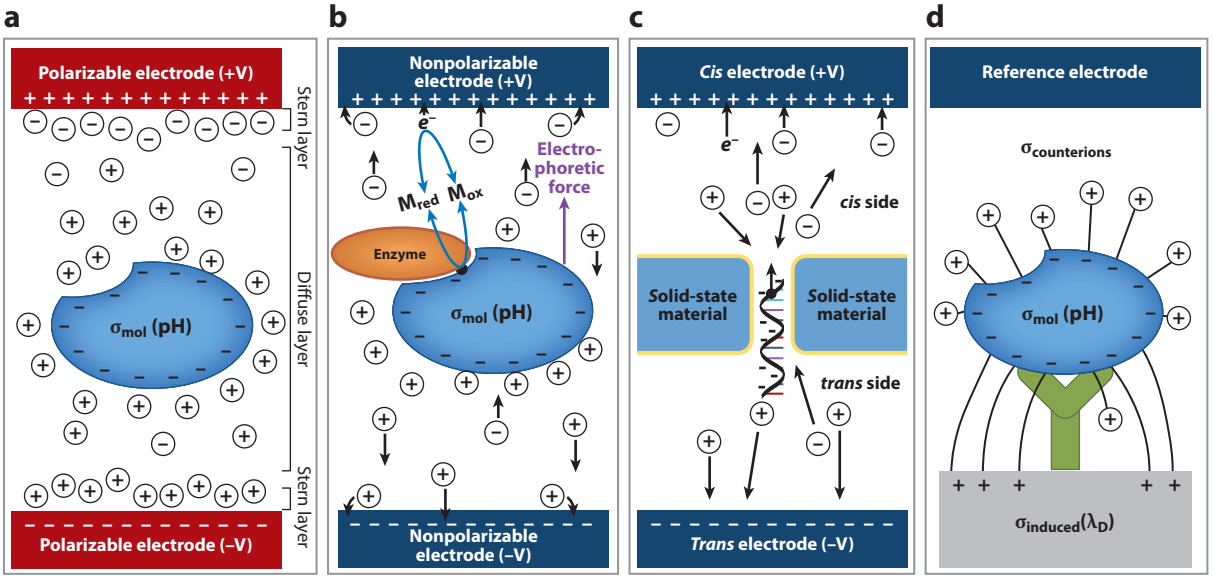


Figure 2

Electrode use in biosensors. (*a*) Polarizable electrodes. These electrodes do not allow ion exchange with the medium. At equilibrium, no current flows in this system, because the electrodes are shielded by counterions in the Stern layer. (*b*) Nonpolarizable electrodes. These electrodes allow ion exchange between the electrode and the solution, resulting in faradaic current flow and an electric field between the electrodes. Modulations in the faradaic current due to redox reactions at the electrode surface are measured by amperometric biosensors. The electric field can also be used to apply electrophoretic forces to charged molecules in solution. (*c*) Nanopore sensors. A nanoscale gap separates two chambers, a *cis* chamber and a *trans* chamber. Voltages applied to nonpolarizable electrodes in these chambers cause ions to flow through the nanopore. DNA passing through the nanopore blocks current flow, resulting in a measurable event. (*d*) Potentiometric sensors. The charge of a bioentity in solution varies as a function of pH. When the pH differs from the molecule's isoelectric point, the entity carries some net charge. Field lines generated by the particle's charge decay exponentially, moving away from the charged surface. Charges that terminate on the sensor surface induce charge movement in the substrate, which can induce a measurable change in the underlying sensor.

The key concern for electrical biosensors is the charge of the target entity. Controlling and maximizing this charge to induce a repeatable signal change are essential to robust sensing. A biological entity's charge originates from a proton's affinity for a particular component in a biomolecule. This affinity is driven by the proton concentration or pH of the solution, the biomolecule's proton dissociation constant (pK), and the biomolecule's environment, which is most often aqueous. Once a biomolecule is placed into an aqueous, ionic solution, the overall charge density on its surface stabilizes to a certain level (**Figure 2*a***). When the pH of the solution is modulated, the net charge changes. At a particular pH, the biomolecule will exist without any net charge. This point is known as the isoelectric point (pI) of a molecule or surface. As the pH of the solution is increased away from the isoelectric point, the amount of free hydrogen in solution decreases and the molecule accrues more and more negative charge. Conversely, as the pH decreases, the molecule becomes more positive. Controlling the pH of a solution enables the end user of a biosensor to tune the biomolecule's charge and control the potential measured signal in an electrical biosensor.

A biomolecule in solution will maintain a specific overall charge; however, the overall ionic environment will affect the apparent charge measured by an electrical sensor. Mobile ions in the solution move to shield and balance charge, which can minimize sensor response. The charge directly at the surface and the surrounding charge shielding are known as the electrical double

layer (**Figure 2*a***). The ions that can be specifically adsorbed directly at the surface make up the Stern layer, which is the layer closest to the charged particle and is typically a few angstroms thick (1). Outside the Stern layer is the diffuse layer, which contains a concentration of mobile ions that decays exponentially according to a Poisson–Boltzmann distribution, resulting in an exponential decay of the induced molecular electric field and the surrounding potential. The distance it takes for the induced electric field from the molecule to decay to $1/e$ of its value at the beginning of the diffuse layer is called the Debye length, which is given by

$$\kappa^{-1} = \sqrt{\frac{\epsilon_r \epsilon_0 k_B T}{2 N_A q^2 I}},$$

where I is the ionic strength of the electrolyte, ϵ_r is the dielectric constant, ϵ_0 is the permittivity of free space, k_B is the Boltzmann constant, T is the temperature in degrees Kelvin, N_A is Avogadro's number, and q is the elementary charge (1).

Minimizing the ionic strength of the solution will maximize the solution Debye length and maximize the measurable signal of the electrical biosensor. Balancing the effect of ionic strength on sensing and biomolecular activities such as binding affinity is essential for a robust electrical biosensor. As an example of the complications presented by the Debye length in potentiometric sensing (**Figure 2*d***), a relevant physiological electrolyte, such as serum (~0.14 M), has a Debye length of around 7 Å (2). Given an antibody's average size of 40–50 Å, a potentiometric biosensor will observe only a small fraction of the antibody's charge. In order to increase the observable signal of an antibody or its bound antigen, the ionic strength of the solution must be decreased roughly 100-fold, the charge must be brought closer to the sensing surface within the Debye layer, and/or the pH must be optimized to increase the biomolecule's apparent charge.

Similar to how an optical biosensor requires a photonic detector, an electrical biosensor requires the use of a charge detector such as an electrode. It is important to carefully choose the right electrode for a given system. Electrodes come in two major types: polarizable and nonpolarizable. An ideal polarizable electrode allows no transfer of electrons or ions to and from solution (i.e., has no faradaic current); an ideal nonpolarizable electrode is one in which such charge transfer through a faradaic current is possible (3). A polarizable electrode experiences similar electrical screening effects as molecules (**Figure 2*a***) and has the same Debye length in a given solution. In this case, when voltages are applied by polarizable electrodes, a charged particle outside a Debye length's distance away from the electrodes will be subjected to minimal electrostatic forces. However, because nonpolarizable electrodes allow faradaic current flow across the solution–electrode interface, ions will pass into the electrodes and electrophoretic forces can be applied to the molecule for manipulation (**Figure 2*b***). Polarizable electrodes are most often used to detect events very close to the electrode surface, such as binding events or local impedance changes. Nonpolarizable electrodes can be used to sense events that perturb the current at the electrode, such as blocking the current path as in a nanopore (**Figure 2*c***) or generating electrons at the electrode as in amperometric biosensors.

1.3. Specifications: Evaluating a Biosensor

Several criteria can be used to evaluate the efficacy of a sensor and describe its performance. Standardization of sensor specifications allows engineers to evaluate how well each sensor works and determines which sensor can be used for which application. A fundamental parameter is the device's resolution, which is the smallest shift in target concentration the device is able to distinguish at a given initial target concentration. Device resolution can be quantified as α/SNR,

where α is a confidence parameter usually chosen to be three or higher and SNR is the device's signal-to-noise ratio. In a real-world sample, where several nontarget biomolecules are typically present along with the target, the device signal is generally considered to be the average raw response in output characteristics to a change in concentration of the target analyte. The sensitivity is the average difference in output from solution one to solution two, divided by the difference between the two concentrations. Sensor noise is generally the sensor's overall average response to anything in the system other than the target analyte, including fluctuations in solution potential, nonspecific adsorption of molecules, and intrinsic device noise. The noise can be quantified by the average output response of the device to switching between two test solutions that contain the identical background, but with the same target analyte concentrations. One of the dominating contributing factors to device noise is the lack of perfect selectivity of the biological interface to the target analyte. Selectivity is the ability of the device to distinguish only the target analyte from a background of all other entities in the solution. A device's limit of detection (LOD) is the lowest concentration the sensor can reliably detect. The device's dynamic range is the ratio of LOD to the highest detectable concentration. Dynamic range, LOD, and resolution are critical criteria for evaluating a biosensor.

The concepts presented in this section, including the basis of electrical charge, how to define a sensor's performance, and the fundamental mechanisms behind different methods of electrical biosensing, provide a necessary background for the electrical methods that can be implemented in next-generation POC platforms. The following sections outline electrical methods that be used to decrease the complexity of POC sensors from sample preparation to detection by eliminating the need for mechanical or optical components.

2. ELECTRICAL METHODS FOR SAMPLE MANIPULATION AND PREPARATION

Although electrically based biological detection techniques have developed significantly in recent years, most commercial lab-on-a-chip systems focus strictly on detection and not on the equally integral initial sample preparation steps (4–6). Sample preparation can be defined as any process necessary to transform the input sample, typically a complex matrix directly from a patient, into a solution compatible with the employed sensing element. To do so, various methods are needed for flowing, mixing, lysing, separation, and concentration. Current methods for sample preparation in centralized commercial systems are typically heavily reliant on manual labor and/or cumbersome equipment. Systems that automate sample preparation are benchtop devices or larger and require large volumes and dedicated lab space. In the POC setting, space, time, and equipment are limited, and as a result, steps such as manual or automated macroscale pipetting and centrifugation are not feasible. Without integrated on-chip sample preparation modules, portable, cost-efficient, fully automated lab-on-a-chip systems will see limited use.

Examples of on-chip loading and sample manipulation exist (7, 8), but their commercialization in POC sensors is limited. Flowing can be accomplished with blister packs, micropumps, syringe-driven flow, or capillary forces, all of which require physical interaction with the sample. Such interaction can result in blockages, air bubbles, or technician exposure to the sample, and it may require larger sample volumes to accommodate pump volumes. Mixing on chip requires either added structures to induce turbulent flow or long channels for diffusive mixing. On-chip lysing can be accomplished with thermal techniques, which require external temperature control, or with chemical techniques, which require additional reagents. A wide variety of techniques can be used for concentration and separation on chip; these include, but are not limited to, the use of filters, membranes, flow profiles, pH gradients, and antibody-based affinity. All of these techniques, again,

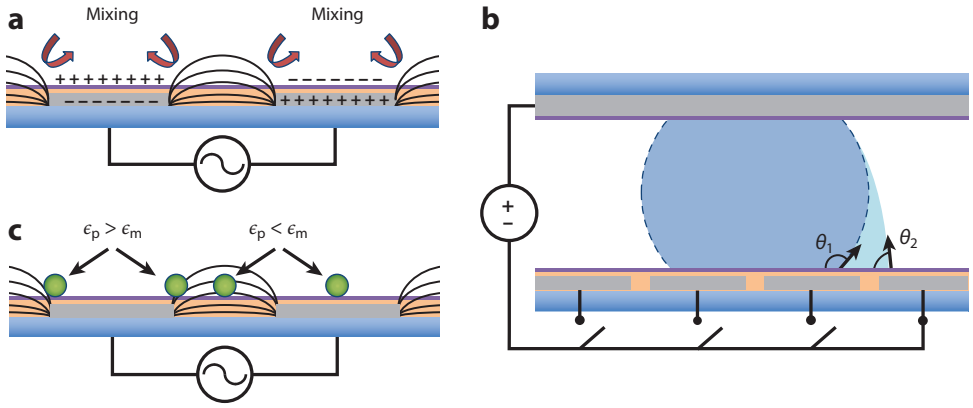


Figure 3

Electrical sample preparation. (*a*) ac electroosmosis for flowing and mixing. An ac signal applied between two electrodes induces charge accumulation at the electrode surface. At the edges of the electrode, the electric field interacts with the charged layer to generate counterrotating vortices above the electrodes. (*b*) Electrowetting on dielectric. A voltage is applied between the top electrode and the addressable bottom electrode, altering the contact angle of the droplet. This change in angle causes the droplet to deform and move in the direction of the reduced contact angle. (*c*) Dielectrophoresis (DEP) for separation. An ac signal applied between two electrodes results in a nonuniform field, which causes beads or cells in solution to localize to areas of either high–electric field gradients and strength at electrode edges (positive DEP) or low–electric field gradients and strength between electrodes or in the middle of electrodes (negative DEP).

require additional structures or specialized external forces implemented on chip. Electrically based sample preparation methods that utilize on-chip electrodes for contactless sample manipulation could provide an opportunity to integrate flowing, mixing, cell lysing, and concentration through the use of simple structures that are already present on the chip.

2.1. Flowing and Mixing via Applied Electric Fields

Application of an electric field to generate fluid movement offers a simple, contactless method that utilizes electrode structures easily integrated with the chip surface. In electroosmosis (EO), application of an electric field perpendicular to a microfluidic channel causes the movement of mobile charges in the diffuse portion of the electric double layer formed at the microfluidic device's surface (9). This movement near the channel surface induces flow in the bulk, resulting in net movement. Normally, fluid movement takes place as counterrotating vortices between the electrodes, allowing the reagents to mix (**Figure 3*a***). To induce net flow, one can use a traveling wave of ac signals down a channel or a dc offset, which causes asymmetric charging of electrodes and a resultant net flow (10, 11). Similar to the more commonplace dc electrophoresis, dc EO requires nonpolarizable electrodes and a faradaic current to drive fluid movement. This process causes heat generation, electrolysis, and pH variations, which can negatively affect biomolecules. At low voltages, ac EO does not induce or require faradaic reactions and provides significantly higher flow rates of several hundred micrometers per second (12). EO eliminates the need for pumping elements for flow, thereby reducing chip footprint and increasing portability.

Application of electric fields to liquids also alters the contact angle at the fluid–dielectric interface. This phenomenon, known as electrowetting on dielectric (EWOD), has garnered increasing interest in recent years as a means to implement large-scale, digital microfluidics applications (13–15) by enabling sample movement, mixing, and separation with only applied fields (**Figure 3*b***).

Here, a solution is placed between two parallel plate electrodes coated with a dielectric. The top electrode serves as a ground, whereas the bottom surface includes multiple, individually addressable electrodes arranged in an array. A droplet is introduced to the parallel plate channel, and a potential is applied between the top and bottom electrodes. This applied potential changes the interface potential between the solution and the dielectric and alters the droplet's contact, causing a pressure difference across the droplet and causing the droplet to move. Arranging droplets in a parallel formation and programming switching between electrodes allow for multiple-droplet movement and mixing. EWOD-based purifications of targeted biomolecules from a complex solution utilize systems that typically employ magnetic beads to tag a targeted analyte (16). The magnetic beads are then held in place while the sample droplet is moved, wash buffers are brought in, and finally an elution buffer is used to release the biomolecules for downstream sample analysis. EWOD offers a method to generate and control individual sample volumes that avoids the complexity of microfluidic valving structures or the need for oil-based encapsulants.

2.2. Lysing via Electroporation

Electroporation is a cell lysis technique that utilizes either an ac or dc electric field to create a potential across the cell membrane. Pores form in the cellular membrane once the transmembrane potential exceeds a threshold of ~0.2–1.0 V (17). These pores either remain open (irreversible electroporation) or reseal (reversible electroporation). Factors that contribute to the variation in size and number of the pores thus formed include the duration and field of applied electrical field, cell type, developmental stage, medium, and cellular dimensions. Numerous examples of electroporation of cells in microfluidic devices targeting POC applications have been published (18, 19). Jokilaakso et al. (20) demonstrated extraction of cellular contents from single cells, and Bao et al. (21) demonstrated cell type–specific electroporation for selective elimination of 98% of circulating tumor cells in blood. With other methods, cell lysing can be achieved chemically (9), enzymatically (10), thermally (12), and mechanically (22). However, electroporation offers some advantages over other techniques. It can alleviate the need for added lysis reagents, eliminate downstream inhibition of processes by incompatible reagents, and reduce complexity by eliminating the need for high-temperature thermal lysis or mechanical perturbation (20, 23). Additionally, without inhibitory chemicals or high temperatures, electroporation causes the release of cellular contents, enabling molecular analysis of released contents with minimal induced chemical or thermal damage.

2.3. Separation and Concentration via Applied Electric Fields

Electrical methods can be used to manipulate cell contents by use of ac or dc fields. Electrophoresis uses dc fields applied by faradaic electrodes to manipulate biological molecules. The traditional methods include gel electrophoresis and capillary electrophoresis, both of which have used for biomolecule separation and analysis for decades (24). In the last 20 years, researchers have pushed the boundaries to smaller, faster, less expensive microcapillary electrophoresis systems implemented on biochips for such applications as biomolecule separation (25), DNA separation (26), and protein separation (27). These systems utilize microfabricated capillaries that require smaller sample volumes, have shorter run times, and can have integrated detection capabilities. For example, capillary electrophoresis separations have been integrated with impedance, potentiometric, and amperometric detection modules (25, 28). However, note that the speed of dc electrophoresis is dependent on voltage and ionic strength.

Pohl & Hawk's (29) pioneering dielectrophoresis (DEP) research demonstrating manipulation of polarizable particles in a nonuniform ac electric field in the 1950s has since been adapted for use

in biochips for filtration (30), concentration (31), capturing (32), and patterning of cells (33). In DEP (**Figure 3*c***), a nonuniform electric field exerts a force on a particle in a direction dependent on the comparative complex permittivity of the particle (ϵ_p) and of the medium (ϵ_m). For cells, this phenomenon can be based on cell type (34), cell cycle phase (35), or viability (29).

For these electrical methods, voltages above a certain threshold cause electrolysis, which can lead to undesirable effects such as degradation of target molecules or surface functional layers, and the high ionic strength needed for tight banding must be compatible with downstream detection techniques (36). However, in comparison to mechanical methods, these electrical techniques can reduce complexity by eliminating the need for physical components such as filters, specialized pumps for achieving specific fluid profiles, or reagents for capture or elution after concentration.

2.4. Perspectives

As described in the next section, much progress has been made toward the development of new detection methods; however, without adequate sample preparation, lab-on-a-chip usage will be limited. Electrical techniques for flowing, mixing, lysing, concentration, and separation can offer simple alternatives to commonly used modules that often require extra components, reagents, specific external forces, or other additional complexity. Typically, these techniques require only patterned microelectrodes and applied voltages that are easily integrated with most electrical biochips, offering the potential for seamless integration with other downstream device components. However, to date these techniques offer only a subset of the capabilities required for full on-chip sample preparation. In addition, several of the techniques described require low–ionic strength solutions for full efficacy, which is fundamentally at odds with the reality of inputting highly complex bodily fluids. The future of on-chip sample preparation will most likely consist of a combination of these simple electrical techniques with other techniques. Researchers who can balance the issues of biological compatibility, sample volume, and electrical characteristics will benefit from the use of electrical components for sample preparation.

3. OVERVIEW OF ELECTRICAL BIOSENSORS

Biosensors are often categorized on the basis of how an analyte of interest induces a response that can be transduced into a signal. For electrical biosensors, the method of signal generation can be potential changes, impedance changes, or current changes. **Figure 2** is a general overview of these different sensing methods. **Figure 2*b*** demonstrates how a target analyte can react with another entity, such as an enzyme, in solution to generate redox mediators and thus perturb the measured current between the two electrodes. This change in current can be correlated to the concentration of the target analyte, an example of an amperometric sensor. Even if such a chemical reaction were not used, the field lines between the two electrodes can be perturbed by the presence of a target entity, especially if the entity is quite large with high impedance—for instance, a cell. This is an example of an impedance biosensor, in which the continuously measured impedance of the solution is modulated by the presence of a target entity between the two electrodes. This category includes Coulter counters and their nanoscale versions, nanopores, as shown in **Figure 2*c***. Finally, the affinity-based biosensor shown in **Figure 2*d*** is an example of a potentiometric biosensor, in which the intrinsic charge of the target analyte induces electric fields that interact with the sensor, modulating the potential on the surface.

In this section, we discuss the prominent electrical biosensor device mechanisms—impedance, potentiometric, and amperometric sensors. Each subsection provides an overview of the most prominent published literature, followed by a perspectives discussion, which focuses on the

commercialization possibility of each technology and its future potential. We evaluate each device according to five critical metrics (graded on a scale of low–medium–high): (*a*) ease of fabrication, (*b*) amenability to multiplexed detection, (*c*) complexity of input solution, (*d*) sensitivity, and (*e*) commercialization status.

3.1. Impedance-Based Biosensors

Impedance-based schemes for biosensors utilize two electrodes in solution, which can be either faradaic or nonfaradaic. The measured impedance is a complex quantity that can be used to approximate resistance, capacitance, and inductance values. When a target entity interacts with the field lines between the two electrodes and alters the resistance, capacitance, or inductance, the magnitude of the induced measured impedance change can be correlated to the concentration of the target entities. In order to measure this impedance, the current response to an applied ac voltage is typically measured and divided by the applied voltage to extract the impedance of the system. One of the primary constraints for impedance sensors is the requirement that the target entities or resulting chemical reactions significantly affect the impedance of the surrounding system. The measurement of impedance can be optimized for very large targets such as whole cells, for very small interrogation volumes, for systems where the target entities are constrained to close proximity to the electrodes, or for systems where chemical reactions catalyzed by the target entity can influence the surrounding solution. This requirement limits the applications of impedance-based biosensors. However, the simplicity and ease of fabrication of these techniques offer very attractive advantages to many applications where the target entity is large compared with the interrogation volume, as in the case of whole-cell counters in microfluidics.

3.1.1. Microscale impedance sensors. Microscale solution-based impedance biosensors utilize electrodes that are micrometers or smaller in dimension. These sensors can be used for cell counting applications, ranging from detection of bacteria to monitoring of a patient's health status through a complete blood count. These devices can monitor the overall change in impedance of a solution containing a certain concentration of cells either as the cells are lysed or as they exhibit characteristic changes as part of normal metabolic activity. The use of smaller electrodes can increase the overall sensitivity of the sensor, decrease the LOD, and increase the potential for portability. However, it is very important to demonstrate that such sensors yield high precision and accuracy of measurement as well as good correlation to control measurements. Liu et al. (37) demonstrated the electrical detection of the germination of spores of *Bacillus anthracis* by using interdigitated electrodes. Cheng et al. (38) used impedance spectroscopy for the selective counting of $CD4^+$ T cells from whole blood by utilizing an antibody-functionalized capture chamber. In this method, red blood cells are lysed, $CD4^+$ T cells are captured in a microfluidic chamber, the solution is exchanged for a low–ionic strength solution, and the captured cells are lysed. The cellular lysate alters the solution impedance. The magnitude of this change can be correlated to the number of captured cells.

Alternatively, the devices can count cells individually as they pass between the measuring electrodes and induce changes in the local impedance, utilizing the Coulter principle. Using this method, Watkins et al. (39) developed a microcytometer chip for the same target $CD4^+$ cell counting applications. This chip utilized an integrated initial red blood cell lysis module followed by a differential capture counting scheme in which the cells were counted as they entered and exited a capture chamber. Within the capture chamber, $CD4^+$ cells bound to immobilized antibodies, resulting in an exit count that differed from the entrance count. This technique has been demonstrated using clinical samples from patients with human immunodeficiency virus (HIV) and has

shown comparable results to those of large-scale fluorescence cytometers. Using a similar method, van Berkel et al. (40) differentiated cell types on the basis of their impedance signature as cells and particles flow over electrodes. This method requires only one or two on-chip sample preparation steps and can be combined with antibody capture techniques for surface antigen interrogation. Microscale impedance sensors offer a simple way to count elements that are large compared with the sample interrogation volume, and they are playing a major role in the development of POC cell counting.

3.1.2. Nanopore sensors for single-molecule DNA detection. Nanopores, which are nanoscale analogues of Coulter counters, are devices that measure the ionic current between two sides of a nanoscale opening (1–50 nm) (41–44). As DNA (~2 nm in diameter) translocates through the pore, the resistance across the nanopore, measured by the two electrodes, is altered, inducing fast-current pulses that can provide information about the electrical characteristics of the molecules translocating through the pore. For DNA, the nanopore must be sufficiently small (3–10 nm in diameter) so that current blockage signal rises above the inherent noise in the system. Modifications or bound proteins increase the diameter of the DNA complex, thereby changing the current blockage characteristics (45, 46). With nanopores, detection of single-molecule translocation events can be used to monitor DNA sequences, detect DNA without amplification steps, reduce reagent usage down to single-molecule probing, enable epigenetic studies of the effect of DNA methylation on DNA transcription, and monitor the presence of DNA-bound proteins (47).

Nevertheless, various technological barriers must be overcome for nanopores to reach their full potential (48). For example, the DNA translocation times for solid-state nanopores need to be increased from 1–3 μs per nucleotide to values in the millisecond-per-nucleotide range, where sensitive electrical measurements can be performed to identify individual bases with high SNR (47). In addition, the spatial resolution needs to be increased so that single nucleotides can be probed electrically, and the DNA extraction and concentration steps need to be integrated with the nanopores so that small volumes or numbers of molecules can be used without the need for amplification.

Several schemes have been proposed to increase translocation times. Iqbal et al. (49) demonstrated a solid-state nanopore functionalized with hairpin-loop DNA to increase the interaction of the translocated DNA with the pore. The electronics of the detection system were also optimized to filter out high-frequency noise and reduce the detectable translocation time limit (50). Venkatesan et al. (51, 52) used aluminum oxide in nanopore membranes, resulting in improved stability with slower transport, due to the positive surface charge, and with a higher SNR, due to reduced noise properties. Forming nanopores in electrically conducting graphene could enable electrical or electrochemical measurements with very high spatial resolution because the thickness of a single layer of graphene is approximately the same as the thickness of a single nucleotide (45).

Companies including Oxford Nanopore Technologies, Nabsys, and Genia are working to commercialize nanopore technologies for sequencing applications due to their ability to directly measure long strands of DNA without the need for amplification. Although many challenges lay ahead, the advantages of single-molecule sensing without amplification, labels, or optics make electrically based nanopores very attractive candidates for next-generation sequencing technologies.

3.1.3. Affinity-based sensors. Affinity-based biosensors rely on a functionalized surface with an immobilized probe molecule that can specifically capture the target analyte from solution. The kinetics and theory of receptor–ligand binding, important for affinity biosensors, have been well studied with the Langmuir adsorption isotherm (53). Affinity-based impedance biosensors, which monitor changes in impedance depending on target binding, can be divided into faradaic and nonfaradaic sensors. Faradaic impedance sensors monitor a change in the charge transfer

resistance at the electrodes upon binding of the target molecules (54). Faradaic DNA sensors can detect 15-mer DNA targets down to 1 nM (55), as well as various proteins with aptamer probes (56, 57). Investigators recently detected microRNA (miRNA) down to 2 pM in various sources, including cancer cell lines and serum (58). However, faradaic sensors require redox-active species and dc voltages, which may induce air bubbles from electrolysis as well as heat generation (54). These factors limit such sensors to specialized applications in which current flow in the solution does not adversely affect signal output. Nonfaradaic impedance sensors do not rely on redox-active species; instead, they measure the capacitance component of impedance when there is no charge transfer (59). As target molecules bind to the surface, the measured capacitance is modulated.

Sensors with on-chip electrodes usually use interdigitated electrodes (IDEs), in which fingers of metal are patterned on chip with periodic spacing. As the feature size of the electrodes is reduced to the comparable size of the relevant analytes, the sensor response is improved (54). IDEs have been used to detect DNA in the nanomolar range (60), DNA by-products of polymerase chain reaction (PCR) effectively down to the picomolar range (61), and antibodies in the picogram-per-milliliter range (62). A recent study demonstrated a novel method for concentrating DNA by monitoring the impedance of an evaporating droplet containing DNA using a nonfaradaic impedance measurement. This approach enabled detection of an initial concentration of 60 aM of DNA (63).

3.1.4. Perspectives. The core strength of impedance-based biosensing devices is their simplicity of fabrication. However, the target entity must significantly affect the overall impedance of the solution, which requires large target entities, higher concentrations, or smaller interrogation volumes (in the case of nanopores). In addition, more robust and repeatable surface functionalization procedures will be crucial for affinity-based impedance biosensors. In the future, impedance-based techniques may be employed in specific POC applications such as global health, where portability and cost restrictions prohibit the use of standard optical techniques but where better sensitivity or multiplexing capabilities are not critical.

The various aspects of affinity-based sensors can be summarized as follows.

- Ease of device fabrication: high. Very simple fabrication techniques can be used to construct these devices. In addition, several examples of this technology require no extra buffers or solutions (such as enzymes or labels), other than a redox-active species for faradaic sensors. In general, these devices are much simpler than other technologies.
- Amenability to multiplexed detection: medium. Several examples of this technology, such as Coulter counters and nanopores, have a limited capacity for multiplexing; however, affinity-based impedance sensors enable multiplexing by using specific capture probes.
- Complexity of input solution: low to medium. Most impedance sensors need a lower-conductivity solution to operate so that the target analytes significantly influence the measured solution properties. Several studies have demonstrated cell counting from human blood, but they incorporated significant sample processing steps (39).
- Sensitivity: medium. Most nucleic acid and protein detection is typically in the high-picomolar-to-nanomolar range, which is not significantly better than that of enzyme-linked immunosorbent assays (ELISAs) or DNA microarrays (59).
- Commercialization status: low to medium. Aside from the established field of Coulter counters, there are no examples of commercialized POC impedance biosensors. However, development of several such products is under way (e.g., Daktari's CD4 counting platform), and several start-up companies (including Oxford Nanopore Technologies) are working to commercialize nanopore technology.

3.2. Potentiometric Biosensors

When any electrode surface is placed in contact with an ionic solution, a potential drop (ψ_0) forms between the surface and some distance into the solution. This voltage is a strong function of surface charge. When an affinity-based biosensor captures charged target analyte molecules on a surface, ψ_0 is affected. A potentiometric biosensor uses ψ_0 at an electrode surface to detect the presence of biomolecules. Similar to affinity-based impedance methods, potentiometric biosensors offer the possibility of increased specificity and multiplexed detection due to affinity-based capture schemes when compared with bulk impedance or amperometric sensors. The requirement of small sample volumes or large target entities for impedance biosensors can thus be avoided with this technology. However, potentiometric sensors are typically more complicated to fabricate, functionalize, and measure. In particular, achieving stable and repeatable surface chemistries for affinity-based biosensors requires further standardization and optimization. Most importantly, due to electric double-layer charge screening, the ionic strength of the measurement solution must be optimized and target entities must be as close to the surface as possible to significantly induce sensor response.

3.2.1. Electrolyte insulator semiconductor systems. Electrolyte insulator semiconductor (EIS) systems, when combined with a reference electrode immersed in solution, form a structure similar to the fundamental two-terminal metal-oxide semiconductor (MOS) capacitor, with the metal gate replaced by the reference electrode and the ionic fluid. This type of system is essentially the same as the system shown in **Figure 2*d***, with a semiconductor material as the substrate. As charged target molecules bind to the surface, the charge induces an opposite-charge split into two components: the counterion cloud in the double layer and carriers in the semiconductor. These components can be modeled as two capacitances in parallel, the double-layer capacitance C_{DL} and the device capacitance C_{device}. The voltage across these capacitors is ψ_0, and the change in ψ_0 due to the binding of the target on the surface shifts the capacitance voltage. The charge at each capacitor interface is determined by the relative magnitudes of the two capacitors; the larger capacitor mirrors most of the charge of the target molecules. C_{DL} increases as the ionic strength of the solution increases, robbing the device of transducer charge and decreasing overall sensor response. Studies have demonstrated detection of various molecules with EIS capacitor structures down to the nanomolar range (64–66), but most of the research in the field has focused on field effect transistors (FETs) for biological detection.

3.2.2. Nanoscale field effect sensors. Ion-sensitive FET (ISFET) sensors are three- or four-terminal devices that add source and drain terminals to the EIS capacitance structure. Here, the output characteristic is the current measured between the transistor's source and its drain (I_{DS}). Fifty years of fabrication development, the intrinsic device gain of a transistor, the ease of performing current measurements in large arrays, and the demonstrated sensing of various biological entities make FETs an attractive option for POC sensing (67–69).

NanoFET sensors are ISFETs that have been scaled down to the nanoscale in thickness and width. By reducing the size of the devices close to the order of targeted molecules, the LOD can be reduced. In a landmark publication, researchers used silicon nanowires 20 nm in diameter that were formed using bottom-up vapor–liquid–solid growth, a method not suitable for scale-up (70). The devices were used to detect DNA and proteins down to the femtomolar range (71, 72). Stern et al. (73) used a top-down fabrication process with electron-beam lithography to create silicon nanowire FETs (25 nm thick, 50 nm wide) from silicon-on-insulator (SOI) wafers, enabling detection down to 10 fM of streptavidin and 100 fM of mouse immunoglobulin A. Another group reported the specific detection of miRNA with top-down-fabricated nanowires by using peptide nucleic acid probes down to 1 fM (74), multiplexed detection of the cardiac biomarkers from

human serum down to 100 fg/mL (75), and detection of carbohydrate–protein interactions down to 1 fg/mL (76). Dorvel et al. (77) demonstrated specific miRNA detection from buffer down to 100 fM by using hafnium oxide as a high-ϵ_r dielectric for silicon nanowire sensors 50 nm in width and 30 nm thick. The use of a high-ϵ_r dielectric allows the use of a thicker gate oxide without sacrificing the gate oxide capacitance.

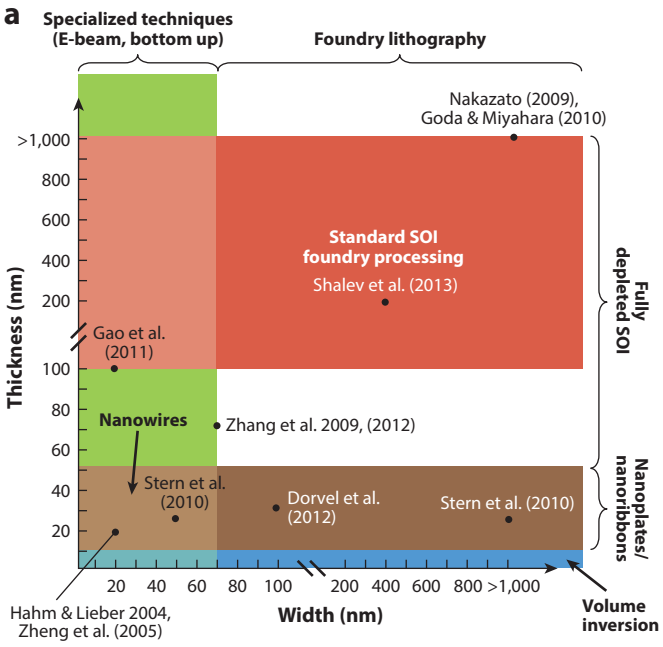
Investigators have also developed a method to transfer nanowire FETs to plastic flexible substrates (78). Carbon nanotubes have been used as FET biosensors because of their biocompatibility and high theoretical sensitivity, given that all the atoms of carbon nanotubes are present at the surface (79). Graphene has been used as a sensing membrane for FET sensors due to its high conductivity and atomic layer thickness (80), but several challenges remain in this young field, including standardization of preparation and surface conjugation difficulties.

There are several reports of FET sensors demonstrating sensing from complex matrix solutions as a result of three main obstacles. First, the large amount of charged entities in more complicated samples can lead to an abundance of false positives without high specificity of the capture probe. Increasing salt concentration and optimizing the pH of the solution can improve specificity. Second, surface receptors can degrade rapidly with exposure to complex matrices, such as blood or serum (69). Third, physiological solutions have an ionic strength of around 100 mM, which corresponds to a Debye length of 0.7 nm. Because charged entities are further away from the surface, this charge is screened by ions, minimizing the induced electric field in the sensor. For reference, a monolayer of typical silanes used as the first anchor layer in all common oxide surface functionalization schemes is around 0.8 nm thick (81). Most schemes therefore anchor charged targets far beyond the Debye length in a physiological solution (82). To alleviate this issue, most researchers use diluted blood or sensing buffers with low ionic strength, given that the Debye length is inversely proportional to the square root of the ionic strength. However, molecular affinity and specificity are reduced without stabilizing salts. Either device sensitivity (high salt, low Debye length) or device specificity (low salt, low probe affinity) must be sacrificed. Significant sample preparation steps will be needed to transform complex sample inputs such as blood into low–ionic strength buffers that allow the devices to sense target entities.

To address this issue, investigators have proposed the use of a microfluidic purification chip (MPC) for detection from whole blood (83). Human blood is flowed into the MPC, which contains immobilized antibodies specific to the target biomarkers to be detected. After being washed to reduce the ionic strength of the solution, the target biomarkers are released and detected by the nanowire chips in a low-salt solution to maximize signal strength. Specific detection was demonstrated from human blood spiked with as little as 2.5 ng/mL of prostate-specific antigen and 30 units/mL of cancer antigen 15-3 (CA 15-3). Makowski & Ivanisevic (69) have summarized studies claiming detection in blood and serum, but several of these schemes employed labels or failed to address the issue of ion screening in high–ionic strength solutions.

FETs have also been used to monitor the by-products of chemical reactions on chip. Ion Torrent, a next-generation sequencing company, described an integrated semiconductor device with a completely nonoptical detection method (84). The chip (with a cost below US$100) has more than 1.2 million FET pH sensors with circuitry to address each individual transistor, as well as circuitry for additional signal processing. PCR beads containing the fragments of the template DNA to be sequenced are loaded into individual wells with the pH sensor in a row–column array, and sequencing by synthesis is performed. As each of the four nucleotides is introduced sequentially to the wells, polymerase adds bases to the nascent strand, hydrolyzing the incoming nucleotide's triphosphate and producing hydrogen ions that can be detected by the underlying FET sensors.

Much of Ion Torrent's intellectual property has been licensed from a POC company named DNA Electronics. DNA Electronics combines on-chip signal processors, control circuitry,



b

Molecule(s)	LOD	Solution	Reference
DNA	fM	Low ionic strength	Hahm & Lieber (2004)
PSA, CEA, mucin-1	fM	1 μM phosphate buffer	Zheng et al. (2005)
Streptavidin	fM	1 mM sodium bicarbonate buffer	Stern et al. (2010)
PSA, CA 15.3	pM	Human blood	Stern et al. (2010)
miRNA	fM	0.02X SSC	Dorvel et al. (2012)
miRNA	fM	0.01X SSC	Zhang et al. (2009)
cTnT, CK-MM, CK-MB	<fM	Human serum	Zhang et al. (2012)
cTnI	fM	0.01X PBS	Shalev et al. (2013)
DNA	fM	0.1X PBS	Gao et al. (2011)
DNA	μM	1 mM phosphate buffer	Nakazato (2009)
BSA	nM	1.5 mM phosphate buffer	Goda & Miyahara (2010)

Figure 4

Dimensions of field effect transistor (FET) sensors. (*a*) A survey of FET sensor studies, organized according to device thickness and width. Widths below 70 nm require specialized fabrication techniques. Thicknesses below 100 nm are difficult to produce with foundry processing. Most studies used nanowire devices <70 nm in width and <50 nm in thickness. Volume inversion, where the entire physical device is enriched with carriers, should yield optimal sensitivity, but none of the studies cited lie ins this regime. (*b*) The biological entity detected in each study, as well as the ionic buffer used for sensing. Sensing buffers are optimized at low ionic strength to maximize the Debye length in solution while maintaining binding efficiency and specificity of the biorecognition element for the analyte of interest. Abbreviations: BSA, bovine serum albumin; CA, cancer antigen; cTnT, cardiac-specific isoform of troponin T; LOD, limit of detection; miRNA, microRNA; PBS, phosphate-buffered saline; PSA, prostate-specific antigen; SOI, silicon on insulator.

on-chip heaters, and temperature sensors for the detection of pH changes induced by PCR (85). This technology offers a nonoptical replacement for quantitative PCR and can increase portability and reduce cost of nucleic acid amplification–based assays. By incorporating on-chip electrical heating elements, this platform can further enable POC DNA diagnostics. In addition, by sensing the ions produced by the molecules of interest instead of the intrinsic charge of the molecules themselves, these technologies bypass the issues associated with Debye screening of molecules.

In addition to the difficulties of detection from physiological solutions addressed previously, FET technology faces challenges relating to standardization (69, 86) and mass fabrication. To date, few studies have utilized commercially produced devices for the direct label-free sensing of target analytes. At the heart of this problem is the issue of device geometry—specifically, the question of the necessity of nanostructured devices. **Figure 4** illustrates a survey of published studies according to device width and thickness and shows the relevant sensing parameters. Sections of width and thickness have been delineated according to different difficulties of fabrication. For example, any device with a width below 70 nm requires specialized techniques, such as electron-beam lithography or bottom-up growth. Few studies have focused on standard SOI, represented in **Figure 4*a***. It will be important to determine the resolution and dynamic range necessary for the target applications to learn whether foundry-fabricated sensors can meet these specifications.

Theoretical frameworks attempting to address this issue have been put forward (87–90). They argue that device geometry and detection time are inextricably linked. For example, diffusion-limited transport of a target analyte to the sensor is a function of device width; the density of analytes on a planar structure is proportional to the square root of time, compared with a linear relationship for nanowire structures (87). However, nanostructured sensors have lower LODs because less charge is contained in the active layer. This model predicts that, given a detection time of 100 s, the LOD should be around 1 pM for nanowires and 100 pM for planar ISFETs. It argues that the femtomolar detection limits reported by various studies are actually statistical anomalies from the tail ends of distributions of device measurements. In the future, it will be important for research groups to provide statistical distributions of detection events instead of illustrating the results from single devices.

3.2.3. Perspectives. The power of potentiometric sensors is exemplified by the work of Ion Torrent and DNA Electronics and by the prevalence of handheld ISFET pH sensors. The scalability and versatility of the semiconductor manufacturing process available at Taiwan Semiconductor Manufacturing Company, Samsung Foundry, and GlobalFoundries, among other companies, can translate into massively multiplexed biochips. In the future, potentiometric sensors will offer the potential for low per unit sensor costs, massive multiplexing, and very low LODs. However, there are factors hindering further development of the field, including a significant lack of standardization of fabrication techniques, surface functionalization, and measurement schemes. One of the most significant challenges is the requirement for low–ionic strength solutions to increase the Debye length and reduce charge screening. FET sensors may replace DNA microarrays and ELISAs with devices that are cheaper and more portable; have higher sensitivity; and require smaller sample volumes for many applications, including drug discovery, early cancer detection, food safety, and global health.

FETs can be summarized as follows.

- Ease of device fabrication: low. Particularly with FET sensors, fabrication is very complicated and still quite expensive. However, after initial challenges are surmounted, this technology will be capable of mass fabrication, which will reduce unit costs. The lack of a label is an attractive feature of FET sensors, reducing overall cost and complications associated with the modification of target analytes.
- Amenability to multiplexed detection: high. The possibility of combining large arrays of FET devices with many different target analytes on the same chip offers the greatest potential for multiplexing out of all the technologies covered in this review. Research by Ion Torrent has shown that thousands of different reactions can take place on a single chip. However, multiplexed intrinsic molecular sensing has yet to be demonstrated with a commercial device.
- Complexity of input solution: low. The greatest challenge posed by FET technology is the trade-off between sensitivity and selectivity. Most sensors require highly purified buffer solutions with low ionic strength. Until this problem is adequately resolved, significant sample preparation will be needed.
- Sensitivity: high. With nanoscale thickness and widths, LODs have been demonstrated in the femtomolar or even attomolar range, far below that of standard techniques. However, some issues remain in interpretation and standardization of these data.
- Commercialization status: high or low. These sensors can be divided into two categories: ion-sensitive technologies (high) and intrinsic molecular charge–sensing technologies (low). ISFET pH sensors, including handheld devices, have been successfully commercialized during the past few decades. In addition, Ion Torrent and DNA Electronics have employed FETs for commercial products by detecting pH changes caused by nucleic acid amplification

reactions. These examples illustrate the extensive commercial potential of potentiometric sensors. However, thus far, no companies have offered a commercial product for intrinsic molecular charge detection (e.g., charge of DNA, proteins), although many start-ups are attempting to do so. There is a significant gap between reported academic research in this area and commercial success.

3.3. Amperometric Biosensors

Research on amperometric biosensors dates back to the 1960s (95), and thousands of papers on this topic have been published. Amperometric biosensors take advantage of enzyme-driven redox reactions to specifically detect targeted biomolecules in solution. In this method, an enzyme that catalyzes the generation of redox reaction components can be free in solution, immobilized at an electrode surface, or immobilized in a matrix that also contains the necessary mediators. The introduction of a medium containing substrates subject to the enzyme's catalytic behavior causes a reaction and generates a product, which is then oxidized at the surface of an integrated electrode. This process results in electron generation and a faradaic current in the underlying electrodes—the latter of which is measured and then correlated to the concentration of the targeted biomolecule.

Of all the technologies covered in this review, amperometric sensors are the most widespread, probably because of the simplicity and repeatability of the technique, wherein two electrodes measure a faradaic current generated in the presence of the enzyme, substrate, and mediators. However, this technique can be used only with very specialized targets when there is a known enzyme that can catalyze a reaction to produce changes in the detected current. At present, this approach limits these sensors' applications to a minute subset of biological targets.

3.3.1. Background. Blood sensors represent the gold standard of amperometric biosensors and potentially all biosensors. This technology has introduced the world to the potential of biosensors, and it is used by patients with diabetes on a daily basis. Given its ubiquity, the glucose meter represents a good starting point for introducing the fundamentals behind amperometric biosensors.

Amperometric glucose sensors monitor the current glucose concentration in a droplet of a patient's blood. Traditionally, this method utilizes a glucose oxidase–catalyzed reaction, which generates hydrogen peroxide. A subsequent electrical signal from the oxidation of hydrogen peroxide or reduction of oxygen is measured. A three-electrode system consisting of reference, working, and counterelectrodes is employed to accurately measure the current generated. A stable potential is maintained with the reference electrode versus the working electrode, while current is measured between the working electrode and the counterelectrode. For the reference electrode to remain stable, no current should be allowed to pass through it. This method can be extended to a wide variety of other analytes, as long as an enzyme can be found that will cause an oxidation–reduction reaction and enable the subsequently generated electrons to be sensed. In order for the generated electrons to be sensitively detected, the enzyme must be localized at the electrode surface, which is often accomplished through immobilization techniques.

To date, there have been three generations of amperometric biosensing modalities, each of which uses the redox reaction in a unique way (96). The first generation resembles the following reaction:

$$\text{glucose} + \text{GOx(FAD)}\ \text{gluconic acid} + \text{GOx(FADH}_2\text{)}$$

$$\text{GOx(FADH}_2\text{)} + \text{O}_2\text{Ox(FAD)} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 2\text{H}^+ + \text{O}_2 + 2e^-,$$

where electrons are sensed from an enzyme's by-product oxidation–reduction (**Figure 5*a***). This method has the advantage of simplicity, but if the by-product is naturally occurring in the sample

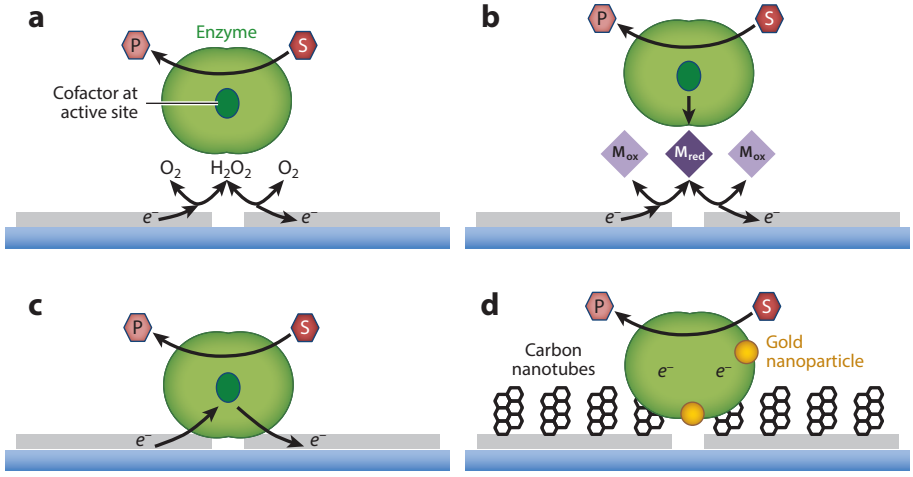


Figure 5

Generations of amperometric biosensors. Each generation relies on an enzyme catalyzing a reaction to convert a substrate (S) to a product (P), such as β-D-glucose to D-glucono-1,5-lactone in glucose sensors. The generations differ in which component of the reaction induces a measurable current. (*a*) The first generation relied on an electroactive by-product, such as hydrogen peroxide in glucose sensors, to generate a measurable current through oxidation–reduction at the electrode. (*b*) To overcome the need for environmental oxygen and response variations that varying levels of oxygen can induce, the second generation introduced electroactive mediators, such as ferrocene, to more specifically and robustly shuttle electrons from the enzyme to the electrode. (*c*) The third generation removed the need for by-products and mediators entirely by utilizing direct electron transfer from the active site of the enzyme to the electrodes. This method is limited for glucose sensors because the active site of glucose oxidase is ~13 Å from the outside of the enzyme. (*d*) To overcome this shielding and facilitate electron transfer, generation 3.5 introduced nanostructures, such as carbon nanotubes or gold nanoparticles bound to the enzyme, to bring conductive elements closer to the enzyme active site and directly transfer electrons to the electrodes.

(as oxygen is for glucose sensors), variations in oxygenation of blood can cause high background noise and reduced resolution. The second generation of sensors (**Figure 5*b***) bypasses this problem by using a molecular mediator, such as ferrocene for glucose sensors, to mediate electron transfer between the enzyme cofactor and the electrode. **Figure 5*c*** depicts the third, most recent generation, which relies strictly on direct electron transfer from the active site of the enzyme to the electrode surface (96). This modality can greatly reduce the complexity of the required reagents and increase specificity, but the greater distances (>8 Å) between the active site and the electrode can significantly reduce the rate of electron tunneling and, therefore, sensitivity. This method has seen limited use in glucose sensors because glucose oxidase's active site is centrally located and is at least 13 Å from the enzyme's edge (97). To overcome this issue, researchers have turned to nanostructures to increase the conduction of electrons from the active site to the electrode. Carbon nanowires bring the electrode closer to the active site of the enzyme (**Figure 5*d***). Alternatively, conductive nanoparticles can be bound to the enzyme, thereby increasing the electron transfer rate to nearby electrodes. These systems have not yet been implemented in commercial devices, but initial results are promising.

3.3.2. Research examples. Even though amperometric biosensors were invented more than 50 years ago, new innovations continue to improve these sensors' performance. The innovations involve either the incorporation of nanotechnology or the development of a third generation

that utilizes direct electron transfer modalities. With regard to the first type of innovation, nanotechnology can be incorporated through (*a*) modification of macroelectrodes with nanostructures such as nanoparticles, nanopillars, or carbon nanotubes; (*b*) incorporation of nanoelectrodes; and (*c*) modification of biomolecules with nanomaterials such as nanoparticles or nanotubes.

Modification of macroelectrodes with nanostructures has become increasingly popular in recent years. Specifically, carbon nanotubes have seen a variety of uses including, but not limited to, detection of cancer biomarkers (98), glucose (99, 100), lactate (101), glutamate (102), horseradish peroxidase (103), and bisphenol A (104). Carbon nanotube sensors improve the sensitivity of detection by increasing the available surface area and potentially by altering the activity and electron transport of the targeted biomolecule by bringing the electrode surface closer to the enzyme's active site (105, 106). The use of nanoelectrodes confers unique advantages on amperometric biosensors, as shown recently by the detection of glucose (107, 108) and L-dopa (109). The increased surface area and small dimensions of nanoelectrodes increase the sensors' sensitivity and enhance mass transport through the use of hemispherical diffusion fields (110).

Modification of biomolecules with nanoparticles is an emerging area of study. As our understanding of enzyme function continues to improve through advances in folding simulations and sequencing, nanotechnologists will be able to modify proteins to increase activity and the rate of electron transfer from the active site (111–113). Meredith et al. (111) recently modified multiwalled carbon nanotubes with anthracene to aid the orientation of laccase. In doing so, they brought the active site closer to the electrode, enabling better direct electron transfer.

3.3.3. Toward in vivo electrochemical sensors. Amperometric biosensors that are integrated with the human body enable continuous and less invasive monitoring of biologically relevant components, such as glucose and lactate. A new class of commercially available glucose sensors are placed just under the skin. They work by monitoring glucose levels in interstitial fluid and can simplify the management of diabetes through implementation of a minimally invasive, continuous monitoring system. Additionally, sensors can be placed on the skin or on a contact lens for continuous monitoring of biomolecules through sweat or tears (114).

Even though most research has focused on glucose monitoring for diabetics, progress has also been made in monitoring lactate. For instance, Jia et al. (115) designed an electrochemical sensor system to be worn as a temporary tattoo—complete with working, reference, and counterelectrodes and an enzymatic layer. The enzymatic layer includes lactate oxidase, which converts lactate from sweat into pyruvate and, in the process, generates electrons for an amperometric measurement. Such systems can be employed to monitor a wide variety of biomolecules in a minimally invasive manner, and when placed on the skin, they avoid many of the biocompatibility issues associated with implantation.

3.3.4. Perspectives. Amperometric biosensors present a unique opportunity. Their simple design and the large background of research are conducive to biosensor development. Amperometric biosensors are limited by their multiplexing capability and sensitivity. Third-generation sensors that utilize (*a*) direct electron transfer to eliminate mediators and increase multiplexing capability and (*b*) nanotechnology to increase sensitivity will address these issues, but their commercialization prospects are still limited. Further standardization of direct electron transfer measurement techniques, as well as a greater understanding of nanomaterial fabrication and enzyme structure and kinetics, is necessary for this field to mature. Until third-generation sensors develop further, applications will continue to focus on scenarios in which measurements must be taken regularly but the sensitivity is limited to the micromolar range—for example, measurements of glucose levels, lactate levels, and groundwater contamination from arsenic or nitrates.

Amperometric biosensors can be summarized as follows.

- Ease of device fabrication: high. First- and second-generation sensors utilize a simple design of interdigitated electrodes. Their cost is only slightly higher than that of other types of sensors due to the need for incorporated enzymes and mediators.
- Amenability to multiplexed detection: low. The specific nature of the enzyme-catalyzed reaction limits the potential for detection of multiple analytes.
- Complexity of input solution: high. The specific nature of the reaction, as well as the inclusion of reaction mediators, allows commercial amperometric biosensors to utilize complex matrices such as blood, tears, and saliva.
- Sensitivity: low. Typical LODs are in the micromolar range. These limits provide relevant data for some analytes, but the sensors are not sensitive enough for many applications, such as typical DNA and cancer biomarker detection.
- Commercialization status: high. The ubiquity of blood glucose sensors represents the commercialization potential of amperometric biosensors.

4. FUTURE PERSPECTIVES

An ideal biosensor has minimal complexity while yielding the most desirable data. Portable glucose sensors for patients with diabetes represent one end of the spectrum of commercially available biosensors (**Figure 6**). Portable glucose sensors are typically quite inexpensive, yield quantitative results in minutes, and require almost no infrastructure, but they are useful for only one specific application: the detection of glucose. At the other end of the spectrum, tools such as next-generation sequencing machines or DNA microarrays yield abundant information for a variety of applications, such as the sequence of an entire genome for disease pathogenesis. However, sequencing machines with such high accuracy are expensive, take a few weeks to yield results, and require highly trained staff and advanced lab equipment. **Figure 6** shows that there is a significant void of commercially available devices, which deliver a high amount of information per test with lower complexity. To fill this void, researchers have envisioned the development of POC devices—cheap, portable devices capable of providing data quickly and accurately at a patient's bedside without the need for specialized technical staff or expensive equipment (116, 117). Electrical biosensors are playing an important role in the future of POC biosensors, but opportunities remain for greater translation of research to the bedside. In this section, we provide an overview of the current status of this field and show how and where low-complexity electrical biosensors can have the greatest impact.

4.1. Current Trends and Unmet Needs

In the subsections below, we discuss the industry trends and unmet needs for POC biosensors.

4.1.1. Commercial products. The POC diagnostics market was worth US$13.4 billion in 2010 and is expected to increase to $16.5 billion in 2016 (117). By far the most successful commercial biosensor has been the amperometric glucose sensor. Glucose test strips for home use are produced on a massive scale, approaching 10^{10} strips per year (116). Companies offering commercial products include Roche Diagnostics (Accu-Chek systems), Johnson & Johnson (LifeScan meters), Abbott Diabetes Care (FreeStyle system, Precision system, i-STAT device), and Trividia Health (Sidekick and TRUEtrack systems). These portable systems have given rise to an entirely new level of at-home personal care, making it easier for millions of diabetes patients across the world. These sensors satisfy virtually all the ideal requirements for POC sensors, including fast time to results (~5 s), small sample volumes (down to 300 nL), inexpensive cost per text, and ability to operate without technical expertise (116). The latest systems, such as Abbott's FreeStyle Navigator, MedTronic's

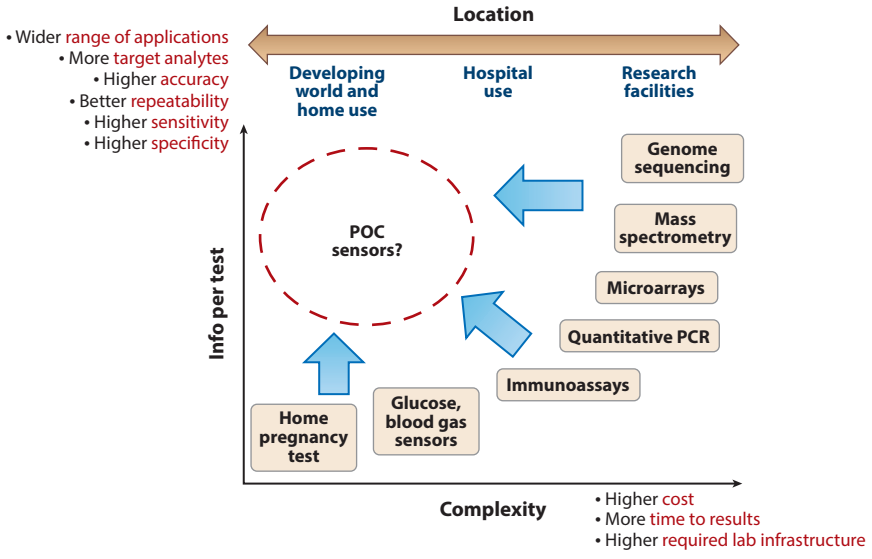


Figure 6

Classifications of diagnostic tests. A trade-off usually exists between increased complexity and information yielded per diagnostic test. As the complexity of the test increases, possible locations for testing may be restricted only to hospitals or research facilities. Point-of-care (POC) sensors aim to fill the void (*dashed red circle*) by offering lower-complexity tests that provide a large amount of useful information. Abbreviation: PCR, polymerase chain reaction.

MiniMed Paradigm REAL-Time Revel, and Dexcom's G4 PLATINUM, enable continuous glucose monitoring with insertion of an electrode just underneath the skin. Similar technologies are used for analyzing blood chemistry, cardiac markers, cholesterol levels, and urine chemistry. These technologies exemplify the potential of POC devices in the health care industry; however, applications are limited due to the specific nature of the enzymatic reaction. Some concerns exist about the accuracy of detection from POC sensors (118) and the efficacy of electrical POC devices to standard techniques for making treatment decisions; these concerns have focused on Abbott's versatile i-STAT device due to its ubiquity (119, 120). We conclude that there is a significant opportunity to improve the accuracy of POC devices to align with currently used techniques.

4.1.2. Academia to industry. There is a large gap between the huge academic output of biosensor research and commercial successes (121, 122), for many reasons. First, investigators need to focus more on detection in complex matrix solutions. Specifically, better standardization of metrics such as LOD, resolution, and dynamic range need to be quantified in solutions such as human blood, serum, urine, and saliva. Such frameworks have already been established by, for instance, the Clinical and Laboratory Standards Institute, a nonprofit organization that is one of the primary authorities on unified standards for various lab practices in health care. For example, LOD is properly determined through statistical analysis of the responses of many samples, including determination of the limit of blank in the application's complex matrix solution.

Second, sample preparation and the elements surrounding the sensor component should be emphasized. POC sensors are not useful if they require considerable off-device preparation with expensive laboratory equipment or skilled technical staff. Third, surface conjugation techniques for affinity-based biosensors need to be optimized and standardized. A systematic comparison between

various approaches is needed, with clear specification goals such as achieving the highest density of molecules and high specificity for the target analyte, and other technology-specific requirements such as the closest attachment to the surface. Finally, further analytical and simulation studies will be necessary for us to better understand the complexity of surface conjugation to affinity-based biosensors. All of these issues need to be addressed by universities, start-up companies, and industry leaders so that we can close the gap between academia and industry in the near future.

4.1.3. Global health and resource-limited settings. The development of POC devices for resource-limited settings has become a very important goal in the twenty-first century (116, 123). This area of research has attracted substantial funding from various organizations, including the Bill & Melinda Gates Foundation and the US Global Health Initiative. Infectious diseases, including HIV, tuberculosis, and malaria, have attracted the most attention. In contrast to the situation in resource-rich settings, where portable, cheap devices requiring minimal technical expertise and laboratory equipment are more of a luxury than a necessity, in resource-limited settings such devices are often the only option. In these settings, simplicity and cost are the two most urgent considerations. Electrical POC biochips have been used in many of these devices to satisfy these requirements, most notably with technology similar to the glucose sensor and with impedance measurements in fluidic devices. Improving global health will continue to be a key application for electrical biochips in the future.

4.1.4. Personalized medicine and biomarkers. During the past decade, the rise of cheaper and faster sequencing techniques has given rise to a new paradigm for health care called personalized medicine, in which treatment strategies are tailored to a patient on the basis of his or her individual available biomolecular information—in particular, the concentration of relevant biomarkers. The discovery of new biomarkers can help in both (*a*) the ongoing quest to gain a complete understanding of disease pathways and (*b*) improvement of patient survival rates by enabling early detection of disease. Biomarkers are becoming increasingly important for cancers, neurodegenerative diseases, cardiovascular diseases, autoimmune and inflammatory disorders, traumatic brain injury, inherited disorders, and prenatal screening.

Semiconductor fabrication enables massively scaled multiplexing, allowing one to test for many different biomarkers with high redundancy. Redundancy is essential because the number of measurements is directly correlated to resolution and LOD. Affinity-based electrical biochips may evolve into versatile plug-and-play platforms, where the same technology can be applied to a wide variety of analytes, all based on the probe–target capture model. Additionally, the advantages of low cost, portability, lower sample volume, faster response times, and elimination of labels will ensure that electrical biochips occupy a significant portion of the biomarker detection market.

4.2. An Exciting Path Ahead

As we move into the future, biosensors will tend to move away from heavily equipped research laboratories and toward more distributed use in hospitals, in doctors' offices, at homes, and on or in the patient. Both in vivo and in vitro biosensors will exist for a range of applications. Sampling from breath, skin, or body fluids could be used to categorize the in vitro sensors because the source of the sample can drive the technologies to be used. Long-term in vivo sensors remain a grand challenge; new, flexible sensors that are mechanically compliant with tissues and skin are an important new advance toward in vivo applications. The enormous potential for biosensing capabilities combined with wearable devices could dramatically increase the frequency of diagnostic testing in the near future, which would enable the integration of diagnostics with therapeutics. The seamless

integration of electrical techniques with biological processes seems inevitable, and important progress toward this ambitious and fruitful goal is under way.

DISCLOSURE STATEMENT

R.B. discloses financial interests in Daktari Diagnostics and ElectroCyt, Inc. Both companies are aiming to commercialize electrically based point-of-care diagnostics technologies. R.B. also provides consulting services to Oxford Nanopore Technologies. B.R. discloses financial interests in ElectroCyt, Inc.

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