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Recent Advances and Progress in Development of the Field Effect Transistor Biosensor: A Review

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The vital utilization of biosensors in different domains has led to the design of much more precise and powerful biosensors, since they have the potential to attain information in a fast and simple manner compared to conventional assays. The present review describes the basic concepts, operation, and construction of biosensors and presented an ideology that choice of categorization, selection of immobilization method and advantages are crucial factors for an efficient and commercial biosensor. Amongst various biosensors, the field effect transistor (FET)-based biosensors have shown much more potential and immense advantages such as high detection ability and sensitivity for both neutral and charged biomolecules and, hence, have been explored comprehensively in the present review. This paper discusses the current challenges in device design by mainly focusing on the quantitative and qualitative performance parameters such as sensing surface properties, signal-to-noise ratio and various other factors, since consideration of these factors will eventually address the crucial concerns related to device design and practical limitations. The critical measures to translate the commercialization of biosensors in the market at a high pace have also been discussed. Hence, the discussion on device challenges illustrates that there is a scope of improvement in the areas such as short-channel effects, specificity and nanocavity filling factor for revolutionary advances in FET-based biosensors. Optimal selection of design rules and biosensing material has the potential to feature the next generation of biosensors. The present paper reports that following integrated multidisciplinary approaches and switching to nanotechnology in designing of FET-based biosensors can offer a lot of improvements in the practical key factors (such as low cost and reliability) and opportunities for the biosensors in the marketplace.

Key words: Biosensor, field effect transistor (FET), immobilization, sensitivity, nanocavity, dielectric modulated

INTRODUCTION

After development of the first oxygen enzyme-electrode biochemical-based sensor (in 1962),[1] researchers have brought together the concepts of different fields to develop more reliable, efficient, and sophisticated biosensors. Since then, biosensors have gained much interest in a number of fields such as environmental monitoring, genetic screening, sports surveillance, agriculture, marine sector, food pathogen and adulterant detection, medical diagnostics, and Internet of things (IoT)-based applications.[2,3] However, the application of biosensors is not limited to these domains, but is also

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widely employed in the fields of bioelectronics and biotelemetry.[4] The immense potential of biosensors in sensing a variety of biomolecules in healthcare and the medical field has led to the evolution of newer tools and technologies to provide point-of-care testing (POCT) facilities, especially in remote areas. These sensing components are capable of sensing real-time signals such as release or production of different biomolecules like glucose or lactate.[5] There is no doubt that in the near future, biosensors will play a crucial role in the neurology field in measuring the neurotransmitter activities and receptor functions. The ease of use and low energy consumption in biosensors, in comparison to laboratory-based conventional assays,[3] i.e., laborious techniques such as liquid chromatography and mass spectrometry and ion chromatographic methods, has made it possible to develop biosensors for different applications. For example, glucose biosensors have replaced the traditional Fehiing's method for monitoring saccharification and fermentation.[2] Although conventional methods offer high precision and selectivity,[6] their inability for in situ applications, time-consuming nature (require sample preparation and sample pre-treatment), demands of energy and extra circuitry, high cost, and need of experienced investigators demand an easy solution such as biosensors for detection. Basically, biosensors are constructed to: (1) detect or recognize biological molecules, (2) characterize novel molecular interactions between different biomolecules and enzymes of interest, and (3) quickly identify a component within a target analyte and quantify the abundance as well as scarcity of molecules of interest. A biosensor has three elements: (1) bio-recognition element that can be an enzyme, antibody, or a tissue, (2) a transducer that converts the output of bio-elements obtained in the form of physical quantities (mass, charge) into measurable electrical signals (current, voltage), and (3) a signal processing unit for amplifying and further processing of the signal.[7] In addition, a data acquisition system can also be utilized to digitize the sensor output. The bio-recognition element is in direct contact with the transducer which makes the biosensor a self-contained integrated device for carrying out different quantitative analyses. The construction of biosensors which includes fabricating materials, transducing devices, and immobilization methods demands multidisciplinary research in chemistry, biology, and engineering fields. The usage of different fabricating materials and transducing devices has categorized the biosensors into different subclasses as detailed in the following section.

Categorization of Biosensors

According to various terminologies, biosensors are referred to as immunosensors, biochips, biocomputers, and glucometers.[8] The categorization of biosensors depends upon several approaches such as the nature of the bio-recognition elements, transducers, and detection methodologies. These subclasses of biosensors have been considered as per the requirements of the present review; however, a detailed elaboration about different categories of biosensors can be found in Ref. 6,7 The bio-recognition elements used in biosensors, depending upon their mechanism, can be the bio-affinity and biocatalytic type.[9,10] In the affinity type of biosensors, the receptor/bio-recognition element is weakly bound to the target biomolecule, leading to a physicochemical change that is measured by the transducer. The different bio-recognition elements in bio-affinity sensors can be antibodies, receptor molecules, nucleic acids, and molecules viz. aptamers and biofilms. The antibodies such as antigens can be low-molecular-weight analytes, proteins, and microorganisms, such as drugs, hormones, insulin, and toxins. The biological receptor molecules can be physiological, pharmacological, and toxicological analytes such as nicotine and bungarotoxin. Nucleic acids, such as ethidium, are used for detection of specific sequences and intercalators. On the other hand, biocatalytic types of sensors convert the combination of a biological element and substrate/analyte to an auxiliary substrate and measures the steady-state concentration of the bio-formed/lost during the biocatalytic reactions. It involves elements viz. cells, tissues, enzymes, organelles, and microbes, such as microorganisms, and genetically modified microorganisms. The catalytic elements are reusable, highly specific, and generally speed up the binding and sensing process. Next, the kind of transducer can also define the type of the biosensor, such as electrochemical-based biosensors, optical-based biosensors, and field effect transistor (FET)-based biosensors. The electrochemical and optical biosensors offer high specificity and low detection limits which make them suitable candidates for real-time processing.[11,12] However, the introduction of simpler and potential platforms such as FETs has gained more value due to their high sensitivity and rapid screening and, thus, they are explored in the present paper.

Detection techniques have also categorized the biosensors into two categories: (1) label-based and (2) label-free biosensors. In label-based detection, the term 'label' is a tag given to any foreign molecule attached chemically or temporarily which enhances the number of binding sites to detect the desired analyte.[13] The labels are more easily detectable by electrochemical sensors.[14] Although label-based methods are accurate and have wide detection limits, their usage leads to difficulties in multiplexing and alterations in intrinsic properties of target molecules; for example, an incorrect label can lead to the development of a drug with side effects. In practice, these methods require high assurance that the label does not interfere with the target analyte/probe interaction process and block any important

active site. On the contrary, label-free detection, without any need for a labeling analyte or modification of the sample, investigates the interactions and physical or chemical properties of target analytes by monitoring and relying solely on their intrinsic physicochemical properties such as affinity constants, refractive index, viscoelasticity, dielectric permittivity, charge, and conductivity. Basically, these molecular linking properties bind the target analyte to the biosensor and, thus, this binding is detected by the label-free approach.[15,16] Label-free technology is more advantageous and undergoing greater progress than label-based technology. Some of the advantages are: (1) high throughput, (2) high sensitivity, (3) low consumption of the sample/analyte with minimal damage, (4) high precision and simplicity, (5) easy on-chip integrations, (6) enables use of natural analytes and ligands, and (7) cheaper method compared to label-based (no reagent/label cost, no lab safety/waste disposal cost). Hence, the label-free biosensing method has become a systematic and universal approach for assay development at micro-scale which is highly flexible for remote diagnostics and field applications. Although the commercial presence and detection accuracy of label-free detection make it an ideal choice for biosensing applications, the label-free methods are competing to provide better reliability, high signal-to-noise ratio (SNR), and low instrumentation.

Among the different transducers and label-free biosensors, FET-based biosensors appear to be a suitable choice which has the potential to offer a miniaturized and more commercialized platform in the field of biosensing. Therefore, the current research work has invested more efforts in exploring the FET-based biosensors. Before that, the biosensing process and its different methods employed in the biosensors are discussed in the next subsection, since the coupling of the target biomolecules on the sensor surface is the first step to initiating the process of sensing biomolecules.

Immobilization Techniques

The process of binding the target biomolecules with the receptors or directly with the sensing surface of the device is referred to as immobilization. Immobilization is done to obtain analyte stabilization so that its activity can be retained for longer periods.[17] The immobilization methods exploit multidisciplinary approaches (i.e., input from chemistry, biological, and engineering fields) to couple the biomolecules in a direct or indirect way.[18] The direct method utilizes covalent coupling in which the target molecule is covalently and directly fixed to the FET (transducer) surface through physical or chemical methods. The direct method can immobilize a wide range of analytes such as pure proteins (purity level $> 50\%$). However, the heterogeneous nature of the coupling decreases the binding to the target molecules and reduces the reusability of the sensor surface.[9] In the indirect method, a capture method is utilized in a way that the target molecule is fixed to the carrier first and then covalently coupled to the surface of the sensor. This coupling method is more advantageous than the direct method as the target molecule can be used in an unpurified or crude form, such that regeneration of the sensor surface is possible, there is lower heterogeneity during binding, and target analytes are less likely to be inactivated.[19] Hence, the method is widely used. The commonly used immobilization methods can be physical and chemical in nature.[20] The physical method comprises adsorption which is the oldest and simplest method that involves the binding of biomolecules on the sensor surface with little or no conformational change in the center of the active surface,[6] as shown in Fig. 1a. This technique involves non-covalent and weak binding forces such as van der Waal's forces, hydrogen bonding, and electrostatic interactions between the biosensing analyte and surface.[18] It is a simple and low-cost method, but the stability of the target molecule is poor, and binding forces are dependent on pH, temperature, ionic strength, concentration, and properties of the target molecule. Due to these reasons, this methodology is not adopted widely at present. Moreover, the non-covalent bonding between target analyte and biosensor in adsorption enables the analytes to flood out from the surface of the biosensor. This attenuates the responsivity and, eventually, the life span of the biosensor. In order to overcome these issues, the techniques with chemical bonds are preferred, which includes:

(1) *Covalent attachment* This method involves the development of covalent bonds between the target molecule and the sensor surface,[21] as shown in Fig. 1b. Covalent bonds can be formed by classic amide coupling reactions, or click chemistry. It has wide preference, high stability, minimum diffusion resistance, strong binding, minimal analyte leakage, and is manageable at low temperature (zero degrees). However, the operational procedure is tedious, and the surface is non-regenerable. Moreover, uncontrolled retention of the biomolecule affects the event recognition domain.

(2) *Cross-linking/affinity* The linking of target molecules with the sensor surface is done by using compounds/reagents with two or multiple functional groups, such as glutaraldehyde, which can bind two different molecules under different conditions.[22] As shown in Fig. 2a, the links formed between target analyte and biosensing surface lead to the formation of three-dimensional cross-linked aggregates. This is advantageous as the analyte loss is minimized, but the toxic chemicals lead to harsh treatment of the analyte, and cross-linking can cause significant alterations in the active/binding site.

Although the chemical methods have a long life span, they are complex, time-consuming, and hazardous. To counter this, the entrapment/

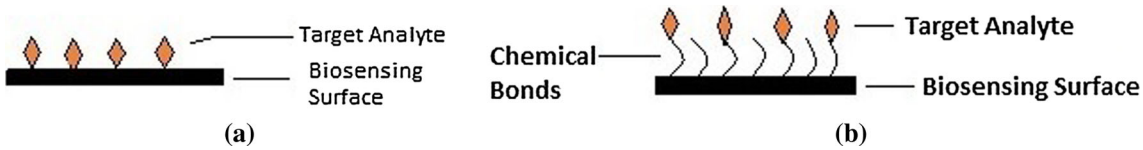

Fig. 1. (a) Adsorption and (b) covalent attachment of a target analyte on the biosensing surface.

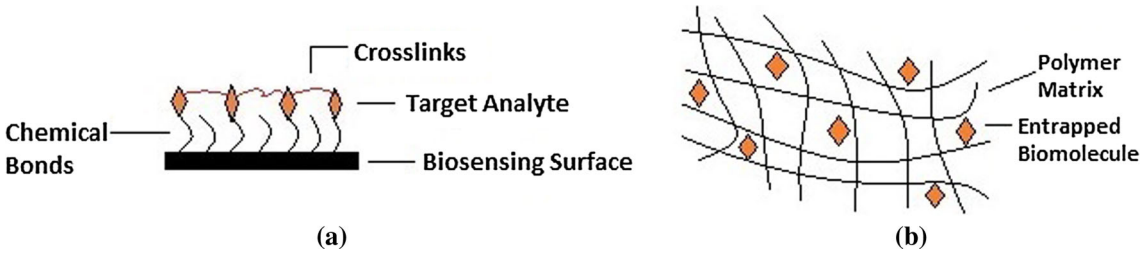

Fig. 2. (a) Cross-linking between the target analytes on the biosensing surface, (b) entrapment of biomolecules within the polymer matrix.

encapsulation method combines all the advantages of physical and chemical methods and eliminates their drawbacks. In the entrapment immobilization method, the target molecules are not directly coupled to the sensor surface, but restricted or caged by covalent or non-covalent coupling within the layers of a polymer matrix or a membrane in such a way that the matrix allows substrate penetration while retaining the desired molecules.[23,24] For example, in a galactose biosensor, the galactose oxidase is entrapped into a polyvinyl formal membrane. A more detailed description of these techniques can be easily found in many of the review studies.[17,25] The method is shown diagrammatically in Fig. 2b. It is a cheap method with minimal loss of analyte activity and maintains biomolecule integrity upon immobilization. However, it has a higher response time and a diffusion barrier (i.e., substrate cannot be diffused deep into the polymer matrix). This method needs advances which can avoid enzyme leakage into the surrounding regions, and mass transfer resistance and improvement in the structure of polymer matrix to allow deep penetration of substrate.

The life span, performance, sensitivity, selectivity, efficiency, structure, and commercial certainty of a biosensor depend upon the right choice of immobilization technique. Selection of the most appropriate and strategic type of immobilization method depends upon: (1) compatibility between the bioreceptor and immobilization method, (2) the nature of the target biomolecule, (3) transducer type, (4) application, (5) cost, (6) reproducibility and difficulty of the process, and (7) other trade-off parameter considerations such as maximum sensitivity or maximum stability. In some cases, even the combination of different immobilization methods has also been implied for enhancing selectivity, activity, sensitivity, and stability of the sensor. For example, an analyte can be pre-immobilized by physical adsorption or covalence before final immobilization by entrapment in a polymer (e.g., a gel). One thing which needs to be ensured is that the selected method should not interfere with target molecule's activity or block any of its active site. Thus, the choice needs to be done wisely such that the chosen method avoids loss of molecular activity and does not change the nature of the target molecule at the binding site.

In the following sections, the present paper has overviewed the FET biosensors and highlighted the progress of FET-based biosensors. The quantitative and qualitative parameters affecting the performance and design of biosensors have been discussed. Furthermore, the current challenges listed in the existing design methodologies have opened numerous platforms for optimizing the design rules of FET-based biosensors. It has reflected the areas that need to be worked upon in order to endow a FET-based biosensor with much more standardization and commercialization.

FET-BASED BIOSENSORS

FET-based biosensors are suitable candidates for a variety of transducers in the label-free category. They have received much more recognition in recent years due to their vital advantages such as good scalability, ultrasensitivity, rapid real-time detection, inherent amplification, lower power requirements, direct electrical readout, and mass production at inexpensive rates in comparison to surface plasmon resonance, microcantilever sensors, fluorescence devices, and other methods.[2,26] Moreover, the availability of mature manufacturing techniques such as the complementary metal-oxide semiconductor (CMOS) process further provides the advantage of miniaturizing, parallel sensing and allowing integration with other circuits and systems which is important for practical development of biosensing devices. It is widely preferable in case the target biomolecules carry electrostatic charges

or bioactivities which changes the electrostatic potential of the device.

Basic Operation

A FET-based biosensor has three electrodes: source, drain, and gate such that the region between the drain and source acts as a biological recognition element that interacts with the target analyte/biomolecules and senses their presence, concentration, and electrical activity. The biosensor then directly transforms the biological information into a measurable signal.[27] Afterward, depending on the application, the obtained signal can be displayed, amplified, stored, and processed or sent to the cloud for further operations.[28] The operation of an FET-based biosensor can be summarized as[29,30]: (1) A change in the concentration of the analyte leads to change in the charge near the sensor interface (given as $d\rho$); (2) This shift in the charge induces a change in effective gate voltage (given by dV_{EG}; (3) This difference in effective gate voltage leads to changes in the drain current (given by dI_D), which can also be evaluated from the I–V characteristics. Mathematically, the sensitivity of the sensor can be represented in the form of an equation given as:

$$\underbrace{\frac{dI_D}{I_D}}_{\text{Sensitivity}} = \underbrace{\left(dc.\frac{d\rho}{dc}\right)}_{\text{i}} \cdot \underbrace{\left(\frac{dV_{EG}}{d\sigma}\right)}_{\text{ii}} \cdot \underbrace{\left(\frac{dI_D}{dV_{EG}}.\frac{1}{I_D}\right)}_{\text{iii}} \quad (1)$$

Here, dc represents an infinitesimal change in the analyte concentration, $d\rho$ represents an infinitesimal change in the charge density at sensor's surface, dI_D represents the change in drain current value on recognition of target analyte, and $I_{D\ is}$ the drain current in a steady state (when the sensor surface is exposed to a reference/blank sample). It has been reported that detection using electrical FETs is based on charge interaction and the permittivity shift effect.

Background and Progress

In the 1970s, an ion-sensitive FET (ISFET) was developed as the first electrical FET sensor based on the charge interaction effect (i.e., detecting charged molecules) for pH measurement (H^+ ion concentration).[31] The ISFET utilizes a metal oxide semiconductor (MOS) with one physical difference in the metal gate electrode which is replaced by a series combination of a dielectric layer, electrolyte, and reference electrode. Basically, it consists of a silicon substrate (say p-type) with two n-doped regions (i.e. source and drain) which are separated by a short channel that is covered by the gate dielectric layer which acts as a sensing membrane, as shown in Fig. 3. The metal gate replaced by a dielectric layer can be a single layer of SiO_2 or double layer of SiO_2-Si_3N_4, or SiO_2-Ta_2O_5.[19,32] In the first reported

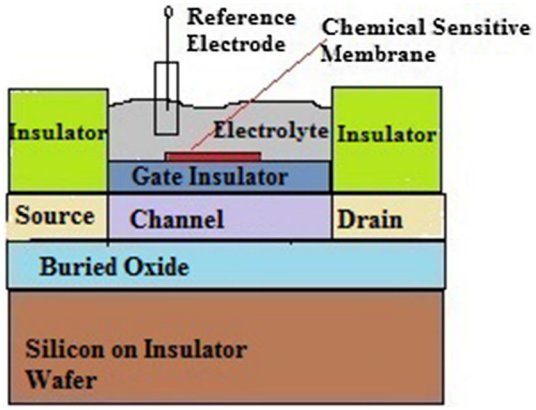

Fig. 3. Basic schema of an ISFET biosensor with a chemical-sensitive membrane deposited on the gate insulator.

ISFET sensor, SiO_2 was used as the gate insulator/dielectric layer.[33] In comparison to a single layer, the double layer provides more stability, current performance, sensitivity, and selectivity. The functioning of ISFET-based sensors is based on the concept of modulation of the drain current due to the supply of positive bias potential at the ISFET's gate terminal which is generated upon sensing the specific ion concentration. The change in amount of drain current can be converted to the concentration of the specific ions. It is clear that an ISFET integrates the sensing surface and an amplifier in one device to provide an output with high current and low impedance value. In literature, more than hundreds of publications, on different platforms viz. international biomedical research, biotechnology letters, sensors, biosensors, and bioelectronics, have explored the ISFET-based bioelectronic sensors.

In 1978, Cheung introduced the immunoFET concept (a label-free, direct electrical immuno-sensing ISFET) in which the gate electrode is modified by stabilizing charged molecules viz. antibodies/antigens on it for direct modulation of the ISFET's drain current.[34] For example, Caras and Janata utilized an ISFET for detection of penicillin.[35] It has many advantages like faster response time, good amplification properties, cost-effectiveness, robustness, multifunctional nature, and can be easily integrated with electronic devices such as a metal oxide semiconductor FET (MOSFET).[36] However, these devices suffer from some issues such as low sensitivity (due to formation of an ionic double layer on exposure of the channel to a solution), poor differentiation between weakly charged and neutral biomolecules, and transistor breakdown while sensing chemicals that prohibit their wide commercialization. In contrast, dielectric-modulated (DMFET) devices[32,37] are capable of detecting both charged as well as neutral biomolecules in even a small volume of sample with high detection sensitivity and fast screening. Its working principle is related to the high responsiveness to the dielectric constant of the

biomolecule as well as the charge effect, i.e., charge carried by biomolecules at the Si-SiO_2 interface, such that upon introducing negatively charged biomolecules (say DNA) into the cavity, the charge effect dominates over the dielectric constant effect. On the contrary, the dielectric constant affects the device parameters, such as sensitivity, in case of neutral biomolecules (for example, biotin-streptavidin binding).

In a DMFET, the concept of utilizing the nanogap as the biosensing material has attracted much attention with its fast, less time-consuming, low cost, and real-time detection nature. The gap, in order of nanometers, formed between the two electrodes (i.e., gate and gate oxide) by deposition of a stack of metal and dielectric layers is referred to as the nanogap in biosensors. On the basis of gap formation, the biosensing devices can be categorized as planar and vertical nanogap devices. The devices with a planar nanogap (both the electrodes are horizontally separated by 50–100 nm), formed using techniques such as lithography, are not capable of providing high throughput and, therefore, are less preferred. On the contrary, the devices with a vertical nanogap, formed using thin-film deposition or wet-etching techniques, has vertically situated electrodes. The vertical nanogap devices are widely preferred due to their low cost, high sensitivity, more uniform electric field, and controlled thickness.[38] The DMFET device, as shown in Fig. 4a and b, has a vertical nanogap positioned at the gate dielectric edge for sensing the target biomolecules. This vertical cavity is basically formed between the gate and gate oxide (say SiO_2), at both sides (i.e., source and drain junction) to fill in the target analytes. The selection of cavity length is a crucial factor as it affects the device parameters (drain current sensitivity, responsivity, electric field, and trans-conductance). The nanocavities provide efficient immobilization and are extremely sensitive, since the nanomolecules such as DNA, proteins, or amino acids occupy a reasonable percent of volume in the nanocavity.[39] The biomolecules confined in the nanogap can modulate the electrical properties at the device junction, such as impedance, resistance, capacitance, and charge field effect. The nanogap concept has introduced numerous signal transduction technologies in FET-based biosensors. Moreover, nanogap-based devices are advantageous in comparison to electrochemical deposition-based devices which requires more complex fabrication processes.

The absorption of biomolecules leads to modification of the electrostatic coupling between the gate and channel. After the absorption, the enhanced cavity length increases the threshold voltage in comparison to the air-filled cavity ($\Delta V_{TH} = V_{TH}$ (biomolecule-filled) $- V_{TH}$ (air-filled)). Thus, the changes in the threshold voltage of the biosensing device can detect the presence as well as the orientation of the biomolecules in the cavity. The specific binding without any label as well as presence of the biomolecules in the nanogap (either fully or partially filled) can be detected by tracing the changes in the threshold voltage.[39,40] For example, the shifting of threshold voltage towards a positive side marks the presence of the negative charges in the cavity. This shift in threshold voltage (ΔV_{TH}), providing the DMFET device sensitivity, is a function of two effects: (1) charge of target analyte (not in the case of a neutral analyte) and (2) modulation effect of the gate dielectric. The target analyte can be a negatively charged biological species such as bacteria and DNA or positively charged molecules such as glucose oxidase (GOx) or charge-neutral entities such as viruses and proteins. The ΔV_{TH} can be expressed in the form of an equation given as:

$$\Delta V_{TH} = \frac{Q_{TA}}{\frac{1}{C_{TA}} + \frac{1}{C_{OX}} + \frac{1}{C_A}} \tag{2}$$

where Q_{TA} is the charge of the target analyte, C_{TA} is the capacitance of the target analyte ($\epsilon_A \epsilon_{TA}/l_{TA}$), C_{OX} is the capacitance of oxide ($\epsilon_A \epsilon_{OX}/l_{OX}$), C_A is the capacitance of air (ϵ_A/l_A), ϵ is the dielectric constant, and l is the thickness of the target analyte, oxide, and air. The n-channel DMFET can identify neutral biomolecules or biomolecules bearing positive charges, whereas a DMFET with p-type channel can detect negatively charged biomolecules. The

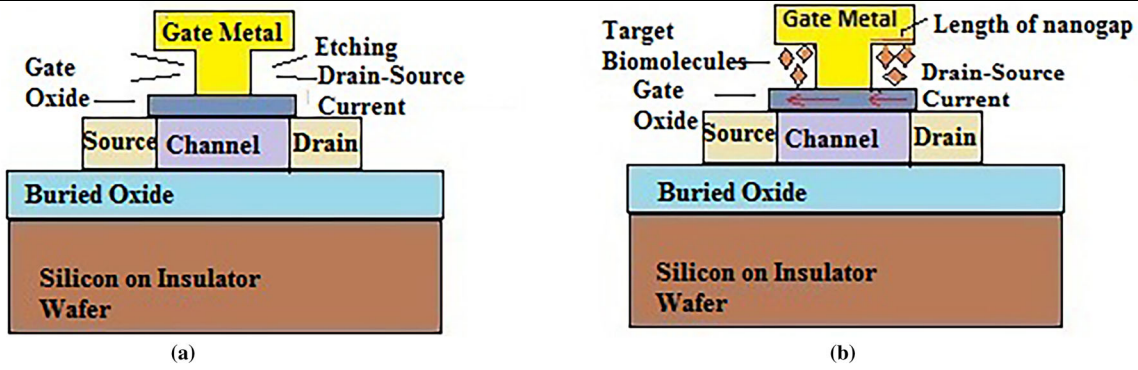

Fig. 4. Structure of DMFET biosensor reflecting (a) nanogap etching, and the (b) nanogap-filling phenomenon.

shift in V_{TH} is not collaborative due to both effects in the *n*-type channel, whereas it is in the same direction for a *p*-type channel. This makes a *p*-channel DMFET highly sensitive and, thus, more preferable than an *n*-channel DMFET.

Current Scenario

The advancements in the field of FET-based biosensors are undergoing revolutionary changes and leading to a wide range of FET-based biosensors to overcome the problems of electrochemical and optical biosensors. Although electrochemical sensors offer miniaturization, low cost, and portability, the requirement of a reference probe in the circuit poses problems.[41,42] Optical biosensors offer label-free detection, but the apparatus complexity and expensive fabrication process have led to the development of more simple options such as quartz crystal microbalance, or FET-based biosensors. With the growth in technology the improvement within the field of FET-based biosensors has led to development of extended-gate FETs (EG-FETs), DMFETs, nano-wire FETs, tunneling FETs (TFETs), organic FETs (OFETs), and organic electrochemical transistors (OECTs). For example, the issue in MOSFET-based biosensors is that their response time cannot be improved further, since the subthreshold slope (SS) is very high (60 mv/decade). The TFET-based biosensors, based on the band-to-band tunneling mechanism, has resolved this issue as their SS value is low (i.e. 37 mV/decade) and can be easily reduced further.[43] The EG-FET with usage of aptamers has enhanced the detection sensitivity without any need for optical assistance and shown suitability in the clinical diagnostics field.[44] The DMFETs have better performance compared to other FET-based biosensors. Some of the improved figures of merit are: (1) high sensitivity and specificity, (2) high SNR, low-cost fabrication, and fast screening, (3) more compatibility with conventional CMOSs and adaptability to read out circuits for on-chip integration, and (4) more design flexibility. Among DMFETs and dielectric-modulated tunneling FETs (DMTFETs), the DMTFETs have better sensitivity and lower SS and, thus, are widely employed in biosensing applications.

In addition to the novel inventions, the different combinations of sensor structure and sensing materials have resulted in a myriad of FET-based biosensors. The structural design variations in FET biosensors have enhanced the sensor performance as well as device parameters and extended the application field. For example, a charged-plasma base gate underlap dielectric modulated junctionless TFET (DM-JLTFET) has the potential to provide superior sensitivity and low cost for developing biomedical sensors.[45] The different properties of the materials such as high charge mobility or mechanical strength have also added diversity into the field of FET biosensors. The graphene FET-based biosensors, extensively covered in Ref. 46, offer high throughput and wide detection range along with other exceptional properties such as high carrier mobility and optical transparency. Similarly, the nanowire FETs also offer a broad limit of detection with high sensitivity. Among the organic conducting materials, the OFET and OECT biosensors have provided the possibility of integration with flexible electronics and wearable devices and, thus, are called electronic sensors.[41,42] For example, a sweat sensor which measures ion concentration in human sweat for healthcare monitoring applications is designed using an OECT.[47] Although the response time is slow in OECT devices, their operation stability at low voltages (< 1 V), wide detection limits (up to femtogram), and compatibility with aqueous environments such as sweat or tears has enhanced their application in bioelectronics and biosensors. The sensitivity is highly improved in an OECT, since it can amplify the small input changes. The application of OECTs is not limited to biological sensing, such as cell monitoring, but has extended to the neural recording platform which is beneficial for brain–machine interfaces.[48] The capability of delivering a label-free response by using a simple electronic read-out set-up and employing printed circuit technologies gives an edge to OFETS over OECTs and optical biosensors.[49] The features such as electronic nature of output signal, high sensitivity level, high integration flexibility, low-cost fabrication, and disposable strip-type sensing systems make OFETs suitable and the biggest strength for POCT applications. Altogether, due to immense advantages of FET-based biosensors, they have become competitive candidates for POCT applications compared to bulky optical-based in vitro diagnosis (IVD) instruments. This suggests that the current efforts are more directed and active towards incorporating nanotechnology by exploiting different nano-materials and nano-structures for miniature and highly efficient FET biosensors. The optimal selection of structural design and material has the potential to feature the next generation of semiconductor devices and create opportunities for the biosensing devices on wearable devices.

Research in the domain of FET-based biosensors with a focus on integrating multidisciplinary approaches (for example, combining knowledge of science with technology and engineering fields) has opened a gateway for a wide range of novel sensor architectures. The multidisciplinary efforts, unlike conventional specialties, lead to much more innovative development of biosensors. The integration of knowledge from multiple interdisciplinary platforms have the potential to accelerate the growth of biosensors which can revolutionize various fields such as biomedical, military, and industry. However, the practical implementation of biosensors lack in generalized ideology of the structure, systematic assessment, and proper evaluation due to

variations in the FET structures in terms of dimensions, shape, and size of nanogap, target analyte properties, sensing component, and testing environment. These unsolved hurdles are the barriers to technical maturation and commercial acceptance of the biosensors. Moreover, a common "design rule" for FET-based sensors has not been established yet. Once the performance parameters of the device as explained in the following section are optimized, one can design highly efficient and reliable biosensors.

Challenges in Device Design for High Performance and Future Scope

FET-based biosensors have shown quantitative analysis of biomolecules, high sensitivity, and fast response. Nevertheless, great challenges remain to construct FET-biosensors for laypersons with features such as miniature size, low cost, portability, simple operation, commercial availability, regenerability, and reliability in addition to optimized sensitivity and stability. The major areas which demand improvement and development are:

Quantitative and qualitative performance metrics First, the validation of both qualitative and quantitative figures of merit is an essential step to prove the capability of biosensors in processing the data. In order to benchmark the device structure, ensure high performances, and, eventually, the sensitivity (effective change either in threshold voltage or drain current, on adsorption/sensing of the target biomolecules[50]) of the device, it is mandatory to work more on the parameters as discussed below:

(1) *Short-channel effects (SCEs)* SCEs deeply affect the performance of sensing devices, i.e., sensitivity,[51,52] and are more severe in thicker oxide layers. SCEs decrease the threshold voltage and drain current characteristics of FET biosensors due to its principal phenomenon of thermionic emission which results in low sensitivity of the device. In order to overcome SCEs:

- Many device engineering approaches have been employed such as gate dielectric engineering or asymmetric channel doping.
- Various alternative structures have been proposed such as embedding a channel deep into the substrate, or developing a silicon-on-insulator (SOI) FET.

(2) *Immobilization technique* The immobilization technique needs to be chosen such that the selected method should generate a robust and reliable sensor surface that has low absorption, high binding efficiency, and high specificity/selectivity of target analytes. The properties of the sensor surface decide the method of immobilization that can be employed for better operational stability. The selected method should enhance the uniformity, density, stability, reusability, and distribution of the sensing surface. The usage of polymers and nanomaterials has the potential to achieve immobilization of analytes, allowing high sensitivity and high detection limit of the sensing device. Thus, selection of the right immobilization technique can help in commercializing cheap and efficient FET biosensors.

(3) *Specificity* The specificity, or selectivity, is a characteristic that is equally important as sensitivity to characterize the biosensor structure. The absorption of the biomolecules on the sensor surface (i.e., channel) changes the device characteristics. Moreover, it has been reported that the unmasked channel leads to absorption of the target biomolecules, thus leading to interference of the non-required biomolecules, i.e., non-specificity. In order to achieve high specificity, the channel should either be functionalized directly with specific receptors/linkers or masked with any polymer/dielectric layer with the same dielectric constant as that of the target biomolecule to enable functionalization. The high value of surface specificity broadens the range of biological molecules and pathways for investigation. Thus, it provides a bigger and better platform for understanding the biological interactions.

(4) *Signal-to-noise ratio (SNR)* SNR refers to the ratio of the actual signals transduced from target biomolecules to the noise signals from non-target biomolecules. Basically, SNR reflects the fidelity and depends upon the impedance matching between different subsystems of FET-based biosensors.[7] If the noise signal is generated from the testing environment, then it is called signal-to-background ratio (SBR = $\Delta I_D/I_D$, where ΔI_D is the change in drain current before and after adding the target biomolecules, and I_D is the drain current before adding bio-targets).

(5) *Nanocavity filling area (N_{FA})* The sensitivity and performance of the FET-based biosensor depends upon the nanogap capacity and its filling criterion.[50] Despite the shape and size of target biomolecules, the device analysis is generally carried out with an assumption that the nanocavity is fully filled by the biomolecules or air. Even after this assumption, many vacant spaces remain in the cavity region of the devices, thereby affecting sensitivity. The N_{FA} can provide the nanocavity area actually filled by the biomolecules and computed by using the following equation:

$$N_{FA} = \frac{P_{FA}}{F_{FA}} \times 100 \quad (3)$$

where N_{FA} represents nanocavity filling area, P_{FA} is partially filled area, and F_{FA} is fully filled area. As shown in Fig. 5, there can be different ways in which a fully filled or partially filled nanocavity can be characterized, i.e., uniform and non-uniform way.

With a fully filled cavity, the device is more sensitive to charge density as well as dielectric constant, since sensitivity is positively correlated

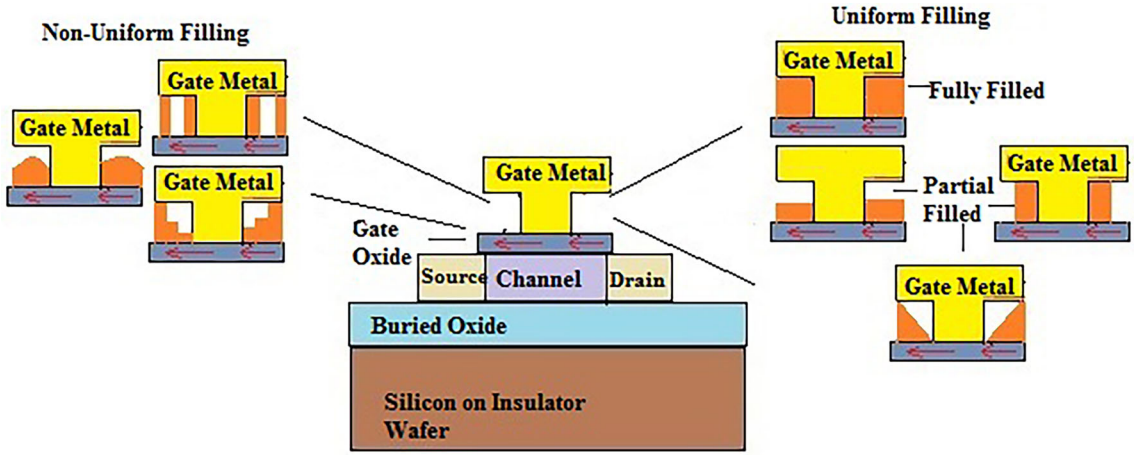

Fig. 5. Typical structure of a DM-FET biosensor reflecting different ways of non-uniform and uniform filling of the nanocavity.

with these two effects. If a partially filled nanocavity is considered, factors such as orientation/position (horizontal or vertical) of the biomolecules and variation in surface coverage affect the sensing performance and response of the device. On comparing the surface potential, it is found that surface potential is more effective in case the cavity is filled horizontally in comparison to when the cavity is filled vertically. However, the surface potential is higher in a fully filled cavity in comparison to partially filled cavity. Similarly, the change in other electrical parameters such as drain current and subthreshold slope shows low sensitivity in the case of a partially filled cavity. From a construction point of view, the usage of a doping process while forming nanogaps can also enhance and improve the sensitivity and other performance metrics.

(6) *Regeneration* Most of the sensors are for one-time use only, which limits the reproducibility as well as the reliability of the sensors. The biosensors with poor regeneration ability are even less demanded in real-time applications. For example, in the medical field, a biosensor that does not need to be replaced after every use is highly desirable. In order to enhance the sensitivity of the biosensor, regeneration is a mandatory step. The process of regeneration refers to the restoration of the sensing surface to its original state (before the analysis of target analyte). The regeneration of the sensor surface depends upon the immobilization technique employed: in the case of direct immobilization, the bound analyte will be removed, and in the case of capture method-based immobilization, the target as well as bound analyte will be removed during the regeneration process. Washing procedures and regenerating buffers can also be employed in the regeneration process. The requirement and selection of appropriate buffers depends upon various conditions such as[52]: (1) strength of the interaction; (2) type of interaction between the bio-recognition element and the target biomolecule (such as covalent coupling, or hydrogen bonding, or electrostatic forces); (3) type of target biomolecule such as antibodies or small molecules; (4) sensitivity of biomolecules to the environmental conditions, for instance, the sensitivity of antibodies to changes in pH leads to dissociation of the bounded biomolecule; (4) compatibility of the bio-recognition element and target molecule, for example, interchanging these two elements can lead to improved reusability. Previous studies have utilized a gentle protein elution buffer and NaOH solution to strip away the bound as well as background biomolecules from the biosensor surface.[53,54] However, if the dissociation rate of the target analyte is very fast, then the sensing surface can be restored by normal washing without any need for buffers. In order to make the regeneration process more efficient, or nearly ideal, a number of conditions need to be met. The conditions are: (a) the response of same analyte needs to be captured over multiple regeneration cycles, (b) the device stability, compatibility, and regeneration are tested by comparing the output current value with initial baseline current value.

(7) *Dynamic properties of target biomolecule* The target biomolecules can range from picometer to nanometer, and the biosensors are highly sensitive and responsive to detect even a single molecular interaction. More efforts are made in confining the target species of nanosize for enhancing the sensitivity, selectivity, and performance metrics of biosensors. The extensive properties of nanomaterials such as high specific surface area, increased aspect ratio, and high stability (chemical and thermal) can lead to the development of novel FET biosensors. For example, miRNA, a single-strand RNA with 20–25 nucleotides, has the potential to find the diagnostic and prognostic biomarkers of neurological disorders, and diseases such as diabetes and cancer. Also, contaminated target analytes, the presence of other charges near the target analyte, and non-targeted analytes on the sensor surface affect the sensitivity. The integration of different types of materials with heterogeneous

structures can bring out new possibilities in developing biosensors with simultaneously high flexibility, portability, and sensitivity.

The charge of the target biomolecules also plays an important role in determining the sensitivity and responsivity. The absorption of negatively charged biomolecules enhances the sensitivity, whereas the absorption of positive charges diminishes the sensitivity. In case of positive charges (with low-k values), the depletion layer width narrows down in the channel, and the gate electrode reduces its control over the channel. In order to enhance the gate controllability and responsivity, the positive charges with high dielectric values need to be considered for biosensing. Moreover, the high dielectric values also reduce the side effects of positive charges.

(8) *Biological sensing surface* The biosensor materials play an important role in deciding the sensor miniaturization, power consumption, and performance metrics. The surface needs to exhibit high specificity, stability (under normal storage conditions), and low variations between the analysis and evaluation. Depending upon the element/material incorporated in the biological sensing surface, a wide range of target materials can be recognized. For example, carbon nanotubes (CNTs) can sense proteins, enzymes, antibodies, viruses, short peptides, and nucleic acids.[55] The sensing surface can improve the performance of the biosensor by enhancing some features such as the use of CNTs to improve the enzyme adsorption and immobilization. The approaches for scaling and preparation of the nano-materials as elements in signal transduction are progressing, but their reproducibility and economic acceptability are still in initial stages.

There needs to be a consensus on these performance parameters for comparing the proposed devices with other available biosensors in order to benchmark the device design. In the future, along with these figures of merit, the consideration of structure aspect is very critical, as a properly designed biosensor would not affect the device sensitivity. For example, the sensitivity can be enhanced by: (1) opting for TFET-based biosensor structures with subthreshold swing steeper than 60 mV/dec, or (2) efficient surface immobilization, or (3) varying geometrical parameters such as reduction in the diameter of nanonowire which improves the g_m/I_d ratio, which in turn will raise sensitivity.

Commercial viability The next biggest challenge is the cost factor, since the adoption and wide acceptance of FET biosensors for most applications in every domain (especially in pharmaceuticals) depends upon the cost of the device. The challenging gap between the innovative research ideas at the academic level and commercialization is due to the cost factor. The label-free methodology offers a tremendous advantage of reduced cost over label-based detections. The cost parameters depend upon: (1) transducer, (2) packaging, (3) testing, (4) manufacturing, (5) additional reagents, (6) temperature control requirement, and (7) industrial processing techniques. Thus, the primary concern for commercialization needs to be the traditional bottom line that is developing low-cost biosensors, while improving some of the performance metrics. Possible ways to reduce the cost of the biosensors can be:

(1) *Miniaturization of the device* The use of nanostructured materials and design approaches can miniaturize the biosensors and reduce the cost due to fewer requirements of much energy, materials, and efforts. For example, the use of silicon nanowire, graphene, and other two-dimensional materials in FET biosensors[56,57] has not only reduced cost but also provided attractive features such as miniaturization, lightweight, and multiplexed detection.

(2) *Usage of organic materials* Implementation of organic materials in forming a transducer by replacing the channel dielectric layer with polymer can yield low cost due to reduced fabrication costs, power consumption, simplicity, and high flexibility. Organic thin-film FETs[58] have provided an alternative and a possible solution for cost-effective biosensors.

(3) *Regeneration and mass production* Regeneration and mass production of standardized sensors by utilizing inexpensive methodologies and materials also offers a hand in making biosensors cost-effective. Henceforth, a multidisciplinary team comprising chemists, engineers, biologist, and technicians is needed to address the sensor development from the very beginning in order to design and manufacture a biosensor which is cheap, portable, and capable of being used by semi-skilled operators.

Apart from the cost factor, the practical issue such as poor reliability (i.e., quality assurance during fabrication at large scale and homogeneity) also hinders the commercialization of FET biosensors and needs to be resolved. Characteristics of Si-SiO_2 interface directly contribute to the reliability of a biosensor. For example, damages such as hot carrier-induced damage or stress-induced damage at the Si-SiO_2 interface can degrade the reliability of a biosensor. Some of the issues occurring within the oxide such as traps, fixed charge, or defect generation also lead to poor reliability and poor optimization of the device. The parameters discussed above can enhance the performance and reliability of the biosensor. In addition, structural changes such as extended-gate FETs can improve the reliability by providing a metal sensing layer on the sensor surface.

The recommended changes, if implemented, can provide better opportunities for development of a new generation of the FET-based biosensor technologies. These biosensing modalities will become mature and beget scientific and commercial success. Moreover, improving the biosensors would benefit the industries as well as consumers.

CONCLUSION

The striking advantages and diverse usage of biosensors in dynamic real-time applications in comparison to conventional assays highlight the potential of biosensors in the practical domain. Selection of the detection methodology, and choice among different immobilization methodologies, which depends upon the type and traits of the sensor surface, can lead to the development of efficient and commercial biosensors. One of the candidates in the label-free approach employing efficient immobilization by using a nanocavity is the FET-based biosensor. The progress achieved in FET-based biosensors to date is a result of integrated multidisciplinary approaches beyond the conventional efforts. However, the overall progress in FET biosensors is modest due to factors such as SCEs, regeneration, N_{FA}, and specificity. These factors, in addition to cost and reliability, need to be improved to enhance the sensing performance as well as to benchmark the design of FET-based biosensors. The assistance of nanotechnology (such as graphene) and organic materials in FET biosensors can reduce the cost factor, and facilitate production of commercial miniaturized devices. Thus, the present paper has made an attempt to provide the challenges as well as recommendations that can assist the upcoming researchers who want to reconnoiter in the field of biosensors. In the near future, point-of-care diagnostics can create a revolution in the healthcare domain if the solutions related to commercial cost and reliability factor of FET biosensors gets adopted practically and widely.

REFERENCES

1. C.L. Clark Jr, and C. Lyons, *Ann. N.Y. Acad. Sci.* 102, 29 (1962).
2. P. Mehrotra, *J. Oral Biol. Craniofac. Res.* 6, 153 (2016).
3. S. Patel, R. Nanda, S. Sahoo, and E. Mohapatra, *Biochem. Res. Int.*, 3130469 (2016).
4. J.C. Dutta and S. Roy, *Am. J. Biomed. Sci.* 3, 176 (2011).
5. B.N. Giepmans, S.R. Adams, M.H. Ellisman, and R.Y. Tsien, *Science* 312, 217 (2006).
6. C.S. Pundir, S. Lata, and V. Narwal, *Biosens. Bioelectron.* 117, 373 (2018).
7. S.K. Arya, A. Chaubey, and B.D. Malhotra, *Proc. Indian Natl. Sci. Acad.* 72, 249 (2006).
8. S. Cheng, K. Hotani, S. Hideshima, S. Kuroiwa, T. Nakanishi, M. Hashimoto, and T. Osaka, *Materials* 7, 2490 (2014).
9. H. Du, C.M. Strohsahl, J. Camera, B.L. Miller, and T.D. Krauss, *J. Am. Chem. Soc.* 127, 7932 (2005).
10. A. Hasan, M. Nurunnabi, M. Morshed, A. Paul, A. Polini, T. Kuila, and A. A. Jaffa, *BioMed. Res. Int.* 2014, 18 (2014).
11. P. Damborský, J. Švitel, and J. Katrlík, *Essays Biochem.* 60, 91 (2016).
12. T. Osaka, M. Datta, and Y. Shacham-Diamand, *SSBM* (2009).
13. A. Syahir, K. Usui, K.Y. Tomizaki, K. Kajikawa, and H. Mihara, *Microarrays* 4, 228 (2015).
14. D.A. Hall, J. Ptacek, and M. Snyder, *Mech. Ageing Dev.* 128, 161 (2007).
15. S. Ray, G. Mehta, and S. Srivastava, *Proteomics* 10, 731 (2010).
16. E. Stern, A. Vacic, N.K. Rajan, J.M. Criscione, J. Park, B.R. Ilic, and T.M. Fahmy, *Nat. Nanotechnol.* 5, 138 (2010).
17. A. Sassolas, L.J. Blum, and B.D. Leca-Bouvier, *Biotechnol. Adv.* 30, 489 (2012).
18. C. Liu, C. Xu, N. Xue, J.H. Sun, H. Cai, T. Li, and J. Wang, *MEMS Sensors-Design and Application*, ed. S. Yellampalli (Rijeka: IntechOpen, 2018), p. 49.
19. R. Halai and M. Cooper, *Label-Free Biosensor Methods in Drug Discovery*, ed. Y. Fang (New York, NY: Humana Press, 2015), p. 3.
20. A. Poghossian and M.J. Schöning, *Electroanalysis* 26, 1197 (2014).
21. J. Haccoun, B. Piro, V. Noel, and M.C. Pham, *Bioelectrochemistry* 68, 218 (2006).
22. S. Singh, P.R. Solanki, M.K. Pandey, and B.D. Malhotra, *Sensors Actuat. B: Chem.* 115, 534 (2006).
23. S.K. Sharma, R. Singhal, B.D. Malhotra, N. Sehgal, and A. Kumar, *Biotechnol. Lett.* 26, 645 (2004).
24. S. Datta, L.R. Christena, and Y.R.S. Rajaram, *Biotech* 3, 7932 (2013).
25. B. Brena, P. González-Pombo, and F. Batista-Viera, *Immobilization of Enzymes and Cells*, ed. J.M. Guisan (Totowa, NJ: Humana Press, 2013), p. 15.
26. Y.C. Syu, W.E. Hsu, and C.T. Lin, *ECS J. Solid State Sci. Technol.* 7, Q3196 (2018).
27. S. M. Sze, and K.K. Ng, (Wiley, 2006).
28. M. Kaisti, *Biosens. Bioelectron.* 98, 437 (2017).
29. K. Shoorideh and C.O. Chui, *IEEE Trans. Electron. Dev.* 59, 3104 (2012).
30. X. Chen, Z. Guo, G.M. Yang, J. Li, M.Q. Li, J.H. Liu, and X.J. Huang, *Mater. Today* 13, 28 (2010).
31. P. Bergveld, *IEEE Trans. Bio-med. Eng.* 5, 342 (1972).
32. P. Bergveld, *Sensors Actuat. B: Chem.* 88, 1 (2003).
33. B. Palan, F.V. Santos, J.M. Karam, B. Courtois, and M. Husak, *Sensors Actuat. B: Chem.* 57, 63 (1999).
34. P.W. Cheung, *Theory, Design and Biomedical Applications of Solid State Chemical Sensors* (Boca Raton: CRC Press, 1978), pp. 165–173.
35. S. Caras and J. Janata, *Anal. Chem.* 52, 1935 (1980).
36. M. Yuqing, G. Jianguo, and C. Jianrong, *Biotechnol. Adv.* 21, 527 (2003).
37. H. Im, X.J. Huang, B. Gu, and Y.K. Choi, *Nat. Nanotechnol.* 2, 430 (2007).
38. A.K. Okyay, O. Hanoglu, M. Yuksel, H. Acar, S. Sülek, B. Tekcan, and M.O. Guler, *Microsyst. Technol.* 23, 889 (2017).
39. C.H. Kim, C. Jung, K.B. Lee, H.G. Park, and Y.K. Choi, *Nanotechnology* 22, 135502 (2011).
40. C.H. Kim, C. Jung, H.G. Park, and Y.K. Choi, *Biochip J.* 2, 127 (2008).
41. L. Torsi, M. Magliulo, K. Manoli, and G. Palazzo, *Chem. Soc. Rev.* 42, 8612 (2013).
42. D. Sarkar, and K. Banerjee, in *70th Device Research Conference IEEE* (2012), p. 83.
43. T. Goda and Y. Miyahara, *Biosens. Bioelectron.* 45, 89 (2013).
44. R. Narang, M. Saxena, R.S. Gupta, and M. Gupta, *IEEE Electron. Device Lett.* 33, 266 (2011).
45. G. Wadhwa and B. Raj, *J. Electron. Mater.* 47, 4683 (2018).
46. M. Donnelly, D. Mao, J. Park, and G. Xu, *J. Phys. D Appl. Phys.* 51, 493001 (2018).
47. Y. Kim, T. Lim, C.H. Kim, C.S. Yeo, K. Seo, S.M. Kim, and M.H. Yoon, *NPG Asia Mater.* 10, 1086 (2018).
48. E. Macchia, M. Ghittorelli, F. Torricelli, and L. Torsi, in *7th IEEE International Workshop* (2017), p. 68.
49. P. Yu, L. Bai, W. Li, C.G. Elósegui, J. Fei, and L. Mao, *Front. Chem* 7, 313 (2019).

50. K. Shoorideh and C.O. Chui, *Proc. Natl. Acad. Sci.* 111, 5111 (2014).
51. A. Porwal, and C. Sahu, in *IEEE Computer Society Annual Symposium on VLSI* (2018), p. 281.
52. H.K. Hunt and A.M. Armani, *Nanoscale* 2, 1544 (2010).
53. I. Sarangadharan, A.K. Pulikkathodi, C.H. Chu, Y.W. Chen, A. Regmi, P.C. Chen, and Y.L. Wang, *ECS J Solid State Sci. Technol.* 7, Q3032 (2018).
54. B. Ibarlucea, L. Römhildt, F. Zörgiebel, S. Pregl, M. Vahdatzadeh, W. Weber, and G. Cuniberti, *Appl. Sci.* 8, 950 (2018).
55. I. Capek, *Carbon Nanotubes-Growth and Applications*, ed. M. Naraghi (Rijeka: IntechOpen, 2011), p. 75.
56. S. Naseh, M.J. Deen, and C.H. Chen, *Microelectron. Reliab.* 46, 201 (2006).
57. S.J. Lee, C.H. Choi, A. Kamath, R. Clark, and D.L. Kwong, *IEEE Electr. Device Lett.* 24, 105 (2003).
58. A.N. Sokolov, M.E. Roberts, and Z. Bao, *Mater. Today* 12, 12 (2009).