Biologically Coupled Gate Field-Effect Transistors Meet in Vitro Diagnostics
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ABSTRACT: In this paper, recent works on biologically coupled gate field-effect transistor (bio-FET) sensors are introduced and compared to provide a perspective. Most biological phenomena are closely related to behaviors of ions and biomolecules. This is why biosensing devices for detecting ionic and biomolecular charges contribute to the direct analysis of biological phenomena in a label-free and enzyme-free manner. Potentiometric biosensors such as bio-FET sensors, which allow the direct detection of these charges on the basis of the field effect, meet this requirement and have been developed as simple devices for in vitro diagnostics (IVD). A variety of biological ionic behaviors generated by biomolecular recognition events and cellular activities are being targeted for clinical diagnostics as well as the study of neuroscience using the bio-FET sensors. To realize these applications, bioelectrical interfaces should be formed between the electrolyte solution and the gate electrode by modifying artificially synthesized and biomimetic membranes, resulting in the selective detection of targets based on intrinsic molecular charges. Various types of semiconducting materials, not only inorganic semiconductors but also organic semiconductors, can be selected for use in bio-FET sensors, depending on the application field. In addition, a semiconductor integrated circuit device is ideal for the massively parallel detection of multiple samples. Thus, platforms based on bio-FET sensors are suitable for use in simple and miniaturized electrical circuit systems for IVD to enable the prevention and early detection of diseases.

1. INTRODUCTION

Biological phenomena are interestingly related to behaviors of ions and biomolecules. This is why biosensing devices that enable the detection of ionic and biomolecular charges contribute to the direct analysis of biological phenomena in a label-free and enzyme-free manner. Potentiometric bio-sensors such as biologically coupled gate field-effect transistor (bio-FET) sensors, which allow the direct detection of these charges based on the field effect, have this capability. That is, bio-FETs are suitable for use as simple devices for in vitro diagnostics (IVD), which can be used to monitor a person’s overall health to help cure, treat, or prevent diseases. Various biological ionic behaviors generated by biomolecular recognition events (e.g., antigen–antibody interactions, DNA hybridization, and enzymatic reactions) and cellular activities (e.g., neuronal transmission, cellular respiration, extracellular acidosis, and autophagy) are being targeted for clinical diagnostics, as well as the studies of cell biology and neuroscience, using bio-FET sensors (Figure 1). To realize these applications, bioelectrical interfaces should be formed between the electrolyte solution and the gate electrode by modifying artificially synthesized and biomimetic membranes, resulting in the selective detection of targets based on intrinsic charges on the gate surface (Figure 1). Various types of semiconducting materials, not only inorganic semiconductors but also organic semiconductors, can be selected for use in bio-FET sensors, depending on the application field (Figure 1).

A solution-gate ion-sensitive FET (ISFET) was proposed for detecting ions in biological environments. In this device, an electrolyte solution was assumed to induce the interfacial potential between the solution and the gate insulator instead of a metal gate in a metal-oxide-semiconductor (MOS) transistor, although it was necessary to use a reference electrode in the solution. A gate insulator is often composed of oxide or nitride membranes such as Ta₂O₅, Al₂O₃, Si₃N₄, and SiO₂; therefore, hydroxyl groups at the oxide or nitride surface in a solution undergo the equilibrium reaction with hydrogen ions through protonation (−OH + H⁺ ⇌ −OH₂⁺) and deprotonation (−OH ⇌ −O⁻ + H⁺) because the change in the surface charge is detected from the change in pH based on the principle of the field effect. This is why the original ISFET sensor is still utilized as a pH sensor. Such ISFET sensors mostly have a silicon substrate, but a variety of semiconducting materials have recently been applied to pH-sensitive ISFET sensors.

Figure 1. Conceptual structure of biologically coupled gate field-effect transistor (bio-FET).

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which require the gate surface in contact with the electrolyte solution to be covered by functional groups such as hydroxy groups, carboxy groups, and amino groups.4−9 For example, a transparent amorphous oxide semiconductor (a-InGaZnO) was utilized as the channel between the transparent conductive source and drain indium tin oxide electrodes, on which SiO2 was thinly coated as the gate insulator, in a pH-sensitive ISFET.7 This transparent ion-sensitive thin-film transistor (ISTFT) can continuously monitor cellular respiration as a change in pH while clearly observing cellular morphologies under an inverted microscope. In accordance with the concept of pH-sensitive ISFET sensors, ion-sensitive membranes (ISMs) with ionophores were coated as bioelectrical interfaces on the gate of ISFETs to selectively detect cations (e.g., Na+, K+, and Ca2+) or anions (e.g., Cl− and F−).10,11 Also, enzyme-immobilized membranes coated on the oxide gate induced a change in pH, which was detected using a pH-sensitive ISFET sensor on the basis of enzymatic reactions such as penicillinase−penicillin, urease−urea, and glucose oxidase−glucose reactions.12−14 Moreover, the concept of direct immuno sensing using an ISFET sensor was proposed for label-free monitoring of antigen−antibody interactions that overcome classical immunoassay.15 In the case of an immuno-ISFET sensor, antibodies are mostly immobilized on the gate surface to selectively detect antigens on the basis of intrinsic molecular charges, but counterions shield these charges from the gate surface because antibodies are often large. That is, electrolyte solutions include equal numbers of positive and negative ions, which results in a neutral condition in the solution. On the other hand, deviation of the positive or negative charge density is found in the vicinity of the gate surface depending on the surface charges, which form a diffusion layer on the gate surface, the thickness of which is defined as the Debye length. The Debye length in a physiological solution has been calculated to be less than 1 nm. This means that a large antibody (>1 nm) will react with an antigen located at a distance exceeding the Debye length, which cannot be detected by an immuno-ISFET sensor.16 The detection limit for immuno-ISFET sensors affects the development of the entire range of DNA-based ISFET sensors for single-nucleotide polymorphism (SNP) genotyping and DNA sequencing based on intrinsic molecular charges.17−30 However, a semiconductor integrated circuit device has achieved the massively parallel detection of multiple samples such as human genome sequences.31 Each gate is composed of a pH-sensitive ISFET, which directly detects the change in pH based on DNA extension reactions and reads 500−600 base pairs on a gate. On the other hand, cell analyses with ISFET sensors can also be realized with a cultured-cell-coupled gate ISFET sensor.32−40 Simply, some ions enter or exit cells through ion channels to maintain cellular homeostasis and control neuronal activities. These ionic behaviors based on cellular functions should be detected using cultured-cell-coupled gate ISFET sensors. In this paper, recent works on bio-FET sensors are introduced and compared to provide an overall perspective based on the above background on ISFET sensors.
2. DNA-COUPLED GATE FETS

2.1. Label-Free DNA Molecular Recognition Based on Intrinsic Molecular Charges. Single-stranded oligonucleotide probes are chemically immobilized at the gate surface to enable DNA molecular recognition using ISFET devices.\textsuperscript{17–30} Oxide or nitride membranes are often used as the gate insulator, which have hydroxy groups at the surface in a solution. Silane coupling reagents, which have reactive groups, are covalently bound to hydroxy groups at the oxide or nitride gate surface. Then, oligonucleotide probes, the terminus of which is modified by reactive groups, are linked with the reactive silane-coupled monolayer at the gate or using a spacer molecule between them. In a single-stranded DNA probe-tethered gate ISFET, changes in electrical properties are caused by the change in the density of molecular charges based on DNA molecular recognition events such as hybridization. In fact, the electrical detection of DNA hybridization based on a change in the threshold voltage ($\Delta V_T$) was realized using DNA probe-tethered gate ISFETs in several previous studies.\textsuperscript{21,22,24,25} $\Delta V_T$ shifted in the positive direction at a constant drain current ($I_D$) because the density of negative charges of the target DNA at the gate surface was increased by DNA hybridization. Such DNA molecular recognition is believed to have been realized by bound/free (B/F) separation. This means that the electrical measurement was performed in the same buffer solution before and after the DNA molecular recognition event, which contributed to the evaluation without changing the ionic strength in the solution.

In general, equal numbers of ions with positive and negative charges exist in an electrolyte solution, but a bias of charges is found near the gate surface depending on the surface charges. That is, charged molecules away from the gate surface are assumed to be shielded from the gate surface by counterions in an electrolyte solution, whereas those near the gate surface can be recognized from the change in the interfacial potential between the solution and the gate, which depends on the change in the concentration of target molecules. The distance from the gate surface at which molecular detection can occur strongly depends on the ionic strength in an electrolyte solution and is known as the Debye length based on an electric double layer (EDL). Ionic behaviors, which contribute to electrical signals, are often discussed by considering an EDL composed of two layers: the Stern layer of the ionic monolayer and the diffusion layer, which balances electrical forces with ion diffusivity.\textsuperscript{41–46} As shown in Figure 2a, 17-base target DNA, the length of which was ideally calculated as 5.78 nm, was detected as a change in threshold voltage of $\Delta V_T = 12$ mV owing to hybridization in a 25 mM phosphate buffer solution, where the Debye length was roughly calculated to be $\approx 2$ nm at most. In addition, extended DNA molecules with 21–61 bases obtained by the enzymatic reaction with DNA polymerase were detected as $\Delta V_T$ in a 25 mM phosphate buffer solution, but the electrical signals were not quantitatively obtained for the extended DNA molecules with a base length of over 51 (>17.34 nm), as shown in Figure 2b.\textsuperscript{28} This means that there is a limit to the base length that can be detected with an ISFET sensor. However, the extended DNA molecules were found to have a linear relationship between $\Delta V_T$ and the base length up to a base length of 41, despite a Debye length of nm order in the electrolyte solution. The Debye length from the gate surface may be a rough standard for elucidating the detection limit of DNA molecules because single-stranded DNA molecules do not necessarily stand up owing to their flexibility on the gate surface, and their structure is changed to a helix structure by hybridization with the target DNA. On the other hand, the ionic environment around DNA molecules was considered to affect the equilibrium reaction between hydrogen ions and hydroxy groups at the oxide surface according to the results of a molecular dynamics (MD) simulation.\textsuperscript{43} This may mean that biomolecules such as DNA have a diffusion layer around themselves, although the effect of such an ionic environment on the electrical signal should also depend on the ionic strength in the electrolyte solution. That is, various biomolecular recognition events may be detected as a change in pH at the oxide gate when the ISFET sensors are used, as shown in Figure 2c. Although the effect of the ionic environment around biomolecules may still be experimentally unclear, MD simulation is a good tool for predicting such ionic behaviors at electrolyte solution/biomolecule/gate interfaces.\textsuperscript{42,43,45,46}

DNA molecular recognition based on hybridization and extension reactions contributes to the application of DNA analyses such as single-nucleotide polymorphism (SNP) genotyping and DNA sequencing in a label-free manner based on intrinsic molecular charges.\textsuperscript{24,27–29} SNPs indicate single-base mismatches in genes that are related to various diseases and drug effects. Here, the control of the reaction temperature for hybridization allows one base mismatch in genetic sequences to be discriminated. A double-stranded DNA molecule undergoes the equilibrium reaction with each single-stranded DNA molecule at a constant temperature that depends on the base sequence, that is, the number of GC base pairs. Therefore, the rates of double-stranded DNA molecules with different base sequences can be compared among them on the basis of $\Delta V_T$ when the measurement temperature is maintained at the melting temperature ($T_m$) of a specific DNA sequence. In fact, the blood coagulation factors of genes were targeted for SNP genotyping with DNA probe-tethered gate ISFETs, where normal or mutant oligonucleotide probes were chemically immobilized. At $T_m$ for the normal base sequence or the mutant base sequence, the difference in $\Delta V_T$ among the normal sample, the mutant sample, and the mixed sample was found to depend on the rate of the hybridization reaction.\textsuperscript{24} In addition, SNP genotyping was also performed on the basis of the DNA extension reaction on a DNA probe-tethered gate ISFET (Figure 2d).\textsuperscript{28} Single-stranded DNA probes, the base length of which was less than that of the target DNA, were designed for a normal sample and a mutant sample so as to include one base mismatch at the end, and then the normal target DNA or the mutant target DNA was hybridized with each probe while focusing on whether the base at the end of probe molecules was matched or mismatched with the target DNA after hybridization. The matched base pair at the end was extended by the enzymatic reaction with dNTP so that the increase in the density of negative charges of the extended DNA was detected using the ISFET sensor. On the other hand, the mismatched base pair at the end did not generate an electrical signal because there was no extension reaction at the gate. Thus, SNP genotyping can be realized from the change in the intensity of the electrical signal of an ISFET based on DNA molecular recognition events.

2.2. Nonoptical Potentiometric DNA Sequencing. As described in the previous section, DNA extension reactions with dNTPs on a gate were directly detected using the DNA complex-tethered gate ISFET sensors. This is why, by
ions generated as byproducts temporarily concentrated in the tethered on the gate oxide surface. Therefore, the hydrogen probes were immobilized on acrylamide beads without being in the order of dGTP, dATP, dTTP, and dCTP. The DNA between the DNA complex and the substrates was performed hybridized on the bead. Then, the polymerase reaction diameter well with the gate, where target DNA molecules were analyzed.31 This method was based on the detection of ionic

Figure 3. (a) Concept of label-free DNA sequencing based on intrinsic molecular charges with DNA probe-tethered gate ISFETs. (b) Change in threshold voltage (ΔV_T) after each single-base extension reaction at the gate surface. Each deoxynucleotide is incorporated into the probe–target duplex on the FET in the following order: dCTP, dATP, dGTP, and dTTP. (c) Conceptual architecture of a well, a bead with DNA probe–target duplexes, and the underlying sensor and electronics. (d) Array of 50 × 50 sensing wells on an ion chip. The brightness represents the intensity of the incorporation reaction in individual sensor wells. (e) First 100 flows from one well. Each colored bar indicates the corresponding number of bases incorporated during nucleotide flow. Credit: from ref 27, reprinted with permission from Wiley (a, b); from ref 31, reprinted with permission from Springer Nature (c–e).

extending each substrate (dCTP, dGTP, dATP, and dTTP) in this order, label-free DNA sequencing based on intrinsic molecular charges can be realized (Figure 3a).27 The base sequence of DNA probe molecules tethered at the gate was a complementary sequence for part of the target DNA, where the nonhybridized sequence of target DNA should be read without labeled materials. As shown in Figure 3b, values of ΔV_T according to the expected base pairs were obtained following the addition of substrates in the order of dCTP, dGTP, dATP, and dTTP together with DNA polymerase. The magnitude of the electrical signals corresponded to that of extended molecular charges, for example, the signal shift for three bases was assumed to be about 3 times larger than that for a single base. However, a limit of detection is expected, that is, “the Debye length limit”, for DNA molecules with longer base lengths, as described in the previous section, when extended DNA molecular charges are left at the gate surface owing to the B/F separation. In 2011, we had a breakthrough extending each substrate (dCTP, dGTP, dATP, and dTTP) in this order, label-free DNA sequencing based on intrinsic molecular charges with DNA probe-tethered gate ISFET devices based on the complementary metal-oxide semiconductor (CMOS) process, which resulted in massively parallel DNA sequencing followed by a cost-effective and high-speed gene analysis.31 This method was based on the detection of ionic charges, that is, not negative charges of extended base pairs but positive charges of hydrogen ions generated by enzymatic reactions as byproducts.31,47 As shown in Figure 3c, a single bead (2 μm) with DNA probes was confined in a 3.5 μm diameter well with the gate, where target DNA molecules were hybridized on the bead. Then, the polymerase reaction between the DNA complex and the substrates was performed in the order of dGTP, dATP, dTTP, and dCTP. The DNA probes were immobilized on acrylamide beads without being tethered on the gate oxide surface. Therefore, the hydrogen ions generated as byproducts temporarily concentrated in the

3. CULTURED-CELL-COUPL ED GATE FETS

3.1. Cell/Gate Nanogap Interface for Cell Sensing with ISFET Sensors. Living cells take glucose and oxygen to yield ATP for energy; then, carbon dioxide as a byproduct is produced in a series of reactions in cellular respiration. Carbon dioxide dissolves in a solution, resulting in the generation of hydrogen ions on the basis of the equilibrium reaction. That is, the culturing of cells induces changes in pH in the culture medium, with the change in pH depending on the cellular activity. This is why an ISFET sensor can detect a change in pH based on a cell culture in real time. Such an effect of cellular respiration on the change in pH in a culture medium is...
Figure 4. (a) Schematic illustration of hydrogen ion behavior around a cell cultured on a substrate. The phospholipid fluorescein was used as an extracellular pH indicator (probe) and fixed to the plasma membrane on the external side of a cell. (b) Change in interfacial pH at the interface between the cell and the substrate as a function of incubation time. (c) Change in surface potential (ΔV_out) for each cell detected using a cell-coupled gate ISFET sensor. (d) Change in interfacial pH (ΔpH_int) at the cell/gate nanogap for each cell detected using a cell-coupled gate ISFET sensor. ΔpH_int was calculated from ΔV_out in (c) using the average pH sensitivity of 56 mV/pH for the ISFETs used in this study. (e) Schematic illustration of the cell/gate nanogap interface. (f) a-InGaZnO-based ISTFT coated on the bottom of a cell culture dish. (g) Photograph of a single mouse embryo on the gate of an ISFET sensor. (h) Real-time and noninvasive monitoring of a single mouse embryo with the ISFET sensor. Credit: from ref 37, reprinted with permission from The Royal Society of Chemistry (a, b); from ref 39, reprinted with permission from American Chemical Society (c–e); from ref 7, reprinted with permission from American Chemical Society (f); from ref 35, reprinted with permission from American Chemical Society (g, h).

ΔV_out was translated to ΔpH using the pH sensitivity (e.g., 56 mV/pH) based on the calibration curve, as shown in Figure 4d. The pH responsivity of ISFET sensors ideally follows the Nernstian response (59 mV/pH at 25 °C). In this case, the Nernst equation is strictly reflected by the coefficient β (=2n²N_aK_a¹/²/kTɛ), where n is the valence of hydrogen ions, N_a is the site density of hydroxyl groups at the oxide membrane, K_a is the equilibrium constant between hydrogen ions and hydroxyl groups, k is the Boltzmann constant, T is the absolute temperature, e is the elementary charge, and C_EDL is the capacitance of the electric double layer. The site density N_a of a Ta_2O_5 surface was reported to be about 10¹⁵/cm², which was sufficiently high for β/(β + 1) to be assumed as 1. Therefore, the Ta_2O_5 film exhibits the ideal Nernstian response. The ΔpH of cancer cells was several times higher than that of normal cells. This means that this device enabled the label-free, real-time, and noninvasive monitoring of microenvironmental pH behavior based on extracellular acidosis around cancer cells in the long term and in situ. Using the cultured-cell-coupled gate ISFET, a change in the interfacial pH at the cell/gate nanogap was also found for embryo activity, allergic responses, chondrocyte organization, and autophagy as well as canceration, on the basis of cellular respiration.

The illustration shown in Figure 4e shows the conceptual principle of cellular respiration detection using a cultured-cell-coupled gate ISFET. Some proteins in a culture medium are expected to be significant and to be directly detected at the interface between cells and the gate surface, that is, the cell/gate nanogap interface. Previous works revealed a gap of approximately 50–150 nm at a cell/substrate interface, where focal or nonfocal regions of contact between membrane proteins and substrates were observed by total-internal-reflection fluorescence microscopy. In fact, the phospholipid fluorescein [N-(fluorescein-5-thio-carbamyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine], which is composed of a pH-dependent fluorescein and a phospholipid, was inserted into a cell membrane from the outside (Figure 4a). The interfacial pH at the cell/gate nanogap shifted to the acidic side for living cells (Figure 4b), which was higher than that around the cell/bulk solution interface after replacing the culture medium with a fresh one with sufficient nutrients. This means that hydrogen ions generated by the reactions in cellular respiration upon the uptake of nutrients concentrated in the extracellular acidic side for living cells (Figure 4b), which was higher than that around the cell/bulk solution interface after replacing the culture medium with a fresh one at 0 h in this graph. ΔV_out for living cells gradually increased after changing the medium. The positive shift of the electrical signals indicated an increase in the numbers of hydrogen ions with positive charges in the source follower circuit, that is, a decrease in pH at the cell/gate interface; this is because the ISFET sensor used was sensitive to pH variation.
nonspecifically adsorbed at the gate surface, to which cells adhere through adhesion proteins. These proteins prevent other ionic biomolecules from coming in contact with the gate surface, but smaller hydrogen ions easily bind to the gate surface through the nonspecifically adsorbed proteins, where the equilibrium reaction between the hydrogen ions and hydroxyl groups at the oxide gate contributes to the change in the charge density at the gate, which depends on pH. That is, cultured-cell-coupled gate ISFETs should be insensitive to most subsequently generated biomolecules, which means that nonspecifically adsorbed proteins on the gate suppress nonspecific signals based on biomolecules contributing to interference, but are sensitive and selective to ΔpH in accordance with the Nernstian response. Also, hydrogen ions generated by cellular respiration concentrate in the cell/gate nanogap, which contributes to the amplification of electrical signals based on ΔpH. Thus, cultured-cell-coupled gate ISFET sensors are the most suitable devices for the nonoptical monitoring of cellular respiration, although it is often considered that they cannot be used to detect biological components in culture media or whole-blood samples owing to the Debye length limit. Moreover, an ISTFT coated on the bottom of a cell culture dish (Figure 4f) monitored cellular respiration as a change in pH, whereas cellular morphologies were clearly observed under an inverted microscope.

Recently, assisted reproductive technology (ART) has been expected to be used as a therapeutic method for sterility. Engineers in addition to obstetricians are expected to ensure the success of ART programs. For in vitro fertilization (IVF) in ART programs, how to evaluate embryo quality and how to select an embryo in good condition are significant problems. Morphological evaluation has been widely used to rank embryo quality because microscopy analysis is noninvasive and useful in predicting pregnancy rates. However, the standard of classification for embryo quality seems to vary among operators because it is a subjective method. Moreover, elective single embryo transfer will be recommended in the future to prevent multiple pregnancies. Therefore, a novel principle for evaluating the quality of a single embryo quantitatively and noninvasively in real time is required for practical use in ART. Among the various reported cellular activities, the ISFET sensors allow the cellular respiration of a single mouse embryo obtained by IVF to be noninvasively monitored on the gate for about 100 h in real time, as shown in Figure 4g,h. It was found that the frequency of birth for mouse embryos cultured on the gate, which was composed of an oxide membrane (Ta2O5), was similar to that on a conventional cell culture dish. Therefore, the safety of an embryo cultured on a gate was confirmed by the transplantation of embryos cultured on the gate into recipient mice. Thus, the quality of transplatable embryos can be noninvasively evaluated with ISFET sensors. That is, a platform based on cultured-cell-coupled gate ISFET sensors is suitable for use as a noninvasive and real-time monitoring system to evaluate the safety and quality of transplantable cells in the fields of regenerative medicine and pharmaceutical discovery.

3.2. Neuron/Semiconductor Interfaces. As shown in the previous section, the live monitoring of cellular respiration requires a relatively long time of a few days to a week depending on the cellular function. On the other hand, the action potential of nerve cells can be monitored in less than a second. As shown in Figure 5a,b, the action potential of a nerve cell cultured on a SiO2 gate surface was controlled using a patch clamp pipet. Here, the ionic flow through ion channels (Na+ or K+) induced by stimulation with intracellular potentials should be detected at the cell/gate nanogap interface. The output potential of this FET device was based on the change in the ionic current and depends on the conductance at the cell/gate nanogap interface. That is, a change in pH is unlikely to have been found around the interface in a short time, but the flow of Na+ or K+ through the ion channel at the cell membrane was detected as a change in the extracellular potential on the gate, although the oxide membrane is expected to interact with such cations that contribute to the change in the charge density at the gate, which depends on the gate surface charge (pK, defined as log10K, where K is the acid dissociation constant) and the concentration of cations. Such potential detection of neurons was also realized using nanowire (NW) transistor arrays (Figure 5c,d). In particular, NW–axon junction arrays were integrated and tested in at least 50 “artificial synapses” per neuron, although some concerns were pointed out about the physical rationale for the sign and amplitude obtained in the NW recordings.
cultured on the membrane but also human bodies, although this may not be a critical problem when devices are placed on the skin for a certain period (about 30 min). Therefore, a biocompatible ISM is highly expected to result in the selective detection of ions such as Na\(^+\), K\(^+\), and Cl\(^-\) based on neuronal functions using cultured-neuron-coupled gate ISFET sensors. Moreover, a biocompatible ion-sensitive arrayed NW transistor may enable the high-resolution and highly selective analysis of a neural network over a long time. This may also be accomplished by flexible and stretchable electronics when well-designed chemical modifications are combined on the sensor surface.

4. SMALL-BIOMARKER SENSING WITH CHEMICALLY MODIFIED GATE FET BIOSENSORS

A small biomarker can be a target for clinical diagnostics. Blood glucose level is the index used when diabetic patients give themselves a shot of insulin, which is electrochemically detected using an enzyme electrode. On the other hand, cortisol in saliva has recently been utilized as a stress biomarker to check health conditions using a colorimetric method based on an enzymatic reaction. Although enzymes and antibodies have been utilized in a wide range of scientific fields and their applications have been recognized as the global standard, the use of these biomacromolecules is problematic owing to their lack of stability, high-cost and time-consuming production, and the difficulty of quality control of their production. Therefore, an artificial and functional membrane based on a standard concept should be used as a platform providing molecular recognition sites for small biomarkers. Moreover, FET-based biosensors can be used to directly monitor biomolecular charges. Therefore, the enhanced detection sensitivity is expected even for small biomarkers without labeled materials, and the detection selectivity for small biomarkers will be improved by the modification of artificial membranes that selectively induce molecular charges. That is, a platform based on well-designed gate FET biosensors may be suitable for a nonoptical and enzyme-free sensing system to selectively detect small biomarkers.

4.1. Molecularly Imprinted Polymer (MIP) Biointerfaces. Molecular imprinting is based on a template polymerization technique involving the copolymerization of a cross-linker and a template molecule covalently or noncovalently bound to a functional monomer. After removing template molecules, cavities are formed in the polymer matrix, which are complementary to the template molecules in terms of shape and size, and capable of template molecular recognition, as shown in Figure 6a. An MIP functional membrane is often utilized as a receptor of a target biomolecule, particularly in the absence of its enzyme and antibody. Such MIP biointerfaces have been modified on the gate of FET sensors as well as electrochemical devices such as impedimetric electrodes and other label-free biosensors such as quartz crystal microbalance and surface plasmon resonance biosensors. Among them, vinyl phenylboronic acid (PBA) has been copolymerized as a functional monomer bound to small biomarkers based on diol binding in MIP biointerfaces of FET biosensors. A PBA-MIP-coated gate FET sensor was applied to the selective detection of glucose molecules, as shown in Figure 6b. In fact, the stability constant \((K_a)\) of PBA with glucose was found to markedly increase to \(K_a = 1192 \text{ M}^{-1}\), which was 2 orders of magnitude higher than \(K_a = 4.6 \text{ M}^{-1}\) obtained by nonelectrical detection methods. In a similar manner, the PBA-MIP-coated gate FETs were used to selectively detect dopamine and oligosaccharides in this application, the Langmuir or multi-Langmuir adsorption isotherm equation was fitted to the change in surface potential \((\Delta V_{out})\) versus the concentration of the small biomarkers,
considering the change in the density of molecular charges of PBA (ΔQ_{MIP}) caused by the adsorption equilibrium of the analytes with the vinyl PBA-copolymerized MIP membrane (\(SM + PBA \rightleftharpoons SM-PBA^–\)). The MIPs used in these studies included (hydroxyethyl)methacrylate as the main chain monomer, which were the hydrophilic polymers even before adding small biomarkers such as glucose. This is the reason why the MIPs generally include a large number of water molecules, that is, the change in the permittivity of the MIPs was almost negligible even after adding small biomarkers such as glucose. It is also because the cross-linking density in the MIPs was controlled to make them relatively rigid. Therefore, the swelling rate of the MIPs was relatively low owing to their intrinsic hydrophilic property and relatively high cross-linking density, which means that the change in the capacitance of the MIP membranes (ΔC_{MIP}) was almost negligible. As a result, ΔV_{out} was estimated from ΔQ_{MIP} on the basis of the equilibrium reaction between the PBA-based MIP and the small biomarkers, enabling potentiometric adsorption isotherm analysis.\(^7\) In addition, a well-defined polymer film, whose thickness is precisely controlled by surface-initiated atom transfer radical polymerization, contributes to the quantitative detection of signals by FET biosensors and provides valuable information for the fabrication of novel bioanalytical devices.

4.2. Aptamer-Based Biointerfaces. Aptamer molecules are widely utilized for biosensors as selective receptors to target molecules, which are composed of single-stranded oligonucleotides or peptides.\(^74,75\) As long as the sequence of aptamers is identified by an in vitro selection method termed systematic evolution of ligands by exponential enrichment,\(^76\) aptamer-based biointerfaces are available for the selective detection of biomolecules as signal transduction interfaces with biosensors, particularly, in the absence of enzymes and antibodies for the target biomolecules. However, the isolation of an aptamer for a specific target may require considerable time and expense. In fact, aptamer-based biointerfaces have recently been utilized for the selective detection of biomolecules using solution-gate FET sensors, and small biomarkers were detected by overcoming the Debye length limitation.\(^77\) The key point in small-biomarker sensing is that the change in the molecular structure of the aptamer is caused by its binding to small biomarkers on the gate surface. Aptamer molecules have negative charges owing to the phosphate groups on the side chain, such as those in DNA or RNA. These negative charges should be detected by FET devices on the basis of the same principle as that of DNA molecular recognition discussed in section 2. For example, the negatively charged backbones of dopamine aptamers with a stem–loop structure are expected to move near the gate surface owing to structural reorientation based on the selective binding of dopamine, which means that the negative charges of dopamine aptamers are assumed to enter the diffusion layer that is less affected by counterions (Figure 6c). On the other hand, not all aptamer molecules necessarily induce negative charges at their backbones near the gate surface by aptamer–target binding, but the density of such molecular charges near the gate surface may decrease in some structural reorientations (a change from the state of lying down to standing up) (Figure 6d), resulting in a reverse shift of the electrical signal.

5. CONCLUSIONS AND OUTLOOK

In the development of novel biosensing devices, the structural components of the biological target, the signal transduction interface, and the detection device must be considered. Most biological phenomena are closely related to ionic behaviors. This is why the detection of ionic and biomolecular charges is the key to the direct analysis of biological phenomena in a label-free and enzyme-free manner. Therefore, semiconductor-based potentiometric biosensors based on the solution-gate FET have strong potential as detection devices. A variety of semiconducting materials can be chosen for use in bio-FET sensors, depending on the application. What characteristics are required for devices used in applications? These surely include superior accuracy, stability, sensitivity, a dynamic range with a limit of detection, selectivity, biocompatibility, flexibility, transparency, and miniaturization. In particular, the development of bio-FETs with new materials has recently been reported.\(^77\) Furthermore, such device performances are controlled by introducing bioelectrical interfaces, which can be formed on the basis of well-designed polymerization techniques or biomimetic materials, as discussed in Section 4. On the other hand, hydrogen ions produced or released as a result of biological events are attractive as a detection target of the bio-FET sensors; that is, we can use a reliable characteristic of ISFET sensors, namely, pH responsivity.

To begin with, bio-FET sensors should also be applied to semiconductor integrated circuits to measure multiple samples simultaneously. This is one of the advantages of utilizing semiconductor technology. The nonoptical DNA sequencing discussed in Section 2 was a breakthrough based on the CMOS technology. The novel applications expected for arrayed devices also include cellular analyses and small-biomarker sensing. In this case, an enormous quantity of detected data is assumed including complicated information; therefore, the data analysis based on a bioinformatics method may be desirable according to the omics approach.

The Debye length limit is a fatal disadvantage of semiconductor-based biosensors, but some new methods to overcome the problem have been reported.\(^73,77,81,82\) In contrast, the Debye screening effect can be utilized to suppress electrical signals caused by nonspecific adsorption in the case of cellular analysis, as described in Section 3. That is, proteins in a culture medium, which nonspecifically cover the gate surface, prevent other ion biomolecules from coming in contact with a gate surface; thus, these unexpected biomolecules adsorbed on the protein layer are not detected because their charges are shielded by counterions larger than the Debye length. As a result, smaller hydrogen ions can be more specifically detected on the basis of the equilibrium reaction with hydroxy groups at the oxide gate surface. This principle is also applied at a polymeric nanofilter biointerface on a gate surface, which prevents low-molecular-weight molecules acting as sources of interference from approaching the gate surface, resulting in the suppression of nonspecific electrical signals.\(^83\) That is, low-molecular-weight molecules acting as sources of interference remain at a distance longer than the Debye length, whereas a target small biomarker reacts with the gate surface passing through the polymeric nanofilter biointerface.

Platforms based on bio-FET sensors, which originate from electronics, are suitable for use in miniaturized and cost-effective systems to directly measure biological samples in the field of in vitro diagnostics.
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Notes

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