TOPICAL REVIEW

Field-effect detection using phospholipid membranes

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Abstract
The application of field-effect devices to biosensors has become an area of intense research interest. An attractive feature of field-effect sensing is that the binding or reaction of biomolecules can be directly detected from a change in electrical signals. The integration of such field-effect devices into cell membrane mimics may lead to the development of biosensors useful in clinical and biotechnological applications. This review summarizes recent studies on the fabrication and characterization of field-effect devices incorporating model membranes. The incorporation of black lipid membranes and supported lipid monolayers and bilayers into semiconductor devices is described.

Keywords: field-effect devices, black lipid membranes, supported lipid monolayers, supported lipid bilayers

1. Introduction
The binding of charged species to semiconductor materials results in an accumulation or depletion of carriers within the semiconductor. The relationship between the electronic properties of the semiconductor and the electrostatic potential at the solution–semiconductor interface is employed for potentiometric sensing using field-effect devices. Field-effect devices can serve as label-free sensors and have a unique feature compared with other label-free devices, such as a quartz crystal microbalance with dissipation monitoring (QCM-D), namely that sensor integration and miniaturization are possible in combination with micro- or nanoelectronics. In addition, most biological processes involve electrostatic interactions that may potentially be monitored by field-effect devices. Owing to these substantial advantages, considerable attention has been devoted to the field-effect monitoring of biomolecules. The common sensing principle involves the immobilization of recognition elements on a device surface. The selective binding or reaction of target molecules with the recognition elements is detected as a change in the electrical properties of the semiconductors. There are various combinations of analytes, recognition elements and semiconductors. Recent examples of detected biological events include protein binding [1–6], enzymatic reactions [7], DNA hybridization [8–10], DNA or RNA hybridization with peptide nucleic acid (PNA) [11, 12], and the binding of other biologically related molecules [13].

Black lipid membranes [14, 15] and supported lipid monolayers and bilayers [16] are useful model systems for elucidating the properties and functions of biological membranes. Black lipid membranes are lipid bilayers formed across a small aperture in a hydrophobic support. Black lipid membranes are fluid lipid membranes formed across a small aperture in a hydrophobic support. Such membranes act as an electrical insulator that separates two aqueous compartments, and therefore they are often used to investigate the formation of ion channels [17–20]. On the other hand, supported lipid bilayers are fluid lipid membranes directly deposited on a solid surface. Because they can be readily formed and are even more stable than black lipid membranes, supported lipid bilayers have been widely used...
for understanding membrane behavior and for engineering biotechnological architectures. For instance, research interest has focused on lipid diffusion [21], the interaction of bilayers with support surfaces [22–24], the properties of lipid domains [25], protein binding to bilayers [26–30], the reconstitution of membrane proteins [31–41], bilayer patterning [42–44] and the electrophoretic separation of constituent molecules [45–47].

In this review, we describe recent examples of the fabrication and characterization of field-effect devices incorporating black lipid membranes and supported lipid monolayers and bilayers (figure 1). Although the sensing principle can be extended for monitoring cells, such as nerve cells [48], we have focused on sensor systems consisting of model membranes. Since the incorporation of transmembrane proteins into electronic devices is potentially of great importance for future applications, methods for reconstituting membrane proteins into supported lipid bilayers are also briefly described in the final section.

2. Black lipid membranes

The behavior of ion channels embedded in black lipid membranes is usually investigated using electrophysiological measurements. Bernards et al have demonstrated that electrochemical transistors are capable of detecting ionic current through black lipid membranes [49]. The transistors consisted of conductive polymers attached to a glass substrate and a Teflon support fixed above the polymer film (figure 2). The conductive polymers were composed of poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS). Black lipid membranes were formed by the painting method on the aperture in the Teflon support. Gramicidin was incorporated in the lipid membranes to vary the membrane permeability. When a gate voltage was applied across the bilayer, the transport of monovalent ions was observed as a change in the source–drain current flowing through the polymer film. The current change is caused by ion drift into the polymer layers. Gramicidin channels are less permeable to divalent ions than monovalent ions. With the reported electronic system, the monovalent and divalent ions were distinguished via a difference in the source–drain current.

Another example has been reported by Rentschler and Fromherz. They described a silicon-based field-effect transistor (FET) system that allowed the detection of ion current through black lipid membranes [50]. Their system consisted of an array of multiple FETs to which a polyimide groove with a depth of \( \sim 80 \) µm was attached. Black lipid membranes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and glycerol monooleate were formed across the groove, thus separating a bulk aqueous solution from a solution exposed to the gate surface. Ag/AgCl electrodes were fabricated on both sides of the transistor array. By applying a voltage between the two Ag/AgCl electrodes, the ion transport through gramicidin ion channels was successfully observed in the voltage profile of the FET array. The voltage profile was dependent on the peptide density in the membranes.

3. Supported lipid bilayers and monolayers

Ion-sensitive field-effect transistors (ISFETs) may be useful for monitoring biological events accompanied by changes in local pH or other ion concentrations. Ottenbacher et al have reported the detection of lactose using lactose permease and a pH-sensitive FET [51]. Lactose permease is a transmembrane protein that facilitates the transport of lactose across lipid membranes. This transport is coupled to the passage of protons. In the study by Ottenbacher et al, unilamellar
vesicles incorporating lactose permease were adsorbed on an Al$_2$O$_3$-gated FET. The pH-sensitive device was then exposed to a lactose solution. The presence and absence of lactose was distinguished from the transistor output signals.

The application of pH-sensitive FETs was also reported by Misra et al [52]. Silicon nanowire (SiNW) transistors were combined with a lipid bilayer by coating the NW with a continuous supported bilayer (figure 3). Two types of ion channel peptides, gramicidin and alamethicin, were incorporated in the supported bilayers to demonstrate the detection of chemically gated and voltage-gated ion transport. The lipid coating on the NW resulted in a decrease in the FET response to a pH change, because the lipid membrane blocked proton transport between the solution above the outer leaflet of the bilayer and the hydration layer below the inner leaflet of the bilayer. When gramicidin was incorporated in the supported bilayer, the pH response of the FET device recovered owing to the proton transport through the ion channels. The gating properties were varied by the addition of Ca$^{2+}$ because the conductance of gramicidin pores is reduced by Ca$^{2+}$. When alamethicin was incorporated, the device response to a pH change depended on the voltage because the transport of alamethicin is voltage gated.

In our recent studies, a bilayer surface charge was detected using a Si$_3$N$_4$/SiO$_2$/Si structure and capacitance–voltage (C–V) measurements (figure 4). C–V data were used to calculate the flat-band voltage. The flat-band voltage change that resulted from the formation of a supported bilayer on the Si$_3$N$_4$ surface was dependent on the ratio of the charged lipids, the salt concentration and the lipid surface coverage [53]. The magnitude of the voltage change was also affected by the type of cations contained in the buffer [54]. We concluded that the salt ions that are specifically bound to the device surface and the bilayer charge both contribute to the signal generation mechanisms.

The binding of charged peptides or proteins to a monolayer or bilayer surface has also been monitored with field-effect devices. Lud et al have demonstrated the detection of peptide and protein binding to a lipid monolayer using a silicon-on-insulator (SOI)-based thin-film resistor [55]. Phospholipids bearing a Ni-nitrilotriacetic acid (NTA) headgroup were incorporated in a monolayer attached to the device to allow the binding of histidine (His)-tagged peptides and proteins to the monolayer surface (figure 5). The peptides consisted of six histidine residues and a different
number of aspartic acid residues. The exposure of the monolayer surface to peptide solutions yielded a change in the output voltage of the semiconductor device. The magnitude of the voltage change depended on the number of charged residues contained in the peptides. The binding and release of His-tagged green fluorescent protein (GFP) were also observed as a voltage shift.

The application of single-walled carbon nanotubes for detecting protein binding to a bilayer surface has been demonstrated by Zhou et al [56]. Supported bilayers incorporating biotinylated lipids were formed on an FET based on carbon nanotubes. The conduction of the nanotubes was affected by the binding of streptavidin to the bilayer surface.

Transparent semiconductors enable the characterization of lipid membranes by both optical and field-effect measurements. Because membrane properties, such as lipid diffusion, can be readily examined by fluorescence microscopy, optically accessible materials are potential candidates for membrane sensing devices. Ang et al have demonstrated the detection of channel-forming peptides using an FET that was fabricated using nanocrystalline diamond [57]. Diamond is optically transparent and has p-type surface conductivity when exposed to an aqueous solution. The formation of supported lipid bilayers on the diamond gate surface was examined by employing fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS) and atomic force microscopy (AFM). From these data, lipid diffusion within the supported bilayers was found to be dependent on the diamond surface roughness. A channel-forming peptide, magainin II, was then added to the supported bilayers to demonstrate the field-effect detection of peptides. The sensing principle is based on the diffusion of negative ions from the bulk solution to the diamond surface and the modulation of the channel current caused by the change in surface charge density. Ang et al showed that the output signals varied with the magainin II concentration.

Supported phospholipid bilayers with high electrical resistance are not readily obtained because defects are often formed within the bilayers. To avoid defect formation and employ the highly resistant membrane for the electrical isolation of a semiconductor device from an aqueous solution, Fromherz et al coupled giant unilamellar vesicles to FETs [58]. Giant unilamellar vesicles were sedimented on gates coated with poly-L-lysine. With this system, a sheet resistance of 100 GΩ was achieved along the junction.

4. Future directions

One of the issues that need to be addressed is probably the incorporation of transmembrane proteins in field-effect devices, because membrane protein-device hybrids may be useful for clinical and biotechnological applications. Supported lipid bilayers provide a better framework than black lipid membranes for reconstituting membrane proteins because supported bilayers are more stable and can be easily manipulated by chemical methods. Therefore, several strategies have been explored for reconstituting membrane proteins into supported bilayers. The simplest reconstitution procedure is the direct fusion of proteoliposomes onto unmodified solid surfaces [59, 60]. However, direct vesicle fusion cannot easily cope with membrane proteins that have large peripheral domains. A water film between a bilayer and an underlying solid surface has a typical separation of only 1–2 nm [61–63]. Large peripheral domains therefore come in contact with the surface, resulting in the immobilization and denaturation of the membrane proteins. To avoid these problems, polymer-supported membrane systems have been suggested [41, 64–71]. The polymer layers decouple the membranes from the underlying surface and also avoid the nonspecific adsorption of proteins. Several types of membrane proteins have exhibited mobility within polymer-supported bilayers [31, 32, 36–38, 63]. Other ways of reconstituting membrane proteins involve the binding of membrane proteins to the substrates in a selective orientation and their incorporation in a lipid bilayer using detergent removal [33–35, 39, 40, 72–74]. The combination of these reconstitution techniques and field-effect devices may provide new biosensing platforms. Although much effort has been devoted to the functional reconstitution of membrane proteins, general protocols that can be applied to various proteins have not been established. The development of reconstitution strategies and the integration of transmembrane proteins into the electronic systems will provide more opportunities for biomembrane sensing.

5. Summary

We have summarized several studies on the incorporation of black lipid membranes and supported lipid monolayers and bilayers in field-effect devices. These studies have mainly focused on the detection of ion transport through peptide ion channels, the intrinsic charge of lipid monolayer and bilayer surfaces, and the binding of aqueous peptides and proteins to lipid monolayers and bilayers. We must await further studies on the incorporation of transmembrane proteins in electronic devices and their characterization by field-effect devices before we can develop membrane-based biosensors.

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