



Biomedical Applications of Lipid Membrane-Based Biosensing Devices

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Abstract

The investigation of lipid films for the construction of biosensors has recently given the opportunity to manufacture devices to selectively detect a wide range of compounds of clinical interest. Biosensor miniaturization using nanotechnological tools has provided novel routes to immobilize various “receptors” within the lipid film. This chapter reviews and exploits platforms in biosensors based on lipid membrane technology that are used to detect various analytes of clinical interest. Examples of applications are described with an emphasis on novel systems, new sensing techniques, and nanotechnology-based transduction schemes. The compounds that can be monitored are urea, cholesterol, glucose, toxins, antibiotics, microorganisms, hormones, etc. Finally, limitations and future prospects are presented herein on the evaluation/validation and eventually commercialization of the proposed sensors.

Keywords

Lipid film-based biosensors · Nanotechnological platforms · Graphene electrodes · ZnO nanowalls and nanowires · Clinical analysis

7.1 Introduction

Nanobiosensors have been widely used for the monitoring and determination of biomolecules (Chandra 2016; Nikolelis and Nikoleli 2018), and it is expected that this technology in combination with the recent nanotechnological advances will

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further promote the therapeutic applications in personalized diagnostics. Biosensor nanotechnologies include a wide range of devices that were developed to monitor biomolecules of clinical interest such as glucose, urea, uric acid, cholesterol, calcium ions, etc. The importance of nanomaterials in biosensing mechanism and the various physicochemical techniques that were used to exploit the mechanism of signal generation were described in previous reports (Nikolelis and Nikoleli 2018; Mahato et al. 2018; Prasad 2014). In accordance probe fabrication techniques, analytical characteristics of the biosensing devices such as selectivity, sensitivity, interferences, and evaluation; and validation for commercialization of these biosensors were extensively discussed in these books and reports (Nikolelis and Nikoleli 2018; Mahato et al. 2018; Prasad 2014; Chandra et al. 2011, 2017).

Since Mueller et al.'s works on bilayer lipid membranes (BLMs) (Mueller et al. 1962), devices that were based on lipid membranes to monitor food toxicants, environmental pollutants, and compounds of clinical interest have increased tremendously. However, the "black" lipid membranes that were based on the technique of Mueller et al. were very unstable and broke under an electrical and mechanical field and were unstable outside a KCl electrolyte solution, and this has influenced their practicality. During the last decade, a number of advances to prepare stabilized lipid-based devices were explored, and this has given the opportunity to construct biosensors to detect food toxicants and environmental pollutants in real samples and in the field. The advantages of lipid film devices are summarized as follows: the membranes are biocompatible and therefore can be used to be implanted in the human body, they are fast-responding devices with response times of seconds, they have high sensitivity and can detect biomolecules in the mM concentration range, they also have high selectivity with minor interferences which allow them to be used in real samples such as human serum, etc. Their size is small due to nanotechnological advances; they can have portability, hold a large number of advantages toward the liquid and gas chromatographic units which are bulky, and have a high cost to buy them; and their analysis times are too long on the order of sometimes of days.

This work describes the platforms of nanosensors based on lipid membranes that were investigated to detect compounds of clinical interest. The chapter provides novel routes for the design and nanofabrication of lipid film-based biosensors for the rapid detection and monitoring of compounds of clinical interest such as urea, cholesterol, glucose, antibiotics, hormones, toxins, etc.

7.2 The Preparation of Lipid Membranes

The methods of the preparation of lipid membranes have been extensively described in the literature and mainly of biosensors that are based on lipid films and are not prone to electrical or mechanical interferences and breakage (Nikolelis et al. 2006, 2008a).

These techniques can prepare lipid film biosensors that are stable in air and outside an electrolyte solution for period of times of more than a month; therefore these devices can be extensively used for practical applications. These biosensing devices

have been used in electrochemical experimentation. An exception is the development of stabilized polymerized lipid films on a filter paper that switch on and off their fluorescence and therefore belong to optical biosensors. Tien and Salamon (1989) in the past have suggested a simple and reliable technique for the construction of bilayer lipid membrane (sBLM) that was stable outside an electrolyte solution and was not prone to electrical or mechanical breakage; these sensors were prepared at a tip of a freshly cut tip of a Teflon-coated metallic wire, and their stability is due to the nascent metallic surface. The method has used a Teflon-coated stainless steel metal wire (0.1–0.5 mm in diameter) which was cut to provide a nascent surface while it was inside in lipid solution of phosphatidylcholine in a solvent of chloroform using a miniature guillotine. The tip of the wire is covered with the lipid solution that turns into a lipid membrane; when is placed in an electrolyte (i.e., 0.1 M KCl), the lipid film spontaneously thins into a self-assembled lipid bilayer membrane (sBLM) is formed.

7.3 Methods for the Preparation of Two Most Important Platforms of Stabilized Devices Based on Lipid Films

During the last decade, the construction of stabilized lipid film-based biosensors that do not break when electrical or mechanical shock is applied and are stable outside an electrolyte solution has been the investigation of a number of reports; these investigations will provide devices that can be commercialized due to their practical applications. Nanotechnological advances have provided a route to construct devices that their size is less than 1 μm size and therefore belong to the class of nanosensors. Techniques for the construction of this class of biosensors based on lipid films are further described and have a number of advantages such as ease of construction, rapid response times, small size high selectivity and sensitivity, and most importantly are stable outside an electrolyte solution that will allow them to be eventually commercialized.

7.3.1 Stabilized Lipid Films Formed on a Glass Fiber Filter

A route of the construction of stabilized in electrolyte lipid membranes was first reported by Nikolelis et al. group, and these films were prepared on glass Whatman filter disks (Nikolelis et al. 2008a); this has permitted a large number of evaluation and validation in real samples, i.e., the continuous monitoring of aflatoxin M_1 in milk and cheese products (Andreou and Nikolelis 1998). The lipid membranes were constructed on a GF/F glass microfiber, which has 0.9 μm of diameter and 0.7 μm nominal pore size (Andreou and Nikolelis 1998; Nikolelis et al. 1995).

Two Plexiglas blocks that were divided by a Saran-Wrap partition film (10 μm thick) folded in half (the Saran-Wrap film had a hole having 0.16 mm of radius) were used as the electroanalytical unit to construct these stabilized lipid film devices; a glass GF/F microporous fiber disk (WhatmanTM, UK) was centered in the hole

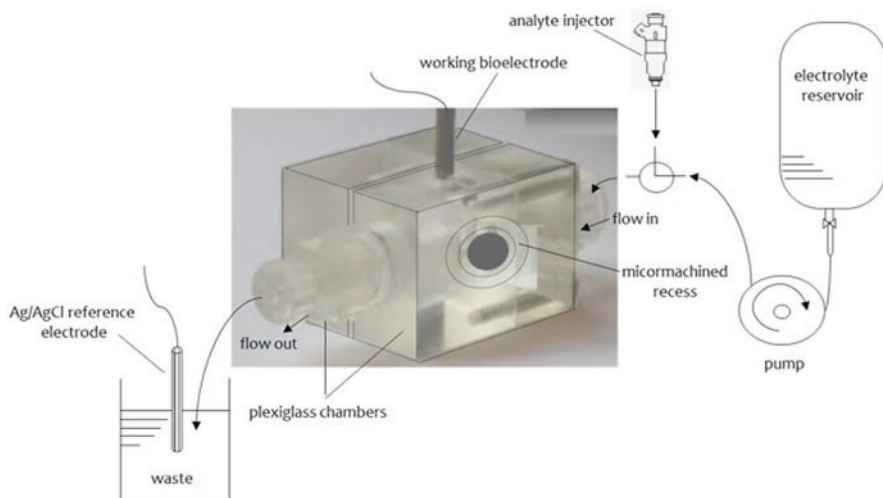


Fig. 7.1 A diagram of the setup used for the construction of lipid membranes that were stable in electrolyte. (From Nikoleli et al. 2018)

between the two plastic Saran-Wrap films. This plastic Saran-Wrap film was clamped between the two Plexiglas block units. The shape and dimensions of these chamber blocks were as follows: one of them was circular with a radius of 0.5 cm and depth 0.5 cm, and the other one was cylindrical. An Ag/AgCl electrode which acted as a reference was positioned in the wastes. This chamber was connected with a carrier electrolyte flow system. The cylindrical chamber had a circular upper hole (diameter 0.2 cm²) and an elliptical lower hole. An Ag/AgCl electrode was positioned at the center of the cylindrical cell, and a 25 mV was applied between the two reference electrodes; at the same time, the current was measured using a Keithley instrument. A volume of 75 microliters samples were injected that contained the analyte. This experimental setup was inside a grounded Faraday cage. A simplified diagram of the instrumentation used is provided in Fig. 7.1. The stabilized in electrolyte solution lipid membranes were formed by established procedure (Andreou and Nikolelis 1998; Nikolelis et al. 1995): Once the lipid films were formed, the current was brought down at the pA levels, and gramicidin D is used to provide the bimolecular structure of these bilayers.

7.3.2 Polymer-Supported Bilayer Lipid Membranes

The polymeric stable in air lipid membranes were constructed as previously reported (Nikolelis et al. 2006, 2008a). UV irradiation and not heating at 60 °C is preferable, because the latter deactivates protein molecules. Differential scanning calorimetry and Raman spectrophotometry have shown that it is required 4 h to finish the polymerization.

7.3.3 Polymeric Lipid Membranes Supported on Graphene Microelectrodes

Graphene nanomaterials have been extensively utilized in biosensors because of their advantages such as enhanced physicochemical properties (mechanical, electrical, and thermal), high biocompatibility, and low toxicity. The large surface-to-volume ratio minimizes the device size and provides rapid response times and lower sensitivity without biofouling. Our group has constructed a device that was composed from a stabilized lipid membrane on graphene electrodes (Nikoleli et al. 2012; Bratakou et al. 2015). These nanodevices were used for the rapid determination of food toxicants, environmental pollutants, and compounds of clinical interest (Bratakou et al. 2016, 2017; Karapetis et al. 2016).

The preparation of graphene electrodes was extensively reported (Bratakou et al. 2016, 2017; Karapetis et al. 2016). *N*-methyl-pyrrolidone (NMP) was mildly sonicated for about 7.5 days and centrifuged at 700 rpm for 120 min. This dispersion was poured onto a copper wire (0.25 mm in diameter) which was positioned on a GF/F microfiber disk, and the solvent was slowly converted into vapor. The copper acted as the connection for the electrochemical experiments.

The method of preparation of the lipid membrane biosensors was previously described in detail (Bratakou et al. 2016, 2017; Karapetis et al. 2016): The stable lipid membranes were constructed following the polymerization stage as above.

“Receptor” molecules were placed in the lipid film devices before they were polymerized by injecting 15 μL of the “receptor” solution on the filter. The filter-supported polymeric BLMs were finally mounted onto the electrode.

7.3.4 Polymerized Lipid Membranes on ZnO Electrodes

Nanostructured ZnO is a promising material for the construction of nanoelectrodes for food, environmental, and clinical applications because it has a large number of advantages such as low cost, ease of preparation, biocompatibility, and catalytic surface activity. Other advantages include high isoelectric point (IEP) and nanostructured ZnO electrodes have high sensitivity and small size. IEP of ZnO is 9.5 which is higher than the IEP of a large number of biomolecules, and therefore it can be used as a matrix to immobilize these compounds through electrostatic bonding. ZnO nanoelectrodes has widely been used for the construction of devices to detect medical important compounds such as cholesterol, glucose, L-lactic acid, uric acid, metal ions, and pH.

7.3.4.1 Potentiometric Biosensors Based on ZnO Nanowalls and Stabilized Polymerized Lipid Film

The unmodified ZnO nanowall electrodes on an aluminum foil can be constructed by the sonochemical technique of Nayak et al. (2012) which briefly is as follows: Unimolar solutions of zinc nitrate hexahydrate, with almost 100% purity, and $\text{C}_6\text{H}_{12}\text{N}_4$ are mixed using 350 rpm for 5 min. An Al coated wire was then placed in this solution and sonicated for about 10 m. The electrode was then washed with distilled water and placed in a N_2 atmosphere.

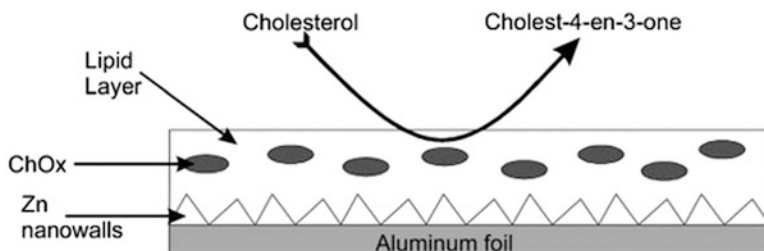


Fig. 7.2 A simple diagram of the device design. (From Psychoyios et al. (2013) with permission)

The preparation of the polymeric stable lipid membranes for the determination of cholesterol has been previously described (Psychoyios et al. 2013), and an extensive procedure was previously has been provided (Psychoyios et al. 2013).

Cholesterol oxidase was placed in these lipid membranes before these films were polymerized by placing 15 μL of the enzyme suspension on these filter disks using a microliter syringe.

The final stage to construct the device was to encapsulate the polymeric lipid membranes onto the wire that contained the electrode. Figure 7.2 shows a simplified diagram of the device.

7.3.4.2 Potentiometric Biosensors Based on Lipid Stabilized Membranes ZnO Nanowires

The biosensor was constructed it was previously reported (Usman Ali et al. 2011). This paper describes in detail the preparation of these electrodes (Usman Ali et al. 2011; Vafae and Youzbashizade 2007).

The enzyme (uricase) was placed in the lipid membranes before the membranes were made polymeric as it was previously described (Tzamtzis et al. 2012). These sensors were used in flow injection analysis (FIA) experiments. The FIA system used was previously described in detail (Andreou and Nikolelis 1998; Tzamtzis et al. 2012). The design of the experimental apparatus was reported previously in literature (Andreou and Nikolelis 1998; Nikoleli et al. 2018; Tzamtzis et al. 2012). The Ag/AgCl reference electrode was immersed in the waste of the carrier electrolyte solution, whereas the ZnO electrode was placed into the cylindrical cell.

7.4 Practical Applications of Lipid Membrane Devices in Biomedical and Clinical Analysis

7.4.1 Applications of Lipid Film Devices Based on Polymeric Lipid Membranes

A synthetic “receptor” (calixarene) was prepared and immobilized on lipid membranes on glass microfiber filters. Calixarene was inserted into the lipid structure and provided a signal which was adequate to rapidly determine insecticides rapidly,

with a sensitive and selective response, and was used to determine these compounds in real samples of fruits and vegetables (Nikolelis et al. 2008b). Similar devices were constructed to selectively and rapidly determine food hormones (i.e., naphthalene acetic acid) in fruits and vegetables (Nikolelis et al. 2008c) and a zinc in water (Nikolelis et al. 2009).

A report that exploits the construction of a receptor for the fast FIA monitoring of zinc and used stabilized lipid films on a methacrylate polymer on a fiber filter disk with an incorporated receptor (Nikolelis et al. 2009). This receptor was prepared by replacing the hydroxyl groups of resorcin into phosphoryl groups. This nanosensor was prepared specifically for the FIA monitoring of zinc and was based on these air stabilized lipid membranes that were polymeric. The nanobiosensor can determine zinc in a drop (75 μL) of the sample. The analyte (i.e., zinc) was injected into the flowing electrolyte streams of 0.1 M KCl electrolyte. A complex formation between the phosphoryl receptor and Zn^{2+} takes place and causes pre-concentration of the analyte at the lipid film/solution interface which in turn causes changes in the electrostatic fields and phase structure of the lipid films; as a result we have obtained ion current transients, and the peak height of these signals was correlated to the analyte concentration. The response times were on the order of ca 5 s, and Zn^{2+} could be determined at very low levels of concentration (i.e., nM detection levels). The analytical curve was linear in the concentration range $1.00 \times 10^{-7} - 1.20 \times 10^{-6}$ M with detection limits of 5.00×10^{-8} M and a rsd of less than 4%. Potent interferences were examined including a wide range of other metals, lipids, and proteins. As an analytical demonstration and evaluation of this technique, trace concentrations of Zn(II) were successfully determined in real samples of waters.

7.4.2 Applications of Graphene-Based Devices

A potentiometric urea lipid membrane-based minisensor on graphene has been appeared recently (Nikoleli et al. 2012). A potentiometric urea device based on lipid film technology on graphene nanosheets has been constructed; a simplified setup of this biosensor is shown in Fig. 7.3. The main characteristics of this biosensor are excellent reproducibility, sensitivity, selectivity, reusability, and rapid response times; the slope of the electrode is ca. 70 mV/decade over the urea logarithmic concentration range which can be determined from 1×10^{-6} M to 1×10^{-3} M.

The interactions of cholera toxin with polymeric lipid membranes with incorporated ganglioside GM1 were reported in the literature (Nikoleli et al. 2011). An injection of cholera toxin in the flowing streams of a KCl 0.1 M carrier solution provided a current signal. The peak height of the ion current signal was correlated with the concentration of cholera toxin in the sample solution and had detection limits of 0.06 μM .

The response times and detection limits were improved using polymerized lipid membranes on graphene nanosheets (i.e., response times of 5 min and detection limits of 1 nM) (Karapetis et al. 2016). The construction of this sensor was easy and has shown excellent reproducibility, reusability, selectivity, long shelf life, and a

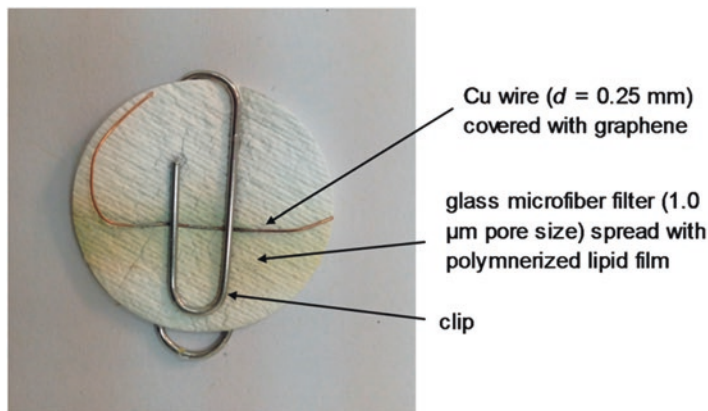


Fig. 7.3 Picture of the lipid film device on graphene minielectrode which was used for the potentiometric detection of urea. (Reprinted from Nikoleli et al. 2017)

slope of 60 mV/decade of toxin concentration. The method was evaluated/validated in lake water samples.

An electrochemical biosensor for the determination of saxitoxin based on graphene nanosheets with stable in air lipid membranes and immobilized anti-STX was provided in the literature (Bratakou et al. 2017). An excellent selectivity, sensitivity, and detection limits (1 nM) for the determination of saxitoxin with rapid response times (i.e., 5–20 min) were noticed. The sensor was easily constructed, lasted long periods of time with a slope of 60 mV/decade over saxitoxin concentration. The method was evaluated/validated for the determination of STX in lake waters and shellfish.

7.4.3 Applications of the ZnO Nanoelectrode-Based Devices

A potentiometric cholesterol device was constructed by immobilizing cholesterol oxidase into polymerized lipid membrane on ZnO nanowalls (Psychoyios et al. 2013). The enzyme was codeposited into the lipid membrane prior to polymerization on the ZnO nanowalls surface and provided a sensitive, selective, stable, and reproducible cholesterol device. The electrode slope was 57 mV/decade of cholesterol. No interferences were noticed by ascorbic acid, glucose, urea, proteins, and lipids. The nanosensor device has shown biocompatibility and could be implanted in the human body.

A uric acid electrochemical device was reported in the literature by immobilizing the enzyme uricase into polymerized lipid membranes on Zn nanowires (Usman Ali et al. 2011). The enzyme was codeposited with the lipid membrane prior to polymerization on the surface of the electrode. The biosensor was sensitive, selective, stable, and reproducible. The presence of a cationic lipid in membranes has increased

the electrode slope by twofold. No interferences were observed by the presence of ascorbic acid, glucose, urea, proteins, and lipids.

ZnO nanowires (NW) were tailored for the immobilization of glucose oxidase in order to fabricate a glucose sensor (Zang et al. 2007). The high specific surface area and isoelectric point provide the electrode efficient immobilization of high concentration of acidic enzymes. The apparent Michaelis constants were adjusted by tailoring the thickness of the GOD/ZnO nanowire layer and the enzyme loading in the nanowires. Through this route, linear region of sensitivity and reaction rates could be obtained. The long-term stability of this biosensor was high due to the inorganic ZnO NW.

Well-aligned ZnO nanowires were constructed on gold-coated plastic substrates using a low-temperature aqueous chemical growth method (Hsu et al. 2017). These arrays had 50–130 nm diameters and were applied to construct a urea biosensor using urease within the concentration range 0.1 mM to 100 mM with logarithmic response. The electrode slope was 52.8 mV/decade for 0.1–40 mM of urea, and response times were less than 4 s; this urea biosensor had excellent selectivity and reproducibility and shown no response to interferents such as ascorbic acid and uric acid, glucose, and K(+) and Na(+) ions.

Well-aligned ZnO nanowires decorated with Pt nanoparticles (NPs) were recently used to construct a nonenzymatic glucose biosensor (Miao et al. 2016). The use of Pt NPs decoration increased the sensitivity by tenfold. The high specific surface area and isoelectric point (IEP) of ZnO have provided the electrode biocompatibility. A similar glucose biosensor on silicon NWs (ZnO/Si NWs) was also reported in the literature (Fung et al. 2017). These nanowire nanocomposites have shown an excellent amperometric sensitivity to glucose ($129 \mu\text{A}\cdot\text{mM}^{-1}$), low detection limits (12 μM), and good stability, reproducibility, and selectivity in the presence of common interferents.

A ZnO NWs/Au electrode was constructed by immobilizing DNA for the fast detection of breast cancer 1 (BRCA1) gene (Mansor et al. 2014). This DNA biosensor was able to detect the target sequence in the concentration range between 10.0 and 100.0 μM with a detection limit of 3.32 μM . A sensitive and selective label-free DNA ZnO NW device which was based on a Schottky contacted was also reported in the literature (Cao et al. 2016). The performance of this device was greatly increased by the use of piezotronic effect (Cao et al. 2016).

7.5 Conclusions and Future Prospects

The present paper describes the recent platforms which are based on lipid membranes and used for biomedical applications for the rapid detection of analytes of clinical interest. These technologies include the construction of stable in solution and in air and are supported on microfiber glass filters and are polymerized on graphene and ZnO microelectrodes. The polymeric lipid film devices can be portable and used in the field. These biosensors have detection limits in the nM concentrations. It is expected soon to commercially prepare units for market production.

The results have exhibited that these lipid membrane-based detectors can be stored and used after remaining in the air for periods of 1 month and can be easily constructed at low cost. The response times of these nanosensors are on the order of s and are not bulky and much cheaper than chromatographic units; these detectors can be complimentary to LC and gas chromatographic instruments for biomedical applications in clinical analysis. These toxicants include toxins, metals, hormones, urea, glucose, cholesterol, etc. with high sensitivity and selectivity, rapid response times, portability, etc.

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