SM Journal of Nanotechnology and Nanomedicine

Review Article

Nanotechnological Advances of Lipid Film Based Biosensors

Georgia-Paraskevi Nikoleli*

Hellenic Army Academy Department of Physical Sciences, Vari GR-16673, Attica, Greece

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 food toxicants, environmental pollutants and 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 in food, environmental and clinical chemistry to detect various toxicants. 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 insecticides, pesticides, herbicides, metals, toxins, antibiotics, microorganisms, hormones, dioxins, etc.

Keywords: Lipid film based biosensors; Nanotechnological platforms; Graphene electrodes; ZnO nanowalls and nanowires; Food toxicants; Environmental pollutants; Clinical analysis

Introduction

A chemical sensor is a device that transforms the chemical information of the concentration of a specific analyte into an analytically useful signal. Chemical sensors consist of two components: a chemical recognition element ("receptor") and a physicochemical transducer. The recognition system translates the chemical information (i.e., concentration of the analyte) into measurable physical signal. The physical transducer provides the signal from the output domain of the recognition element into an electrical, optical, or piezoelectric, etc. domain. A biosensor is a self-contained integrated device which is capable of providing specific quantitative analytical information using a biological recognition element (e.g., enzymes, antibodies, natural or artificial receptors, cells, etc.), which is retained in direct spatial contact with a transduction element.

Nanotechnology deals with the generation and alteration of materials to nanosize (10^{-9} m). Nanomaterials are the materials which have dimensions between 1-100 nanometres. The size constrains of these materials makes them very special as they have most of their constituent atoms located at or near their surface and have all vital physicochemical properties highly different from the same materials at the bulk scale. Nanomaterial based biosensors represent the integration of material science, molecular engineering, chemistry and biotechnology can considerably improve the sensitivity and specificity of biomolecule detection and have great potential in application such as molecular recognition, food and environment monitoring, clinical analysis and pathogen diagnosis.

The early 1960's attempts to reconstitute lipid bilayers in vitro gradually established a lipid membrane technology path, quite intriguing, in the sense that the dynamic and complex nature of biological membranes could be actually simulated with a lipid solution, some skill and a set of affordable instrumentation, yet highly demanding in expertise when the extremely fragile lipid bilayer produced, freely suspended between two electrolyte interfaces, should remain intact for the duration of an experiment [1]. Nowadays lipid membrane constructs can be shaped in many architectures (bilayers, multilayers, mixed layers, branched, doped, nanodiscs, tethered, functionalized, etc.) to serve, efficiently and effectively, a dual purpose: analytical detection [2], and simulation studies [3]. Both research fields benefit from the capability to reproduce some properties of biological membranes and package them into sensing platforms for biosensor devices or membrane interaction studies, respectively. Interestingly, the former field uses the term *artificial lipid membranes* [2] while the latter exploits similar principles and methods to yield *biomembranes* or *membrane mimics* [3]; the different connotations refer to the scopes of research and not the research tool per se.

The number of devices based on lipid membranes that were used to monitor food toxicants, environmental pollutants and compounds of clinical interest has increased tremendously for the last two decades. During the last decade, a number of efforts to prepare stabilized lipid devices were successful 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 have an appropriate biocompatible structure with fast response times, high sensitivity and selectivity, small size, portability, and provide advantages as compared with the bulky liquid chromatographic (LC) instruments. The new generation

Submitted: 08 November 2019 | **Accepted:** 22 November 2019 | **Published:** 25 November 2019

***Corresponding author:** Georgia-Paraskevi Nikoleli, Hellenic Army Academy Department of Physical Sciences, Vari GR-16673, Attica, Greece, Email: dnikolel@chem.uoa.gr

Citation: Nikoleli GP (2019) Nanotechnological Advances of Lipid Film Based Biosensors. SM J Nanotechnol Nanomed 3: 7.

of stabilized lipid membrane nanosensors has the potentiality to construct site-specific analytical devices with respect to analytical performance, operational stability and response.

The biosensing concept of the bilayer lies on the interaction of the target analyte with a biological element or system. This pairing might not always be known or attainable; this is true for many environmental pollutants such as pesticides, herbicides and insecticides [1,4]. More insight is required on the interaction of these species with the lipid membranes in order to devise bioelement-free sensing strategies. In order to grasp in full membrane functionality, the freely-suspended original platforms have been revisited and partly re-engineered with cushioned floating bilayers [5]. The concept that is expected to be realized in the near future is a free-standing, freely-suspended bilayer that is close to the transducer surface without interacting with it.

Lipid membrane technology reduces the complex membrane properties and processes to well-controlled and defined interactions between biological moieties, lipids and transducers (Figure 1). Although the very nature of biological membrane still remains elusive [6], it is easy to isolate specific membrane properties for drug permeability [7], or protein-lipid interaction studies [8] or even to control and manipulate nano-processes on the lipid bilayer by changing macro-parameters (pH, temperature, ionic strength, lipid composition, etc.) [9]. Biological moieties (enzymes, antibodies, receptors, ligands, DNA, etc.) can be easily immobilized on the surface of lipid membranes [2] or embedded into the lipid organization [8], using thermodynamically driven self-assembly processes or more precise techniques, such as patterning [10] or surface printing [11]. The lipid micro-environment presents a compatible setting for biological species to retain their full functionality while experimented upon. Further, the physical state of the lipid membrane offers an intrinsic signal transduction and amplification mechanism, perfectly fitted for electrochemical sensing: when the biological moiety, attached on or embedded in the membrane, interacts with the target analyte, lipid-protein and lipid-lipid interactions are affected to a degree sufficient to disrupt the lipid organization [1]; this affects the flux of ions through the membrane and can be readily detected as current alterations.

Methods for the Preparation of Stabilized Biosensors Based on Lipid Membranes

During the last decade, the construction of stabilized lipid film based biosensors that are not prone to electrical or mechanical shock 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 1μm size and therefore belong to the class of nanosensors. Below we describe the techniques for the construction of this class of biosensors based on lipid films 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 be commercialized.

Stabilized lipid films formed on a glass fiber filter

The route of the construction of stable in electrolyte lipid films was first reported by Nikolelis et al., group and these films were formed on glass fiber filters [12,13], this has permitted a large number of applications in real samples, ie. the continuous monitoring of aflatoxin M_1 in dairy products [12]. The lipid films were prepared on a glass fiber disk ie.GF/F glass microfiber, 0.9 cm in diameter and 0.7 μm nominal pore size [12,13].

The method of preparation of these stable in electrolyte lipid films has extensively been described [12,13]. A diagram of the set-up that has been used is given in Figure 2. The stabilized in electrolyte solution lipid membranes were formed by established procedure as follows [12,13]. 10 μL of a lipid solution in hexane was placed with a microliter syringe at the electrolyte surface in the cylindrical cell. The level of this solution was brought below the 0.32 mm hole and then raised again within a few seconds. 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.

Polymer-supported bilayer lipid membranes

The polymeric stable in air lipid membranes were constructed as previously reported [14,15]. 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 4h to finisg the polymerization.

The construction of these devices has been described in the literature and briefly is as follows [14,15]. 0.07 mL of methacrylic acid, 0.8mL of ethylene glycol dimethacrylate, 8 mg of 2,2'-azobis-(2-methylpropionitrile) and 1.0mL of acetonitrile are added in 0.8 mL of a suspension that contained 4% w/v DPPC in a solvent of n-hexane [which evaporates and the lipid membranes are "solvent-free"). Then nitrogen is allowed to pass through this mixture and a sonication follows. A volume ot 0.15mL of this suspension is placed on the microfilter (Whatman, UK, GF/F microfiber glass disk haning diameter of 0.9cm and pore size of 0.7μm) and the filters are irradiated with a UV deuterium lamp. The experimental instrumentation was the same as in Figure 1. These membranes were stable in storage in air for at least one month and can be used in flow through experiments.

Polymeric lipid membranes supported on graphene microelectrodes

Graphene nanomaterials have been used in biosensors because of their advantages such as enhanced physicochemical properties (mechanical, electrical and thermal), large surface-to-volume ratio, 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 a copper wire that contained graphene nanosheets [16,17]. These nanodevices have been used for the rapid determination of food toxicants, environmental pollutants and compounds of clinical interest [18-20].

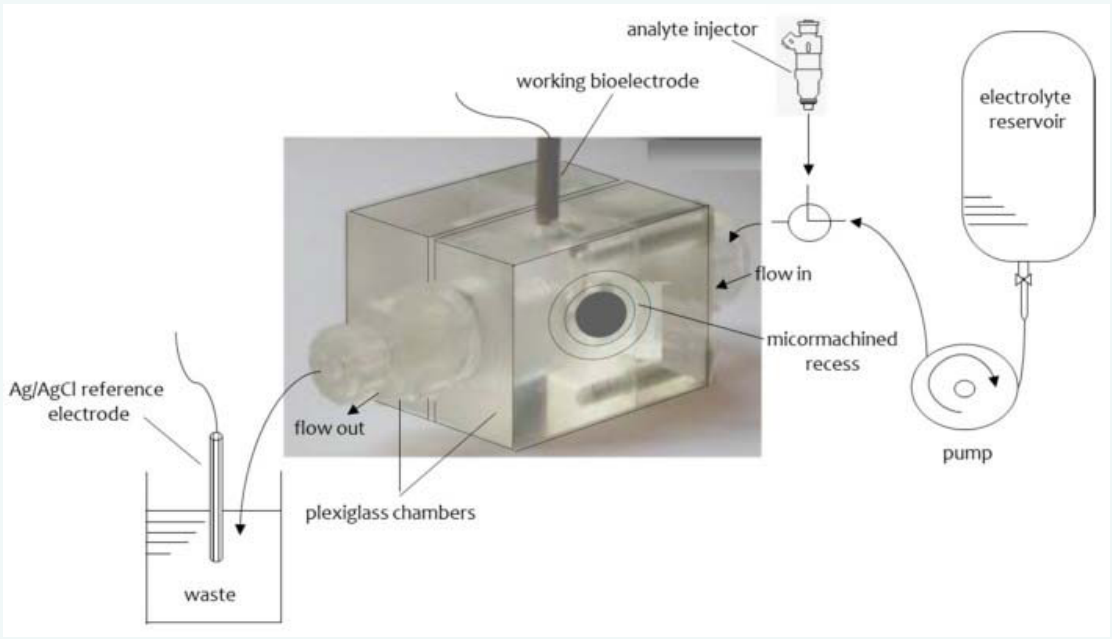

Figure 1 A diagram of the set-up for the formation of lipid membranes that were stable in electrolyte solution.

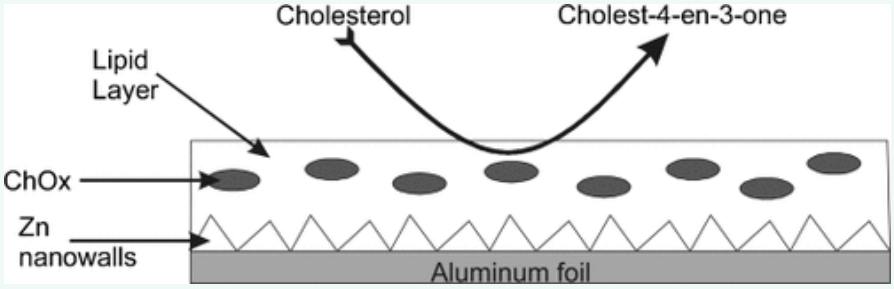

Figure 2 A simple diagramm of the device design (from ref. 13 with permission).

The preparation of graphene microelectrodes has been extensively described in the literature [18-20]. *N*-methyl-pyrrolidone (NMP) was mildly sonicated for 180 hours and centrifuged at 700 rpm for 2 h which provides a homogeneous dispersion (~0.4 mg/mL). This dispersion was poured onto a copper wire (0.25 mm in diameter) which was placed on a glass microfiber filter and the solvent was evaporated. The copper acted as the connection for the electrochemical experiments.

The method of preparation of the lipid membrane biosensors was previously described in detail [18-20]: The stabilized lipid films were prepared by polymerization, as described above

The "receptor" molecules were incorporated in these lipid film devices prior to polymerization by injecting 15μL of the "receptor" suspension on the polymerization mixture. The filter-supported polymeric BLMs were finally mounted onto the copper wire that contained the graphene nanosheets.

Polymer lipid films supported on ZnO microelectrodes: Nanostructured ZnO is a promising mqterial 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), nanostructured ZnO electrodes have high sensitivity and small size, high surface area and high electrical transport. Note that the electrical transport ZnO properties depend on its crystal structure, surface polarity, etc. and these propertied can be modeled. The 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 important analytes such as cholesterol, glucose, L-lactic acid, uric acid, metal ions, and pH.

Potentiometric biosensors based on ZnO nanowalls and stabilized polymerized lipid film: The unmodified ZnO nanowalls electrodes on an aluminum (Al) foil can be constructed by the well known sonochemical technique of Nayak et al. [21].

The preparation of the polymerized stabilized lipid films for

the determination of cholesterol was previously described [22]. Cholesterol oxidase was incorporated in these lipid membranes prior polymerization by spreading on the microfilter 15μL of the enzyme suspension with the polymerization mixture using a microliter syringe.

The final stage to construct the device was to encapsulate the filter-supported polymerized lipid film onto the wire containing the ZnO-nanowalls electrode. Figure 3 shows a simplified diagram of the device.

Potentiometric biosensors based on lipid stabilized membranes ZnO Nanowires: The sensor electrodes were constructed following the method as previously [23]. The plastic substrate was affixed on a support in the vacuum chamber of an evaporator. Firstly, the titanium film was deposited (thickness ca. 10nm) and then 50nm of gold was deposited on the surface of the plastic substrate. The gold coated electrode (4cm long and 1 mm width) was rinsed with acetone and then by de-ionized water and dried at room temperature. A chemical approach was used to prepare the ZnO nanowires on the electrode [24]. The electrode was inserted twice into a solution that contained zinc acetate (2 min) and then was dried in the air. The electrodes then were inserted in a 025 M Zn $(NO_3)_2$ $6H_2O$ solution that contained 0.025 M hexamethylenetetramine and placed an oven (2-4 h, 90°C). The ZnO nanowires were finally rinsed with de-ionized water and dried at room temperature. The diameters were 80–150 nm and were uniform.

The enzyme (uricase) was incorporated in the lipid membranes prior to polymerization as previously described [25]. Construction of the uricase device was finalized by encapsulation of the filter-supported polymerized lipid film onto copper wire containing the ZnO nanowires.

Practical Applications of Lipid Membrane Biosensors

Applications of lipid film sensors based on glass microfilter

An atrazine lipid film biosensor was reported in the literature which was based on microfiber glass filters betwwin two Saran-Wrao™ partition in which the lipid was deposited [26]. A transient ion current signal (duration of s) was the result of interactions of atrazine with the lipid membranes and appeared within 1 min following the injection of atrazine into the bulk electrolyte solution. The introduction of an anionic lipid (35% DPPA) in membranes and of Calcium ions in the electrolyte solution increased the sensitivity of the technique. Similarly, the use of platelet-activating factor (PAF; an ether analog of PC) in membranes increased greatly the sensitivity.

A FIA technique of mixtures of the triazine herbicides simazine, atrazine and propazine using a mixture of PC/DPPA in glass fiber filter-supported lipid films was reported in the literature [4]. When a sample containing a mixture of these herbicides was injected into the flowing carrier KCl electrolyte solution, a transient ion current signal (duration of s) appeared in less than 2 min following the injection. The peak heights of these signals were linearly correlated to the herbicide concentration, μM detection limits. The signal did not decreased but remained practically constant even when repetitive cycles ocf injections were performed. The time of appearance of the transient signal varied on the hydrophobicity of each herbicide and increased to the order of simazine, atrazine and propazine, thus allowing the simultaneous determination and analysis of these triazines in mixtures.

Applications of lipid film devices based on polymeric lipid membranes

The stable in air lipid film devices were applied for the determination of carbofuran using flow injection analysis (FIA) [27]. The technique was based on the degree of inhibition and reactivation of enzyme upon substrate injections. Carbofuran could be detected between 10^{-7} - 10^{-9} M. Interference studies were made and involved proteins and lipids which are food components. No interferences were noticed from these compounds. The device was evaluated/ validated using real food samples (ie., fruits, vegetables and dairy products). The recovery studies have shown satisfactory results between 96% and 106%, which shows that there are no interferences from the sample matrix.

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 [28]. Similar devices were constructed to selectively and rapidly determine food hormones (ie., naphthalene acetic acid) in fruits and vegetables [29] and a zinc in water [30].

Applications of graphene based devices

A potentiometric urea lipid membrane based minisensor on graphene has been appeared recently [16]. A potentiometric urea device based on lipid film technology on graphene nanosheets has been constructed; a simplified set up of this biosensor is shown in Figure 3. The main characteristics of this biosensor are

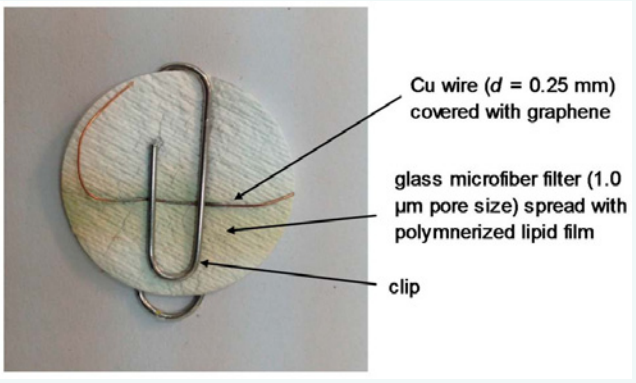

Figure 3 Picture of the lipid film device on graphene minielectrode which was used for the potentiometric detection of urea.

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.

A nanosensor for naphthalene acetic acid (NAA) was reported in the literature and was based on polymerized stable in air lipid films devices in which auxin-binding protein 1 receptor was codeposited with the lipid film [18]. The sensor was evaluated in reals samples of fuits and vegetables. Using a flow through experimental set up, NAA was injected into the flowing 0.1 M KCl electrolyte solution and the flow has stopped. An ion current transient was obtained; the peak height was related to the concentration of NAA with mM detection limits and analysis times of ca. 5 min. Interference studies have shown no interferences from compounds normally found in foods and vegetables (eg, lipids, proteins, ascorbic acid, etc). The sensor has been used to determine NAA in fruits and vegetables with quite satisfactory results.

A potentiometric carbofuran minisensor on graphene in which lipid films were polymerized was provided in the literature [17]. This device was applied to construct a carbofuran chemical sensor by codepositing resorcin [6] arene selective receptor. The detection limits were at the nM levels with times of response of 20 s. The device was constructed easily and shown excellent characteristics such as good reproducibility, reusability, selectivity, long shelf life; the electrode slope was 59 mV/decade and carbofuran was logarithmically related in the range 10^{-6} to 10^{-3} M.

The electrochemical interactions of cholera toxin with polymerized lipid films with incorporated ganglioside GM1 was reported in the literature [32]. An injection of cholera toxin in the flowing streams of a KCl 0.1 M carrier electrolyte, provided a current signal. The peak height of the ion current signal was correlated with the concentration of cholera toxin in the sample solution with detection limits of 0.06μM.

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) [19]. The construction of this sensor was easy and the sensor has shown good reproducibility, reusability, selectivity, long shelf life and sensitivity of 60 mV/decade of toxin concentration. The method was applied 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 [20]. An excellent selectivity, sensitivity and detection limits (1 nM) for the determination of saxitoxin with rapid response times (ie., 5-20 min) and were noticed. The sensor was easily constructed and had adequate storage stability with a slope of 60 mV/decade over saxitoxin concentration. The method was applied for the determination of STX in lake water and shellfish samples.

Applications of the ZnO nanoelectrode based devices

A potentiometric cholesterol device was constructed by immobilizing cholesterol oxidase into polimerixed lipid membrane on ZnO nanowalls [22]. The enzyme was codeposited into the lipid membrane prior 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 present biosensor has shown biocompatibility and could be implanted in the human body.

An uric acid electrochemical device was reported in the literature by immobilizing the enzyme uricase into polymerized lipid membranes on Zn nanowires [23]. 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 two-fold. No interferences were observed by the presence of ascorbic acid, glucose, urea, proteins and lipids.

ZnO nanowires (NW) was tailored for the immobilization of glucose oxidase in order to fabricate a glucose sensor [33]. 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 [34]. These arrays had 50-130 nm diameters and were applied to construct a urea biosensor using urease within the concentration range 0.1mM to 100mM with logarithmic response. The electrode slope was 52.8mV/decade for 0.1-40mM of urea and response times were less than 4s; this urea biosensor had excellent selectivity, reproducibility and shown no response to interferents such as ascorbic acid and uric acid, glucose. K(+) and Na(+) ions.

Well-aligned ZnO nanowires decorated with Pt nanoparticles (NPs) were recently used to construct a non-enzymatic glucose biosensor [35]. The use of Pt NPs decoration increased the sensitivity by 10-fold. The high specific surface area and isoelectric point (IEP) of ZnO has provided the electrode biocompatibility. A similar glucose biosensor on silicon NWs (ZnO/Si NWs) was also reported in the literature [36]. These nanowire nanocomposites has shown an excellent amperometric sensitivity to glucose (129 $\mu A\cdot mM^{-1}$), low detection limits (12μM), good stability and reproducibility and selectivity in the presence of common interferents. Recent works applied a roll-to-roll flexographic printing technique was used to construct a three electrode telectrochemical unit that consisted of ZnO NWs [37]. This unit exhibited a sensitivity of 1.2 ± 0.2 $\mu A\cdot mM^{-1}\cdot cm^{-2}$ and the calibration graph was linear over the glucose concentration between 0.1 to 3.6 mM.

A ZnO NWs/Au electrode was constructed by immobilizing DNA for the fast detection of breast cancer 1 (BRCA1) gene [38]. This DNA biosensor was able to detect the target sequence in the

concentration range between 10.0 and 100.0μM with a detection limit of 3.32μM. A sensitive and selective label-free DNA ZnO NW device which was based on a Schottky contacted was also reported in the literature [39]. The performance of this device was greatly increased by the use of piezotronic effect [39].

Conclusions

Regardless the scope of research, lipid membrane platforms has become indispensable tools in biosensing and cell studies. Electrochemistry combined with photonics will most certainly provide an optimal for guiding lipid formation and monitoring membrane interactions [40]. Many challenges lie ahead, mostly referring to the minimization of matrix effects, membrane reproducibility and sensor reliability.

The development of lipid-based platforms into nature-mimicking gustatory or olfactory arrays can rely on current chip integration techniques but the extremely low detection limits required can be only achieved with ligand-gated ion channels, provided that technology advances in controlling protein orientation will become available in the near future. Synthetic or engineered channels, with built-in guidance systems has been recently proposed [12] and might prove to be quite useful for mass production.

The paper describes the recent platforms which are based on lipid membranes and used for food, environmental and clinical analysis. 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 one 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 in-field applications for the rapid detection of food and environmental toxicants and in clinical analysis. These toxicants include toxins, carbamates, hydrazines, hormones, polycyclic aromatic hydrocarbons, glucose, cholesterol etc with high sensitivity and selectivity, rapid response times, portability, etc.

References

1. Siontorou CG, Nikoleli GP, Nikolelis DP, Karapetis SK. Artificial lipid membranes: past, present, and future. Membranes. 2017; 7: 38.
2. Mazur F, Bally M, Städler B, Chandrawati R. Liposomes and lipid bilayers in biosensors. Adv Colloid Interface Sci. 2017; 249: 88-99.
3. Su ZF, Leitch JJ, Lipkowski J. Electrode-supported biomimetic membranes: An electrochemical and surface science approach for characterizing biological cell membranes. Curr Opin Electrochem. 2018; 12: 60-72.
4. Gould SB. Membranes and evolution. Current Biol. 2018; 28: 381-385.
5. Ebert A, Hannesschlaeger C, Goss KU, Pohl P. Passive permeability of planar lipid bilayers to organic anions. Biophy J. 2018; 115: 1931-1941.
6. Vacek J, Zatloukalova M, Novak D. Electrochemistry of membrane proteins and protein–lipid assemblies. Curr Opin Electrochem. 2018; 12: 73-80.
7. Nikoleli GP, Nikolelis DP, Evtugyn G, Hianik T. Advances in lipid film based biosensors. TrAC Trends Anal Chem. 2016; 79: 210-221.
8. Andreou VG, Nikolelis DP. Flow injection monitoring of aflatoxin M_1 in milk and milk preparations using filter-supported bilayer lipid membranes. Anal Chem. 1998; 70: 2366-2371.
9. Nikolelis DP, Siontorou CG, Andreou VG, Krull UJ. Stabilized bilayer-lipid membranes for flow-through experiments. Electroanalysis. 1995; 7: 531-536.
10. Nikolelis DP, Raftopoulou G, Nikoleli GP, Simantiraki M. Stabilized lipid membrane based biosensors with incorporated enzyme for repetitive uses. Electroanalysis. 2006; 18; 2467-2474.
11. Nikolelis DP, Raftopoulou G, Chatzigeorgiou P, Nikoleli GP, Viras K. Optical portable biosensors based on stabilized lipid membrane for the rapid detection of doping materials in human urine. Sens Actuators B Chem. 2008; 130: 577-582.
12. Nikoleli GP, Israr MQ, Tzamtzis N, Nikolelis DP, Willander M, Psaroudakis N. Structural characterization of graphene nanosheets for miniaturization of potentiometric urea lipid film based biosensors. Electroanalysis. 2012; 24: 1285-1295.
13. Bratakou S, Nikoleli GP, Nikolelis DP, Psaroudakis N. Development of a potentiometric chemical sensor for the rapid detection of carbofuran based on air stable lipid films with incorporated calix [4] arene phosphoryl receptor using graphene electrodes. Electroanalysis 2015; 27: 2608-2613.
14. Bratakou S, Nikoleli GP, Siontorou CG, Nikolelis DP, Tzamtzis N. Electrochemical biosensor for naphthalene acetic acid in fruits and vegetables based on lipid films with incorporated auxin-binding protein receptor using graphene electrodes. Electroanalysis. 2016; 28: 2171-2177.
15. Karapetis S, Nikoleli GP, Siontorou CG, Nikolelis DP, Tzamtzis N, Psaroudakis N. Development of an electrochemical biosensor for the rapid detection of cholera toxin based on air stable lipid films with incorporated ganglioside GM1 using graphene electrodes. Electroanalysis. 2016; 28: 1584-1590.
16. Bratakou S, Nikoleli GP, Siontorou GC, Nikolelis DP, Karapetis S, Tzamtzis N. Development of an electrochemical biosensor for the rapid detection of saxitoxin based on air stable lipid films with incorporated Anti-STX using graphene electrodes. Electroanalysis. 2017; 29: 990-997.
17. Naval AP, Katzenmeyer AM, Gosho Y, Tekin B, Islam MS. Sonochemical approach for rapid growth of zinc oxide nanowalls, Applied Physics A. 2012; 107: 661-667.
18. Psychoyios VN, Nikoleli GP, Tzamtzis N, Israr MQ, Willander M. Potentiometric cholesterol biosensor based on ZnO nanowalls and stabilized polymerized lipid film, Electroanalysis. 2013; 25: 367-372.
19. Usman Ali SM, Alvi NH, Ibupoto Z, Nur O, Willander M, Danielsson B. Selective potentiometric determination of uric acid with uricase immobilized on ZnO nanowires, Sens. Actuators B. 2011; 152: 241-247.
20. Vaface M, Youzbashizade H. Production of zinc oxide nanoparticles by liquid phase processing: an investigation on optical properties. Mat

Sci For. 2007; 553: 252-256.

21. Tzamtzis N, Psychoyios VN, Nikoleli GP, Willander M, Qadir Israr M. Flow Potentiometric Injection Analysis of Uric Acid Using Lipid Stabilized Films with Incorporated Uricase on ZnO Nanowires, Electroanalysis. 2012; 24: 1719-1725.

22. Bratakou S, Nikoleli GP, Nikolelis DP, Psaroudakis N. Development of a potentiometric chemical sensor for the rapid detection of carbofuran based on air stable lipid films with incorporated calix [4] arene phosphoryl receptor using graphene electrodes. Electroanalysis. 2015; 27: 2608-2613.

23. Bratakou S, Nikoleli GP, Siontorou CG, Nikolelis DP, Tzamtzis N. Electrochemical biosensor for naphthalene acetic acid in fruits and vegetables based on lipid films with incorporated auxin-binding protein receptor using graphene electrodes. Electroanalysis. 2016; 28: 2171-2177.

24. Karapetis S, Nikoleli GP, Siontorou CG, Nikolelis DP, Tzamtzis N, Psaroudakis N. Development of an electrochemical biosensor for the rapid detection of cholera toxin based on air stable lipid films with incorporated ganglioside GM1 using graphene electrodes. Electroanalysis. 2016; 28: 1584-1590.

25. Bratakou S, Nikoleli GP, Siontorou GC, Nikolelis DP, Karapetis S, Tzamtzis N. Development of an electrochemical biosensor for the rapid detection of saxitoxin based on air stable lipid films with incorporated Anti-STX using graphene electrodes. Electroanalysis. 2017; 29: 990-997.

26. Vaface M, Youzbashizade H. Production of zinc oxide nanoparticles by liquid phase processing: an investigation on optical properties. Materials Science Forum. 2007; 553: 252-256.

27. Tzamtzis N, Psychoyios VN, Nikoleli GP, Willander M, Qadir Israr M. Flow Potentiometric Injection Analysis of Uric Acid Using Lipid Stabilized Films with Incorporated Uricase on ZnO Nanowires, Electroanalysis. 2012; 24: 1719-1725.

28. Nikolelis DP, Andreou VG. Electrochemical transduction of interactions of atrazine with bilayer lipid membranes. Electroanalysis. 2005; 8: 643-647.

29. Nikolelis DP, Raftopoulou G, Simantiraki M, Psaroudakis N, Nikoleli GP, Hianik T. Preparation of a selective receptor for carbofuran for the development of a simple optical spot test for its rapid detection using stabilized in air lipid films with incorporated receptor. Anal Chim Acta. 2008; 620: 134-141.

30. Nikolelis DP, Ntanos N, Nikoleli GP, Tampouris K. Development of an electrochemical biosensor for the rapid detection of naphthalene acetic acid in fruits by using air stable lipid films with incorporated auxin-binding protein 1 receptor, Protein and Peptide Lett. 2008; 15: 789-794.

31. Nikolelis DP, Raftopoulou G, Psaroudakis N, Nikoleli GP. Development of an electrochemical chemosensor for the rapid detection of zinc based on air stable lipid films with incorporated calix4arene phosphoryl receptor. Int J Environ Anal Chem. 2009; 89: 211-222.

32. Nikoleli GP, Siontorou CG, Nikolelis DP, Bratakou S, Karapetis S, Tzamtzis N. Biosensors based on lipid modified graphene microelectrodes. Carbon. 2017; 3: 9.

33. Nikoleli GP, Nikolelis DP, Tzamtzis N. Development of an electrochemical biosensor for the rapid detection of cholera toxin using air stable lipid films with incorporated ganglioside GM1. Electroanalysis. 2011; 23: 2182-2189.

34. Zang J, Li CM, Cui X, Wang J, Sun X, Dong H, Sun CQ. Tailoring Zinc Oxide Nanowires for High Performance Amperometric Glucose Sensor, Electroanalysis. 2007; 19: 1008-1014.

35. Ali SMU, Ibupoto ZH, Salman S, Nur O, Willander M, Danielsson B. Selective determination of urea using urease immobilized on ZnO nanowires. Sens Actuators B. 2011; 160: 637-643.

36. Hsu CL, Lin JH, Hsu DX, Wang S.H, Lin SY, Hsueh TJ. Enhanced non-enzymatic glucose biosensor of ZnO nanowires via decorated Pt nanoparticles and illuminated with UV/green light emitting diodes. Sens. Actuators B. 2017; 238: 150-159.

37. Miao F, Lu X, Tao B, Li R, Chu PK. Glucose oxidase immobilization platform based on ZnO nanowires supported by silicon nanowires for 9glucose biosensing. Microelectron Eng. 2016; 149: 153-158.

38. Fung CM, Lloyd JS, Samavat S, Deganello D, Teng KS. Facile fabrication of electrochemical ZnO nanowire glucose biosensor using roll to roll printing technique. Sens. Actuators B. 2017; 247: 807-813.

39. Mansor NA, Zain ZM, Hamzah HH, Noorden MSA, Jaapar SS, Beni V, et al. Detection of Breast Cancer 1 (BRCA1) gene using an electrochemical DNA biosensor based on immobilized ZnO nanowires. Open J Appl Biosens. 2014; 3: 9-17.

40. Cao X, Cao X, Guo H, Li T, Jie Y, Wang N, Wang ZL. Piezotronic effect enhanced label-free detection of DNA using a schottky-contacted ZnO nanowire biosensor. ACS Nano. 2016; 10: 8038-8044.