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REVIEW

Impedimetric Biosensors

JIAN-GUO GUAN,[1]* YU-QING MIAO,[1,2] AND QING-JIE ZHANG[1]

State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan 430070, P.R. China[1] and College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, P.R. China[2]

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Electrochemical impedance spectroscopy (EIS) is a sensitive indicator of a wide variety of chemical and physical properties. An increasing trend towards the development of impedimetric biosensors is being currently observed. Impedimetric techniques have been performed to characterize the fabrication of the biosensors and to monitor the catalyzed reactions of enzymes or the biomolecular recognition events of specific binding proteins, lectins, receptors, nucleic acids, whole cells, antibodies or antibody-related substances. However, little attention has been paid to reviewing this interesting research area. Herein, impedimetric biosensors are reviewed and many novel designs are discussed.

[**Key words:** impedimetric biosensors, biosensors, electrochemical impedimetric spectroscopy, electrochemical biosensors]

Electrochemical impedance spectroscopy (EIS) combines the analysis of both the resistive and capacitive properties of materials, based on the perturbation of a system at equilibrium by a small amplitude sinusoidal excitation signal (1, 2). The potential of EIS is that the impedance of the system can be scanned over a wide range of alternative current (AC) frequencies. The equivalent circuit models are useful for the interpretation of impedance spectra, and it is a valuable technique for the characterization, analysis and study of coatings, batteries, fuel cells and corrosion phenomena. It has also been used extensively as a tool for investigating electrode kinetics, conducting polymers, semiconductors, sensors, animal and plant tissues and general material.

Biosensors are constructed for monitoring a biological reaction at the surface of electrodes. A variety of biomolecules have been used as basic detection elements of AC impedimetric biosensors with different degrees of success. In particular, enzymes, antibodies, nucleic acids, cells and microorganisms have been immobilized onto the surface of electrodes to develop impedimetric biosensors. Various strategies have been performed to design potentially useful impedimetric biosensors. In this regard, Katz and Willner (2) recently reviewed, in detail, the probing of biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy, as well as impedimetric immunosensors, DNA-sensors, and enzyme biosensors.

Many capacitive biosensors have been reported as impedimetric biosensors. Detailed information about capacitive biosensors was recently reviewed by Berggren *et al*. (3). Besides that the content of our review is similar to that of Refs. 2 and 3, here we introduce both impedimetric techniques, which are usually used for the characterization of the fabrication of the biosensors and for monitoring the analytes, and impedimetric biosensors giving some typical examples, in a simple, straightforward and easily comprehensible manner. In particular, the new development of cell- and microorganism-based impedimetric biosensors is included.

I. ENZYME BASED IMPEDIMETRIC BIOSENSORS

Impedance spectroscopy provides an effective method to probe the electric features of surface-modified electrodes. However, the recording of a full impedance spectrum within a broad region of frequencies is time-consuming. Thus, impedimetric techniques are only used as a characterization method for most of the enzyme-based impedimetric biosensors, and indirect monitoring strategies are generally adopted.

A biosensor for collagenase detection was developed, which detected the change in impedance caused by the proteolytic digestion of gelatin-coated interdigitated gold electrodes (4). Enzyme degradation of the layer produced a rapid rise in impedance when a critical thickness was reached. The change in impedance with protease digestion was correlated with solubilization of the gelatin layer from the sensor surface. The response time of the device could be greatly reduced by stirring the fluid around the biosensor and it seemed to be useful for the detection of collagenase activity. However, the ability to detect the gelatin coating on the sensor was severely impaired by the presence of electro-

* Corresponding author. e-mail: guanjg@mail.whut.edu.cn
phone: +86-27-8721-8832 fax: +86-27-8787-9468

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lytes. The implications of these findings for further biosensor development were discussed by Saum *et al*. (4).

Many enzyme-based impedimetric biosensors were used in the modification of electrodes with Langmuir–Blodgett (LB) methods, since impedimetric techniques were suitable for the characterization of LB films.

LB films containing butyrylcholinestrase (BuChE) were fabricated to realize an ion-sensitive field-effect transistor (ISFET) for the detection of organophosphorus pesticides in water (5). Enzyme/stearylamine-mixed LB films were immobilized onto the gate of the ISFET after treatment of glutaraldehyde vapor to improve the stability of the LB film. The deposition of LB film on Si/SiO_2 changed the impedance of the system. The suggested equivalent model corresponded to the Si/SiO_2/LB film-electrolyte interface. The thickness of the LB film could be determined to be about 60-nm by a fitting program, which was in good agreement with the ellipsometry results. The fabricated biosensors could be used to detect trichlorfon.

Yea *et al*. (6) described a supported bilayer lipid membrane (s-BLM) doped with platinum (Pt) nanoparticles as a biosensor. Impedance spectroscopy on a bare glassy carbon (GC) electrode and an s-BLM-modified electrode with and without Pt nanoparticles in the presence of $[Fe(CN)6]^{3-/4-}$ showed that the bare GC electrode exhibited an almost linear line that was characteristic of a diffusion-controlled step of the electrochemical process. With respect to the modified electrodes, the Nyquist complex plane plots of the s-BLM with and without doping with Pt nanoparticles were characterized by part of a single semicircle in the high-frequency domain, which meant that doping of the s-BLM with Pt nanoparticles caused the membrane capacitance to increase and the membrane resistance to decrease. The Pt nanoparticle array in the s-BLM electrocatalyzed the reduction of oxygen (O_2), which found practical application in the construction of a glucose biosensor.

Peptide-tethered lipid membranes (tBLMs) on a gold support were designed as a biomimetic system to investigate integral membrane proteins (Fig. 1) (7). As an example, cytochrome *c* oxidase (COX) from bovine heart was incorporated into the preformed peptide tBLM. Impedance spectroscopy was used to study both the quality of lipid films and ion transport across them. It indicated the poor quality of the peptide-supported lipid mono- and bilayers where the electrode was only partially (70%) covered with a bilayer and approximately 30% with defect domains of a monolayer

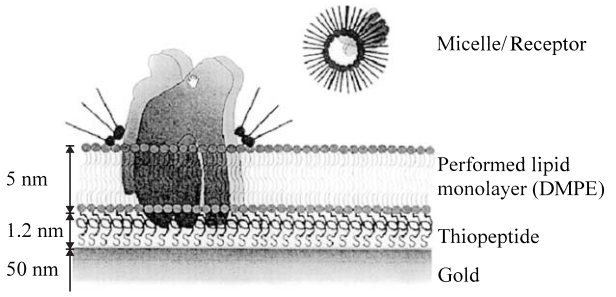


FIG. 1. The incorporation of cytochrome *c* oxidase in a preformed lipid bilayer (7).

of peptide or peptide-lipid. Impedance spectra were correlated quantitatively with concentrations of substrate and inhibitor. The system could thus be promising for use in biosensor applications. In similar research by the same team (8), H^+-ATP synthases from chloroplasts were incorporated into tBLMs. The activity of the protein was demonstrated by impedance spectroscopy. The resistance decreased due to proton transport across the lipid film, which occurred as a consequence of adenosine triphosphate (ATP) hydrolysis. Changes in the impedance spectra due to the addition of ATP were fully reversible.

II. IMMUNO-BINDING BASED IMPEDIMETRIC BIOSENSORS

Affinity-binding based impedimetric biosensors are attracting increasing interest, since they are direct and label-free electrochemical immunosensors and have many potential advantages with respect to speed, the use of unskilled analysts and the potential development of multi-analyte sensors. In recent years, many novel designs of affinity-binding-based impedimetric biosensors have been reported.

Ma *et al*. (9) reported an impedance immunosensor for human mammary tumor associated glycoprotein. Antibody was immobilized onto a gold electrode by spontaneous adsorption. The change in the electrochemical characteristics took place during the binding of the specific antigen. The charge-transfer resistance (R_{ct}) calculated from the semicircle in the Nyquist plot increased due to the formation of the stable antigen–antibody complex.

Conductive polymers are good matrixes for the immobilization of biomolecules. Sargent and Sadik (10) investigated the mechanisms of antibody–antigen (Ab–Ag) interactions at conducting polypyrrole (PPy) electrodes using impedance spectroscopy techniques. The theory of charge generation and transportation in the heterogeneous polymeric interface was proposed to explain the current flow during Ab–Ag binding. According to this mechanism, the current obtained at the antibody-immobilized conducting polymer electrodes occurred via the following steps: (i) diffusion of ions to the electrode; (ii) charge transfer at the porous PPy/membrane interface; (iii) migration through the polymer PPy membrane, and (iv) adsorption/desorption of the Ag at the PPy/solution interface.

The adsorption/desorption process in step iv was considered to be the rate-determining step. This step could be controlled through the appropriate choice of electrical potential, which confirmed that the Ab–Ag interaction was largely influenced by the applied potential.

An electrodeposited biotinylated polypyrrole film was described as an immobilization matrix for the fabrication of impedimetric immunosensors (11). Biotinylated antibody (anti-human IgG) was attached to the free biotin groups on the conducting polypyrrole film with avidin as a coupling reagent. As shown in Fig. 2, the second semicircle diameter in the Nyquist plot increased with increasing antigen concentration, especially at low frequency, which could be chosen for concentration-dependent impedance measurements. This immobilization method allowed a highly reproducible and stable device to be obtained. The resulting immunosensor

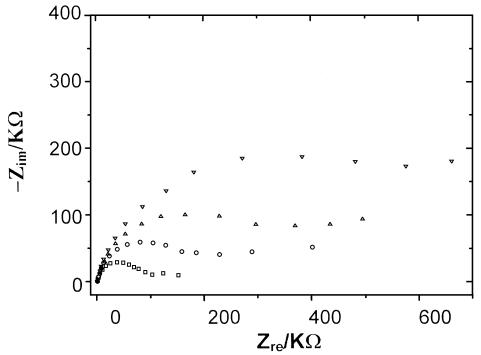


FIG. 2. Complex plane impedance plots for an antibody modified electrode at a potential of −1.4 V vs SCE and AC signal of 5 mV, in PBS (squares), without antigen injection; after antigen injection at different concentrations: circles, 10 ng/ml; positive triangles, 50 ng/ml; inverted triangles, 100 ng/ml (11).

had a linear dynamic range of 10–80 ng/ml of antigen and a detection limit of 10 pg/ml. Again using ploypyrrole as the immobilization matrix, Geoffrey *et al*. (12) reported an impedance-based reagentless bioaffinity sensor. Two charge-transfer processes were observed with the antibody-loaded polypyrrole films, which were assigned to polaronic conduction at low frequencies and electronic conduction at high frequencies. The affinity reaction did not lead to any significant alteration in the Bode plot. However, a possible binding-dependent response, observed as a decrease in peak polaronic phase angle, occurred when a redox cycle (−0.1 V to −0.9 V to −0.1 V) was performed on the film following exposure to the appropriate analyte. This result might be attributed to the realignment of the polymer chains around the protein after binding.

Protein multilayer films were also investigatd by impedance spectroscopy in the presence of a redox probe (Fig. 3) (13). The multilayer film was composed of avidin and biotin-labeled antibody (bio-Ab) on a gold surface, which was prepared by layer-by-layer assembly technology. A significant difference in the impedance spectra was observed upon the stepwise formation of the multi-layers. R_{ct} increased gradually, since the avidin and bio-Ab were both nonconductive and their multilayer films blocked the electron-transfer of $[Fe(CN)6]^{4-/3-}$. A good linear relationship between R_{ct} and the layer number also indicated the uniform construction of avidin/bio-Ab multilayer films.

Impedance spectroscopy was used to characterize the structure of biomaterial layers on the gate surface of ISFET devices (14), and to elucidate antigen-antibody binding interactions on the gate interface. The ability to characterize the thickness of layered protein assemblies on the gate interface of ISFET devices by means of impedance spectroscopy not only provided a method for the structural characterization of the systems, but also yielded an analytical method to probe and sense bio-recognition events that occurred on the gate surface of the ISFET.

Nanoscale fabrication may be useful in improving the response performance of impedimetric biosensors. Some helpful attempts have been reported. Van Gerwen *et al*. (15) developed nanoscale interdigitated electrode (IDE) arrays for impedimetric biosensors (Fig. 4). Electrode widths and

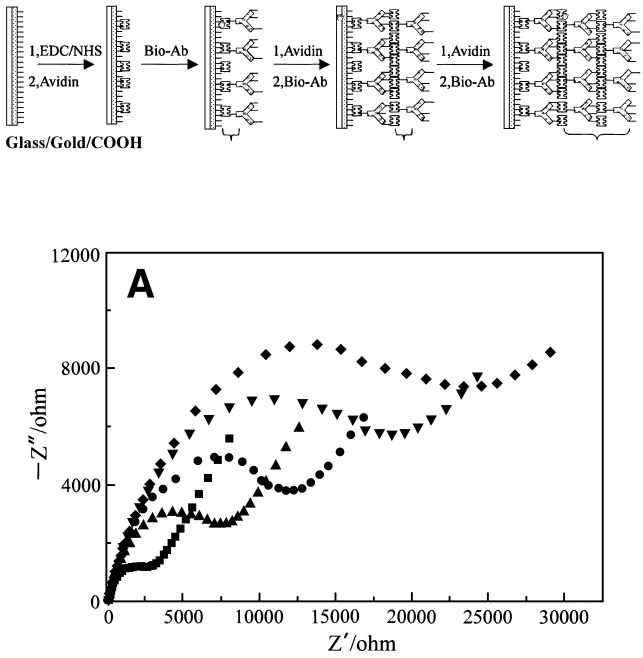


FIG. 3. A layer-by-layer construction of avidin/biotin-labeled-antibody on gold surface and complex plane impedance plots of avidin/biotin-labeled-antibody with increasing numbers of outmost bio-Ab layers from 1 to 5 at 0.24 V vs Ag/AgCl in 10 mM PBS + 0.15 M KCl + 5 mM $[Fe(CN)6]^{4-/3-}$ solution. Frequency range is between 0.1 and 100,000 Hz with a signal amplitude of 50 mV (13).

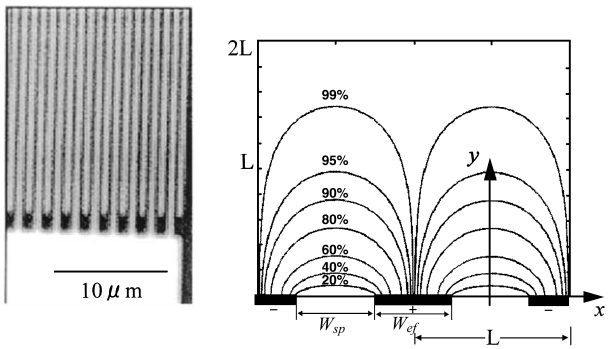


FIG. 4. SEM of IDE and calculated current comprisement of IDE. A curve of, *e.g*., 80% means that 80% of the current flows beneath the curve (15).

spacings from 500 to 250 nm were achieved. The nanoscale electrodes only scanned the region about 100 nm above the surface and thus showed an improved sensitivity, in comparison with conventional electrodes. The effect was theoretically analyzed by calculating the electric field between the interdigitated electrodes. For example, for electrodes with widths and spacings of 250 nm, 80% of the current flowed in a layer not higher than 250 nm above the surface. The model and characterization in different KCl solutions also showed that the impedance was almost completely determined by the process close to the surface, and the characteristics of the bulk did not appear in the signal. Glucose oxidase (GOD) was immobilized on a silanized surface for the detection of the affinity binding of biomolecular struc-

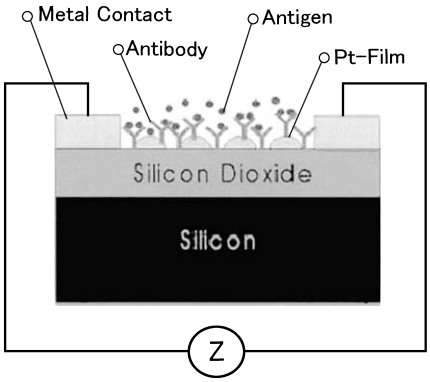


FIG. 5. Cross-sectional diagram of ultrathin platinum film immunobiosensor (16).

tures (*e.g.*, antigens, DNA) by impedimetric measurements. After silanization, the double layer impedance of the sensor was well represented by a constant phase element. The measurement of this parameter facilitated the monitoring of the immobilization of GOD *in situ*. It was found to be influenced by the biomolecules near the surface of the IDE rather than by the bulk of the solution, which determined the capacitance.

A novel immunobiosensor based on a discontinuous nanoscale platinum layer was also described by Pak *et al*. (16) (Fig. 5). The film was about 25 Å thick and consisted of a discontinuous layer with channels 20–30 Å wide. Monoclonal antibodies were bound to the sensor surface by silane. The impedance at fixed frequencies across the film was used to track modification and binding at the surface. The impedance increased 55% at 20 Hz during the activation of the surface with anti-alkaline phosphatase (anti-AP). Binding of alkaline phosphatase (AP) to the prepared surface resulted in a further increase of 12%. The sensors behaved in a distributed network or similar to a transmission line, since the Pt film of the sensor comprised interconnected islands. It was likely that conduction flowed through the interconnected network of islands and the double layer capacitances of each island contributed to the overall sensor response.

A novel concept for detection by impedimetric biosensors was described by Katz *et al*. (17). The biocatalyzed precipitation of an insoluble product on the electrode surface was used as an amplification path for immunosensors. Faradaic impedance spectroscopy and chronopotentiometry were used to follow the precipitation processes. An antigen-immobilized electrode was used to sense the dinitrophenyl antibody. An antibody–HRP conjugate was applied as a biocatalyst to yield the insoluble product of the enzyme reaction. Impedance analysis showed the precipitation of an insoluble, insulating product on the surface of the electrode which introduced a barrier for the electron transfer at the electrode interface, resulting in an enhanced electron-transfer resistance.

The use of biological receptors in biosensors has well-known limitations including low stability of the biological species, difficulties of immunization by small antigens, and low chemical and thermal stabilities. Hence, there is a strong tendency to replace biological receptors with artificial receptors. Molecularly imprinting polymers (MIPs) are intrinsically stable and robust, facilitating their application under extreme pressure, temperature, pH or organic solvents. They are also cheap to produce and can be stored in a dry state. MIPs have been developed not only for small organic molecules, for example, pesticides, amino acids, steroids and sugars, but also for proteins and cells (18). This technique could be used to develop biosensors of wide definition. Panasyuk-Delaney *et al*. (19) reported an MIP-based impedimetric biosensor. The herbicide desmetryn was used as a template. The adsorbed layer of benzophenone, irradiated by UV light, initiated a radical polymerization near the surface. The electrodes coated with the MIP displayed fairly specific binding of desmetryn, as detected by the decrease in the capacitance of the electrode. Only small capacitive effects were observed on addition of terbumeton or atrazine, while metribuzine caused a capacitance decrease similar to that caused by desmetryn. Molecular imprinting (template polymerization) is considered as a promising and inexpensive alternative. However, the slow diffusion of analytes into thick MIP layers results in slow response kinetics, typically in the timeframe of hours.

III. NUCLEIC ACID-BASED IMPEDIMETRIC BIOSENSORS

A hanging mercury drop electrode was used to sense differences between single-stranded and double-helical conformations of nucleic acids and synthetic polynucleotides (20). From the frequency dependence of the impedance of the electrode double layer represented in a complex plane impedance plot, the electric equivalent circuit of the electrode covered with adsorbed DNA layer was determined. It was concluded that the desorption of denatured ssDNA was accompanied by higher dielectric losses than the desorption of native dsDNA, which could be explained by the higher flexibility of ssDNA than that of the dsDNA. Similar research has also been reported by the same group (21).

Electroactive indicators have frequently been used to characterize single-stranded (ss) and double-stranded (ds) DNA by AC impedance spectra. Zhao *et al*. (22) investigated the impedimetric spectra of a bare gold electrode, ds-DNA/Au and ssDNA/Au in a solution of $Co(bpy)_3^{3+}$. Their results clearly showed that $Co(bpy)_3^{3+}$ concentrated at DNA-modified electrodes due to its interaction with the surface-anchored dsDNA or ssDNA, which conversely confirmed that dsDNA and ssDNA could be immobilized onto the gold electrode surfaces by adsorption. Ferrocenium hexafluorophosphate (FcPF6) was also used as an electroactive indicator in another study (23). Single-stranded hepatitis B virus (HBV) DNA was immobilized on a gold electrode surface via a carboxylate ester as a linkage between the 3′-hydroxy end of the DNA and the carboxyl group of thioglycolic acid (TGA) self-assembled monolayer. The immobilization and the hybridization reaction on the surface were evidenced by the interactions of Fc^+ with ss- and dsDNA monitored by AC impedance spectra. The mechanism of the interaction between FcPF6 and HBV ss- or ds-DNA immobilized on the electrode surface was found to be electrostatic.

Brett *et al*. (24) described the formation of triple helix DNA on an electrode surface by the characterization of an impedimetric technique. When a glassy carbon electrode containing immobilized dsDNA was immersed in a solution containing ssDNA and a potential of 0 or +1.4 V was applied, migration of ssDNA occurred. The interaction between dsDNA and ssDNA led to the modification of the DNA conformation. It was probable that the interaction of ssDNA from the bulk solution with the surface-bound dsDNA formed portions of triple helix DNA on the electrode surface, which was supported by the impedance and the voltammetric experimental results.

Electrochemical impedance measurement was also used to characterize the immobilization of oligonucleotides on the surface of Si/SiO_2 substrates modified with either an aminosilane or a glycidoxysilane (25). Each step of the implementation of the oligonucleotides immobilization (hydroxylated silica, silanization, oligonucleotide coupling) could be monitored by following the evolution of the out-of-phase impedance curves. The shifts along the voltage axis corresponded to changes of the flat band potential of the semiconductor, which reflected the field effect inside the structure due to the variation of electric charges at the dielectric/electrolyte interface. Electrochemical impedance measurement showed that the GPTS/amine method yielded a denser and more robust immobilization of oligonucleotides on the Si/SiO_2 substrates than that using the aminosilane.

Bilayer lipid membranes (BLMs) composed of surfactant molecules are biocompatible with natural cell membranes and have similar physical properties. Membranes of the uncharged surfactant Brij-52 self-assembled onto a gold electrode were very stable and could be used as impedimetric transducers for the detection of affinity interactions (26). As a result of the introduction of a hydrocarbon chain bound with oligonucleotide pentadecathymidylate (dT15) into the hydrophobic region of the surfactant bilayer or the adsorption of antibodies on the bilayer surface, the immobilization of oligonucleotide or antibodies was carried out. The specific DNA coupling caused a decrease in the real part of impedance and the antibody–antigen interaction an increase in the real part. Therefore, the complementary coupling led to a decrease in membrane resistance, which might be caused by local violations of the inner bilayer order due to the hydrocarbon chains of modified oligonucleotides. At the same time, the large antigen binding increased the thickness of the interfacial layer on the electrode surface and further shielded ion permeation. This effect increased the real part of the impedance. The results obtained facilitated the development of impedimetric affinity sensors for clinical analysis or for the detection of various environmental pollutants.

An electrochemical study of hypoxanthine and inosine 5′-phosphate will provide an understanding of the oxidation mechanism and adsorption of purine bases and purine nucleosides on the surface of electrodes. Also, improved knowledge in this field will aid the development of DNA-biosensors and help to explain oxidative damage caused to DNA by hazardous compounds. Complex plane impedance spectra for hypoxanthine were studied at different applied potentials (27). At the potential corresponding to the oxidation of hypoxanthine, well-defined semicircles were observed. When applying a potential in the zone of oxidation, R_{ct} increased to three times its initial value. However, at the open circuit, there was only a very small increase in R_{ct}. This demonstrated clearly that adsorption was not spontaneous and that it was necessary to apply a potential for the adsorption to occur, and the adsorption was thus associated with the oxidation process. This suggested that the adsorbed species were electroactive oligomer products of oxidation which blocked the electrode surface. The clarification of the mechanism of oxidation in this work has shown the possibility of the detection of these compounds in aqueous media and the usefulness of electrochemical impedance for probing the electrode process.

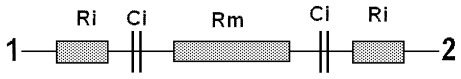
IV. CELL- AND MICROORGANISM-BASED IMPEDIMETRIC BIOSENSORS

One common application of electrical impedance is in the field of microbiology, as a means to detect, quantify and even identify bacteria (28). The measurement of impedance growth curves permits rapid detection of microbial proliferation. Roughly, about 1×10^3 to 3×10^7 cells/ml are required to produce a detectable change in the impedance curves (29).

Theoretical analysis of the electrode–electrolyte interface during bacterial growth has been performed by many scientists (29). The basic principle of impedance microbiology can be very simply described as in Fig. 6, where the impedance between two electrodes is modeled by a series circuit. The circuit includes the medium conductance G_m, the interface conductance G_i and the capacitance C_i of each electrode, and also G as the total conductance. In general terms, C_i represents the double layer capacity of the electrode–electrolyte interface (Felice, C. J., Ph.D. thesis, INSIBIO-UNT, Tucumán, Argentina, 1995).

Metabolic products created during the growth of microorganisms modified the composition of the medium, changing the ionic content, which, in turn, resulted in conductivity changes of the culture broth (30). Changes also occurred at the electrode–electrolyte interface. Such modifications were proportional to the concentration of viable microorganisms, which could be monitored by impedimetric techniques.

Applications of microorganism-based impedimetric biosensors have been mainly found in the detection and quantification of microorganisms in milk and dairy products. In 1978, Cady (31) was one of the first to propose impedance as an alternative method to plate counting for the rapid screening of milk microbial content. After this, several commercially available systems such as Bactometer® (bioMerieux, Hazelwood, MO, USA) (32, 33), Malthus® System (Malthus Instruments, Crawley, West Sussex, UK) (34) and BacTrac® (Sy-Lab, Purkersdorf, Austria) (35) were developed for dairy



$$|Z_{1,2}| = \sqrt{(Rm + 2Ri)^2 + (1/\pi \cdot f \cdot Ci)^2} \qquad R = Rm + 2Ri$$

FIG. 6. Electrical circuit equivalence between two electrodes.

products. The impedimetric technique was also applied to monitor the total microbial count in fish (36), meat (37), wine (38), fruit juices (39) and potable water (40).

The electrical impedance of endothelial monolayers was continuously evaluated under dynamic flow conditions by Depaola *et al*. (41). The results indicated that the alterations in monolayer impedance might be correlated to the changes in endothelial cell morphology and function known to occur in the presence of fluid forces. The observed changes in endothelial impedance were reversible upon flow removal with a recovery rate that varied with the duration of the preceding flow exposure. These results demonstrated that the impedance of endothelial monolayers changed dynamically with flow indicating morphological and/or functional changes in the cell layer. This electric cell-substrate impedance sensing (ECIS)/Flow system provided a continuous evaluation of quantitative, real-time changes in the electrical impedance of endothelial monolayers upon exposure to physiological fluid shear stress forces.

A novel application of impedance is the study of the interaction of mammalian cells with artificial surfaces, which is interesting for both scientific and medical reasons (42). The method, referred to as ECIS, is based on measuring changes in AC impedance of small gold-film electrodes deposited on a culture dish and used as a growth substrate. This method can be used to monitor the attachment and spreading of mammalian cells quantitatively and in real time. The attachment and spreading of epithelial MDCK cells (strain II) on different protein coatings were also investigated by Wegener *et al*. (42) using the ECIS technique.

The biochip or lab-on-a-chip, defined as microfabricated devices used for processing (delivery, processing, and analysis) of biological species has become one of the most attractive research areas for biosensors.

Gómez *et al*. (43) described the fabrication and characterization of a microelectronic biochip for the impedance spectroscopy of microorganisms (Fig. 7). The device consisted of fluidic channels, planar fluidic interface ports, integrated metal electrodes, and a glass cover. The total fluidic path volume was in the order of 30 nl. Electrical impedance measurements for a suspension of a live microorganism *Listeria innocua* injected into the biochip demonstrated an easy detection method for the viability of bacterial cells. The electrical characteristics of a low conductivity suspension medium were altered significantly due to the change of the ion strength from the bacterial metabolism.

Medoro *et al*. (44) presented a lab-on-a-chip for electronic manipulation and detection of microorganisms based on the use of closed dielectrophoretic (DEP) cages combined with impedance sensing (Fig. 8). Both DEP levitation and impedance sensing were based on the same physical properties, *i.e.*, differences in conductivity and permittivity between the particles and the suspending medium. A prototype was given using standard printed circuit board technology by which polystyrene microbeads were trapped, concentrated and quantitated. The presence of particles in the region above the electrodes induced a perturbation of the electric field that could be detected by an impedimetric technique. The proposed approach was suitable for use in the integrated circuit technology, which combined dielectro-

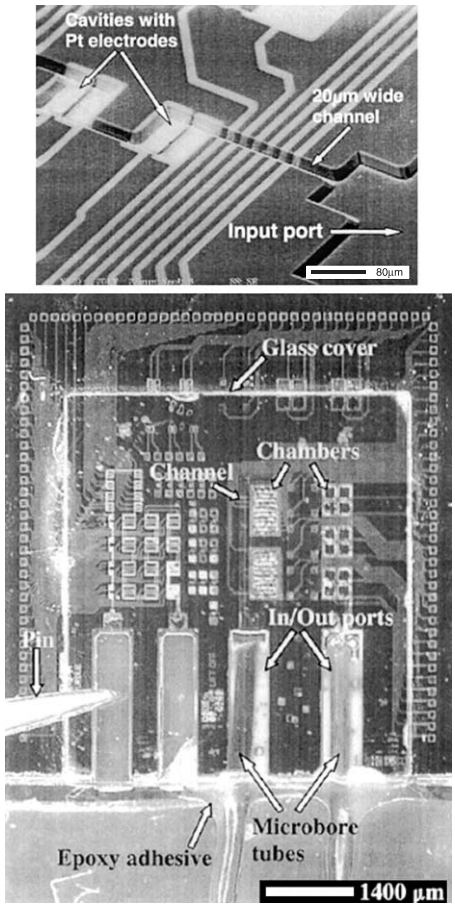


FIG. 7. Scanning electron micrograph of the biochip channels with integrated electrodes and the picture of the completed microfluidic biochip (43).

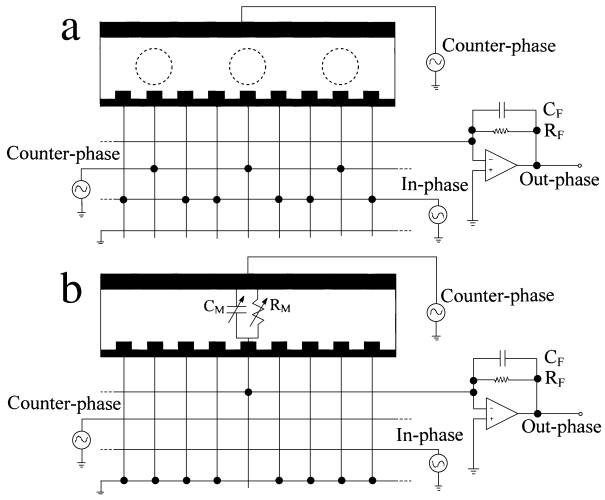


FIG. 8. Circuit connections of the electrodes and the lid: (a) during the actuation, each electrode can be connected, under software control, to an in-phase or a counter-phase stimulus. (b) During the sensing phase, one electrode, with which the measurement is to be performed, is connected to a trans-impedance amplifier and all the other electrodes are connected to the GND; a sinusoidal stimulus is applied to the lid (44).

phoresis with impedance measurement to trap and move particles in the device while monitoring their location and quantity, and allowed manipulation and detection of single cells and reduced the system dimensions.

CONCLUSION

Few studies on enzyme-based impedimetric biosensors have been reported in recent years due to the inherent time-consuming characteristics of impedimetric techniques. Impedimetric techniques are seldom used to detect the substrate or product of an enzyme reaction. They are preferred for the characterization of enzyme-based biosensors.

Many studies on impedimetric biosensors are focused on the monitoring of affinity reactions. In particular, antibody and receptor molecules have been immobilized onto the surface of electrodes, and a change in impedance is detected when the antigen binds to the sensor; but often the binding reaction at the surface is insufficient to produce a large signal change (45, 46) since the affinity interaction does not directly lead to an enlarged signal from the reaction cascade. However, the development of simple label-free immunosensors has been difficult to achieve for electrochemical devices and impedimetric techniques are being used for this purpose, since impedimetric techniques provide a wealth of information regarding surface states. New techniques, such as polymer techniques and nanotechniques, may pave the way for the development of novel affinity-based impedimetric biosensors.

Impedimetric techniques have been used to monitor the amount and activity of microorganisms for many years. Several commercially available instruments are currently to be found. A novel application of impedance is to study the interaction of mammalian cells with artificial surfaces. It could be used to quantitatively monitor the attachment, spreading and propagation of mammalian cells in real time, which is interesting for both scientific and medical reasons. Currently, the development of a biochip or lab-on-a-chip system for impedimetric biosensors is the most attractive research field.

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