

## Chapter 2

# Nanomaterials for Biosensors and Implantable Biodevices

Roberto A. S. Luz, Rodrigo M. Iost and Frank N. Crespilho

**Abstract** The study of biological recognition elements and their specific functions has enabled the development of a new class of electrochemical modified electrodes called biosensors. Since the development of the first biosensor almost 50 years ago, biosensors technology have experienced a considerable growth in terms of applicability and complexity of devices. In the last decade this growth has been accelerated due the utilization of electrodes-modified nanostructured materials in order to increase the power detection of specific molecules. Other important feature can be associated with the development of new methodologies for biomolecules immobilization. This includes the utilization of several biological molecules such as enzymes, nucleotides, antigens, DNA, aminoacids and many others for biosensing. Moreover, the utilization of these biological molecules in conjunction with nanostructured materials opens the possibility to develop several types of biosensors such as nanostructured and miniaturized devices and implantable biosensors for real time monitoring. Based on recent strategies focused on nanomaterials for electrochemical biosensors development, these topics has presented recent methodologies and tools used until nowadays and the prospects for the future in the area.

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R. A. S. Luz  
Federal University of ABC (UFABC), Santo André, 09210-170, Brazil

R. M. Iost · F. N. Crespilho (✉)  
Institute of Chemistry of São Carlos (IQSC), University of São Paulo (USP),  
São Carlos 13560-970, Brazil  
e-mail: frankcrespilho@iqsc.usp.br

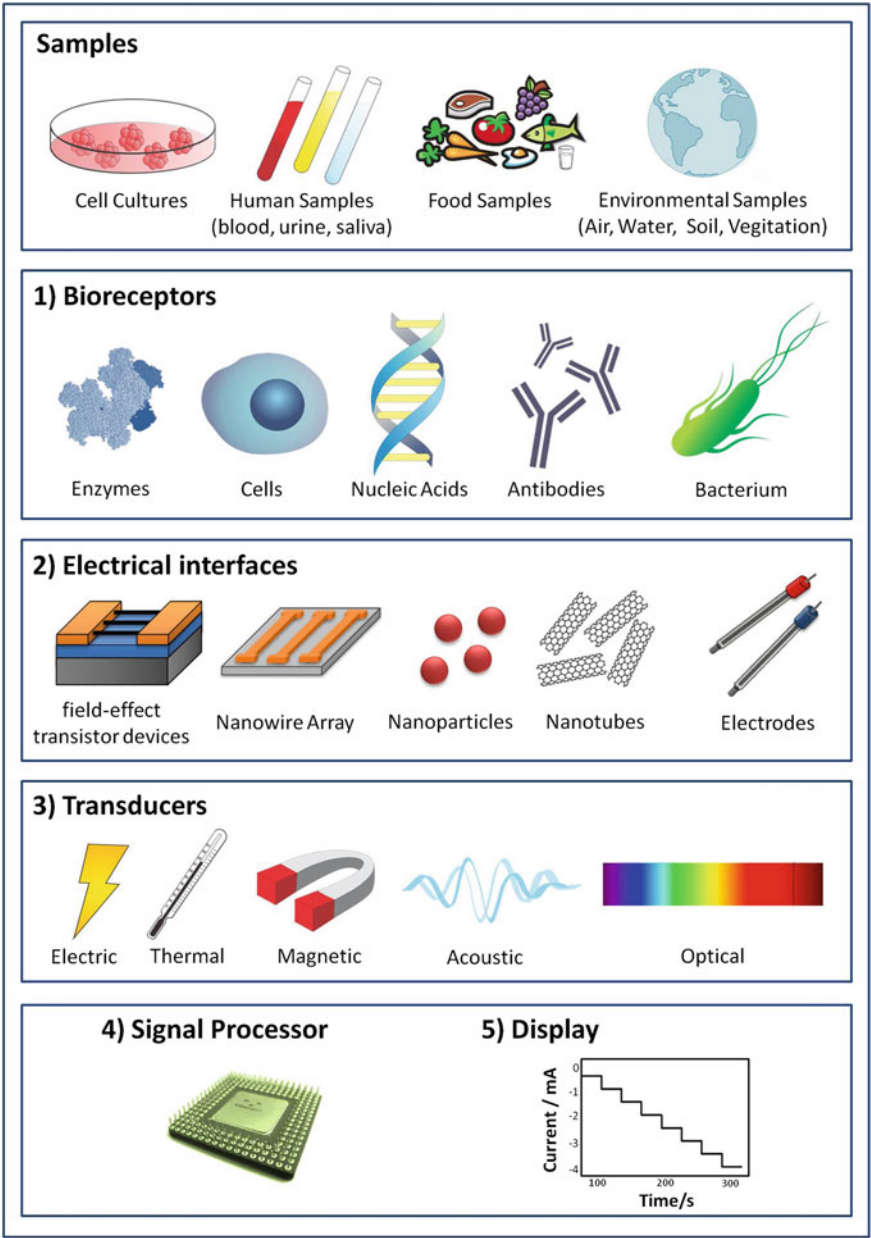
## 2.1 Introduction

There is no doubt that the increase interest for the development of new materials applicable in electroanalytical techniques has been associated with the necessity of control specific molecules present in the environment or in more recently efforts, the human body [1–3]. This includes the possibility to improve the quality of life by development of efficient electrochemical devices and biodevices [2]. More than the use electrochemical devices to detect analytes is the challenge to develop more sensitive and selective electrochemical devices that provide the possibility to detect small quantities of molecules utilizing efficient transducing elements and specific recognition materials for biosensing [5–18]. The so called electrochemical biosensors are based on a specific biological recognition element such as enzymes, antigens or another biological molecule that interacts directly with a transducer element [19].

In general, a typical biosensor is composed for five parts (as illustrated in Fig. 2.1): (1) bioreceptors that bind of specific form to the analyte; (2) an electrochemically active interface where specific biological processes occur giving rise to a signal; (3) a transducer element that converts the specific biochemical reaction in an electrical signal that is amplified by a detector circuit using the appropriate reference; (4) a signal processor (e.g. computer software) for converting the electronic signal to a meaningful physical parameter describing the process being investigated and finally, (5) an proper interface to present the results to the human operator. Currently, the biosensors can be applied to a large variety of samples including body fluids, food samples, cell cultures and be used to analyze environmental samples [20].

The basic principles of electrochemical biosensors are associated with their capability to detect a specific molecule with high specificity. Also, these characteristics are dictated by a better correlation between the biological component and the transducing element. Important advances in these aspects has been achieved with the utilization of several kinds of nanomaterials such as metal nanoparticles [21], oxide nanoparticles [22], magnetic nanomaterials [23], carbon materials [24, 25] and metallophthalocyanines [26] to improve electrochemical signal of biocatalytic events occurred at electrode/electrolyte interface.

Recent advances in bionanoelectrochemistry are being reported about the enormous impact of nanomaterials when utilized as transducing element in modified electrodes [27–30]. Since then, thousands of scientific articles exploring the favorable association between biomolecules and nanomaterials to improve electrical signal originated in biochemical reactions have been published. One interesting example is the use of thin films on electrode surfaces to increase the sensitivity of sensors and biosensors. This sense, the Langmuir–Blodgett was the pioneering technique for the fabrication of thin films formed by transferring an amphiphilic material dispersed at air/water interface to a solid substrate. In particular, the obtention of thin monolayer films is very attractive for enzymes immobilization, proteins, nucleic acids and others [31]. Another interesting technique for fabrication



**Fig. 2.1** Components of a typical biosensor

of thin organic films was developed by Decher in the beginning of 90 decade [32–34] as a simple strategy for fabrication of multilayer films with high control of thickness at nanoscale level. Instead of specific chemical interactions between substrate and

organic molecules the layer-by-layer (LBL) technique is based, basically, in coulombic electrostatic that provides multilayers growth. Such strategy has been reported as an interesting tool for films fabrication with simplicity, which can be applied to several kinds of materials likes polyelectrolytes [35], metallophthalocyanines [36], carbon nanotubes [37], nanoparticles [38] and also biological molecules such as enzymes and proteins [39].

In what concerns the fabrication of electrochemical biosensors, it is unquestionable the importance of nanostructured materials and their implications in biosensors properties. Recent efforts have been made in order to use the nanostructured modified electrodes for monitoring specific biological molecules *in vivo* [40]. Also, the possibility to detect a specific molecule in living organisms at real time has open new paths for controlled of pathogenic diseases and, also, some analytes such as glucose at the human body [41]. Although these new class of electrodes opened the possibility to improve electrochemical biosensors performance, focus has been made in order to fabricate electrochemical devices at nanoscale level for single molecule detection [42]. Moreover, one of the main challenge until nowadays is to detect single events originated by enzymatic reactions utilizing a unique nanomaterial [43].

In this chapter, we describe the recent trends in the field of electrochemical biodevices exploring the principal strategies utilized in the last years to improve signal response of enzymatic biocatalysis and describe briefly the electrochemical characteristics of several nanomaterials when utilized in modified electrodes. The fabrication of nanoelectrodes by some techniques is also explored in this chapter. In addition, we will discuss the many efforts in order to detect specific molecules *in vitro* and *in vivo* and recent advances in the development of implantable biosensors.

## 2.2 Nanostructured Thin Films for Biosensing

Nanostructured thin films have opened the possibility to fabricate electrochemical sensors and biosensors with high power of detection due to intrinsic properties associated with their dimensions at nanoscale level. These interesting properties can be explained based on the organization level obtained when molecular arrangement is obtained at a solid conductor substrate. Also, the materials that can be used include a large range of organic and inorganic materials for films growth. Moreover, the possibility to improve the detection limit in biosensing devices can be also explained by using compatible materials such as natural polymers. The aim objective behind the utilization of these materials is to combine the high power of detection with preservation of the structural integrity of the biomolecules and, also, maintaining their biocatalytic activity.

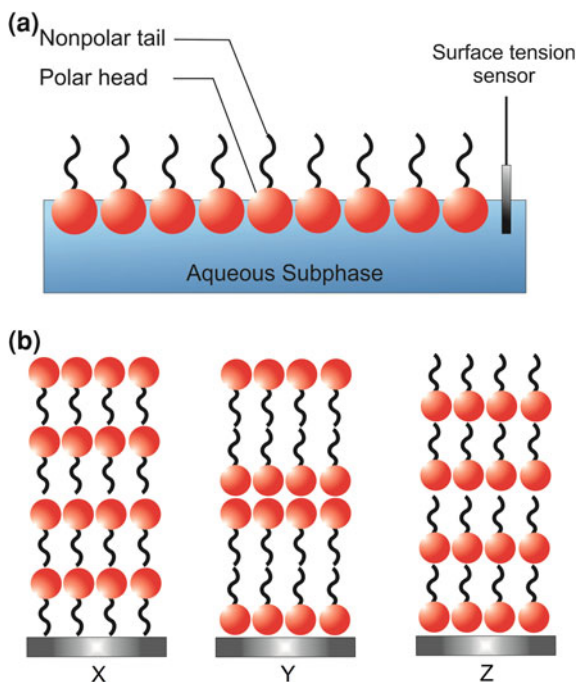
### 2.2.1 *Langmuir–Blodgett and Layer-by-Layer Based Biosensors*

The field of materials science has opened new possibilities towards the utilization of organic, inorganic nanostructured materials and hybrids formed by biological components and nanostructured materials. In parallel, composites has been develop to confer or improve some specific properties which includes the use of metallic nanostructures or organic polymers. In particular, nanostructured organic films has opened a new research area with the aim purpose to obtain interesting properties at nanoscale. Nanostructured thin films has showed great impact in the field of electrochemical biosensors in the past few years with a large range of materials that can be employed in films construction. The study of organic molecules has arised since from 1960s decade with the discovery of their electronic properties and potential application in optic and electronic devices [44]. The major interest behind the utilization of nanostructured thin films for biosensing lies in the possibility to understand biochemical mechanisms and, at the same time, to fabricate mimetic systems based on cellular membranes [45]. The role of the control of depositing monolayers of organic films and their final properties was first studied by Irving Langmuir and Katherine Blodgett in the beginning of XX century [46, 47]. This technique of thin films fabrication is based on the self-organization of amphiphilic molecules at air/water interface in order to diminish the free surface energy and form a dispersed monolayer. The formation of organic monolayers is obtained by dropping of a dilute lipid solution at air/water interface with subsequent solvent evaporation. Also, the more stable monolayer conformation of Langmuir film formed on air/water interface is achieved by application of a horizontal and controlled compression throughout the Langmuir cube. Further, the compression is accomplished by two moves barriers localized at cube and is accompanied by measurement of certain surface properties such water surface tension and surface potential. The surface tension of water with the dispersion of an amphiphilic molecule on water interface can be measure utilizing Eq. 2.1.

$$\pi = \gamma_0 - \gamma \quad (2.1)$$

where  $\pi$  is the measurement of water surface tension change,  $\gamma_0$  is the surface tension of pure water and  $\gamma$  is the surface tension of water with the presence of amphiphilic molecule at air/water interface. Although amphiphilic molecules are common used due to their self-organization at air-water interface, the dispersion of organic or inorganic molecules at interface is not considered to be limited to specific molecules. Moreover the type of substrate functionalization plays an important role for films formation. According to substrate functionalization, the monolayers can be transferred by immersion of substrate through the interface containing the amphiphilic monolayer. Consequently, the transfer of monolayers to the substrate is carried out by successive dipping the substrate in the cube. Also, the interaction during the substrate dipping is based on monolayers functionalization and the Langmuir films with X, Y and Z-type can be obtained [44]. One of

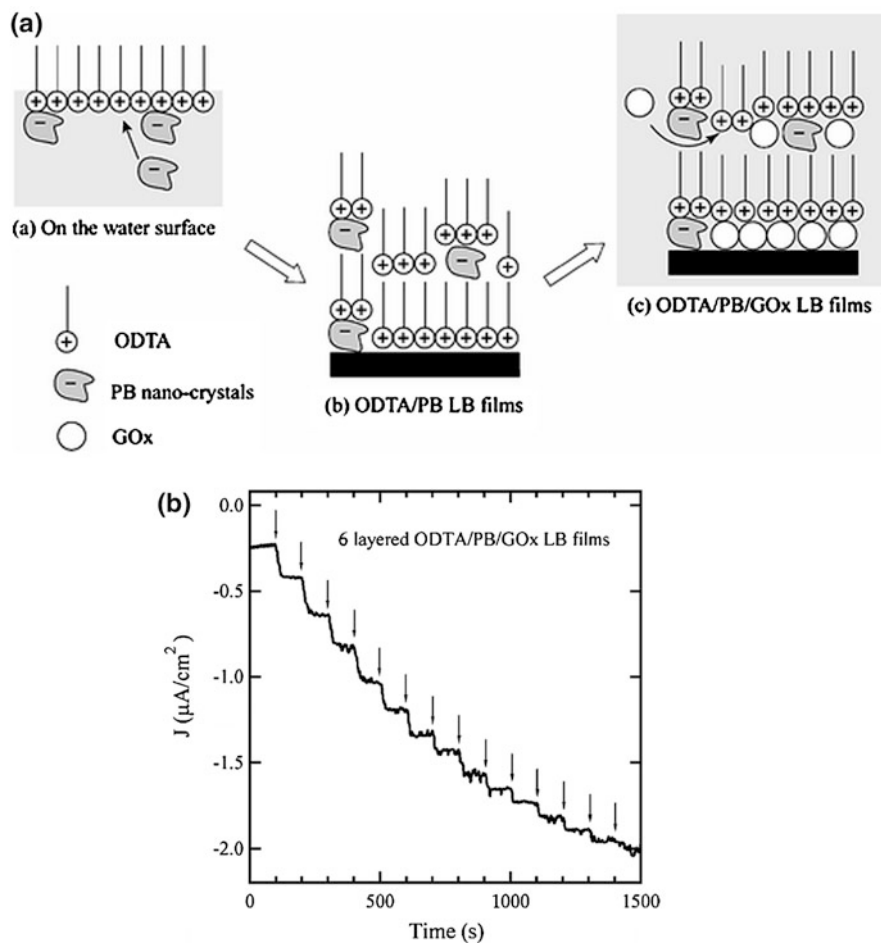
**Fig. 2.2** **a** Schema for a Langmuir Monolayer obtained at air–water interface. **b** X, Y and Z Langmuir-Blodgett films obtained according to substrate and molecules used for films fabrication.



the major and interesting advantage is the possibility to control thickness and roughness by adsorption of multilayer films onto solid substrates. Figure 2.2 shows a schematic representation of a) Langmuir cube and b) the type of monolayer deposition according to the substrate functionalization and molecules used for films fabrication.

In the field of electrochemical biosensors, the utilization of biomolecules such as antibodies, DNA, enzymes or another kind of proteins adhered to Langmuir-Blodgett films confer specificity to the system [48–50]. Concerned the development of modified electrodes for enzymes immobilization, Langmuir-Blodgett films has been considered an important path for biosensors fabrication and many kinds of architectures has been reported in the last decades as very promising approaches for biosensors development. Examples of biosensors development using LB method has been extensively reported on literature for application in several biosensing approaches [51, 52].

Several examples are reported about the determination of glucose using LB method as mimetic membrane platform for enzyme glucose oxidase (GOx) immobilization [18]. As an example, Sun and co-workers [53] reported the utilization of LB films for GOx immobilization utilizing cross-linking agents to improve biological process when enzyme was immobilized at monolayer surface. On another approach, Ohnuki and co-workers [54] reported the use of Langmuir films consisting of octadecyltrimethylammonium (ODTA) and Prussian blue (PB) clusters as platforms for enzyme GOx immobilization. The immobilization



**Fig. 2.3** **a** Scheme of LB films preparation containing ODTA, PB, and GOx. **b** Amperometric response obtained at 0.0 V in a buffer solution at pH 7.0 with ODTA/PB/GOx LB films (6 layers) deposited on a gold electrode. The arrows show the moment of glucose solution injection whose amount corresponds to an increase of  $1 \text{ mmol L}^{-1}$  glucose concentration. Reproduced with kind permission of Ref. [54]

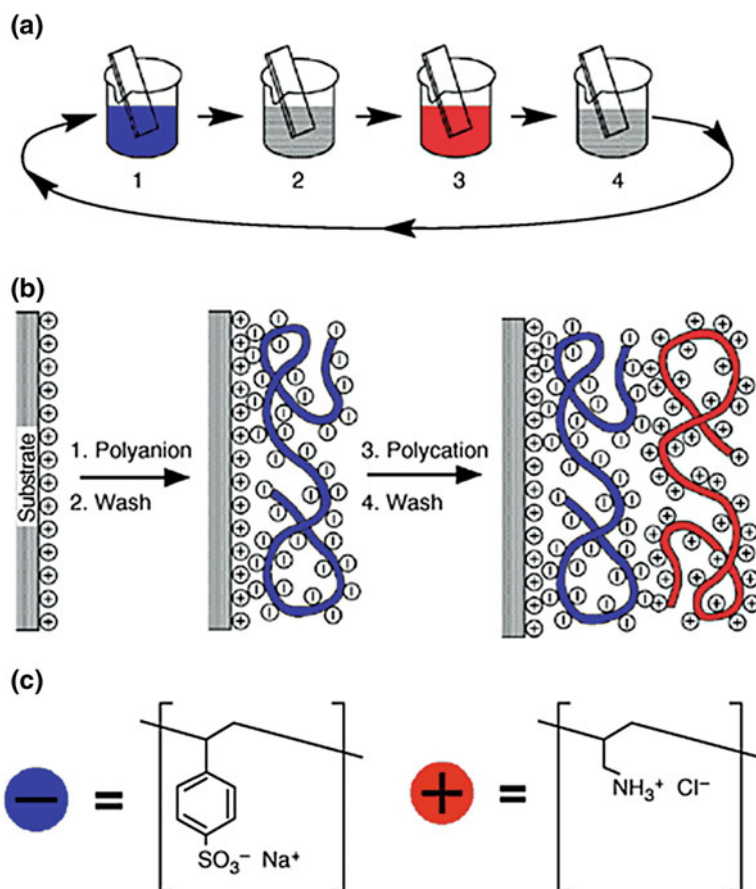
of enzyme GOx was confirmed by FTIR spectra before and after enzyme immobilization with ODTA/PB Langmuir films. The configuration exhibited shows a good amperometric response upon glucose addition with the utilization of 6 layers, electrochemical increase process associated with the presence of PB electrocatalyst. Figure 2.3 shows a schematic representation of ODTA/PB Langmuir films and the amperometric response obtained at 0.0 V (Ag/AgCl).

It is unquestionable that the exploration of self-assembly methodologies has opened new ways for the development of more selective and sensitive electrochemical devices and so on the LB method has provided the fabrication of interesting approaches for biosensors development. Although LB is an interesting route for thin films obtention with high quality, it requires especial experimental conditions and equipments for films growth. The experimental approach to produce organic thin organic films was extended with the utilization of organic polyelectrolytes by Decher [32–34] in the beginning of 90 decade which the principle of growth were based in the auto-organized molecules primarily by coulombic electrostatic adsorption process between polyelectrolytes with oppositely charges. This method for multilayer films obtention is basically described by immersing a conducting substrate alternatively on cationic and anionic polyelectrolyte during a specific time which is washing on solvent solution to remove the excess between each step of adsorption (Fig. 2.4).

One important point about the formation of organic bilayers is the films stability achieved in the association of multilayers due to films growth. The energy of coulombic association is very low when each interaction of ions pairs is availed. However, the global association along polyelectrolyte interaction provides a high stability between polyelectrolytes chain [55]. The explanation for the association between polyelectrolyte multilayers were described utilizing the concept of extrinsic and intrinsic charges compensation when the association of two polyelectrolytes with opposite charges interacts [56]. The intrinsic charge compensation is described by the charge association of polyelectrolytes chains and is the basic explanation for the multilayers formation. At the same time, the charge balance of counter ions or the balance of extrinsic charges occurred with the polyelectrolyte chains during the formation of multilayers. For this purpose, some works described the polyelectrolyte association in terms of doping salt and the thermodynamic constant  $k_{dop}$  of extrinsic charge association, represented by Eq. 2.2 [57].

$$K_{dop} = \frac{y^2}{(1-y)a_{MA}^2} \approx \frac{y^2}{a_{MA}^2} \quad (2.2)$$

where  $y$  is the compensated fraction of polyelectrolyte charge and  $a_{MA}$  is the activity association between cation and anion. Much more details about the multilayers formation was described by several studies with the model of multilayers interpenetration between adjacent monolayers [58]. Jomaa and co-workers [59] utilized neutron reflectivity studies with interdispersed layers of deuterated poly(styrenesulfonate) (PSS) and poly(diallyldimethylammonium) (PDAC) to describe the formation of multilayer films. The exploration of films growth has also been described on literature by controlling several experimental conditions such as pH, concentration of salt or ionic strength, concentration of polyelectrolytes, temperature of the system, the solvent utilized, time of deposition, the nature of substrate and so on [58]. Also, the high control of film properties such as roughness, thickness and films stability can be obtained by controlling these conditions and plays an important role for the quality and stability of multilayers



**Fig. 2.4** **a** Schematic of the film deposition process using slides and beakers. Steps 1 and 3 represent the adsorption of a polyanion and polycation, respectively, and steps 2 and 4 are washing steps. The four steps are the basic buildup sequence for the simplest film architecture  $(A/B)_n$ . The construction of more complex film architectures requires only additional beakers and a different deposition sequence. **b** Simplified molecular picture of the first two adsorption steps, depicting film deposition starting with a positively charged substrate. Counterions are omitted for clarity. The polyanion conformation and layer interpenetration are an idealization of the surface charge reversal with each adsorption step. **c** Chemical structures of two typical polyions, the sodium salt of poly(styrene sulfonate) and poly(allylamine hydrochloride). Reproduced with kind permission of Ref. [32]

achieved. Besides the possibility to obtain thin organic platforms with experimental simplicity compared to other techniques, LBL method has the advantage to incorporate a large range of materials in films fabrication that includes organic and inorganic materials, hybrids formed by materials at nanoscale and biological components. Regards the applicability of thin films for biosensing devices, the incorporation of biomolecules as components for films growth was described by

Lvov and co-workers [60] in the formation of self-organized multilayer films of proteins and polyelectrolytes of mioglobin (Mb) and PSS and by enzyme GOx and poly(ethylene imine) (PEI). Several other works reported the utilization of LBL method for biomolecules immobilization focused on biosensing applications. One important question is about the interaction study of nanomaterials and such biological molecules and their impact on biological process when biomolecules are exposed out of their natural environment [61]. The principal question is about the changes in molecular structure which reflect directly on their biological properties. Moreover, the biological properties reflect directly on biosensors quantification and their capability to respond to a specific molecule. This property has been achieved with the development and utilization of nanostructured thin films that can act as platforms for biomolecules immobilization [36, 62]. The next topic will emphasize the utilization of these hybrid functional materials and their capability to be used as transducer elements in biosensing devices.

## 2.3 Nanostructured Materials for Biosensing Devices

Nanostructured materials are well known as interesting tools with specific physical and chemical properties due to quantum-size effects and large surface area that provides unique and different properties compared to bulk materials. The exploration of these different characteristics provides the possibility to improve biosensors properties and increase the power of detection throughout size and morphology control. Interesting approaches have reported about the high increase in electronic properties when metallic nanostructures are used as components for electrodes modification. These include the utilization of nanostructured materials with specific forms such as 0D (quantum dots, nanoparticles), 1D (nanowires or carbon nanotubes) or 2D (metallic platelets or graphene sheets) orientation that reflects in their final properties. The next topic will be emphasized in biosensors fabrication using metallic nanoparticles (MNPs) as transducing elements on modified electrodes and some interesting electrochemical approaches used to improve biosensing performance.

### 2.3.1 *Nanoparticles-Based Biosensors*

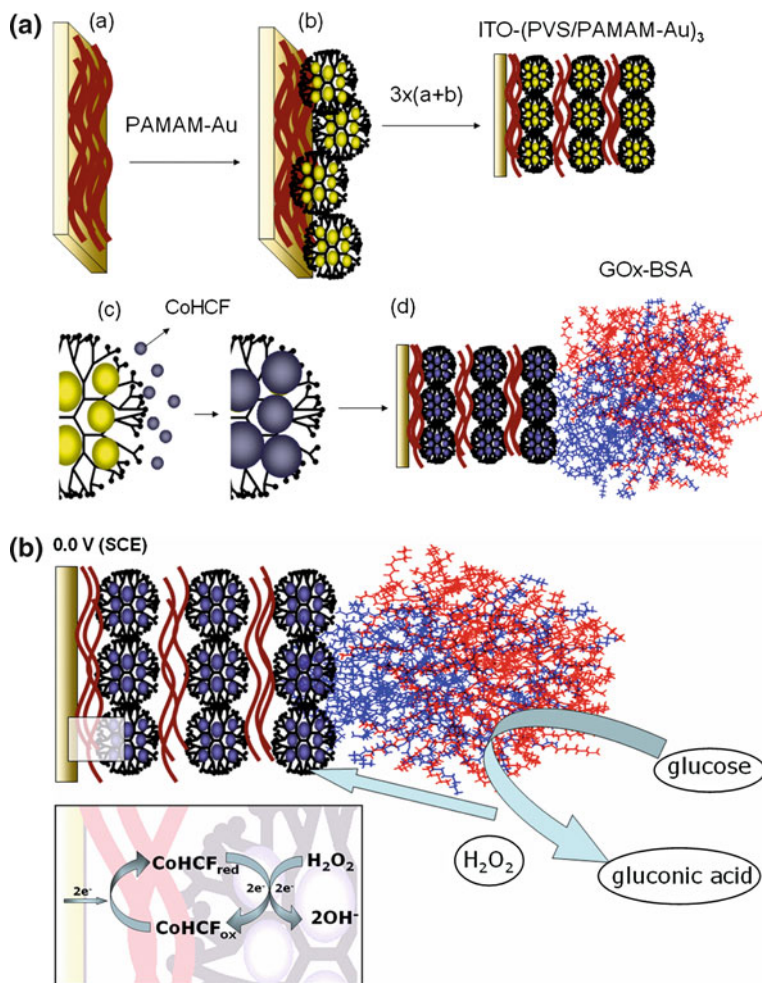
Metallic nanoparticles are very interesting materials with unique electronic and electrocatalytic properties which depend on their size and morphology [63, 64]. The efficiency of electronic and electrochemical redox properties becomes these classes of nanostructured materials very interesting for technological applications. In particular, gold nanoparticles (AuNPs) are much explored materials as components for biosensors development due to the capability to increase electronic signal when a biological component is maintained in contact with nanostructured surface. On the other hand, silver, platinum, palladium, copper, cobalt and others

has extensively explored in biosensors development [65–69]. In particular, the exploration of gold nanostructured materials has provided new paths for enzymatic biosensors development. At the same time, specific organic stabilizers have been used to produce nanostructured materials with different morphologies. Dendrimers are known as organic macromolecules with tridimensional and highly defined structure functionality [70]. The capability of dendrimeric structures to stabilize and maintain integrity of metallic nanoparticles was reported by Crooks and co-workers [71]. As an example, polyamidoamine dendrimers (PAMAM) were used as template for nanoparticles growth or nano reactors with cavities for nanoparticles nucleation. According to functional groups at molecular structure, dendrimers have been subject of intense studies in the field of nanostructured thin films fabrication and also, in the form of hybrids with metallic nanoparticles. An interesting approach was reported recently utilizing hybrids of PAMAM-AuNPs as components in multilayer thin films based on LBL technique to enhance charge transfer in modified electrodes leading to the concept of electroactive nanostructured membranes (ENM) [72]. In this case, PAMAM-AuNP hybrids were assembled utilizing LBL technique in multilayers to produce modified electrodes. The strategy to produce modified substrates is based on self-assembly of polyvinylsulfonate (PVS) as negatively charged polyelectrolyte alternating with the positively charged PAMAM-AuNP hybrids onto ITO (indium tin oxide) conducting electrodes to obtain ENM. Also, the strategy involved the deposition of a redox mediator around metallic AuNPs to enhance charge transfer in modified electrodes (Fig. 2.5).

The capability to increase charge transfer utilizing the LBL approach was investigated with details by electrochemical impedance spectroscopy (EIS) was also evaluated using electrodeposition of different redox mediators (ITO-PVS/PAMAM-AuNP@Me). This approach can be generalized for a wide range of electrochemical devices, including sensors and biosensors. The enhanced charge transport on electrodes based on LBL approach was also explored by electrodeposition of Prussian blue redox mediator (PB) on PAMAM-AuNP nanocomposite [73]. The electrochemical results show kinetic behavior correlation for cathodic current peak for AuNPs showed a non-linear response compared to adsorption time for bilayers formation.

### ***2.3.2 Carbon Materials-Based Biosensors***

Carbon materials have received great attention in the last decades with the emergence of nanoscience area [75]. The utilization of carbon nanomaterials also possibilities the increase on charge transfer in bioelectrochemical devices. These includes the modification of electrodes with several kinds of carbon at nanometer range carbon powder, carbon nanotubes, graphene sheets and carbon capsules [76–78]. The investigation of electronic properties of carbon nanotubes since their discovery by Iijima and co-workers [79] in 1991 are one of the most reported



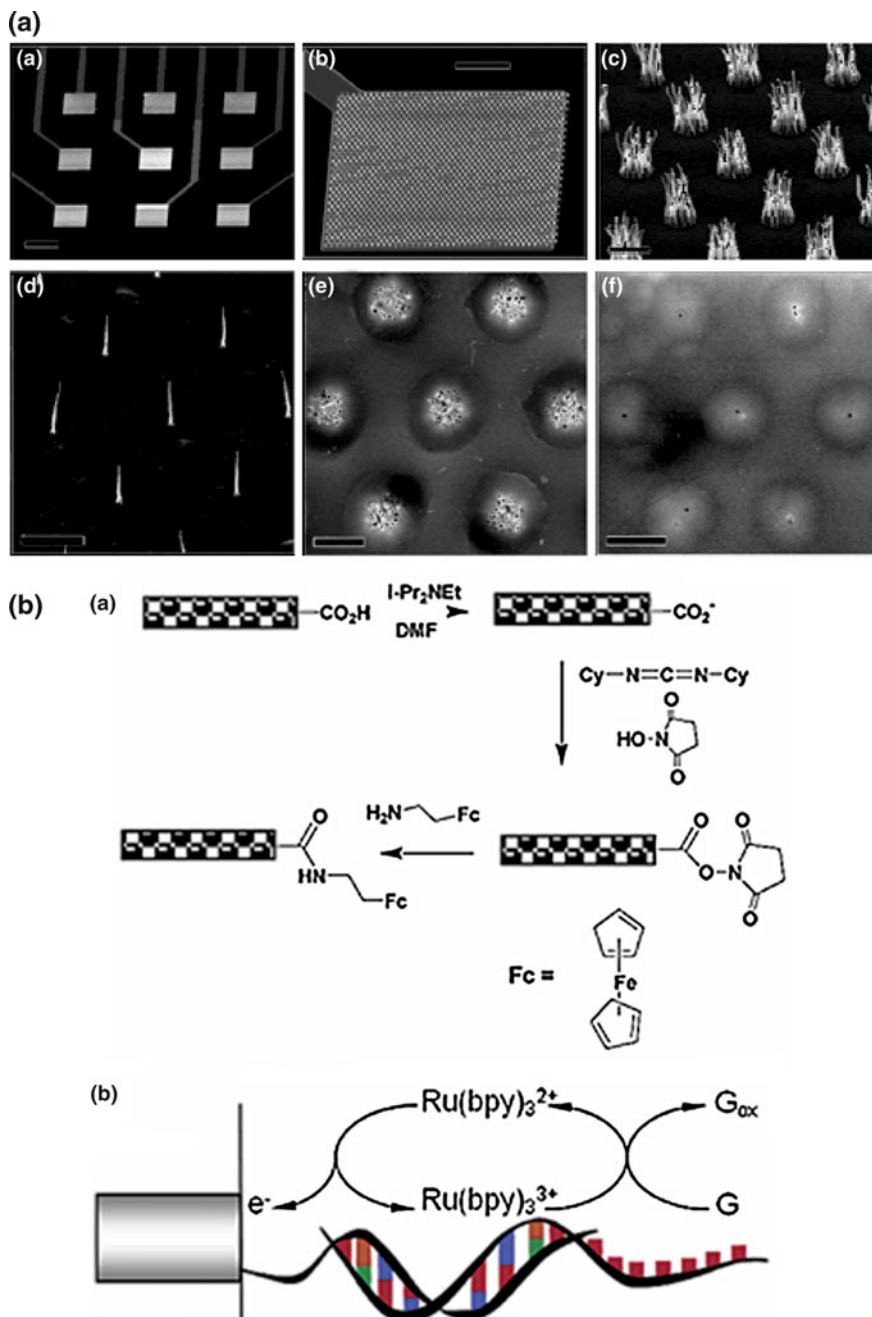
**Fig. 2.5** **a** Schematic fabrication of LbL films comprising PVS and PAMAM-Au. The sequential deposition of LbL multilayers was carried out by immersing the substrates alternately into (a) PVS (a) and PAMAM-Au (b) solutions for 5 min per step. After deposition of 3 layers, an ITO-(PVS/PAMAM-Au)<sub>3</sub>@CoHCF electrode was prepared by potential cycling (c). The enzyme immobilization to produce ITO-(PVS/PAMAM-Au)<sub>3</sub>@CoHCF-GOx (d) was carried out in a solution containing BSA, glutaraldehyde and GOx. **b** Schematic representation of reaction of glucose at ITO-(PVS/PAMAM-Au)<sub>3</sub>@CoHCF-GOx electrode. Reprinted with permission from Ref. [74] Copyright 2007 Elsevier

approaches used to explain their capability to increase the detection limit in modified electrodes. The intrinsic electronic properties of carbon materials can be explained based on the nature of carbon bonding in their allotropic forms. Graphite is the simplest form of carbon-carbon bond with  $sp^2$  hybridization with weak bond energy between adjacent layers and  $\sigma$  bond with and out of plane of  $\pi$  orbitals.

Carbon nanotubes (CNT) are formed by a hollow cylinder formed by a unique carbon sheet forming a single walled carbon nanotube (SWCNT) or concentric carbon sheets with different diameters forming multiwalled carbon nanotubes (MWCNT) with carbon-carbon with  $sp^2$  bonding [75]. The particular cylindrical form of CNT is the principal aspect that provides the quantum confinement effect in the oriented 1D nanostructured materials [80]. These characteristics provide the possibility to increase chemical reactivity and electronic properties of this particular carbon material, which becomes a crucial point for biosensing devices [75].

The electrochemical properties of CNTs have also been considered an interesting point for biosensors fabrication. Initially, edge-planes sites and defect areas present on tubes structures has been the focus of intense studies about their electroactivity. On the other hand, some interesting studies reported about iron impurity present on CNTs and their influence on electrocatalytic activity [81]. Anodic or cathodic pre-treatments have also been employed principally for detection of biological systems. Liu and co-workers [82] reported the preparation of PDDA/GOx/PDDA/CNT-modified glassy carbon electrode self-assembly nanocomposite for flow injection glucose biosensing applications. The modified electrodes was obtained through electrostatic adsorption between adjacent bilayers (LBL method) showing linear response at range of 15  $\mu\text{M}$  to 6 mM and detection limit of 7  $\mu\text{mol L}^{-1}$  for  $\text{H}_2\text{O}_2$ . Another interesting approach was reported recently using nanoarquitectures based on capacitive field effect transistor-modified with LBL of PAMAM and CNTs as sensing platforms for penicillin G detection [14]. The large surface area provide by incorporation of a organic matrix and the increased response with CNT incorporation on the modified transistors exhibited and excellent and faster response upon addition of penicillin on electrolytic media. The same electrode configuration based on PAMAM/CNT arquitectures has also provided the improvement of biosensing effects for glucose biosensor ranges from 4.0  $\mu\text{mol L}^{-1}$  to 1.2 mM and limit detection of 2.5  $\mu\text{mol L}^{-1}$ . Other applications include the utilization of several other arquitectures (Fig. 2.6a) with interesting electrochemical properties upon immobilization of biomolecules such as DNA [83] and antigens for immunosensing applications [84]. Li and co-workers [85] reported the utilization of MWCNT nanoelectrodes highly oriented embedded on  $\text{SiO}_2$  for ultrasensitive DNA detection. The combine of redox species  $\text{Ru}(\text{bpy})_3^{2+}$  mediated guanine oxidation possibilities detection of small quantities of redox substances-based immunosensing applications (Fig. 2.6b).

Graphene sheets (GS) have recently attracted much attention in the field for electrochemical sensing and biosensing areas [86, 87]. The 2D electronic structure of graphene was investigated in detail in several articles due to their potential application as components in a large range of electrochemical devices [88]. The aim advantage of GS is their large surface area when compared to CNTs and consequently, their electrochemical properties can increase enormous when biological molecules are immobilized on electrode surface [25]. One interesting method for synthesis of graphene conductor sheets is based on (chemical, physical or electrochemical preparation) insulator graphene oxides as precursor to form graphene conductor structures. Several studies emphasized changes in structural



**Fig. 2.6** **a** SEM images of **(a)**  $3 \times 3$  electrode array, **(b)** array of MWNTs on one of the electrode pads, **(c)** and **(d)** array of MWNTs at UV-lithography and e-beam patterned Ni spots, respectively, **(e)** and **(f)** the surface of polished MWNT array electrodes grown on  $2 \mu\text{m}$  and  $200 \text{ nm}$  spots, respectively. Panels **(a–d)** are  $45^\circ$  perspective views and panels **(b–f)** are top views. The scale bars are  $200$ ,  $50$ ,  $2$ ,  $5$ ,  $2$ , and  $2 \mu\text{m}$ , respectively. **(b)** **(a)** The Functionalization Process of the Amine-Terminated Ferrocene Derivative to CNT Ends by Carbodiimide Chemistry and **(b)** the Schematic Mechanism of  $\text{Ru}(\text{bpy})_3^{2+}$  Mediated Guanine Oxidation [85]

properties of graphene according to the methodology employed in their fabrication [89]. Shan and co-workers [90] studied the influence of polyvinylpyrrolidone-protected graphene/polyethylenimine-functionalized ionic liquid/GOx in modified electrodes for glucose biosensing. This electrode configuration showed high electrochemical sensibility and biocompatibility when enzyme GOx was immobilized at electrode surface. These two combined properties of biocompatibility and improvement of electrochemical sensibility upon addition of  $\text{H}_2\text{O}_2$  and  $\text{O}_2$  in electrolytic media shows their potential application in biosensor devices. On another interesting approach, Kang and co-workers [91] reported about the utilization of nanocomposites based on GS and chitosan (Ch) organic natural polymer as platforms for glucose sensing. It is well known that Ch is a natural polymer that provides the ability to improve electrochemical redox process when used in modified electrodes. Also, this electrochemical approach has been much studied as promising methods for enzyme immobilization, as in the case of enzymatic biosensors development with sensitivity of  $37.93 \mu\text{A mM}^{-1} \text{cm}^{-1}$  at linear range of  $0.08 \text{ mmol L}^{-1}$  to  $12 \text{ mmol L}^{-1}$ .

Although carbon-based nanostructured materials are relatively a recent area, their impact in the field of biosensors development has been arisen significantly in the last decades as interesting approaches for biomolecules study. Such progress can be attributed to the intense research in nanocomposites development applicable in electrochemistry devices with unique electronic properties. These features includes the utilization of different carbon materials such CNT and GS as platforms for enhance electronic signal between electrode surface and biomolecules such as oxidoreductases enzymes. Concerning the development of more sensitive biosensing devices, the crescent use of nanostructured materials for improve electronic communication of biological materials and electrode surface plays an important role for detection of small quantities of molecular substances.

## 2.4 Miniaturized Devices and Implantable Biosensors

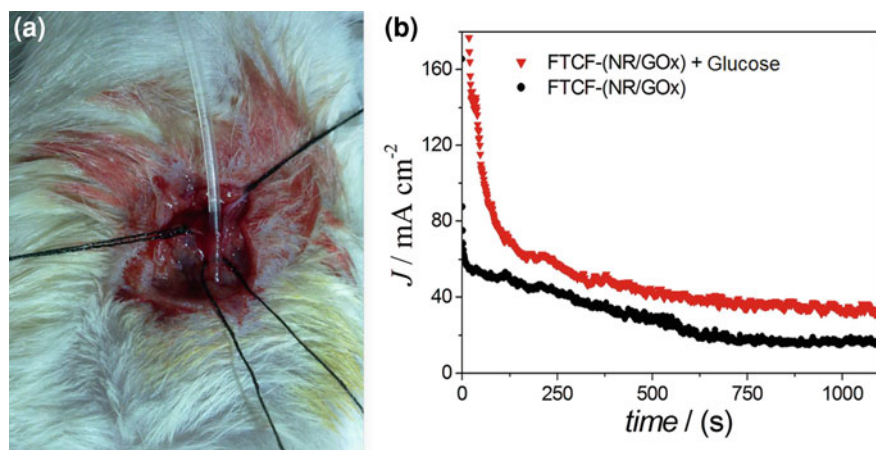
Besides the modification of electrodes surface by nanomaterials, in recent years, some studies have been done in trying to build biosensors and bioelectronics devices with nanometric geometry [42, 92], where the individual 1D structures are applied as working electrodes for current measurements low, typically on the order of femtoamperes (f) and picoamperes (pA). Several types of electrodes such as single-walled carbon nanotubes (SWNTs) [93, 94], boron-doped silicon nanowires (SiNWs) [92] and Sn doped  $\text{In}_2\text{O}_3$  nanowires (ITO-NWs) have been shown to be interesting for building nanodevices [42]. For example, in a pioneer work, Lemay and co-workers [95] performed electrochemical measurements, on reduced scale of redox enzymes to study a small amount of molecules. This approach was based

on lithographically fabricated Au nanoelectrodes with dimensions down to ca.  $70 \times 70$  nm, where was demonstrated successfully for the first time a distinct catalytic response from less than 50 enzymes ([NiFe]-hydrogenase) molecules. These results were obtained using cyclic voltammetry in which were observed a turnover current of 22 fA. However, because of high surface-to-volume ratio and tunable electron transport properties related to the quantum confinement effect present in these nanodevices, their electrical properties are strongly influenced by minor perturbations. This way, when an electrode with nanometer dimensions is used, various types of noises can affect the measurements and compromise the interpretation of the results.

Recently, the noise and distortions are the main factors limiting the accuracy of measurements in devices at low current conditions (sub-pico-Ampere). In experiments using electrodes macro-scale (centimeters, micrometers) problems related noises can be easily overcome by the use of programs for signal smoothing. However, for nanoelectrodes, the use of conventional methods of smoothing of signals can lead to loss of useful information. Thus, many research efforts have been observed in the development of methodologies capable of minimizing the effects of external disturbances in the low currents measurements in nanoelectrodes. In a pioneering study, Goncalves and co-workers [92] reported the development of numerical methods for smoothing signal and noise modeling. Like most of the noise frequency affecting the measurements are known (thermal, flicker, burst and shot noise) smoothing filters were used to promote a better visualization of the useful signal. Numerical methods have proven useful for the treatment of the signal due to its simplicity and speed of processing, allowing the identification of unwanted signals, changes in control parameters related to the final quality of the processed signal and quick view of the desired signal [92].

The miniaturization of electrochemical platforms is an important feature in the development of the new generation of implantable clinical devices for monitoring metabolites at living organisms [96]. The implantable biosensors are presented as ideally devices desirable for the diagnosis and management of metabolic diseases such as, diabetes, which currently is based on data obtained from test strips using drops of blood. Although widely used, this procedure is unable to reflect the general situation of the patient and point out trends and patterns associated with their daily habits. Thus, many studies focused on the development of implantable biosensors for continuous monitoring of several biologically important metabolites have been reported in bioelectrochemical area with the purpose to improve human quality of life and too in recent trends, the capability to generate energy from biomass fuels [97–99]. Figure 2.7, for example, shows a catheter microchip that consists of flexible carbon fiber electrodes modified with neutral red redox mediator (FTCF-NR) being implanted in jugular vein of rat. This system can be used both to monitor glucose levels and for power generation in biofuel cells utilizing enzymes and microorganisms.

Despite promising, the reliability of implantable systems is often undermined by factors like biofouling [100, 101] and foreign body response [102] in addition to sensor drifts and lack of temporal resolution [103]. To minimize such problems,



**Fig. 2.7** **a** Photograph of implanted catheter microchips in jugular vein of rat from *Rattus Novergicus* species **b** Chronoamperometry curves in situ without the addition of glucose (black line) and with addition of glucose (red line)

many researchers have directed their work for the synergism between biosensors and nanotechnology which has led to diagnostic devices more reliable [104, 105].

The prospects of implantable devices and in particular the metabolic monitoring can only be achieved if they can be readily implanted and explanted without the need for complicated surgery. In this sense, for facilitated the implantation, the implantable device should be extremely small, which calls for miniaturization of various functional components, such as electrodes, power sources, signal processing units and sensory elements. This way, miniaturized biosensors can cause less tissue damage and therefore less inflammation and foreign body response [106].

## 2.5 Conclusion

Currently, research in the area of biosensing is conducted not only in the construction of miniaturized devices, faster, cheaper and more efficient, but also in the increasing integration of electronic and biological systems. This way, the future development of biosensors and devices bioelectronics analysis of highly sensitive and specific will require the combination multidisciplinary areas like quantum, chemistry and solid state physics and surface, bioengineering, biology and medicine, electrical engineering, among others. Advances in any of these fields will have significant effects on the future of medical diagnosis and treatment, where the monitoring continuous diseases, prevention methods and development of more effective drugs with side effects minimized will be benefited by biosensing technologies.

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