Polydopamine-modified surfaces in biosensor applications

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Electrode surface modification is a widely used strategy for electroanalytical devices. In addition, biosensing applications require highly biocompatible and properly functionalized surfaces to anchor the biorecognition material. Conventional immobilization methods are usually aggressive, long-time requiring and could be complicated or even not applicable to all surfaces. Thus, easy, efficient and versatile immobilization methods are required in biosensor developments. Polydopamine (PDA), a mussel-inspired coating, has recently attracted considerable attention from researchers. In 2007, Lee et al. reported the first publication using dopamine (DA), with a similar molecular structure to L-DOPA, to obtain an adhesive PDA film. The very reactive residual quinone groups in PDA allow further derivatizations with nitrogen and thiol residues through Schiff base formation or Michael-type addition. This high reactivity and the possibility of covering almost unlimited number of materials offer a great opportunity for further modifications. In the particular case of biosensor development, PDA provides a suitable microenvironment for immobilizing a high density of biomolecules onto the transducer surface, and it turns out to be an easy, convenient and non-aggressive method to prepare biosensors. We will introduce this bioinspired material and its application in sensing and biosensing applications, in this chapter. Firstly, we will present different synthesis approaches, the main proposed polymerization mechanisms, and an overview of their numerous advantages and applications reported in the literature. Finally, recent advances in sensing application including: biosensors, immunosensors, DNAsensors and aptasensors are discussed.

Keywords: Polydopamine; Biosensors; Immunosensors; Electrochemical devices, Electroanalysis

1. Introduction

Surface-functionalization methods are of essential importance for diverse science areas from biomedicine to biosensing, catalysis, water treatment, supercapacitors and batteries applications. Among the different approaches, the most common methods to carry out such functionalization are electro- and photopolymerization, self-assembled monolayer, physical and chemical vapor deposition, plasma polymerization and layer-by-layer deposition. Such modifications allow the control of surface properties and the addition of new functionalities such as biocompatibility, chemical stability, antimicrobial activity, new electrical and optical properties, etc. Nevertheless, these methods may be complex, limited to certain surfaces, laborious, time consuming and sometimes poorly reproducible. Therefore, new and green functionalization methods that allow easy and reproducible surface modifications are needed.

In addition, biosensors devices require highly biocompatible surfaces to anchor biological molecules on the transducer surface without loss of activity. During the immobilization procedure biomolecules have to maintain their structural properties and hence their biological activity (catalytic or bio-affinity properties). In addition, biomolecules must remain tightly bound to the surface and not be desorbed during the use of the biosensor. In this context, adsorption, physical entrapment, cross-linking, affinity interactions and covalent immobilization are the most common methods to immobilize the bioactive molecules [1]. The choice of the most appropriate immobilization technique depends on various factors such as the nature of biomolecule and transducer, the detection method, linear range, detection limits, biosensor stability requirements, etc. Table 1 shows the main advantages and drawbacks of the main immobilization methods. Moreover, the analytical performance of the biosensors depends on the immobilization technique used during the immobilization step [2].

Non-oriented immobilization on polished or non-nanostructured surfaces usually produces lower sensitivities, probably due to lower surface/volume ratio and the greater freedom of orientation of the biomolecules. The latter involves random distribution and poor orientation of the enzyme/biomolecule and may induce conformational changes (especially in the active or the binding site), biomolecule denaturation, difficult of accessibility of the substrate to the active or antigen binding site, etc. [3, 4]. Much effort has been made to develop successful immobilization strategies in order to assure greater sensitivity and stability of biosensors. In this respect, better results are obtained when the biomolecules are properly oriented on the transducer surface via covalent immobilization, self-assembled monolayers (SAMs) or affinity interactions (e.g. between biomolecule and (strept)avidin molecules or lectins) [1]. Therefore, under the oriented method, the active (enzymes) or the binding sites (antibodies, aptamers, DNA probes) are correctly exposed to the bulk solution and enzymatic substrate or antigen may access them.

From the perspective of the polydopamine (PDA) biosensors, PDA has the ability to modify a large number of surfaces and may be used for further modifications or derivatizations, and this is an easy and convenient modification method and provides a suitable microenvironment for highly dense immobilization of biomolecules via covalent bond
with amino, imidazole and thiol residues (through Schiff base formation or Michael-type addition) on the surface of the electrodes while they retain their biological activity [5, 6].

Table 1 Advantages and drawbacks of the most common immobilization methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Binding nature</th>
<th>Advantages</th>
<th>Drawbacks</th>
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<tbody>
<tr>
<td>Adsorption</td>
<td>Weak bonds: hydrophobic, Van der Waals or ionic interactions</td>
<td>Simple and easy</td>
<td>Desorption</td>
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<td></td>
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<td>Cheap</td>
<td>Nonspecific adsorption</td>
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<tr>
<td>Entrapment</td>
<td>Incorporation of biomolecules within a gel or polymeric network</td>
<td>Wide applicability</td>
<td>Diffusion barrier</td>
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<td>Several types of enzymes may be simultaneously</td>
<td>Enzyme leakage</td>
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<td></td>
<td></td>
<td>immobilized</td>
<td>High concentrations of monomer and enzyme</td>
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<tr>
<td>Cross-linking</td>
<td>Enzymes molecules are cross-linked by a functional</td>
<td>Biocatalyst stabilization</td>
<td>High biomolecule activity loss</td>
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<tr>
<td></td>
<td>reactants (e.g. di-aldehyde)</td>
<td>Simple</td>
<td>Diffusion barrier</td>
</tr>
<tr>
<td>Covalent</td>
<td>Chemical binding between functional groups of the biomolecule and support</td>
<td>No diffusion barrier</td>
<td>Coupling with toxic product</td>
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<tr>
<td>coupling</td>
<td></td>
<td>Short time response</td>
<td>Matrix and biomolecule cannot be regenerated</td>
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<tr>
<td>Affinity</td>
<td>Affinity bonds between two affinity partners (e.g. avidin/biotin)</td>
<td>Controlled and oriented</td>
<td>Need specific functional groups</td>
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<td></td>
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<td>immobilization</td>
<td>High cost</td>
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<td>Remarkable selectivity</td>
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2. Polydopamine

Dopamine (DA) is an organic chemical of the catecholamine and phenethylamine families that plays several important roles in the brain and body. It is an amine synthesized by removing a carboxyl group from a molecule of its chemical precursor, 3,4-dihydroxy-L-phenylalanine (L-DOPA), which is synthesized in the brain and kidneys. DA is a catecholamine neurotransmitter related to many physiological processes and neurophysiological disorders such as schizophrenia, Parkinson's and Alzheimer's diseases, as well as several social and addiction behaviors [7]. DA mimics the adhesive proteins (DOPA, tyrosine and lysine) which have attracted wide interest due to their self-polymerization capacity in aqueous phase under weak alkaline conditions [5, 7-9]. Early studies showed that the presence of L-DOPA and lysine-rich proteins were responsible for the extremely robust adhesion of mussels. In 2007, Lee et al. first reported on the use of DA in obtaining an adhesive PDA film over a wide variety of organic and inorganic surfaces [10]. Later on, new PDA properties as well as interesting applications in biosensors, sensors, remediation, biominalization, drug delivery and hyperthermia were reported [5, 7].

Although the structure of this polymeric film has not yet been fully elucidated, it is probably formed by the reaction of the primary amino groups and quinone groups of oxidized DA via the formation of Schiff bases and/or Michael addition adducts [5, 6]. In addition, the reactive quinones at the surface of PDA film may be used as anchoring points for further chemical modification and/or immobilization of biologically active macromolecules (antibodies, enzymes, etc.) [11-18]. PDA-mediated surface modification has been shown to provide versatile platforms for enzyme [19-21], antibody [12-14], aptamer [11, 22] and DNA immobilization [23, 24], efficient and reliable manipulation of human neural stem cells (NSCs) [25], growth factor immobilization [26], stem cell adhesion [27], antibacterial surface preparations [28] and hydroxyapatite crystallization [29].

The primary advantage of PDA is that it can easily be deposited on virtually all types of inorganic and organic substrates, including superhydrophobic surfaces. Another valuable feature of PDA lies in its chemical structure, which incorporates many functional groups such as catechol, amine, and imine groups that can be used as starting points for further covalent modifications [5, 6]. In addition, the very reactive residual quinone groups allow further derivatization with nitrogen and thiol residues through Schiff base formation or Michael-type addition [5, 6, 16, 17, 21]. This high reactivity and the possibility of covering an almost unlimited number of materials offer a great opportunity for biosensor assemblies. In the particular case of PDA biosensors, PDA provides a suitable microenvironment for immobilizing a high and oriented density of biomolecules onto the transducer surface resulting in an easy, convenient and non-aggressive method to prepare biosensors. Furthermore, the biological material is firmly anchored by covalent bonding preserving its catalytic and bio-recognition activity [6, 11, 18, 20, 21, 30, 31].

The most common method used for the production of PDA is the solution oxidation method under slight alkaline conditions [5, 6, 10, 17, 21]. Figure 1 shows a schematic illustration about PDA formation. In the present approach, the monomer, DA is oxidized (using oxygen as oxidant) and spontaneously self-polymerizes under alkaline conditions (pH
This self-polymerization reaction is mild and does not need any complicated instrumentation or harsh reaction conditions.

During the polymerization reaction an evident color change occurs in the solution, changing from colorless to pale brown, and finally turning to deep brown after several hours. Some experimental variables may be tuned to control the thickness and the morphology of the polymeric film such as polymerization time, monomer concentration, used oxidant and buffer nature (phosphate, TRIS, etc.) [32]. However, the maximum thickness of the resulting PDA film in a single reaction step is about 50 nm [32]. Previous authors, using X-ray photoelectron spectroscopy, calculated a linear growth rate of PDA on silica of about 3.6 nm/h [33], although some discrepancies (mainly due to different experimental conditions) have been found by other authors when they obtained PDA-modified magnetic nanoparticles [12, 16, 17, 21]. On the other hand, Ponzio et al. [34], showed that the polymerization of PDA at the air/water interface makes it possible to obtain a polymeric thickness between 30 and 50 nm after just 3 h. This result was justified by the increased oxygen concentration found in the air/water interface respect to those found in the solid/liquid interface used in previous methods. To overcome such limitations, some authors have suggested other methods [5, 6, 11, 20, 32, 35-37]:

1. The use of several polymerization steps, with freshly prepared DA solution in each step
2. The use of copper or ammonium persulfate ions as oxidants (allowing the polymerization of PDA even under acidic conditions, pH = 4)
3. Electrochemical polymerization of DA onto conventional electrochemical electrodes, even in de-aerated solution.
4. The use of enzymes such as Lacasse to catalyze the synthesis of the polymeric film.

Figure 2a shows PDA electrodeposition at constant potential (+0.75 V versus Ag pseudoreference) on a carbon nanotube-modified screen printed electrode (SPE/CNT) for 400 seconds. This figure shows a rapid decrease in current, which is consistent with the self-sealing behavior observed for similar electrosynthesized polymers (e.g. poly-o-phenylenediamine) [38-40]. Figure 2b shows the typical cyclic voltammogram (CV) for the electropolymerization of PDA on a SPE/CNT. A pair of peaks at 0.25/0.05 V is observed. The anodic peak at 0.25V is responsible for DA oxidation to ortho-dopaminoquinone and the cathodic peak at 0.05 V is related to ortho-dopaminoquinone reduction to DA. In successive scans, the peak currents decrease cycle-by-cycle suggesting that a compact and insulating PDA film is formed and coated onto the electrode surface progressively, which leads to the reduction of the voltammetric response. Figure 2c shows the CV, using the ferro/ferricyanide pair as redox probe, for gold SPE with and without PDA. Data confirmed the correct electrodeposition of PDA on the electrode surface, suppressing the redox activity of the redox probe on the surface electrode after PDA electrodeposition.
2.1 Polymerization mechanisms

Since the first evidence of the formation of PDA films in 2007 by Lee and co-workers [10], the polymerization of DA has been considered to follow a similar reaction pathway as the eumelanin synthesis in living organisms [5, 6, 41, 42]. First results, based on FTIR studies, suggested the intramolecular cyclization of DA with the formation of their indole derivatives (see Figure 3a). Therefore, DA was first oxidized to dopamine-quinone followed by intramolecular cyclization via 1,4 Michael-type addition to produce leucodopaminechrome. Thereafter, leucodopaminechrome further suffered from oxidation and rearrangement to form 5,6-dihydroxyindole (DHI), which is easily oxidized to 5,6-indolequinone. These two reaction products were capable of undergoing branching reactions at positions 2, 3, 4, and 7, leading to the formation of multiple isomers of dimers and, eventually, higher oligomers, which self-assemble through the reverse dismutation reaction between catechol and o-quinone to give the cross-linked polymeric film. Figure 3 shows some of the reactions involved during the polymerization reaction and some of the proposed structures.
Although there is evidence for some of the proposed intermediates, the real mechanism is not fully understood due to the difficulties in determining the full intermediates of reaction, their structure and the full reaction process. In addition, little solid experimental evidence has been found for this “eumelanin” model and new mechanisms have been proposed. Nowadays, the debate is centered on the question of whether the structure of PDA is only composed of covalently bonded 5, 6-dihydroxyindole, 5,6-indoledione and dopamine units or whether noncovalent interactions are involved during polymerization too. Liebscher and co-worked [43] proposed that the structure of PDA consists of mixtures of different oligomers, wherein indole units with different degrees of (un)saturation and open-chain DA occur. In addition, Hong and colleagues [44] proposed a mixed mechanism where PDA is a result of the combination of noncovalent self-assembly and covalent polymerization. Figure 4 shows the mechanism proposed by the latter researchers. In order to support this mechanism, they identified a considerable amount of DA that remained unpolymerized and that formed a self-assembled complex with its oxidative product, 5,6-dihydroxyindole (DHI). In contrast, Dreyer et al. [45], suggested that PDA may be an aggregate of monomers, which were cross-linked primarily via strong noncovalent forces, including hydrogen bonding, charge transfer, and π-stacking, similar to other synthetic biological polymers. Finally, Vecchia et al. [46] proposed that the formation of PDA undergoes three main competing pathways and that the final polymeric structure depends on the synthesis conditions. These authors suggested that PDA is formed by planar indole units, amino group, carboxylic acid group, catechol or quinone functions and indolic/catecholic π-systems. All these reactive groups are responsible for the excellent and robust adhesion capability of PDA to a great number or surfaces and could provide a versatile platform for further derivatization reactions and the immobilization of biomolecules. In the present model, these authors proposed that quinone groups are generated slowly at a very low DA concentration (0.5 mM), leading to a higher proportion of cyclized indole units. Meanwhile, at a higher DA concentration (10 mM), the quinone, generated by autoxidation of the catechol groups, might be efficiently trapped by DA increasing the fraction of uncyclized elements.
Fig. 4 (a) two reaction pathways, proposed by Hong et al. [44], for polydopamine (PDA) formation where the polymeric film is obtained by combination of covalent bond structures (obtained by oxidative polymerization) with trimeric complexes of two dopamine (DA) and 5,6-dihydroxyindole (DHI) molecules obtained by physical self-assembly pathway.

2.2 Polydopamine-modified materials

PDA has a broad interesting set of properties. Over the last decade PDA has proven to be useful for a variety of applications such as batteries, catalysis and photocatalysis, remineralization, bioimaging, drug delivery, photothermal therapy and biosensor applications [5, 6]. However, here we will only introduce some of the interesting properties of PDA such as its adhesion properties and chemical reactivity and its application on the generation of novel hybrid materials in the context of biosensor applications.

Research on PDA has been directed towards the construction of PDA-modified substrates based on its inherent adhesive properties. Although the exact adhesion process is not yet clear, researchers think that catechol and quinone groups play a central role in such mechanisms. The adhesion mechanism can be divided into two binding-type mechanisms: covalent and noncovalent. Therefore, substrates with amino and/or thiol groups on their surfaces may be modified via Michael addition and/or Schiff base reactions under basic conditions. On the other hand, on metal or metal oxide surfaces, noncovalent binding interaction such as metal coordination or chelating, hydrogen bonding, $\pi-\pi$ stacking, and quinhydrone charge-transfer complexes may be responsible for its adhesion [5, 6].

The many functional groups found in PDA are able to react with a wide range of biomolecules. Among the different functional groups presented in PDA, the presence of quinone or catechol groups offers an interesting platform for secondary surface functionalization. Therefore, the cross-linking reaction of PDA with amino and/or thiol residues are the most widely investigated reactions in the literature [12, 16, 17, 21, 23, 24]. The reactivity of PDA with amino
residues is a function of the catechol/quinone chemical equilibrium in the PDA matrix and the pKₐ of the amino group [6]. Therefore, under basic conditions, catechol groups may be oxidized into the corresponding quinone, which can then react with nucleophilic amino groups presented in other biomolecules via Schiff base reaction and/or Michael-type addition. On the other hand, other nucleophiles such as thiol residues are most likely to react with PDA through Michael addition reaction. **Figure 5** shows the covalent coupling reactions involved during the immobilization of amino and thiol residues onto PDA films. Both reactions are straightforward, without the need for any harsh reaction and may be produced under slight basic conditions at room temperature. Other important advantages of the use of PDA as an immobilization matrix is that this covalent binding is fairly stable, in marked contrast to N-hydroxysuccinimide (NHS) or maleimide agents, commonly used during biomolecules immobilization.

![Figure 5](image)

**Fig. 5** Covalent bonding mediated by Michael addition and Schiff base reaction involved during the immobilization of biomolecules with thiol (a) and amino (b) groups.

### 3. Biosensors

Thanks to the excellent adhesion properties of PDA, many research studies have reported the straightforward modification of a great diversity of surfaces such as carbon nanotubes, graphene, magnetic nanoparticles, glassy carbon electrodes, etc. Therefore, hydride configurations have been used for developing new and improved materials where PDA serves as a link to the modified surface allowing the covalent conjugation with biomolecules, acting as a reducing agent, allowing the development of inorganic-organic materials or being capable of constituting specific structures (e.g. in Molecular Imprinting Techniques) for sensing applications. In addition, the synthesis and application of core/shell nanostructures, particularly PDA-based core/shell nanoparticles, have recently been reported [12, 16, 17, 21]. For example, PDA-modified iron oxide-based magnetic nanoparticles have attracted much attention in biomedical, analytical, environmental and industrial applications due to their easy and controlled synthesis and surface modification, high biocompatibility and low toxicity. Moreover, their magnetic properties add a new dimension to biomedical and analytical applications such as in immunoprecipitation and separation techniques, drug delivery, immunoelectrochemical and biosensors devices, magnetic resonance imaging and hyperthermia treatments. However, only the application of PDA-modified surfaces in biosensors applications will be introduced in the present section.

Although the first interest in PDA was related to its excellent adhesive properties, some researchers studied the possibility of immobilizing biological material on the PDA structure and its immediate application in sensing devices. Therefore, a large number of publications have recently appeared in the literature describing the application of PDA-modified surfaces in biosensor applications, including CNT; graphene, carbon nanofiber and metallic and bi-metallic composites have been well reported too.

Therefore, Li et al., reported a novel phenolic biosensor prepared on the basis of a composite of polydopamine (PDA)-laccase (Lac)-nickel nanoparticle loaded carbon nanofibers (NiCNFs) [18]. Firstly, NiCNFs were fabricated by a combination of electrospinning and a high temperature carbonization technique. Subsequently, the composite was obtained through one-pot Lac-catalyzed oxidation of dopamine (DA) in an aqueous suspension containing Lac, NiCNFs and DA. The immobilized Lac showed a pair of stable and well-defined redox peaks, and the electrochemical behavior
of Lac was a surface-controlled process. This biosensor was characterized by using catechol as a proof of concept and had a sensitivity of 25 μA mM⁻¹ cm⁻², a detection limit of 0.69 μM, and a linear range from 1 μM to 9.1 mM, as well as good selectivity and stability. In addition, other authors [37] reported the electropolymerization of DA and other catecholamines preoxidized by Lac catalysis as efficient immobilization methods for glucose oxidase (GOx) on Au electrodes for sensitive amperometric biosensing of glucose. The rates of Lac-catalyzed polymerization in aqueous solutions were found to follow the order of dopamine (DA) > L-noradrenaline (NA) >> epinephrine (EP), as examined by visual inspection and confirmed by UV-vis spectrophotometry and electrochemical techniques. Therefore, the DA and NA polymer substrates gave almost identical glucose-biosensing performances that were much better than the EP-based polymer. Another example of the application of laccase enzyme in the development of PDA-modified biosensors was reported by Li and co-workers in 2014 [31]. These authors reported a new PDA-Lac-Fe₃O₄ magnetic polymeric bio-nanocomposite (MPBNCs) prepared through one-pot Lac-catalyzed oxidation of DA in an aqueous suspension containing Lac, Fe₃O₄ nanoparticles and DA. Later on, a PDA-Lac-Fe₃O₄/Au electrode was prepared by simple and efficient magnetic separation/immobilization of the MPBNCs on a magnetic Au electrode for biosensing hydroquinone (HQ), giving a sensitivity of 374 μA mM⁻¹ cm⁻², a limit of detection of 30 nM⁻¹, as well as good selectivity and stability. Finally, such biosensors were also used for HQ analysis in real sample matrices with satisfactory results.

In addition, similar modifications have been successfully applied to other materials such as CNT [36], Tan et al., described the simple preparation of polymer-enzyme-multiwalled carbon nanotubes (MWCNTs) by in situ laccase (Lac)-catalyzed polymerization and its direct application in electrochemical biosensing and biofuel cell applications. These authors developed a hydroquinone biosensor PDA-Lac-MWCNTs/GCE with a sensitivity and detection limit of 643 μA mM⁻¹ cm⁻² and 20 nM, respectively. Moreover, the DA substrate yielded the best biosensing performance, as compared with aniline, o-phenylenediamine, or o-aminophenol as the substrate for similar Lac-catalyzed polymerization.

More recently, Martín et al., reported novel phenolic [17] and peroxide hydrogen [16] biosensors using PDA-modified magnetic nanoparticles (MNPs) and horseradish peroxidase (HRP) enzyme. Firstly, MNPs nanoparticles of about 15-20 nm were prepared by the chemical coprecipitation method under N₂ atmosphere. After being washed and magnetically decanted several times, 500 mg of MNPs were dispersed under continuous stirring in 25 mL of 10 mM DA solution (PBS, pH 8.5) during 3-6 hours. After that, PDA-modified nanoparticles (MNPs@PDA) were magnetically decanted and washed again. HRP was covalently immobilized on the surface of the PDA-modified MNPs via Michael addition and/or Schiff base formation. For this end, 50 mg of MNPs@pDA were dispersed in 1 mg mL⁻¹ HRP solution (2.5 mL PBS, pH 7.4) for 4 h under agitation at room temperature. Afterwards, MNPs@pDA/HRP were used to construct a biosensor for phenolic compounds by capturing the HRP-modified-nanoparticles onto the surface of a magnetic-modified glassy carbon electrode (GCE). Cyclic voltammetry and amperometry were used to study the electrochemical and analytical properties of the biosensor using HQ as a redox probe. Among the different phenolic compounds studied, the biosensor exhibited higher sensitivity for HQ of about 1.38 A Μ⁻¹ cm⁻² and with detection and quantification limits of 0.3 and 1.86 mM, respectively. The analytical biosensor performance for HQ and 2-aminophenol compared advantageously with those of previous phenolic biosensors reported in the literature. On the other hand, this enzymatic nanoconjugate was used to construct a second-generation biosensor (GCE/MNP@PDA/HRP) for H₂O₂ sensing [16]. Under the present approach, the enzyme biosensor showed a high sensitivity of 442.14 mA M⁻¹ cm⁻², a low limit of detection of 182 nM and high stability for 1 month. Other interesting results when developing a H₂O₂ biosensor were reported by Gao and co-workers [15]. These authors developed an electrochemical H₂O₂ biosensor using PDA-entrapped G-quadruplex-hemin DNAzyme. After the physical adsorption of DNAzyme on a glassy carbon electrode, a PDA film was generated depositing a droplet of 10 μL (containing 5 g L⁻¹ dopamine in PBS (pH 8.0)) onto the surface. The proposed biosensor had a linear range with the concentration from 10 μM to 1.5 mM and a detection limit of 2.2 μM for H₂O₂.

Recent works have shown that the immobilization properties of PDA films may be improved by thermal oxidation treatment [21, 35], thus Martin and co-workers described the first amperometric magneto-biosensor for the specific and sensitive detection of glucose based on quinone-rich PDA-modified MNPs [21]. These researchers suggested an easy method to modify PDA-MNPs and to increase the amount of quinone groups by using thermal oxidation. The present approach showed that the amount of Gox immobilized was almost two times higher compared to non-oxidized films and, thus, its sensitivity and LOD were improved against the non-oxidized PDA-MNPs. MNPs@pDA/Gox were used to construct a biosensor for glucose detection by capturing the Gox-modified-nanoparticles onto the surface of a magnetic-modified screen-printed electrode (SPE) using Prussian Blue to detect the H₂O₂ generated during the enzymatic reaction at a low potential. The enzyme biosensor had a sensitivity of 8.7 mA M⁻¹ cm⁻², a low limit of detection (0.02 mM), and high stability for 45 days. Finally, the biosensor was used to determine glucose in blood samples and was checked against a commercial glucometer. Figure 6 shows the full modification protocol, the biosensor ensemble and its response against glucose.
Graphene (GR) is an inexpensive material with good mechanical and biocompatibility, high surface area-to-volume ratio, flexibility, excellent electrical and thermal properties and because of these properties it has received considerable attention in many research areas. Moreover, GR provides an ideal platform to prepare electrochemical sensors and biosensors. Ruan et al. [20] recently reported a glucose biosensor based on PDA-graphene hybrid film modified GOx enzyme electrode. DA, graphene oxide and GOx were mixed and casted on an Au electrode. The as-synthesized enzyme electrodes were characterized by scanning electron microscopy, transmission electron microscopy, Fourier transform infrared and X-ray diffraction spectra. The biosensor gave an excellent amperometric biosensing performance with high detection sensitivity (28.4 μA mM⁻¹ cm⁻²), low response (4 s) and a very low limit of detection (0.1 μM).

Another interesting work using graphene as enzymatic support was reported by Zhou et al. [19]. Theses authors modified graphene oxide (GO) with DA to create a matrix for enzyme immobilization. Firstly, DA was self-polymerized to obtain PDA which was then coated on a GO surface. At the same time, GO was reduced to obtain a PDA/rGO biocomposite and then used to immobilize GOx. Experimental results indicated that the thermal and pH stability, as well as the storage stability and resistance to the denaturing agents of GOx were significantly improved after immobilization. The Michaelis constant of the immobilized GOx was close to that of the free GOx.

Other novel applications for PDA films have been reported in the field of immunosensors, where specific antibodies are used to detect a specific target. In the present approach, Peng et al. [14] reported a novel and highly sensitive label-free electrochemical immunosensor for the detection of alpha-fetoprotein (AFP) based on gold nanoparticles-PDA-thionine-graphene oxide (Au-PDA-THi-GO) nanocomposites. The as-prepared Au-PDA-THi-GO nanocomposites not only provided a favorable microenvironment to maintain the activity of the immobilized biomolecules due to the excellent biocompatibility of PDA, but also increased the loading capacity as a result of the large surface area of nanocomposites. Besides which, the presence of gold nanoparticles enhanced the conductivity and charge-transport properties of the composites. Under optimal conditions, this immunosensor exhibited a wide linear range from 0.1 to 150.0 ng mL⁻¹, a low detection limit of 0.03 ng mL⁻¹, high selectivity, long-term stability and good reproducibility. In the same year, Sun and co-workers [13] developed a sensitive electrochemical immunosensor for 3-bromobiphenyl (3-BBP) detection using a PDA coated Prussian Blue-mesoporous carbon (PDOP/PB/CMK-3) nanocomposite as the substrate platform and a multi-horseradish peroxidase-double helix carbon nanotubes-secondary antibody (multi-HRP-DHCNTs-Ab2) as the signal label. PDOP/PB/CMK-3 nanocomposite effectively enhanced the specific surface for antigen loading due to the three-dimensional structure of the nanocomposites, while PB improved the electrochemical response. Furthermore, PDOP film provided a biocompatible environment to maintain the activity of antigen availability. Under the optimized conditions, the proposed immunosensor showed a good current response to 3-BBP in a linear range from 5 pM to 2 nM with a detection limit of 2.25 pM. In addition, the specificity, reproducibility and stability of the immunosensor also proved to be acceptable, indicating its potential application in environmental monitoring. More recently, Li et al. [30], reported novel carbon encapsulated Fe₃O₄ nanoparticles embedded in two-
dimensional (2D) porous graphitic carbon nanocomposites (Fe₃O₄@C@PGC nanocomposites). Theses authors developed a highly label-free sensitive magnetic immunoSENSOR for the detection of carcino-embryonic antigen (CEA). Firstly, Fe₃O₄@C@PGC nanocomposite was modified by the self-polymerization of DA, acting as both the reductant and template for one-step synthesis of gold nanoparticles. The prepared Au/PDA/Fe₃O₄@C@PGC nanocomposite was then used to immobilize the capture antibody (Ab) using the following protocol: 6 μL of Au/PDA/Fe₃O₄@C@PGC solution (1.0 mg/mL) was fixed on the surface of magnetic-modified glassy carbon electrode (MGCE). After which 6 μL of Ab was anchored on the Au/PDA/Fe₃O₄@C@PGC by the physical absorption and the chemical bonding of Au-N or Au-S bonds. Under the optimal conditions, the immunoSENSOR showed a wide linear range (0.001-20.0 ng mL⁻¹), a low detection limit (0.33 pg mL⁻¹), good reproducibility, selectivity and acceptable stability. Similarly, an electrochemical immunoSENSOR for interleukin-6 (IL-6) detection was reported by Wang and co-workers [47]. Here, Au-PDA-Fe₃O₄ nanocomposites were synthesized by a seed-assisting growth method and PDA was a secondary substrate for the growth of Au nanoparticles. In the presence of IL-6, a rapid decrease in the electrochemical activity of the Au nanoshell on the electrode surface was observed as a result of the formation of the antigen-antibody complex, with a detection limit as low as 0.013 ng/mL and a wide linear range of 3 orders of magnitude. Another noteworthy approach was reported by Martin et al. [12], where PDA-modified MNPs were used to develop a novel amperometric magnetooimmunoassay for the selective determination of Legionella pneumophila SG1. These authors used a specific capture antibody (Ab) linked to the PDA-modified MNPs (MNPs@PDA-Ab) and incubated them with bacteria. The captured bacteria were then sandwiched using a secondary antibody labeled with horseradish peroxidase (Ab-HRP), and the resulting MNPs@PDA-Ab-Legionella pneumophila-Ab-HRP were captured by a magnetic field on the SPE surface. The amperometric response was obtained at -0.15 V after the addition of H₂O₂ in the presence of hydroquinone. The achieved detection limit was 10³ Colony Forming Units (CFUs) mL⁻¹ and it showed good selectivity and stability for 30 days. Besides which, these authors were able to low down a concentration of bacteria (10 CFUs mL⁻¹) by introducing a pre-concentration step before the bacteria immobilization. Finally, the authors suggested that the developed magnetooimmunoassay could be an attractive alternative analytical tool to provide a fast screening (<3 h) of a possible contaminated source taking into account that the gold standard method (culture techniques) for the detection of L. pneumophila takes up to 10 days.

There has recently been much interest in DNASensors, RNAsensors and aptasensors. The latter differs in the use of aptamers, and these sensors are a new generation of synthetic biorecognition elements which challenges antibodies and enzymes in many aspects. Aptamers are artificial oligonucleotide or peptide molecules engineered through repeated rounds of in vitro selection or equivalent, SELEX (systematic evolution of ligands by exponential enrichment), to bind to various molecular targets such as small molecules, nucleic acids, proteins, and even cells, organisms and tissues. Other than their discriminate recognition, aptamers offer advantages over antibodies as they can be completely artificially engineered, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications. Based on the present approach, Zhang et al. [24] have recently reported an electrochemical DNA sensor based on covalent immobilization of an amino-terminated probe DNA onto the PDA-modified SPE. Firstly, PDA was formed via electrochemical polymerization of DA monomer on the SPE surface and after a sandwich-type hybridization reaction, the gold nanoparticle-labeled reporter DNA was bound onto the DNA sensor surface to further induce silver deposition in the presence of silver enhancer solutions. The proposed method detected the target DNA in a linear range from 1.0 pM to 70 pM, with a detection limit of 0.3 pM. The DNA sensor exhibited good stability, selectivity and acceptable reproducibility. Similarly, Dong and colleagues [23] described a sensitive chronocoulometric DNA sensor for the sequence-specific detection of DNA. It was based on a GCE modified with MWCNT, PDA and gold nanoparticles. The signal of the ruthenium (III) hexamine complex, electrostatically bound to the anionic phospho groups of the DNA strands, was measured by chronocoulometry before and after hybridization and taken as a quantitative indication of the hybridization progression. Their results showed that the difference in signal intensity was linearly related to the logarithm of the concentration of the target DNA in the range of 1.0 nM to 10 IM with a detection limit of 3.5fM, presenting excellent sensitivity and selectivity. In addition, Fu et al. [22], described a novel label-free thrombin aptasensor based on multifunctional polymeric bionanocomposites (PBNCS). The present approach, based on the analyte-induced suppression of enzymatic catalysis in PBNCS after the hybridization process, used GOx as the catalytic center. The binding of thrombin to the aptasensor surface significantly hindered the mass-transfer of the enzymatic substrates/products and thus suppressed the enzymatic catalysis efficiency. This aptasensor detected thrombin with a broad detection range (1–100 nM), a detection limit down to 0.1 nM, and acceptable specificity. Finally, a novel approach was reported by Jolly and collaborators [11]. These authors successfully combined aptamers and molecular imprinting to overcome some of the challenges faced by conventional protein imprinting. Thus, a thiolated DNA aptamer, selective for prostate specific antigen (PSA), was complexed with PSA prior to being immobilized on the surface of a gold electrode. Electropolymerized PDA was used to both entrap the complex, holding the aptamer in, or near to, it’s binding conformation, and to localize the PSA binding sites at the sensor surface. Following the removal of PSA, it was proposed that the molecularly imprinted polymer (MIP) cavity would act synergistically with the embedded aptamer to form a hybrid receptor (apta-MIP), displaying recognition properties superior to those of aptamer alone. These results showed that the apta-MIP sensor had high sensitivity with a
linear response from 100 pg/ml to 100 ng/ml of PSA and a detection limit of 1 pg/ml, which was three-fold higher than simple aptamer sensor for PSA.

**Conclusions**

The present chapter is an introduction to polydopamine (PDA) bioinspired polymeric film. Although the structure of PDA has not yet been fully elucidated, it is probably formed by the reaction of the primary amino groups and quinone groups of oxidized DA via the formation of Schiff bases and/or Michael addition adducts. Although the eumelanin model was initially proposed, nowadays the full polymerization reaction is not fully understood and some polymerization mechanisms have been proposed. There is also a discussion of the most noteworthy properties of this polymer and the great potential of PDA-modified surfaces for anchoring biomolecules. From a biosensor perspective, PDA has excellent abilities to modify a large number of surfaces and is an easy and convenient immobilization for polymer and the great potential of PDA-modified surfaces for anchoring biomolecules. From a biosensor perspective, PDA has excellent abilities to modify a large number of surfaces and is an easy and convenient immobilization for biomolecules, via covalent bonding with amino, imidazole and thiol residues, retaining the biological activity. Finally, there is a description of many of the recent works in sensing applications, including enzymatic biosensors, immunosensors, DNAsensors and aptasensors.

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**References**


