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Review Article

Design and Development of Biosensors for Progesterone Detection

Wenwen Sun,[1,2] **Qinchen Xu**,[2] **Jiwei Sun**,[2] **Xiangde Lin**,[3] **and Hongzhi Pan**[2]

[1]*Department of Research, Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, Shanghai 201318, China*
[2]*College of Medical Technology, Shanghai University of Medicine & Health Sciences, Shanghai 201318, China*
[3]*School of Medical Instrument, Shanghai University of Medicine & Health Sciences, Shanghai 201318, China*

Correspondence should be addressed to Hongzhi Pan; panhongzhilaoshi@163.com

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Progesterone (P4) is an important biomarker of various diseases. When P4 level exceeds the normal value, the human body produces a series of problems, including carcinogenic risks. Developing the method for P4 monitoring with accurate, inexpensive, and fast becomes an important topic for researchers. In recent years, the abundance of new materials and synthesis technologies has developed P4 biosensors. Based on functional materials, this paper reviews the recent decade literatures and summarizes the latest progress and applications in enhancing detection of P4. In this study, the functional materials used to manufacture P4 biosensors are mainly divided into three categories: metal and metal-oxide nanomaterials, composite material, and other materials. A composite material refers to a combination of two or three types of materials, including carbonaceous nanomaterials, metal nanomaterials, polymers, and biological materials. The other materials mainly include a combination of special compounds, biomaterials, luciferin materials, and quantum dot materials, of which one or two. The introduction of these new functional materials improves the sensitivity and selectivity of P4 detection. Moreover, this study provides ideas for the future research on improving the performance of P4 biosensor. The future study should put more attentions on enzyme catalysis amplification, cyclic amplification, and DNA isothermal amplification strategies.

1. Introduction

Progesterone (P4) is a small amount of C21 steroid hormone secreted by the corpus luteum, which plays a diagnostic role for threatened abortion, habitual abortion [1–3], and other amenorrhea [4, 5]. The detection of P4 has been one of the main physicochemical indexes of water, milk, mammalian blood, and body fluids, which exhibited the focus in current research.

P4 is ubiquitous in mammals, among which part of P4 in the human body comes from the secretion of the body, and the other part comes from drinking water, food, and drugs. The concentration of P4 in the serum for adult female generally ranges from 0.48 nM to 79.5 nM, but it rises to 731 nM for pregnant women [6]. When the concentration of P4 exceeds the normal level for the human, it will result in a series of problems, even carcinogenic risks [7, 8]. For female, excessive P4 level might cause breast tenderness, constipation, diarrhoea, vomiting, fatigue, body pain, irritability, mood swings, excessive worry, and the abnormal vaginal secretion and urination [9, 10]. For male, the high levels of P4 might negatively affect the secretion of gonadotropin-releasing hormone (GnRH), causing a decrease in the production of testosterone and affecting male sexuality [9, 10]. However, the low level of P4 will also produce negative effects, for example, increasing the risk of a miscarriage in early pregnancy. Therefore, it played an important role in monitoring the concentration of P4 in serum and urine. The research on P4 in water pollution detection should not be ignored. The pathways of P4 into surface water can be divided into four aspects: agriculture, industry, domestic sewage discharge, and surface runoff after rainfall [11]. Studies have shown [12] that it would convert into other steroids in the environment, and some of which were biologically active. When the concentration of P4 was lower than 1 ng/L, it will cause endocrine-disrupting effects on the biota. In

addition to water, P4 also plays an important influence on milk, and the detection method of P4 becomes an indispensable technology in breeding and production. P4 concentration during the oestrus cycle rise and fall depends on the time interval. However, upon establishment of pregnancy, the level of P4 remained increasing, and for this reason, pregnancy determination is via detecting the concentration of P4 (>15 ng/mL) [13], while the low P4 concentration (<5 ng/mL) has been used to determine estrous [14]. Therefore, measuring the level of P4 in cows regularly is helpful for dairy cattle farmers to identify the estrus of cows, grasp the timing of artificial insemination, and monitor the abnormalities during pregnancy [15].

The monitoring method of P4 concentration has been paid attention to by researchers. In past, the traditional detection methods of P4 mainly exhibited chromatography and immunoassay. Chromatography referred to the chromatographic instrument that was used to determine the level of P4 in the sample directly, during which liquid chromatography-mass spectrometry, high-performance liquid chromatography, and gas chromatography-mass spectrometry were used normally. Immunoassay was divided into radioimmunoassay, enzyme-linked immunoassay (ELISA), and immunofluorescence. Both of the detection methods had their own advantages. For the detection of P4 concentration in samples, it exhibited sensitively, accurately, and reliably for chromatography [16], while the immunoassay exhibited strong specificity and high sensitivity. However, considering the high cost, long detection period, and high technical skills for operators, the immunoassay was not perfect for the field detection of P4. Therefore, researchers have focused on exploring the monitoring methods with good selectivity, simple preparation, low cost, simple instrument, easy operation, and fast detection in recent years.

Nowadays, electrochemical biosensors and optical biosensors have been increasingly used to detect P4. With the simple operation, high sensitivity, and relatively low cost, the optical biosensors have attracted the attention of researchers. The optical biosensors for P4 were mainly divided into fluorescence chemical biosensor, fluorescence biosensor, colorimetric adaptive biosensor, photon biosensor, surface plasmon resonance (SPR) imaging photochemical biosensor, quantum dot Forster resonance energy transfer method (FRET) photochemical biosensor, and luminescent resonance energy transfer (LRET) optical signal biosensor. Compared with optical biosensors, the research on electrochemical biosensor was more extensive [17]. The electrochemical biosensor combined the biological analysis technology and the electrochemical detection technology. That is a special type of biosensor where a biological entities was detected by converting the information into an electrical signal, i.e., voltage, current, and impedance [18]. In addition, it exhibited a good sensitivity, high selectivity of biometric identification, real-time, and rapid detection, which provides a new method for P4 analysis. It also displayed high operational stability under dynamic conditions, as well as showed reliable reproducibility [19–22]. With the development of nanomaterials and biotechnology, many new materials have been developed and applied to electrochemical biosensors, which enhanced the electrical signal and increased the sensitivity [23]. Based on the summary of research papers on P4 detection for the past decade, electrochemical biosensors for P4 detection were divided into electrochemical immune biosensors, electrochemical aptamer biosensors (including direct method, competitive method, and sandwich method), nonenzyme electrochemical biosensors, and electrochemical enzyme catalysis nanobiosensors.

For the construction of electrochemical biosensors, the functional materials exhibited great significant role in improving and optimizing the performance of biosensors [24]. The appearance of new materials provided a better detection limit and accuracy for P4 detection. Compared with the disadvantages of the traditional method for P4 detection, such as the need for professional operation, high cost, and long detection time, the emergence of new functional materials has improved the above problems with excellent performance, low cost, and fast detection. Over the past 20 years, many aptamers have been used for a variety of analytes, including bacteria, viruses, proteins and small molecules, and even some ions with high affinity and specificity. In terms of P4 monitoring, AuNPs were the most commonly used metal and oxide nanomaterials [9, 11, 25]. Some composite material, such as Au-CuO-Cu_2O and WO_3NBs@GR also can be used in electrochemical biosensors based on aptamer [26, 27]. AuNPs, nsBiFE, and Thi-GO were found to be the most commonly used functional materials in electrochemical biosensors based on immune [28, 29]. In the electrochemical biosensors based on immune, the enzyme was fixed on the electrode to construct a simple electrochemical enzyme nanoreactor, which had high stability and biological activity. Nowadays, the cases have been found using CYP_3A_4 as a functional material [30]. In electrochemical biosensors based on nonenzymatic, composite materials such as GO-IMZ and WO_3NBs@GR were used to replace enzymes with artificial enzyme active sites to complete the detection task [26, 31].

As a kind of functional materials, metal and metal-oxide nanomaterials were widely used in the field of biosensors due to its excellent properties, such as strong electrical conductivity, large surface ratio, and good biocompatibility. In order to optimize the performance of P4 biosensor, carbonaceous nanomaterials, metal nanomaterials, polymer, and other functional materials were combined to construct biosensors. Normally, it had the characteristics of strong signal, economy, high efficiency, high selectivity, simplicity, and so on. In recent years, apart from metal-oxide nanomaterials and composite materials, researchers have developed a variety of other modification materials to optimize the performance of P4 biosensor. Therefore, the functional materials being used for the manufacture of P4 biosensor in this study are mainly classified into three categories: metal and metal-oxide nanomaterials, composite materials, and other materials. These new functional materials have improved detection sensitivity and selectivity, as well as the performance improvement of optical biosensors and electrochemical biosensors.

Table 1: Performance comparison of progesterone biosensor with metal and oxide nanomaterials.

Type of biosensor	Functional material	Samples	Linear range (μmol/L)	LOD (nM)	References
Electrochemical biosensors based on aptamer	AuNPs	Tap-water	0.0318-0.1911	0.002866	[5]
	Fe_3O_4@SiO_2@TiO_2	Liquid milk	0.000001-0.006	0.0003	[35]
	AuNPs	Water	0.005-0.05	4700	[11]
	AuNPs	Tap-water, serum, urine	0.00089-0.5	0.89	[25]
	AuNPs	Serum, urine	0.00089-0.5	0.89	[9]
Electrochemical sensor	BiFE	Drug samples	400-7900	180	[36]
Electrochemical biosensors based on immunosensor	AuNPs	Female rabbit whole blood	0.0001592-0.3185	26.1783	[50]
	nsBiFE	—	—	—	[29]
Photon biosensor	$Ge_2Sb_2Te_5$, PhC	—	—	—	[34]
Surface plasmon resonance imaging optical biosensor	GAMMA-AuNPs	Milk standard	—	0.001592	[32]
	Magnetic nanoparticles	Milk, artificial milk	—	0.121	[51]

Note that LOD represents the limit of detection.

2. P4 Biosensor Based on Different Functional Materials

In this section, the main functional materials (metal and metal-oxide nanomaterials, composite materials, and other materials) of P4 biosensor are discussed in detail. As shown in Table 1, most of P4 biosensors being modified by special materials had low detection limits and high sensitivity.

2.1. P4 Biosensors Based on Metal and Metal-Oxide Nanomaterials. Metal and metal-oxide nanomaterials were widely used in the field of biosensors due to its excellent properties, such as strong electrical conductivity, large surface ratio, and good biocompatibility. The metal nanomaterials of gold nanoparticles (AuNPs) and new synthetic metal materials, such as $Ge_2Sb_2Te_5$ and nsBiFE, were normally used in the detection of P4.

Considering its excellent properties, AuNPs have been widely used as common materials for P4 detection biosensors. AuNPs are excellent materials for signal transduction due to their aggregation behavior. The dispersion and aggregation form could be detected by the color change from red to violet blue, which could also be determined by the shift of the surface plasmon resonance band to a longer wavelength. Therefore, AuNPs have been used as a perfect material with simple, rapid, and sensitive characteristics for the detection of screening estrogen substances. Du et al. [9] have developed a colorimetric aptamer biosensor to detect the concentration of P4, in which AuNPs were modified by the surfactant cetyltrimethyl ammonium bromide (CTAB). AuNP has become an excellent material for signal transduction due to their aggregation behavior. Separately dispersed AuNP appeared red in the solution, but when it aggregated, it increased the size of particles in the solution, resulting in an increase in absorbance within a certain range with a blue color. In the absence of P4, the aptamer can form a supramolecular with CTAB, thus keeping AuNPs dispersed. However, when P4 existed in the sample, the biosensor would change from red to blue because the P4 aptamer was depleted. An aptamer-P4 complex was formed that dissociated CTAB, leading to the aggregation of AuNP and the change of color.

Daems et al. [32] used an SPR platform to develop in-house fiber optic- (FO-) SPR, which was demonstrated for the first time for the detection of low molecular weight targets. In the FO-SPR, antibodies were detected to competitively bind P4 in the sample or its derivatives of being fixed on the surface of the biosensor. To improve the limit of detection (LOD), the AuNP functionalized with goat anti-mouse antibody (GAM) was used to amplify signal. In addition, the biosensor combined the ease of use and cost-effectiveness of transverse flow analysis, as well as the accuracy, sensitivity, and automation possibility of ELISA detection. Chamas et al. [33] prepared a new electrochemical biosensor, in which P4 was fixed on a gold disk electrode by self-assembly monolayer- (SAM-) bonding chemistry. SAM can avoid potential contaminants and blood interference; moreover, gold nanoparticles can enhance the signal. The biosensor can detect three target hormones simultaneously. The detection linear range was 0.05-100 ng/mL, and the LOD was 8.22 ng/mL.

Much work so far has focused on novel synthetic metal materials for P4 biosensor, and Panda et al. [34] have theoretically studied an unmarked novel photonic crystal biosensor to detect different concentrations of P4 with a (Na_3AlF_6/CeO_2)N/$Ge_2Sb_2Te_5$/Defect/$Ge_2Sb_2Te_5$/(Na_3AlF_6/CeO_2)N multilayer structure. The biosensor adopted an alternate arrangement of Na_3AlF_6 and CeO_2 with a defect layer in the middle, and a thin layer of new phase change chalcogenide material $Ge_2Sb_2Te_5$ (GST) was deposited on both sides of the defect layer to improve the sensing performance. The transfer matrix method (TMM) was used to simulate the transmission

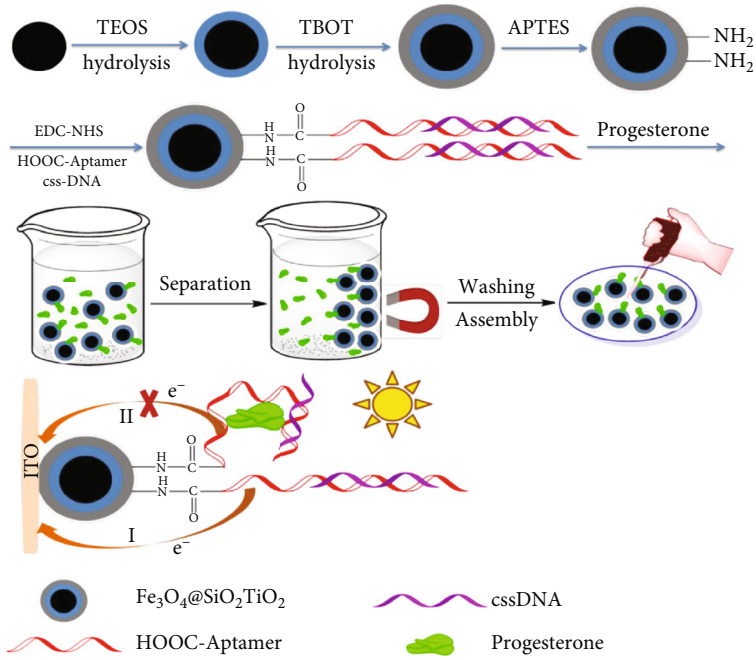

Figure 1: The synthesis of $Fe_3O_4@SiO_2@TiO_2$ modified with aptamer and capture DNA for PEC aptasensing of progesterone [35]. Images reproduced with permission.

spectrum of TE mode. By changing the phase of GST material from amorphous to a crystalline state, the position and strength of the defective film were analyzed. As a result of the high tunability of the defect film wavelength, the detection sensitivity was 1.75 nM. With the advantages of simple structure, easy analysis, and feasible manufacturing technology, the biosensor of this material was of great significance to the accuracy of P4 detection. In addition, some researchers used metal oxides to manufacture P4 biosensors. The superparamagnetic core ($Fe_3O_4@SiO_2@TiO_2$) with magnetooptic dual-function beacon was applied to the design of a novel photoelectric chemistry (PEC) biosensor [35] (Figure 1). The materials combined with specific probes were used to selectively capture progesterone molecules in the sample, conducting magnetic separation and effectively removing complex coexisting substances on the surface of modified electrode. The shelf life of the as-designed PEC sensor was examined by keeping it in a refrigerator at 4°C, and the photocurrent measurements were performed every week. The photocurrent was almost consistent with the initial value after 3 weeks and was still maintained at a level of 94.3% after storing for 4 weeks. This suggested that the fabricated PEC sensor can keep its stability for an even longer period of time. In addition, a relative standard deviation (RSD) of 5.2% was estimated from the calibration plots of seven freshly fabricated PEC sensors, indicating that the as-designed PEC sensors can be repeatedly produced. With the advantages of high efficiency, easy manufacturing, superior selectivity, sensitivity, and repeatability, the biosensor had a broad application prospect in the actual biological environment for P4 monitoring.

Bismuth film, as a new metal material, was widely used. The main reasons were as follows: (1) low electrode cost; (2) high mechanical stability; (3) deposited on various substrates; and (4) used as a new mercury-free electrode material due to the similar physical and chemical properties of bismuth with mercury. Compared with other bismuth-based electrodes and unmodified glassy carbon electrodes, Zidaric et al. [29] developed a nanostructured bismuth film electrode (nsBiFE), which showed superior electrical analysis performance, even for the low level of P4 detection. The specific focus is that it can work in media where the pH was close to the real physiological environment. Most of the electrochemical sensors for P4 were studied in alkaline solutions using metal electrodes and nonmetal electrodes, some of them even in the presence of CTAB to facilitate P4 reduction and its further dimerization. All of these sensors exhibited linear responses in similar or in higher concentration ranges in comparison with the nsBiFE sensor. In addition, the nsBiFE sensor operates in a neutral medium. These characteristics provided the biosensor with a potential future applicability as a simple, rapid, and inexpensive portable monitoring or diagnostic tool.

De Lima and Spinelli [36] developed an out-of-situ bismuth film electrode for the determination of P4 in four drug samples, and the results showed a good agreement with spectrophotometry. During the range of 0.40-7.90 mol/L, a peak current of -1.63 V was generated as the concentration of P4 increased. The detection limit was 0.18 mol/L. In addition to bismuth film, Mn (III)-SB complex membrane as a new metal material was also investigated by Shamsipur et al. [37]. As a new nonimmune sensing method, the electrocatalytic oxidation of P4 in alkaline medium was developed. Manganese chromium complex membrane being used as the electronic medium to assist the catalytic reaction

could effectively avoid the synergistic reduction of unknown interfering species in complex matrix. By comparing GCE and GCE\AuNP for Mn(III)-SB electro-polymerization, it was found that the probability of landing on the GCE surface passing through the energy consumption process was lower than that on the C\Au surface; meanwhile, the probability of Mn(III)-SB deposition on the GCE surface was lower than that on the GCE\AuNPs surface. Therefore, GCE\AuNPs were selected as the functional material. The reproducibility of response toward P4, between three different constructed modified electrodes with same procedure, was studied, and the results showed an RSD of 5.8%. The proposed electrode was stored in ambient condition in closed container for about 3 months and found to retain about 91% of its initial response, indicating it exhibited a good stability. Furthermore, compared with electrochemical immunosensor, the proposed biosensor had significant advantages with simple structure and economy and perfect sensitivity, selectivity, and repeatability. The linear range of this biosensor was 35-800 μmol/L, and the detection limit was 11.4 nM.

2.2. P4 Biosensors Based on Composite Material. In order to optimize the performance of P4 biosensor, carbonaceous nanomaterials, metal nanomaterials, polymer, and other functional materials were combined to construct biosensors. Normally, it had the characteristics of strong signal, economy, high efficiency, high selectivity, simplicity, and so on. From the perspective of biosensor types (electrochemical biosensor and optical biosensor), P4 biosensor modified by composite materials was summarized as follows.

2.2.1. Electrochemical Biosensor Based on Composite Material

(1) Composite of Carbonaceous Nanomaterials and Metal Nanomaterials. On the basis of excellent properties such as simple operation and structure, high efficiency, and wide application fields, semiconductor-carbon heterostructure nanocomposites have attracted wide attention, especially in the preparation of biosensors [38]. The combination of carbonaceous nanomaterials with metal oxide semiconductor nanostructures was beneficial for electrocatalytic applications for providing novel nanocomposites with synergistic effects. In metal oxide nanostructures, metal oxide nanofibers possessed many superiorities, such as flexible surface function, superior mechanical properties, rich response sites, and high porosity. Compared with other geometric nanomaterials, it had a larger specific surface area and stronger efficient catalytic capacity. Samie and Arvand [39] have manufactured GQDs-NiO-AuNF nanocomposites by applying electrostatic spinning technology to synthesize NiO-Au hybrid nanofibers. In the semiconductor transition metal-oxide, semiconductor had a broad application prospect in electrochemical sensors and biosensors because of its large specific surface area, promising biocompatibility, nontoxicity, and no damage. Moreover, compared with single metal oxides, nanocomposites doped with noble metals, such as NiO-Au hybrid nanofibers, showed superior electrocatalytic activity. Its performance range exhibited 0.01-1000 nM, and the detection limit reached 1.68 pM. Trioxide transition metal oxides (TMOs) have attracted extensive attention owing to its excellent physical and chemical properties, in which tungsten trioxide nanoparticles are well known for its electronic and electrocatalytic properties as well as high performance and efficient approaches in energy and sensing applications. With high conductivity and large specific surface area, graphene (GR) could improve the electrocatalytic ability of nanocomposites. Govindasamy et al. [26] prepared an electrochemical nonenzymatic biosensor based on multilayer graphene-covered tungsten trioxide nanospheres (WO_3NBs@GR). The detection linear range of this biosensor was 0.025-1792.5 μM, and the detection limit was 4.28 nM. Compared with the pure electrode and control electrode, the modified nanocomposite electrode showed a clear electrooxidation peak, fully verifying its outstanding electrocatalytic ability and performance. Carbon quantum dots had good performance in chemical sensing, biomedical imaging, electrochemistry, photovoltaics, light-emitting diodes, and nanotechnology. Along with their optical features, carbon dots (CDs) had desired properties such as less toxicity, environmentally friendly nature, inexpensive, and simple preparation processes [40]. Zhu et al. [27] applied carbon dots and graphene oxide (CDs-GO) composites as photoactive materials with combing Au-CuO-Cu_2O synergistically modified electrodes. After covering CDs-GO on the electrode surface, the biosensor was composed of antibody, P4, and aptamer-Au-CuO-Cu_2O coupling. The CDs-GO composites showed considerable cathodic photocurrent response while the aptamer-Au-CuO-Cu_2O bioconjugate combined the advantages of photoactive CuO-Cu_2O heterojunction, plasmonic Au nanoparticles, and high-affinity aptamer. With the incubation of P4 as connection center, enhanced cathodic photocurrent response was obtained. In addition, the reproducibility of this biosensor was assessed by checking the cathodic PEC responses of five independently prepared aptamer-Au-CuO-Cu_2O/P4/Ab/CDs-GO/GCE incubated with 100 nM P4. The RSD was obtained to be 2.6%, indicating a perfect reproducibility of this biosensor. In addition, the photocurrent response of the modified electrodes stored in a refrigerator at 4°C was measured every three days. The results showed that the PEC sensor still maintains at least 94.2% of its initial response after fifteen days of storage, demonstrating satisfactory storage stability of the proposed PEC sensor. Considering the high selectivity, good reproducibility, and good stability, the proposed cathode PEC biosensor has been successfully applied to the detection of P4 in human serum samples. Under the optimal conditions, the detection linear range was 0-180 nM, and the detection limit was 0.17 nM.

(2) Composites of Carbonaceous Nanomaterials and Polymers. Graphene quantum dots (GQDs) had the advantages of low toxicity, easy preparation, high chemical stability, environmental friendliness, high solubility in a variety of solvents, and edge functionalization, which showed a broad application prospect in biosensors, biological analysis, optical dyes, biomedicine, and other fields. In addition, by expanding the contacting area with analytes, GQDs

increased the electrochemical effective surface area with some electroactive analytes. Considering its easy preparation, good thermal stability, low raw material cost, ambient operating conditions, and good biocompatibility, poly(sulfosalicylic acid) (PSSA) is an ideal material for improving the sensitivity of chemical/biosensor materials. The superior performance of GQDs and the advantages of PSSA have attracted more and more attention in the preparation of CP/QDs hybrid materials with high performance. Arvand and Hemmati [41] fixed GQDs doped with PSSA on glassy carbon electrode (GCE) to form GQDS-PSSA composites. The synergistic contribution of PSSA and GQDs made this novel material a candidate for the preparation of high-performance modified electrode materials for trace biological compounds. The GQDs-PSSA/GO nanocomposite/GCE exhibited higher electrocatalytic activity toward oxidation of P4 and higher oxidation peak current attributable to superior conductivity and better site-accessible surface for fast recognition. Therefore, the synergistic contribution of PSSA and GQDs makes a new electrode material which shows precise responses, excellent reproducibility, and long-term stability, in addition to providing good recovery for P4 in human serum samples and pharmaceutical formulation. In addition, due to the stable dispersion of GQDs in polymer layer and improved electrosynthesis of nanocomposite, the reproducibility expressed in RSD obtained 2.68% for P4. Furthermore, to characterize the repeatability of the current response of the GQDs-PSSA/GO/GCE, seven successive determinations of solutions containing 1.0 μmol/L of P4 were made in a day. The result of seven repetitive measurements with the same modified electrode displayed that the anodic peak currents were nearly constant with small RSD values of 1.48% for P4, which revealed a good repeatability. Accompanied by simple and environmentally friendly electrochemical construction strategy, this biosensor opened an economical and effective way to construct nanocomposites and broadened the application of electrochemical biosensors in the green field.

(3) Composites of Carbonaceous Nanomaterials, Metal Nanomaterials, and Polymers. Wang [42] combined a screen-printed Au electrode and a special material of staphylococcal protein A (SPA) to modify electrode as well as simultaneously bind antibodies. SPA was a cell wall protein being isolated from *Staphylococcus aureus* type A, which had six different immunoglobulin binding sites. The Fc segments of immunoglobulin G (IgG) was specifically bonded by five sites, which made the Fab segments (the active center of bonding antibodies and antigenic determinant) expose in the outer layer of the modified membrane and extend to the mobile phase, and thus avoid affecting the reaction of antibody and antigen. Meanwhile, SPA and Au can form a stable SPA-Au complex with the affinity index up to 10^8 L/mol. Furthermore, SPA fixing antibody could bond more antigens with the advantage of geometric orientation; thus, it was often used to fix antibodies on Au electrode. The antibodies being fixed by SPA could ensure the homogeneity and the repeatability of conjugation of the molecular membrane fixed by antibodies, which was recognized as a preferable method of antibody fixation. The electrochemical activity of the biosensor can be improved by the combination of the two electrodes, and the linear range was 0-0.1592 μmol/L. Dong et al. [28] developed an immunosensor based on thionine-graphene oxide (Thi-GO) platform and biotin-avidin system (BAS) (Figure 2) to detect P4 sensitively and selectively with a linear range of 0.02-20 ng/mL. Thi-GO composites with excellent biocompatibility were synthesized and coated to a clear glassy carbon electrode. P4 coating antigen (P4-OVA) was immobilized to the electrode, and then, a sample as well as biotinylated antibody (biotin-P4 Ab) was added. The free P4 can compete with P4-OVA for binding to biotin-P4 Ab. After the further addition of streptavidin-HRP, H_2O_2 was introduced to develop electrical signal for quantitative determination of P4. Among them, Thi-GO composite material and BAS could effectively amplify the signal, which was mainly due to the thionine with positive charged being absorbed onto the surface of GO through electrostatic interaction, and also the thionine coupling with GO by π-π reaction. In addition, the Thi-GO composite exhibited enhanced biosensing properties. Gevaerd et al. [31] developed an imidazole-functionalized graphene oxide (GO-IMZ) as an artificial enzyme active site for the determination of P4. The composite material was immobilized on the surface of GCE to simulate the active site of the enzyme, and the synergistic effect between IMZ and GO reduced the hormone directly. Under optimized experimental conditions, the linear range reached 0.22-14.0 μmol/L, and the detection limit was 64 nmol/L.

As shown in Figure 3, Xu et al. [30] discovered CYP_3A_4 enzyme, which exhibited a natural enzyme substitute for P450. By immobilizing enzyme in nanoparticle graphene foam (PNGF) modified by polydopamine (PDA), it can construct a simple electrochemical enzyme catalysis nanoreactor (CYP_3A_4/PNGFs). Using the unique three-dimensional structure of NGFs and the cross-linking agent PDA, CYP_3A_4 had high stability and biological activity and easily being absorbed by PNGFs. Furthermore, with the changing of the pore size of PNGFs, the enzyme activity of CYP_3A_4 could be regulated effectively. The advantage of CYP_3A_4 was that the pore size was confined in about 65 nM, which was closer to the size of the enzyme. Thus, it showed a higher enzymatic reaction rate and affinity for P4 substrates. This method had potential application value in the rapid detection of steroid hormone metabolism, drug development, and toxicity screening.

(4) Optical Biosensor Based on Composite Material. In the detection of P4, optical biosensors also used composite materials to improve detection efficiency. Tan et al. [43] have synthesized P4 molecular imprinted film (MIF) by field grafting method and then was monitored after being loaded into SPR biosensor. Considering there are many nanosized holes for the adsorption of P4 on the surface of MIF, it can be found a linear relationship between the increase of light intensity and log value of P4 concentration by using SPR biosensor for adsorption monitoring. The P4 molecularly imprinted film synthesized by this biosensor could not be

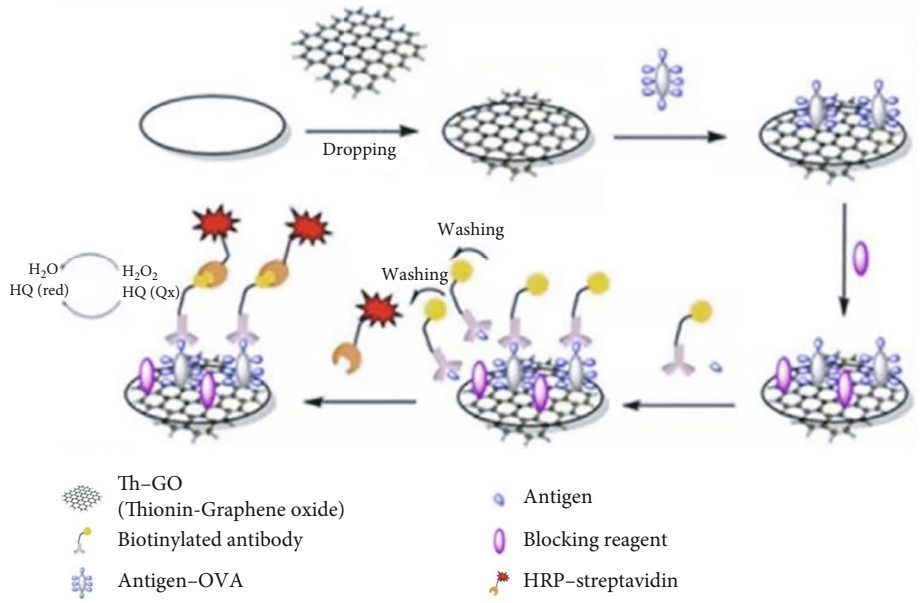

Figure 2: Schematic diagram of structured immunosensor [28]. Images reproduced with permission.

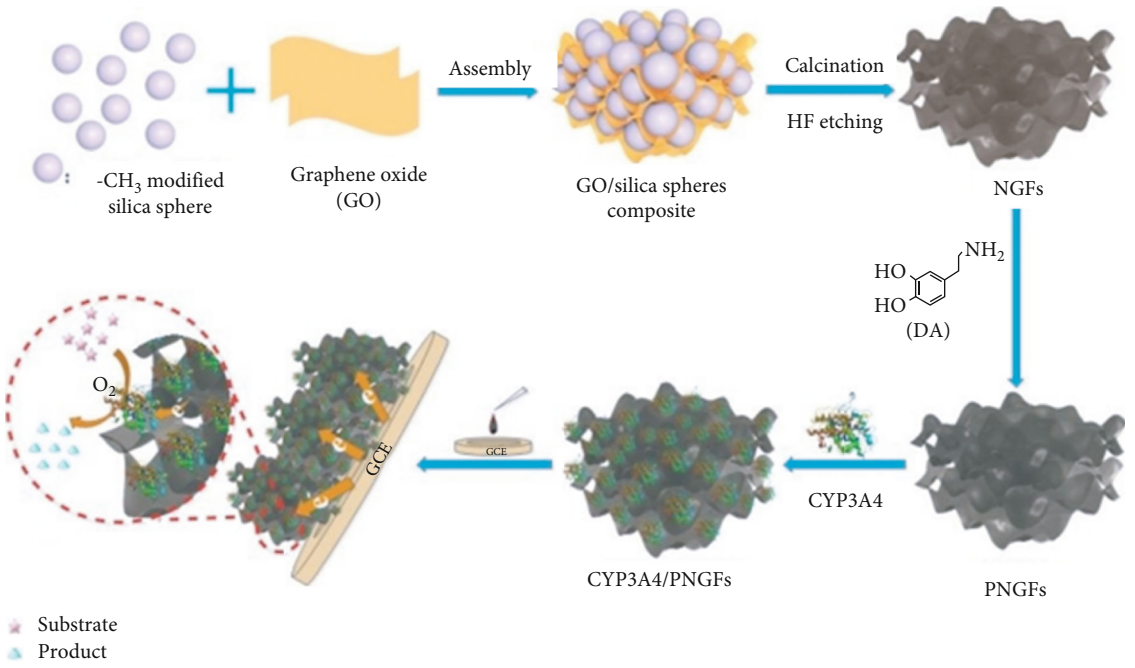

Figure 3: The synthesis of PNGFs and the construction of CYP_3A_4/PNGF nanoreactors [30]. Images reproduced with permission.

disturbed by other analogues and recognize P4 selectively with favorable reusability and stability. The linear range of the biosensor was $1.0 - 1.0 \times 10^3$ pmol/L, and the detection limit was 0.3 pmol/L. Kang et al. [44] reported the first efficient near-infrared to near-infrared (NIR-to-NIR) signal based on a luminescence resonance energy transfer (LRET) system for the detection of P4, chosen as a proof-of-concept target, through homogeneous competitive immunoassay (Figure 4). Upon the NIR irradiation, upconversion nanoparticles (UCNPs) can emit near-infrared (NIR), and the strong penetration and low self-fluorescence in the NIR region, called as the diagnostic window, enhanced the signal-to-noise ratio. In order to enhance the efficiency of LRET, they constructed NIR-to-NIR emitting insert-core/active-shell/insert-ultrathin shell UCNPs ($NaYF_4$@$NaYF_4$:Yb,Tm@$NaYF_4$) to act as an LRET donor and compact progesterone/horseradish peroxidase/IRdysQC-1 (P-HRP-dyes) as an LRET acceptor. Using the developed NIR-to-

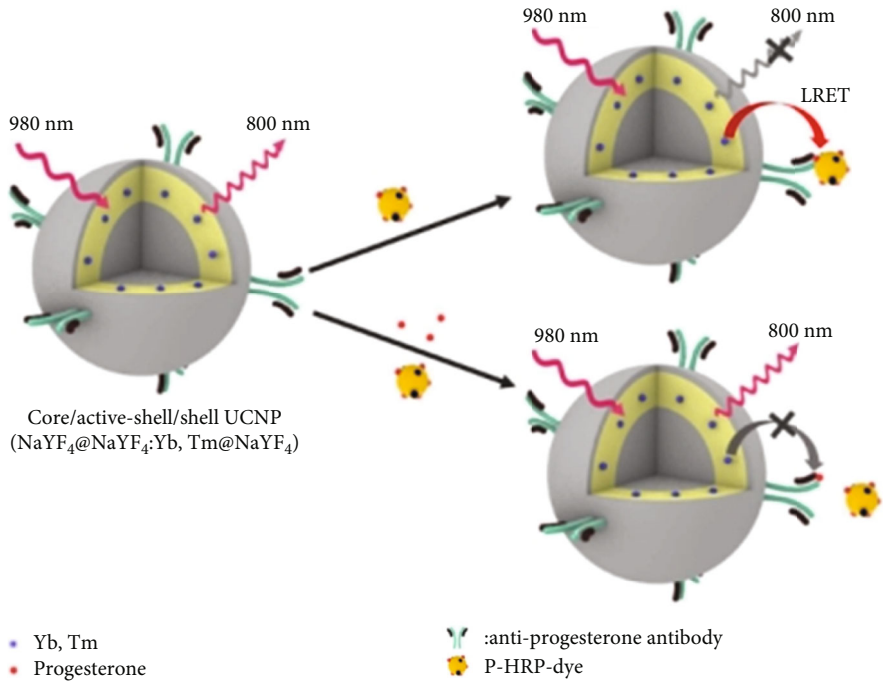

Figure 4: Schematic illustration of NIR-to-NIR signal-based LRET system for detection of progesterone via competitive immunoassay. Inert-core/active-shell/inert-ultrathin-shell lanthanide-based upconverting nanoparticle donors achieved greatly enhanced LRET efficiencies when combined with a compact progesterone/horseradish peroxidase/IRdyeQC-1 complex acceptor (P-HRP-dye), compared with conventional donors and acceptors [44]. Images reproduced with permission.

NIR LRET system, P4 was successfully detected with a background-free signal and low detection limit of 1.36 pg/mL in ten-fold-diluted human serum. The biosensor has the potential to serve as a simple probe for homogenous competitive immunoassay with the ability to rapidly detect biomarkers. Grazon et al. [45] developed a P4 optical biosensor by using the quantum dot-transcription factor-Förster Resonance Energy Transfer (QD-TF-FRET) framework based on SRTF1 (a protein of a transcription factor). After the QDS were coated with amphoteric ions, the transcription factors were autonomously loaded onto the surfaces with containing SRTF1 as FRET receptor markers and bound to the QD-transcription factors in a specified DNA/quantum dot ratio. The P4 concentration was then measured using a tablet card reader. Compared with the current assays based on antibody, the approach to identify and isolate bacterial allosteric transcription factors (aTFs) exist two key advantages. First, the inherent transduction mechanism of aTFs made real-time repeated sensing possible, which convert this into a direct electrical reading. Second, the cost to produce, modify, and evolve of bacterial proteins was low. Tan et al. [46] constructed a portable hormone biosensor, based on duplex molecular recognition coupled with a signal-amplified substrate, for the simultaneous visualization and quantitation of multiple steroid hormones. Based on the different luminescence emissions and specific recognition ability of aptamer-functionalized UCNPs, high selective simultaneous quantification of multiple hormones was realized. Due to a spatially ordered material with excellent light manipulation and obvious enhancement effect, photonic crystal (PC) substrate was used as optical signal amplifier to detect multiple hormones in real time. Here, the researchers immobilized aptamer-functionalized UCNP as the nanoprobes on PC substrates. When the excitation light was displayed on the surface of the PC substrate, the energy can be stored near the surface of the PC substrate by forming electromagnetic field waves, which could provide stronger electric field intensity than the illumination light source. Therefore, the absorption of luminescent materials on PC surface was enhanced, and the luminescent emission was enhanced. These results indicated that the PC dot array could obviously enhance the luminescence intensity of UCNPs. Therefore, the substrate with PC lattice can be used as a promising tool for amplifying luminescence signal and improving detection sensitivity.

2.3. P4 Biosensor Based on Other Materials. In recent years, to better optimize the performance of P4 biosensor, besides metal-oxide nanomaterials and composite materials, researchers have developed a variety of other modification materials. P4 biosensors based on other material modifications were summarized as below.

2.3.1. Special Compounds. Hong et al. [47] have found one chemical biosensor which could recognize P4 by changing the self-aggregation state and display a significant fluorescence state. By screening a series of metabolites in water buffers, they found two compounds (PG-1 and PG-2) with

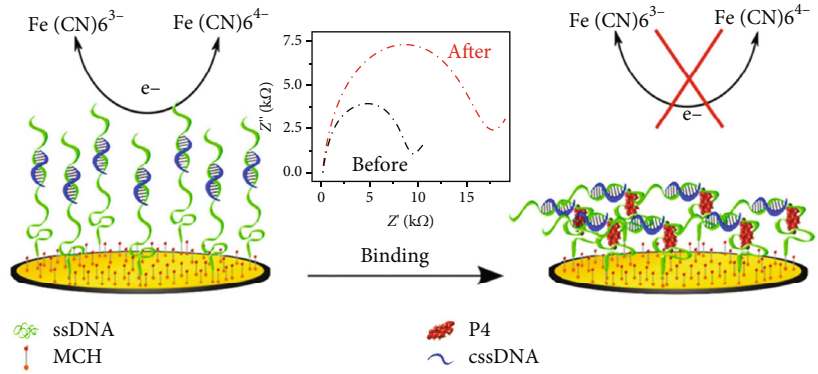

Figure 5: Impedimetric mechanism of the aptasensor [5]. Images reproduced with permission.

specific selectivity for P4. By using dynamic light scattering and transmission electron microscopy to investigate the aggregation properties of the probe, it was found that these two compounds could recognize P4 and produced large aggregates to induce fluorescence enhancement. Dynamic light scattering and transmission electron microscope data supported P4 selective bulk aggregate formation, which was over hundred nanometer size, from self-aggregates of the probe that was approximately tens of nanometer size. Such sensing mode based on the aggregation of fluorophores has not appeared in the detection direction of P4. The characteristics of the biosensor were simple and rapid detection, no need for special equipment and high cost.

2.3.2. Single Biomaterials. Jiménez et al. [5] developed a label-free adaptive sensor (Figure 5). A certain amount of DNA aptamers with high affinity for P4 were selected and identified, in which aptamer P4G13 showed excellent specificity for P4. The aptamers were selected in vitro from an ssDNA library of 1.8×10^{15} oligonucleotides showing a dissociation constant (K_D) in the low nanomolar range. An aptamer-complementary DNA (cDNA) oligonucleotides were tested to maximize the signal gain of the aptasensor after binding with P4. Moreover, the aptamer P3G13 did not show cross-reactivity to analogues similar to P4 such as 17b-estradiol and norethisterone. When the aptamer was hybridized with a short complementary sequence at specific site instead of the pure aptamer, the signal enhance was more significant. Based on the conformational change of P4G13 aptamer, an impedance aptamer sensor for P4 was prepared by self-assembly fixed on gold electrode and combined with P4 to increase electron transfer resistance. The developed aptasensor exhibited a linear range for the concentrations of P4 from 10 ng/mL to 60 ng/mL with a detection limit of 0.90 ng/mL. Moreover, the aptasensor was applied in spiked tap water samples showing perfect recovery percentages.

2.3.3. Luciferin and Biomaterials. A study mentioned that fluorescence-based adaptor biosensors detected P4 through structural transformation mechanism [48]. The biosensor consisted of fluorescein, dabcyl-labeled complementary oligonucleotides, and unlabeled DNA nucleic acid ligands to form a tripartite fluorescence quenching dsDNA duplex. dsDNA duplex underwent conformational changes in the aptamer in the presence of the target molecule, in which fluorescein-labeled DNA and quenching-labeled DNA were released to enhance fluorescence. This novel fluorescent biosensor based on aptamer had high selectivity and good recovery. The detection linear range was 10-100 ng/mL, and the detection limit was 110 pg/mL.

2.3.4. Quantum Dots and Biomaterials. Nanoenhancers (near-infrared quantum dots) could produce energy coupling with oscillating surface plasmas on metal surfaces, Zeidan et al. [49] used it to optimize surface plasmon resonance imaging (SPRi) biosensors for ultrasensitive detection of P4. Nanoenhancers and X-aptamers played a key role in signal amplification. X-aptamer was a small single-stranded DNA molecule containing nucleotides and other micromolecules attached with amino acid functional groups. These chemical modifications made the X-aptamer have great chemical diversity and specificity when binding to its target. In small molecule sensing, accurate recognition and detection of an analyte in near-infrared spectroscopy required highly sensitive capture ligands which allowed efficient binding of analytes in solution to maximize platform signal subpoints. In addition, distinguishing these structurally similar biomolecules that existed in the same complex samples by capturing ligands was required. Therefore, combining the nanoenhanced biosensing function of SPRi with the introduced P4 X-aptamer possessed many advantages compared with the current method of using SPRi and non-SPR immunosensor detection. The biosensor had a positive superiority in the detection sensitivity by the high binding property of X-aptamer and the enhancement of signal by NIR quantum dots. The detection limit was 1.575 ng/mL (5 nM).

3. Discussion

Among the P4 detections in milk samples, the functional material of Fe_3O_4@SiO_2@TiO_2 with an electrochemical

Table 2: Performance comparison of progesterone biosensor with composite material.

Type of biosensor	Functional material	Samples	Linear rage (μmol/L)	LOD (nM)	References
Electrochemical biosensors based on carbonaceous nanomaterials and metal nanomaterials					
Electrochemical biosensors based on aptamer	GQDs-NiO-AuNFs/f-MWCNTs	Human serum samples and drug formulations	0.00001-1	0.00186	[39]
	Au-CuO-Cu_2O	Human serum	0.0005-0.180	0.17	[27]
Electrochemical biosensors based on nonenzymatic	WO_3NBs@GR	Blood	0.025-1792.5	4.28	[26]
Electrochemical sensor based on carbonaceous nanomaterial and polymer modification					
Electrochemical sensor	GQDs-PSSA	Human serum	0.001-6.0	0.31	[41]
Electrochemical sensors based on composite modification of biomaterials with carbon nanomaterials, metal nanomaterials, or polymers					
Electrochemical biosensors based on immune	Thi-GO	Milk	0.00006369-0.06369	—	[28]
Electrochemical biosensors based on nonenzymatic	GO-IMZ	Drug samples	0.22-14.0	64	[30]
Electrochemical biosensors based on enzyme	CYP_3A_4/PNGFs	Laboratory control standard	—	—	[31]
Surface plasmon resonance imaging optical biosensor	Thionine-graphene oxide (Thi-GO) and biotin-avidin system (BAS)	—	—	—	[52]
Optical biosensor based on composite material modification					
Light-emitting resonance energy transfer (LRET) optical signal sensor	$NaYF_4$@$NaYF_4$:Yb, tm@$NaYF_4$	Human serum	—	0.004331	[44]
Optical biosensor (quantum dot Forster resonance energy transfer (FRET) method)	QD-TF-FRET	Artificial urine	—	—	[45]
Fluorescent biosensor	Photonic crystal (PC) and UCNPs	Serum	—	—	[43]
Others					
Electrochemical biosensors based on nonimmune	GCE/AuNPs	Milk	35-800	11.4	[37]
Electrochemical biosensors based on immune	MGO	Progesterone standard sample	0.00000001-1	DPV: 0.00015; CV: 0.00017	[4]

Note that LOD represents the limit of detection.

Table 3: Performance comparison of progesterone biosensor with other materials.

Type of biosensor	Functional material	Samples	Linear range (μmol/L)	LOD (nM)	References
Special compounds					
Fluorescent chemical sensor	PG-1, PG-2	Blood and amniotic fluid samples from pregnant women	—	PG-1 (1210) PG-2 (38.1)	[47]
Biomaterial					
Fluorescent biosensor	CFP, GFP, and DsRed	Monkey serum	—	(EC50) 0.4809	[33]
Luciferin and biomaterials					
Fluorescent biosensor	Fluorescein and Dabcyl	Tap water, urine	0.03185-0.3185	0.3503	[48]
Quantum dots and biomaterials					
Surface plasmon resonance imaging (SPRi) optical biosensor	NIR-streptavidin-coated quantum dots	P4 sample	0.005016-126000	5	[49]

Note that LOD represents the limit of detection.

aptamer sensor showed an excellent linear range and detection limit (linear range 0.000001-0.006 μmol/L, LOD 0.0003 nM) (Tables 1–3). Furthermore, its selectivity was also superior. The maximum interference was no more than 6.8% even when norethisterone at 200 times the concentration of the target progesterone (10 pmol/L), 17β-estradiol (500 times), cortisol (500 times), estrone (500 times), quinolones (500 times), estriol (500 times), and a mixture of these species (each 100 times) was added [35]. In addition, the functional material Thi-GO in the electrochemical immunosensor also showed a good detection range and good selectivity. Among all the analogues, the cross-reactivity was lower than 0.1%, suggesting that these analogues had no capability for the detection of P4 in the real world [28]. Many studies on electrochemical sensors also used AuNPs combining a variety of decoration materials as a functional material for P4 detection to enhance signal and linear range. Compared with electrochemical sensor, the performance of the linear range and the LOD applied to the optical sensor was weak in these two aspects. Moreover, the relevant research on the selectivity of sensors was less.

Among the studies on applying AuNPs with the functional material of fluorescein and dabcyl in an electrochemical sensor on P4 detection in tap water, the linear range and detection limit of AuNPs showed no significant difference in the performance of the electrochemical sensors composed of the two types of functional materials; however, the analysis of the linear range and detection limit of AuNPs alone showed better performance linear ranges (0.00089-0.5 μmol/L) and lower detection limits (0.002866 nM) (Table 1). However, if immunoassay was used, the repeatability and specificity of biosensor were poor, but it would obtain a good selectivity and could be easily separated from potential disruptors (E2, E3, DES, AMP, THI, AZT, and BPA).

4. Conclusions

This paper reviews the advances in biosensors for P4 detection, including electrochemical biosensors and optical biosensors. The various nanomaterials were commonly applied to improve the performance of the biosensor for P4 detection. In this study, we classified the nanomaterials for P4 detection, including metal nanomaterial and the metal-oxide materials, composite materials (the combination of two or three types of materials, including carbonaceous nanomaterials, metal nanomaterials, polymers, and biological materials), and the other materials (special compounds, biomaterials, luciferin, and quantum dots, of which one or two). With strong electrical conductivity, larger surface, and satisfactory compatibility, the metals and their oxide nanomaterial have a relatively mature technical basis in the field of biosensors. Composite material, as a kind of electrode modification material with the characteristics of strong signal, economy, high efficiency, high selectivity, and simplicity in recent years, has attracted the attention of researchers and application field. Other modified electrode materials have the advantages of visualization, multidetection, anti-interference, and easy recovery. In addition, we found that conductive polymer materials have a superior signal amplification effect during the preparation of electrochemical biosensors; however, it has not been used in the detection of P4. Future research on improving the performance of P4 biosensor should put more attention on enzyme catalysis amplification, cyclic amplification, and DNA isothermal amplification strategies.

Data Availability

The dataset is available upon request.

Disclosure

This manuscript "Design and Development of Biosensors for Progesterone Detection" has not been published or presented elsewhere in part or entirety and is not under consideration by another journal. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these.

Conflicts of Interest

We declare that we have no commercial or associative interest that represents a conflict of interest in connection with this work submitted.

Authors' Contributions

Wenwen Sun carried out the methodology, formal analysis, writing—review and editing; Qinchen Xu carried out the investigation and writing—original draft; Jiwei Sun carried out the investigation and writing—original draft; Xiangde Lin carried out the writing—review and editing; Hongzhi Pan carried out the writing—review and editing, and funding acquisition.

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