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Analytical Platforms for Medical Diagnosis: A Study on the Performance and Recent Trends on Aptamer and Antibody Based Biosensors

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ABSTRACT

Antibodies and aptamers play a crucial role to improve the analytical performance of biosensors for medical applications. Besides their natural compatibility with many antigens and pathogens, their biochemical structure selectively binds the analyte providing high sensitivity and selectivity. Accordingly, this minireview presents the recent approaches in the field of immunosensors and aptasensors for clinical diagnoses with a focus on the key features of sensors over the conventional diagnostic assays and the trends in this field of knowledge (point-of-care devices for *in situ* applications, label-free bioreceptors, real-time monitoring of analytes and outstanding transduction techniques).

Abbreviations: SELEX: Systematic Evolution of Ligands by Exponential Enrichment; AG: Any Protein; FAB: Fragment Antigen Binding; LOD: Limit of Detection; PCR: Polymerase Chain Reaction; ELISA: Enzyme-Linked Immunoassays; POC: Point-of-Care; HER2: Human Epidermal Growth Factor Receptor 2; SPEs: Surface of Screen-Printed Electrodes; MIP: Molecular Imprinting Polymers; EIS: Electrochemical Impedance Spectroscopy; CV: Cyclic Voltammetry; PSA: Prostate Specific Antigen

Introduction

Advances in the field of molecular biology and chemistry have driven the studies in biosensing to an important and necessary level. The increasing attention of the population to healthcare summed to the alterations in their alimentary and social habits significantly changed the needs for personal health. Miotto, et al. [1] mentioned that the current context of healthcare demands to "ensure that the right treatment is delivered to the right patient at the right time". In this scenario, the study of biosensors has provided sufficient tools, especially in the last decade, to advise the science of sensitive, rapid and accurate medical diagnostics. Clark and Lyons [2] were the pioneer in the field with the development of an enzymatic biosensor for detection of glucose. Their technology based on the oxidation of glucose by the enzyme glucose oxidase produced gluconic acid, hydrogen peroxide and electrons. This technology inspired unlimited researches up to nowadays and the more known commercial devices are still based on biosensing of glucose (being the first commercially available biosensor for glucose fabricated by the company Yellow Spring Instruments) [3].

Once biological molecules are irreplaceable agents in living beings to make humans and animals to perfectly function, not surprisingly, scientists and research companies devote maximum efforts to mimic the biochemical reactions that naturally occur in the nature. This is the basis of a biosensor. A biological element of recognition is attached to the surface of an electrode material to detect a target molecule by means of their specific sites. Changings in chemical and/or physical properties of the transducer system are thus monitored and associated to the presence or to the concentration of the molecule of interest. Regardless the numerous possibilities of substrate materials, transduction modes and kind of molecules of interest, possibly the study of bioreceptors is represents the golden effort to achieve the two most important characteristics of a tool for diagnosis: sensitivity and selectivity. In light of this context, this work proposes a critical review of the literature on biosensing technologies for medical diagnosis with respect to two of the most important bioreceptors employed in high-performance sensors: antibodies and aptamers. A discussion on the global features of biosensors, their importance and application in medical diagnoses, key aspects of antibodies and aptamers to be employed as bioreceptors are provided herein. This knowledge is illustrated with the most recent trends in current works available in the specialized literature in order to contribute to the field of biosensors and clinical bioassays.

Biosensors and Units of Biorecognition

Sensors are part of our daily lives, inserted in the most diverse equipment's with the most different functionalities. In general, a sensor is a device that transforms a certain physical or chemical property into an analytically measurable signal. In this way we can classify sensors where the variation of a biochemical property generates any signal, these devices we call biosensors, which can be defined according to IUPAC as being "device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" [4] A biosensor consists of two parts, one formed by the biological recognition element (receiver) and the other by the transducer, which can be electrochemical, optical, thermal, piezoelectric, capacitive and field effect. We can classify them, by the different methods of transduction, as well as according to the element's receptor. Here, we will classify them only this. Bioreceptors can be selective or not, but recognition element plays a crucial role in the overall biosensor performance and selectivity toward a particular analyte [5]. Temperature, pH, contaminants, ionic strength, type of solution (buffer solution, body fluids, water) are factors that determine the performance of the biosensors [6,7].

Aptamers / Aptasensors

Aptamers are short and single-stranded nucleic acids (DNA or RNA) with capacity to bind to target molecules with high affinity and specificity [8]. First introduced in 1990, the process of selecting an aptamer is called Systematic Evolution of Ligands by Exponential enrichment (SELEX), from a large oligonucleotide library [9,10]. Aptamers can be selected for a variety of targets, including small molecules, proteins, nucleic acids, microorganisms, cells, tissues, metal ions and chemical compounds [11-13]. With the advantages of small size, high binding affinity, good stability and easy synthesis, aptamers show potential for various applications, such as targeted therapy, detection and clinical diagnoses [14-17]. After selection and characterization, aptamers can be customized for developing sensors [18]. A large variety of aptamer-based biosensors (aptasensors) with various detection strategies have been developed and reported in the literature [19]. In comparison to antibodies, aptamers are smaller units containing oligonucleotides with sizes over 30 oligos [20].

They are similar to monoclonal antibodies in terms of binding affinities, being called synthetic antibodies [21] in addition to other advantages, such as chemical stability and regeneration of its three-dimensional structure even after several cycles of denaturation/renaturation [22]. Its small size allows a greater density of immobilized molecules. They are chemically synthesized, which allows the flexibility of the conformation of their two-dimensional structure, so it can be built for the detection of any antigen, from small molecules, heavy metals, protein, enzymes, microorganisms and cells, with the possibility of adjusting the sensitivity and selectivity [23-28].

Antibodies / Immunosensors

Antibodies (Abs) are proteins that can be employed as valuable tools in laboratory and clinics [29]. Antibodies include those secreted by a single clone of B lymphocytes, termed monoclonal antibodies (mAbs), and those produced by a mixture of various B lymphocyte clones, the polyclonal antibodies (pAbs) [30-32]. In 1975, Kohler and Milstein developed a system for the production of monoclonal antibodies. Abs demonstrate high affinity and specificity to target molecules and have been frequently selected for a wide variety of applications including immunodiagnoses, biomarker detection, immunological research and vaccine quality control [33-35]. Abs can be used to develop a variety of sensors (immunosensors) upon the formation of an antibody-antigen complex [36]. Immunosensors are based on antigen-antibody affinity, where an immunochemical reaction forms a very stable complex. Every protein has an isoelectric point (point where the

global electrical charge is equal to zero) that varies according to the composition of the amino acids, thus determining the magnitude and polarity of that point at a specific pH [37].

One can assume that any protein (Ag), with charge Ch1, and its antibody pair with charge Ch2, the reaction of that system (AgAb) results in a global charge Ch3 which can be described by the following equation:

$$Ag^{Ch1} + Ab^{Ch2} \xleftrightarrow{K} [AgAb]^{Ch3}$$

where K is the binding constant for this complex. This change in electrical charges can ideally be detected, depending on the antigen concentration and the transduction technique used. The ambivalence of this system still allows the use of a biosensor for the detection of an antigen, regarding the possibility of immobilizing an antigen and the antibody becomes the analyte. Abs possess a "Y" shaped structure consisting of two heavy and two light polypeptidic chains bound by S-S bonds with approximately 150 kDa and dimensions of 14 nm x 10 nm x 4 nm [38,39]. The base of this "Y" structure is called fragment crystallizable region (Fc) and is composed by the heavy chains. On the other two extremities, there are the antigen-binding sites, or epitopes, comprising the fragment antigen binding (Fab). The Fab branches exhibit different characteristics (such as the chemical composition, the physical structure and the isoelectric point) as a natural consequence of their properties to bind different analytes [39,40]. At the same time it is advantageous to orientate the immobilization of Abs by the Fc portion because it keep frees the active specific sites (Fab) to bind analytes, the extra protocol to allow this orientation makes the fabrication of oriented antibodies-based sensors more laborious and frequently more expensive.

Key Features on the Performance of Biosensors

The most important characteristics of a biosensor are its selectivity, reproducibility, stability, sensitivity and linearity. The combination of these parameters has been the focus of many researchers specially in the last decade to develop high performance devices for diagnosing molecules of medical interests. These features can be defined as follows:

a. Selectivity: represents the ability of a sensor to present an analytical signal exclusively due to the recognition of the target analyte, not suffering the influence of interfering species at a significant level. Morales and Halpern [41] mention that selectivity is essential in the development of point-of-care biosensors. This is because the testing biological samples are typically very complex and can possess various interfering molecules capable to compete for the bioreceptor sites of the sensor;

b. Limit of Detection (LOD): is the minimum amount of analyte able to generate an output signal distinguishable from the blank signal (analyte absence) [42]. Depending on the level of affinity between the biorecognition element and the analyte, the biosensor can achieve low LODs and meets a broader window of applications in the field of clinical diagnosis. This affinity is expressed in terms of the dissociation constant "KD" (reciprocal of the association constant "KA"), which relates the concentration of free and bound molecules in a solution to provide a sense of strength of these interactions. In this regard, the lower KD is, the higher is the affinity between the bioreceptor and the analyte and, consequently, the lowest concentrations can be detected by the biosensor. IUPAC recommends the use of the equation LOD = 3S/m to calculate LOD, where "S" corresponds to the standard deviation derived from the black measurements and "m" represents the slope of the calibration curve;

c. Sensitivity: despite it is still very common to observe the misuse of this term to designate the LOD, the sensitivity actually refers to the variation of the analytical signal due to the variation of the target analyte. In other words, it is calculated as the slope of the calibration curve and has the unit of the transduction signal (*e.g.* Ampères, Ohms, Volts, degrees, Celsius degrees, Hertz, etc) divided by the unit of concentration [43]. Briefly, the higher is the sensitivity, the higher is the response of a biosensor when it binds an analyte;

d. Stability: capability of keeping the analytical signal robust enough to not suffer the influence of extrinsic agents, such as environmental disturbances, loss of bioreceptors' affinity to the target, molecules degradation over time, etc [38];

e. Linearity: corresponds to the obeyance of the calibration curve to a mathematical expression. Once the linearity is set known, the concentration of the molecule of interest in a certain medium can be predicted and this is the working principle of quantitative accurate biosensors;

f. Reproducibility: can be defined as the ability to provide similar responses under similar conditions of detection.

In addition to those basic analytical properties, some authors also defend the evaluation of the linear range of detection and the response time to validate the performance of a biosensor. The former represents the concentration range of the analyte at which the sensor generates linear output signals, which is important to define whether the working range meets the requirement for a certain application besides helping to calculate the LOD and the sensitivity. The latter is an important reference mainly in medical applications. The response time of a sensor is the time required by

the device to generate the analytical output signal as a consequence of the recognition of the target molecule. It is also frequent in the literature to find this definition as the time required to obtain 95% of the data resulting from the detection [38]. In the context of clinical diagnoses, fast responses of biosensors allow doctors to manage the diseases at early stages, avoiding the spreading of infections and the worsening of the clinical picture of patients.

Within the scenario of the ongoing pandemic of coronavirus disease (COVID-19) for instance, authors defend that the importance of a quick diagnosis lies on fact that SARS-CoV-2 has exhibited higher contagiousness and infection rate if compared to other coronaviruses infections [44]. Furthermore, early diagnosis contributes to fast decisions on medical treatments and quarantine strategies to slow down the spread of the transmission rate.

Traditional Analytical Techniques for Diseases Diagnosis

Diagnosis, detection and prognosis techniques have been studied for several years and many methods for fault detection and diagnosis have been developed [45]. Molecular diagnostics assays use *in vitro* biological techniques for detection. Polymerase chain reaction (PCR) and quantitative PCR are performed to detect and amplify a genetic material (DNA or RNA) from a specific organism, for instance, a virus [46,47]. The advantages of PCR include the high sensitivity, quick performance and the ability to detect less-common organisms. On the other hand, its disadvantages include the supply costs, machinery fees and training expenses [48,49]. At present, PCR assay is regarded worldwide to as the most accurate and reliable test to detect active COVID-19 infections [50,51]. Immunoassays, such as enzyme-linked immunoassays (ELISA) and point-of-care (POC) techniques can be used for detection of antigens or specific antibodies [52]. Currently, immunoassays play a prominent role in the analysis of many clinical laboratory analytes such as proteins [53]. A broad variety of tests detecting specific SARS-CoV-2 antigens and IgA, IgM and/or IgG antibodies were developed [54,55]. Although the classic immunoassays can provide very sensitive and accurate diagnoses, many of them possess some important limitations: high cost, they are time consuming, demand sophisticated equipment and high skilled staff [56].

Recent Trends in Biosensors for Detection of Analytes of Medical Interest

It is worthy notable that the field of biosensing through the design of assays to detect molecules of medical interest has attracted huge attention specially in the last year with the outbreak of COVID-19 around the world. Not exclusively due to the current pandemic, though, numerous researches have been devoted to some special improvements in the analytical sciences in order to ameliorate the performance of the already known technologies. Within the recent literature in this domain, one can easily recognize some trends in the newest biosensors of medical interest: the fabrication of point-of-care devices, the label-free detection, real-time measurements and the advance of electrochemical transducer mechanisms. Under all these trends, the use of antibodies and aptamers as bioreceptor agents seem to properly match the needs and expectations of current diagnoses.

Point-of-Care Biosensors

Point-of-care diagnoses collect several unquestionable advantages over traditional laboratory setups. Not surprisingly, the golden characteristic refers to the possibility of running the test wherever the patient is, on-demand and onsite [57]. It makes the sensors amenable for bedside monitoring, analysis in pharmacies or even by the user himself. Consequently, this kind of device tends to gain increasing visibility in the market. Eguilaz et al. [58] highlight that these devices are even more relevant in resource-limited regions where the access to medical centers is difficult to the majority of the population. Nonetheless, point-of-care devices combine other interesting characteristics, such as (generally) the rapid detection, fewer steps for data/results acquisition, friendly interface, easy transport due to the reduced dimensions and light weight and demand for small sample volumes [57,59]. Concerning this last characteristic, though, there is a strategic point to be taken in account. Depending on the application, the target molecule is present at very low concentrations in the sample of analysis. Thus, a small volume for testing can contain insufficient quantity of analyte in such a manner that the biosensor would not be able to detect it [58].

In this regard, antibody- and aptamer-based biosensors are widely employed to overcome this drawback because of their high sensitivity resultant from the high affinity and selectivity of these molecules. Searching for overcoming the limitations of conventional diagnoses, Ferreira, et al. [60] worked on the development of an aptasensor for detection of breast cancer in undiluted human serum. This kind of cancer is unfortunately responsible for thousands of deaths annually. According to the authors, the diagnosis is mostly based on the detection of tumor markers present in blood or other corporal fluids at concentrations from 15 ng/mL to 75 ng/mL (over the regular healthy range of 2- 15 ng/mL). Ferreira, et al. [60] exploited two functionalization methods to attach Human Epidermal Growth Factor Receptor 2 (HER2) aptamers to the surface of screen-printed electrodes (SPEs). These devices are widely recognized in the literature to serve as useful substrates for designing portable electrochemical sensors, mainly because of their good conductivity, electrical stability in typical electrolytes and reduced dimensions [61]. In a list of 110 recent articles reviewed by Ranjan, et al. [62] on point-of-care biosensors for breast cancer diagnosis, 23% were described the use of antibodies and 5% the

use of aptamers as bioreceptors, which symbolically represents the large employment of these biomolecules in biosensors of medical interests. In this same work, other elements of recognition were described, e.g. enzymes, inorganic probes, DNA, proteins, receptor-ligand complexes and molecular imprinting polymers (MIP).

Label-Free Detection

The topic of label-free sensing in the context of bioassays generally converges to two points: the advantage of reducing the consumption of reagents and the consequent lower number of fabrication steps of biosensors in comparison to golden standard techniques (*e.g.* ELISA and PCR). Label-free sensing mechanisms consist in the direct detection of target molecules by the bioreceptor attached to the transducer substrates, *i.g.*, without the needs for fluorescent chemicals, enzymes and so on [63]. Thus, since the label-free biosensors do not demand extra labels to run the detection, this characteristic nicely meets the requirement of point-of-care biosensors for the simplest incubation protocols and allows the use of unprepared samples at working environments. Among other interesting features, Andryukov, et al. [63] point out the following advantages over label-based similar analytical assays: simpler pattern of detection, lower response time, lower cost of analysis, opportunity to detect small molecules and possibility of multiplexing.

Zhang and Liu [64] mentioned that the success of using DNA in label-free devices based on optical biosensors has inspired the same approach in the aptamer field. However, the authors explain that aptamers can fold DNA and hide its bases, providing slow kinetics of target binding, especially when the target is a small molecule. Therefore and since aptamers possess lower affinity to small molecules (KD around low micromolar units) than DNA (KD approximately in picomolar or low nanomolar), the detection of aptasensors tends to be more challenging, justifying the efforts on label-free sensing to enhance its analytical response. On the other hand, numerous works can be easily found in the field of label-free immunosensors for detection of analytes for highly sensitive diagnoses [65-68].

Real-Time Measurements

The key point of real-time biosensing is the necessity of the sensor to rapidly recognize the target molecule. If so, the output signal will be registered by the transducer source in short time intervals and a variation in its magnitude could be notable as illustrated in Figure 1. This need makes some important well recognized techniques such as ELISA and Luminex assay to fail as real-time methods for *in vivo* applications, since they require laborious and pre-defined longtime steps [69]. Cohen et al. [69] highlight that ELISA, for instance, depends on diffusion processes concerning the interaction between antibodies and antigens in a non-mixed solution, which is associated to a low binding equilibrium constant and makes the response time longer. Typically, this technique requires approximately 3 hours to be performed [70,71]. In this regard, Shengnan, et al. [72] reported the construction of an aptasensor for the real-time detection of vascular endothelial growth factor, one of the most important cytokines present in cancer patients (with average concentration of 434 pg/mL). The authors achieved a LOD of 0.1 pg/mL within a linear detection window from 2 pg/mL to 500 pg/mL. The mechanism of recognition was based on a Chronoamperometry test at the positive redox peak potential of ferrocene-labeled aptamer for 5,000 seconds.

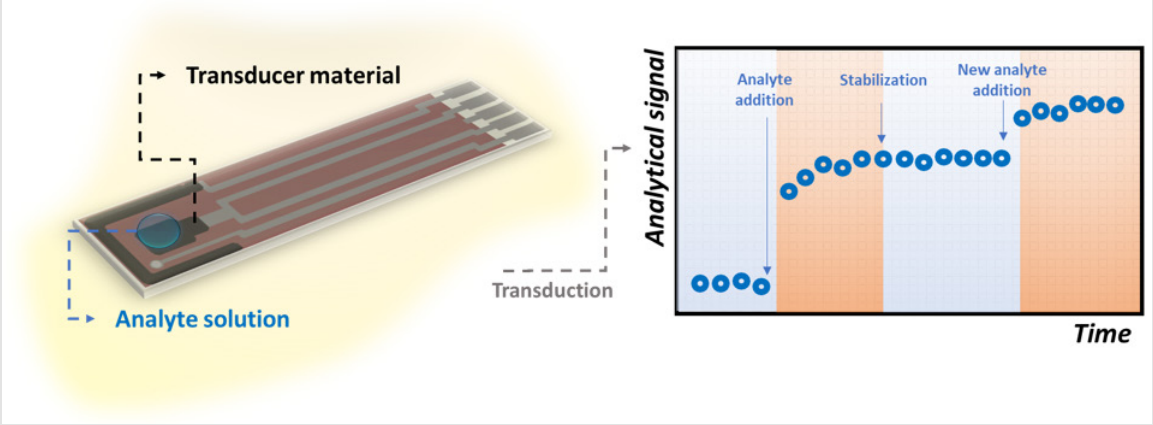

Figure 1: Illustrative scheme referring to the fluctuations of the analytical signal of a biosensor as a consequence of rapid interaction between its bioreceptors and the target molecules.

Also taking advantages of the specificity of aptamers as bioreceptors, Soleimani, et al. [73] manufactured an aptasensor assisted by a computerized monitoring system to detect prostate specific antigen (PSA). To characterize their aptasensor and to construct the calibration curve towards PSA, the authors carried out Electrochemical Impedance Spectroscopy (EIS) and Cyclic

Voltammetry (CV). Due to the steric hindrance of the analyte, the electrochemical signal of the transducer substrate increases over the time when aptamers bind the molecules of PSA. As a result, the findings showed that this kind of setup presented sensitive and rapid response fitting the real application aimed to the diagnosis of patients with prostate cancer.

Electrochemical Transducing

As per the examples of the previous sections, electrochemical mechanisms have progressively illustrated the transduction modes of many biosensors for medical applications. From 2017 to 2019, for example, these devices represented 45% of the published articles in the specialized literature of biosensors [74]. The reason is the collaboration that electrochemical reactions provide to enhance sensitivity, accuracy and response time.

Briefly, in this kind of biosensor the electrical properties of biological molecules and their interaction with electro active surfaces are exploited for assessing the changes in current, potential, charge, impedance, conductivity, etc. Complementarily, depending even on the dimensions of target molecules, the distance from the electrode surface and needs for redox probes, the specific electrochemical technique can be chosen to achieve highest analytical performance [75]. The detection system consists of three (or two) electrodes, of which one is the sensing surface (named working electrode), one is the counter-electrode and the other is a reference. These electrodes must be immersed in a conductivity solution to allow redox processes to occur and charges transfer.

When the detection of the analyte happens and the electrical properties of the surface is altered, an electronic system acts to amplify and manage the resultant data. Traditionally, this last step is performed by a potentiostat interfaced with a software for control of the required parameters. Mishra, et al. [75] pointed out details on the electrochemical aptasensors referring to design strategies and functionalization. The researchers reported that aptamers have been mostly immobilized to gold and carbon-based electrodes via chemical cross-linking with particular attention to ensure biochemical stability, surface coverture and optimal binding affinity. Most common electrochemical techniques used for fabrication of biosensors are CV [76,77], EIS [78-80], potentiometry [81,82] and amperometry [83,84]. When real-time performance is required, time-based assays (such as chronoamperometry, chronocoulometry and chronopotentiometry) well fits medical applications.

Efficiency of Immunosensors and Aptasensors

Cesewski and Johnson [85] point out that, in some cases, the high sensitivity of immunoassays are not enough to detect certain pathogens in the organism. In such circumstances, although these infectious agents are present, they do not generate enough available Abs in the blood, so the concentration of the Abs in the blood are lower than the LOD of the technique, failing the detection. According to the authors, this is a typical situation in which the employment of DNA-based systems is more useful. The biosensors consisting of nucleic acids, for instance, are usually able to recognize low concentrations of pathogens by themselves or through the indirect expression of toxins they release in the infected organism (*e.g.* toxins, other nucleic acids and raised cells. In this regard, Table 1 contains a list of recent researches in the literature of biosensors for medical applications using antibodies and aptamers as bioreceptors. It is worthy notable that these biomolecules facilitate the biosensing of analytes at concentrations as low as some femtograms per milliliter [86-90].

Table 1: Recent developments (from the last two years) in the field of aptasensors and immunosensor for assisting clinical diagnosis.

Analyte	Bioreceptor	Assay of detection	Transducer	LOD	Reference
PSA	Thiol terminated PSA binding DNA aptamer	Square wave voltammetry	Decorated glassy carbon	0.14 pg/mL	[73]
PSA	Anti-PSA antibody	Differential pulse voltammetry	Glassy carbon with iron magnetic NPs	0.45 pg/mL	[86]
Carcinoembryonic antigen (CEA)	Hemin@MIL-88B (Fe)/CEA aptamer1 and Luminol-CEA aptamer2	Chemiluminescence	Fe_3O_4@SiO_2	1.5×10^{-3} ng/mL	[87]
CEA	Anti-CEA antibody	Differential pulse voltammetry	Three-dimensional porous graphene-oxide-supported platinum metal NPs	6.0×10^{-3} ng/mL	[88]
ATP	Adenosine triphosphate (ATP) aptamer	Fluorescence measurement	AuNPs* and carbon quantum dots	20 μM	[89]
Tropomyosin	Tropomyosin-binding aptamer	Colorimetry	AuNPs	40 nM	[90]

Tropomyosin	Rabbit polyclonal anti-tropomyosin antibody	Electrochemiluminescence	Oxidized carbon nanohorns/ Nafion/ Fe_3O_4@Pd nanocomposite	28.16 fg/mL	[91]
Salmonella enterica serovar	*Salmonella*-specific recognition aptamer	Differential pulse voltammetry	Chitosan, carbon nanofibers, AuNPs and graphite	1.223 CFU/mL	[92]
Interleukin-6	3′-thiolated Interleukin-6 specific aptamer	EIS	Glassy carbon modified with p-aminobenzoic acid, p-aminothiophenol and AuNPs	1.6 pg/mL	[93]
Interleukin-6	Anti-Interleukin-6 antibody	Quartz crystal microbalance assay	AuNPs, sulfur-doped graphene quantum dots and hollow ZnSeCdS nanocage	3.33 fg/mL	[94]
Bacillus anthracis	*Bacillus cereus* spores-binding aptamer	EIS	Gold screen-printed electrode	3.0×10^3 CFU/ml	[95]
Hepatitis B surface (HBS) antigen	Anti-HBS antibody	Amperometry	PtPd nanocubes@MoS_2 nanoenzymes	10.2 fg/mL	[96]
Interferon gamma (IFN-γ) and interleukin 10 (IL-10) cytokines	Anti-bovine (IFN-γ or IL-10) antibody	EIS	Aerosol-jet-printed graphene	IFN-γ: 25 pg/mL IL-10: 46 pg/mL	[97]
Cancer antigen 125 (CA125) oncomarker	Anti-CA125 monoclonal antibody	Square wave voltammetry	Polyamidoamine/AuNPs and three-dimensional reduced graphene oxide-multiwall carbon nanotubes	6 μU/mL	[98]

Note: *NPs = Nanoparticles

Regardless the obvious different protocols used to attach antibodies and aptamers to the different transducer substrates, it is worthy notable that the sensitivity of these devices are really high. Besides, in this recent literature is not rare to observe a trend in using label-free molecules to optimize the fabrication step and to allow accessible *in-situ* measurements [91-98]. Nonetheless, it is also evident that many authors have employed electrochemical techniques to ensure accuracy and high performance of biosensors, corroboration the previous discussion brought to this minireview in the section "Recent trends in biosensors for detection of analytes of medical interest".

Conclusion

With the increasing humans needs for accurate, fast and friendly methods for health control, biosensors for medical applications have undergone important changes in the last decade. The immobilization of antibodies and aptamers on transducer substrates for high performance detection has been an exhaustive strategy for the production of biosensors, especially due to the high sensitivity of these bioreceptors. Articles published in the recent literature exhibit LODs in the order of femtograms per milliliter. To this end, added to the intrinsic advantages of antibodies and aptamers, there is a notable trend to search for label-free devices, with less functionalization steps, lower times for the formation of bioreceptor-analyte complexes, under selective and sensitive sensing modes. Thus, much is seen about the use of electrochemical techniques such as CV, EIS and amperometry, although optical and piezoelectric transduction techniques are also present in the field of biosensors for various applications including the ones for medical diagnostics.

It is believed that this specific application demands advanced technologies, especially to shorten the detection time, since early diagnoses are essential for the administration of first aids and precise medications that can enhance the chances of cure and survival of patients (especially those who have less access to health centers). The main challenges in the area still seem to be related to the commercial viability of these devices. Likewise, quite possibly, the prospect of advances in technology is likely to be based on the study of alternative materials and methods to make immunosensors and aptensensors increasingly simple and inexpensive.

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