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New Insights on Optical Biosensors: Techniques, Construction and Application

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Additional information is available at the end of the chapter

1. Introduction

Since Clark´s enzymatic electrode in 1962s [1], biosensors have been proposed for a range of application and, aiming clinical analysis application, the amperometrical, potentiometrical and optical are the biosensors which have achieved most significant development. In concept, optical biosensors are those based on the detection of changes on absorption of UV/visible/Infrared light when chemical reactions occur or on the quantity of light emitted by some luminescent process. Regarding to supramolecular nanostructures and their ability of enhancing the sensing activity when applied to biosensors construction, a very instigating work, presented by Jin Shi et al. [2] showed a way of turning carbon nanotubes into more water-soluble compounds and, consequently, more biocompatible by modifying their surface with a synthetic DNA sequence. In this way, the carbon nanotubes can overlay the biosensor electrode more efficiently, enhancing the biosensing activity. Lieden et al. [3] also took advantage of the properties of nanotubes in biosensing. In their work, they showed that a biosensor can present a rate of detection tree times faster when carbon nanotubes are used in the nanobiosensor construction, preventing the attachment of protein to the device components. The change in electric resistance of carbon nanotubes when proteins touch them is immediate, which confer to the device a fast recognition ability, and leads to an increased efficiency of the biosensor. Yet, other fascinating works are the one presented by

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Chen et al. [4] and the work presented by Park et al.,[5] in which a piezoelectric nanogenerator was developed to feed several devices, including implantable biosensors, which make of this research field very promising, since nowadays, implantable biosensors present the disadvantage of the need to be recharged or replaced when discharged.

In a common sense, biosensor is a device constructed to inform about a system, requiring as less human action as possible. It is formed of sample holder, a biological recognition element which must be selective, a physical transducer to generate a measurable signal proportional to the concentration of the analytes and the signal processing unit, which gives to the analysts graphical, numerical or comparative information that they shall interpret (fig.1). The recognition element can be of almost any type of biological system, from antibodies, proteins and peptides to viruses, microbes, cells and tissues.The selection of the appropriate recognition element considers not only what is the information to be obtained, but also the ease of construction of the devices employing such element and, of course, their durability. As an example, some microbes have been employed instead of antibodies and proteins mostly due to their facile production via cell culture and in vitro stability. Nevertheless, it is evident that microbes might lead to a lack of selectiveness, due to their non-specific metabolisms. Recent research had proposed that highly selective microbial biosensors can be constructed by inducing a desired microbe metabolic pathway and by adapting them to the substrate of interest, using selected conditions of cell-culturing. Also, as alternative to manipulate the selectivity and sensitivity of microbial biosensors at the DNA level, the genetically engineered microorganisms (GEMs) had been proposed. [6]
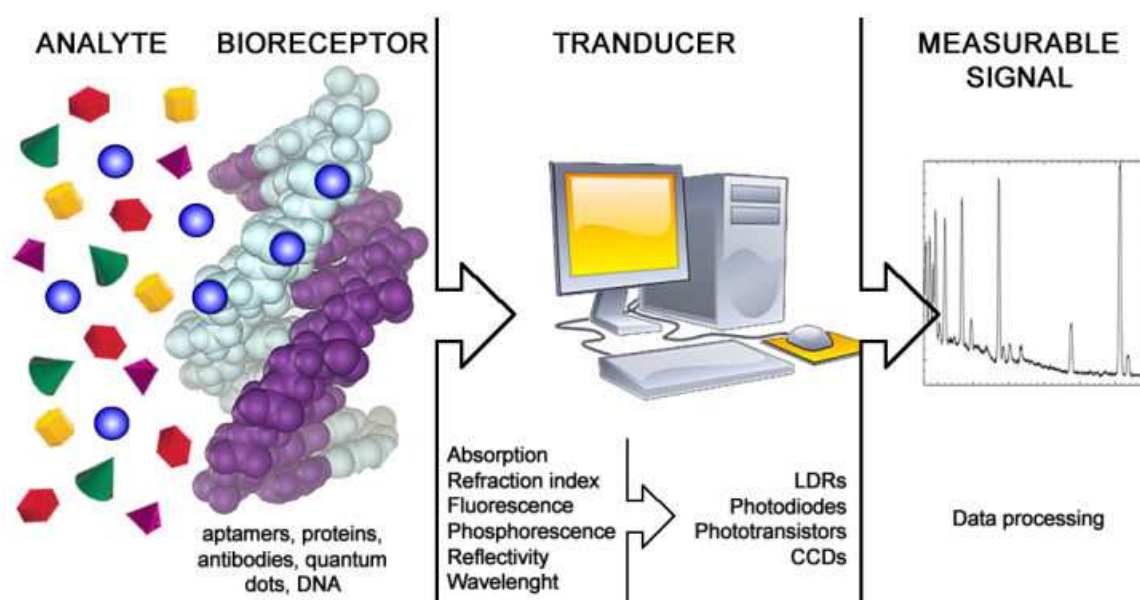

Figure 1. General scheme of a biosensor.

Although it is of extreme importance to accurately choose a recognition element to propose a suitable biosensor, it is of great importance to define a way to turn the recognition event

into a signal that can be detected and interpreted. The transducer figures now as the element which is able to transform the biochemical response into a recognizable physical signal. For example, techniques of immobilizing microorganisms on transducers had played important roles in the fabrication of microbial biosensors. [7] Some traditional methods of immobilization include adsorption, encapsulation, entrapment, covalent binding, and cross-linking, mainly via sol-gel processes and, in special, immobilization of microorganisms in conducting polymers are of great interest due to their unique electrochemical properties, which can be exploited on the transduction function. [8, 9]

A number of possible transducers can be proposed, once the interaction between analyte and recognition element is defined. Available techniques are of a large number and to elect the proper one very often is not a trivial task. Among various sensing techniques, there are piezoelectrical, calorimetrical, enthalpimetrical, DNA microarray, Surface Plasmon Resonance (SPR), Impedance Spectroscopy, Scanning Probe Microscopy (SPM), Atomic Force Microscopy (AFM), Quartz Crystal Microbalance (QCM), Surface Enhanced Raman Spectroscopy (SERS), but electrochemical and optical techniques are the widest used in the development of microbial biosensors, due to their numerous possibilities, which turn possible the construction of a number of selective sensors.Electrochemical biosensors are classified as amperometric, potentiometric, conductometric, voltammetric, depending on which detection principle is employed in the biosensor. In a quick overview on these important devices, an amperometric biosensor is the one that operates at a given applied potential between the working and the reference electrodes. A current signal, related to analyte´s concentration in the sample, is then generated, due to the reduction or oxidation process suffered by an electroactive metabolic product. A conductometric biosensor is that in which a conductivity change is observed upon production or consumption of ionic species involved in the metabolic process. It became a very attractive device due to its enhanced sensitivity and fastness brought about through sophisticated modern analytical techniques. Additionally, they are suitable for miniaturization once it requires no reference electrode in the system. [10] Its disadvantage lies on that all charge carriers lead to a change of conductivity, which directly affects the device selectivity and is known as relatively poor.

The potentiometric biosensor is based on the potential difference between working and reference electrodes. In these biosensors, the measured species is not consumed, as it is in the amperometric biosensor. Its response is on the activity of the species in comparison to the reference electrode, with the output signal recorded in voltage units and, independently of the sensor size, the signal is proportional to natural the analyte concentration. Its great advantage lies on sensitivity and selectivity, if the working electrode is species-selective. However, a highly stable and accurate reference electrode is always a requirement.

The most versatile electrochemical technique applied to biosensors is voltammetry, since both current and potential difference combined, consist on a reasonable system response. Its major advantage its successfully application as a multi-component detector.

2. Optical biosensors

Optical biosensors are those which can sense phenomena related to the interaction of micro-organisms with the analytes and correlate the observed optical signal to the concentration of target compounds, based on the measurement of photons involved in the process, rather than electrons, as in the afore mentioned techniques. More specifically, optical detection is based on the measurement of luminescence, fluorescence, colour changes, by the measurement of absorbance, reflectance or fluorescence emissions that occur in the ultraviolet (UV), visible, or near-infrared (NIR) spectral regions.

Even though the first biosensor reported, the Clark´s amperometric enzyme electrode for glucose [1] is dated of 1962, the first fiber-optic biosensor was described only in 1975, by Lubbers and Opitz [11, 12] and, since then, with the incredible rapidity of new detection techniques development, various optical biosensors have been demonstrated. Among optical properties, fluorescence is by far the most often exploited one by a significant number of techniques, mostly based on parameters such as intensity, lifetime, anisotropy, quenching efficiency, non-radiative and luminescence energy transfer, and so on.

Although fluorescent biosensors have been reported since the late nineties, [13- 15] very often there is a confusion between labelling and the description of a biosensor, which turns the exact moment of the fluorescent biosensor proposal not very clear to point. Nevertheless, amazing development ondetection methods in biosensors, their construction, even at nanoscale, and applications have been reported in the last decade. [16- 22]

2.1. Classes of optical biosensors

Due to the enormous quantity of biosensors already proposed, it is a very hard task to classify them all. Nevertheless, for our objective, it is convenient to classify them into two classes, not based on the detection method, which, of course, must be an optical method, but on the recognition one. With it in mind, optical biosensors can be sub-classified as:

• probing biosensors: this class consists on biosensors that have based their activity in differences of interactions between analyte and recognition element, ruled by affinities. This behavior leads to changes in the optical response that can be measured by several optical methods.

• reacting biosensors: in this class, their optical responses are related to chemical processes, such as chemisorptions, catalytic reactions of any kind, formation of new chemical bonds and so on. These chemical (and definitive changes) can also generate optical changes which are detectable by countless optical methods.

With respect to the detection mechanism, biosensors can be of fluorescence, phosphorescence, reflection, UV/Vis/IR absorbance, lifetime, which is the characteristic measured by Förster Resonant Energy Transfer (FRET) based biosensors, responsible for the wide range of applications in which biosensors had acted lately. Also, refractive indexes changes can be detected by several and modern techniques, such as interferometry and Surface Plasmon

Resonance, which had opened a new and promising field for biosensor research. Further information about them will be presented later in this chapter.

Among all optical properties exploited in biosensors construction, the most common method of detecting and quantifying biological compounds is still based on the fluorescence activity due to the fact that fluorescent properties of most organic fluorophores are susceptible to environment changes, which is indispensable to sensing applications. The most important advantage of these biosensors is that they are proposed for general use, thus, they provide the possibility of multiple compounds detection within a single device, they are able to perform remote sensing and they are ease to build. Nevertheless, there are some requirements for the construction of the fluorescent recognition unit that must be attended, regarded to structure of the fluorophore and to its photophysical activity.

When proposing a biosensor, the hardest task is to address the right fluorophore to compose it, the one that will provide the needed answer. This is because there is a number of fluorescent probes which can be applied on biosensing and a number of device designs that can be proposed within the same objective.

Since in sensing applications, detection is based on changes of fluorescent responses of a particular fluoroprobe, when it is inserted into the analyte environment and interacts with it by a variety of mechanisms, it is of extremely importance to profoundly understand the photophysical behavior of the system and the fluorescence parameters that will be determinant in detection. Once this step is achieved, a biosensor can be built aiming to countless applications, which include immunoassays, nucleic acid detection, cellular and sub-cellular labeling, resonance energy transfer studies, diagnostic assays and disease monitoring and treatment.

Most of fluoroprobes used in biosensors present an environment-dependent luminescent behavior. Therefore, attention must be given to the environment characteristics that may interfere with the photophysics of fluoroprobe-analyte, such as pH dependence of the luminescent response; self-quenching at high concentrations of fluoroprobes; susceptibility to photo-bleaching; short excited state fluorescent lifetimes, which may confer low sensibility to salvation relaxation; small Stokes shifts, which favors self-absorption effects and undesired luminescent emission shifts and short-term stability when in presence of water or in aqueous medium [23, 24].

2.2. Fluorescent biosensors

In fluorescent biosensors, a particularly interesting characteristic that have been more and more exploited is the effect that local interactions can cause on fluoroprobe's electronic excited states, even when no chemical changes are included. With this respect, mechanisms of controlling and orienting these effects are proposed and studied. For example, non-radiative energy transfers leading to a more selective system or a more efficient luminescent response, achieved by combining common organic fluorophores with metallic nanoparticules, metallic complexes or with nanostructures of carbon or peptides have presented interesting results. In our previous work, with leadership of Prof. Alves, [25] diphenylalanine peptide nano-

tubes were physically modified with a fluoroprobe containing a polar head, 1-pyrenyl-carboxylic acid, and their steady-state and dynamic fluorescence responses were determined (fig.2). Also, the mechanism of interaction between peptides to form the supramolecular structure and their interaction with the fluorophore were studied and revealed. By computational simulations, it was shown that the nanotube formation and the fluorophore adsorption are governed by π-stacking interactions, which makes of electrostatic interactions essential. Moreover, since the connection of nanostructured materials, especially biomaterials such as peptides, with solid well-structured surfaces make the manipulation of such structures an interesting alternative for device fabrication. Although the afore mentioned work focused on the supramolecular chemistry of nanostructure obtainment and the role of pH conditions to ensure the right nanotube dimensions, it rose the perspective of constructing an electronic device for biological recognition. Aiming at the application of this nanostructured peptides to a biosensor construction, it is of great importance to determine whether the interaction of the peptide nanostructure with the probable substrates can influence the optical response measured. The first step is to determine the nature of the interaction between the fluorescent peptide nanotubes with Indium-Tin Oxide (ITO) electrode, elected as a good candidate to act as anode in a future biosensor device. The modified Phe-Phe nanotube was then deposited on ITO electrode and the time-resolved fluorescence of this system was detailed studied.

Since the amphoteric ITO is a transparent conductive oxide of low electrical resistivity (10^{-6} to $10^{-4}\Omega.cm$) and small bandgap (of 3.3 eV), high physical-chemical stability and good surface morphology, it is widely used as electrode in a variety of devices, which includes sensors. When applied to biosensors, ITO can interact with protein residues due to –OH groups on its surface, which enables the adsorption of carboxylic and amine residues via hydrogen bonds.

By combining the final fluorescent nanotubes with ITO electrodes, a non-radiative resonant energy transfer (FRET) was detected between the thin layer of mixed oxide and the fluorophore doping in nanotube surface. In fact, the results show that when pyrenyl-doped nanotubes are formed at neutral and higher pH ranges, FRET occurs, leading to a small excited-state lifetime of pyrenyl moieties, but, at lower pH ranges, the excited states of pyrenyl moieties are stabilized and the lifetime rises. This energy transfer process is favored, in this case, by the strong electrostatic interaction between the charged nanotube and the charge transporter ITO of dipole-dipole induced type. When nanotubes are formed in low pH ranges, the final structure presents an overall superficial positive charge, due to protonation of carboxylic and amine groups of peptide residues and carboxylic groups of the fluorophore, which is responsible for a less effective electrostatic interaction between combined peptide structure and 1- pyrenyl-carboxylic acid moieties and the ITO surface. These results contribution laid in terms of the supramolecular control of the structures, showing that they can be designed and actually obtained as desired and many aspects of biosensing activity can be exploited in the same device conception. In this example, both a fluorescent-based biosensor for local environment monitoring and a FRET-based fluorescent biosensor can be designed, depending only on the approach.
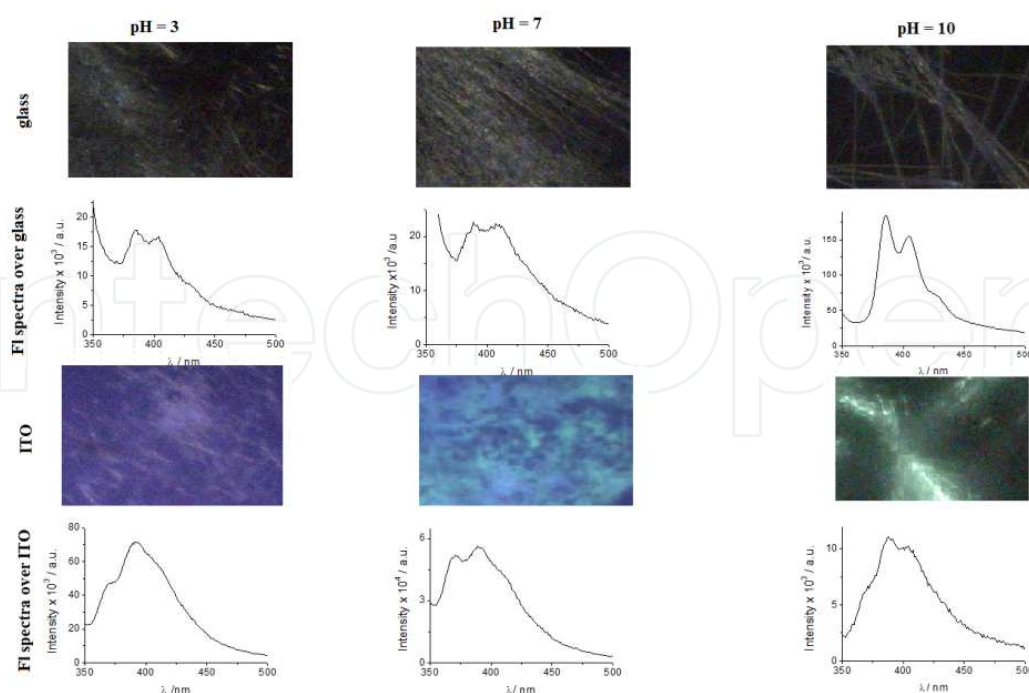
Figure 2. Epifluorescence images (200 times increased) and respective fluorescence spectra of Phe-Phe nanotubes samples obtained at of 0.07% m/m of 1-pyrenyl-carboxilic acid and at distinct pH ranges, deposited over glass and glass covered by ITO substrates.

2.3. FRET-based fluorescent biosensors

FRET is often exploited in biosensors due to the variety of systems that can present this effect and to the quantity of sensing elements that can be employed in the biosensor construction. An example is the use of colloidal luminescent semiconductor nanocrystals, the quantum dots (QDs), to biosensors. These fluorescent compounds are very well known as fluorescent labels for a series of studies, in particular, for imaging of biological and non-biological systems. [26-28] Nevertheless, they also give rise to more robust biosensors, since their unique fluorescent properties can overcome the organic-based fluorophores liabilities.

The major property that inform about the occurrence of non-radiative energy transfer process is changes in the fluorophoreexcited state lifetimes. Due to the variety of compounds that can have their excited state disturbed, several detection techniques can be applied, several device architectures only dependent on the application, rather than in the detection technique, generating biosensors with significant sensitivity and selectivity enhancement, which can be even at the one single molecule limit. FRET is also essential to the conductive polymers, DNA, aptamer or protein-based biosensors and all these possible architectures make use of the interpretations of luminescent signals that reveals several mechanisms for FRET. Indeed, FRET is a resonant process that depends on several conditions of the environment where it takes place. In a short description, this non-radiative excitation energy transfer occurs always that a donor chromophore and an acceptor become close to each other and their electronic energy levels interact, with some pre-requisites. As described by Förster [29],

FRET is determined by a long range dipole–dipole interaction between the donor and the acceptor and his formulations for these events are widely applied from solutions to solid systems, which contain the chromophores of biochemical interest. As an advantage, FRET offers an experimental approach to determine molecular distances through luminescent spectral measurements, which correspond to the efficiency of energy transfer between a donor and an acceptor located at two distinct specific sites, with separation limited to a range of 10–80 Å. Because of the sensitiveness of this technique corresponds to the inverse sixth power dependence of the transfer efficiency to the donor-acceptor distance (equation 1), FRET is assumed to consist of a sensitive technique for detection of global structural alterations. Förster formalism assumes that donor and acceptor are stationary in the timescale of their electronic excited-states lifetimes and, as a consequence, the donor-acceptor separation is static, giving a single distance between them. Nevertheless, the dynamic nature of large systems such as proteins and polymers cannot be ignored and the distances between them are expressed as a distribution. Förster mechanism involves an inductive resonance transfer in which the excitation process creates an electric field around the donor, due its charge transport. As a second oscillator, the acceptor, come closer to the donor, it inductively oscilates and, if it occurs with the adequate frequency, the energy of the donor is transferred to the acceptor. The energy transfer is maximum when both oscillators are similar. To observe this phenomenon, it is necessary that electronic transitions of both donor and acceptor are permitted and then, that coulombic interactions, such as dipole-dipole, which are distance dependent by a factor of R^{-3}, occur. This leads to a probability of occurrence of FRET proportional to R^{-6}. Förster predicts that the energy transfer occurs if there is a coupling between transitions and radiation field, at a rate constant (k_{DA}) given by:

$$k_{DA} = \frac{9000 k^2 \ln 10}{128 \pi^5 n^4 N_A \tau_{DA} r^6} \int \frac{F_D \varepsilon_A(v)}{v^4} dv \qquad (1)$$

In which k^2 describes donor-acceptor dipole relative orientation; N_A is the Avogadro Constant; F_D stands for the donor corrected fluorescence intensity; ε_A is the acceptor molar extinction coefficient; τ_{DA} is the donor fluorescence lifetime; r is the distance between donor and acceptor; n, the refractive index and v the wavenumber.

When the probability of FRET occurrence is 50%, the distance in which it takes place is a reference distance, called Förster Distance (R_0), defined as the distance in which the FRET rate K_{DA} is equivalent to the fluorescence rate of the donor in the absence of the acceptor τ_D^{-1}. They are related by:

$$k_{DA} = \frac{1}{\tau_D} \left(\frac{R_0}{r} \right)^6 \qquad (2)$$

When R = R_0, $K_{DA} = 1/\tau_D$

2.4. DNA – based fluorescent biosensors

Despite specific requirements of the systems of interest, these assumptions can be applied to any measurements that can identify the energies of the electronic transitions involved and the excited states lifetimes. In DNA-based biosensors, high sensitivity detection and real time information are crucial. In their work, Liu and Bazan [30] proposed homogeneous biosensor assays, which were based on the detection of distinct luminescent responses of a water-soluble conjugated polymer and took advantage of its characteristic of self-assemble to improve the biosensor capability over those employing small molecules. In this biosensor, the interaction between the oligonucleotide hybridized with a cationic polythiophene and a single-stranded DNA or a double-stranded DNA in the presence of cationic poly(fluorene-co-phenylene) leads to conformational changes on polymer backbone and to changes in the fluorescent response, as the cationic poly(fluorene-co-phenylene) acts as donor in the fluorescence energy-transfer assay and, hence, to a signal amplification(fig.3). In the presence of the single-stranded DNA, the positively charged polymer interacts with it, but without energy transfer and only emission from the poly(fluorene-co-phenylene) is detected. When interacting with the double-stranded DNA, emission from the poly(fluorene-co-phenylene) decreases and emission from the hybridized oligonucleotide is observed. The signal transduction is then controlled by specific electrostatic interactions.
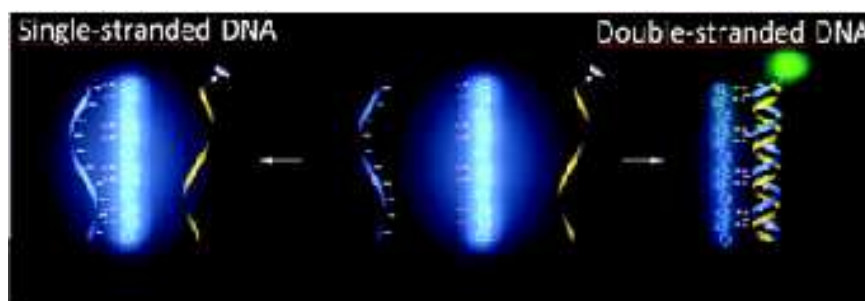

Figure 3. Scheme of the transduction mechanism on Liu´s conductive polymer FRET-based biosensor for DNA detection. Reprinted (adapted) with permission from [30]. Copyright (2004) American Chemical Society.

The approach of employing oligonucleotides in the transduction process of biosensors has become very attractive and popular in such a way that a new class of biosensor has arose: the *aptamer-based* ones.

2.5. Aptamer-based biosensors

Although most of the aptamer-based biosensor utilizes optical methods for detection, it is not exclusive. In fact, they consist of a versatile tool for biosensors, since they behave as efficiently as antibodies, selectively interact with the target and consist of innovative approaches for biosensor construction. There are numbers of aptamers that can be selected from the Systematic Evolution of Ligands by Exponential (SELEX) enrichment, which consists of an *in vitro* iterative process of adsorption, recovery and re-amplification of single-

stranded DNA combinatorial lists. This routine of select an aptamer is necessary due to the specificity of their interactions and the variety of biosensors that can be proposed.

In a recent work, Yildirim et al. [31] showed an environmental application for aptamer-based optical biosensors. In their approach, β-estradiol 6-(O-carboxy-methyl)oxime-BSA was covalently immobilized on an optical fiber surface to develop an aptamer-based biosensor for rapid, sensitive and highly selective detection of 17β-estradiol, a endocrine disrupting compound that is a common water pollutant. In an indirect competitive detection approach, samples of 17β-estradiol were premixed with a fluorescence-labeled DNA aptamer. In the sensor surface, a higher concentration of 17β-estradiol led to a less intense fluorescence of the labeled aptamer, by creating a dose-response curve of 17β-estradiol, with a detection limit as low as 0.6 ng mL^{-1}.

2.6. Quantum dots-based fluorescent biosensors

Some of the quantum dot´s photophysical properties overcome by several orders of magnitude those of common fluorophores. For example, they present very broad absorption spectra, from UV region towards blue-visible region, corresponding to a large wavelength range; their molar extinction coefficients are of hundred times larger than those of small organic fluorophores and can reach values of several millions. [32] Also, they present the ability of tuning their photoluminescence as a function of the core size, which turn possible assign a determined quantum dot for an application.[33, 34] This possibility becomes a great advantage of quantum dots in comparison to organic luminescent polymers, which cannot have their behavior well predicted only by their chain size: it is important to know their solubility and the interaction forces that act in a given system, since their final photophysical properties are intimately related to their chain conformation and, so, to inter and intrachain energy transfer processes.

Regarding to the photoluminescent properties of quantum dots, their tunable fluorescence combined to the very broad absorption lead to a large effective stokes shifts and to the probability of an efficient excitation of a mixed population of quantum dots, at a single wavelength, several nanometers delocalized from their fluorescent maximum. The characteristics of size-tunable luminescence and of broad absorption spectra make of quantum dots suitable for multi-color (or, as usually called multiplexed) immunoassays. Their photostability and sensitivity also make them good options for a number of imunoassays, especially because they provide flexibility to the analytical techniques [35, 36].

In Pinwattana et al. [37] work, quantum dots were conjugated to a secondary anti-phosphoserine antibody in a heterogeneous sandwich immunoassay, acting as labels and generated amplified electrochemical signals, analyzed by square-wave voltammetry, which is not an optical technique, but it demonstrates the amplitude of analytical methods that the choice of quantum dots as actives in biosensors permit. Their experiments consisted of the addition of the model phosphorylated protein, bovine serum albumin, to a primary bovine serum albunmin antibody-coated polystyrene microwells, followed by the addition of a quantum dot labeled anti-phosphoserine antibody. This quantum dot label was then removed by acid at-

tack and the free label was detected, leading to current responses that were proportional to the concentration of the phosphorylated bovine serum albumin.

Since quantum dots are usually obtained from organometallic precursors, they are poorly water-soluble and this is, in some cases, an issue to biosensing, since it seems improbable that, in these conditions, a quantum dot will interact with a biosystem. Nevertheless, there are several methods available to efficiently exchange or functionalize their native organic ligands with a desired ligand that can better both solubility and bioconjugation potential, by either chemical or physical processes. One of the most common modifications is to attach a biomolecule to a functional group on the quantum dot surface, which can be amines, carboxyls or even thiols. In this matter, amines andcarboxyls can be easily modified by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, a common reactant. Thiol groups are usual sites for maleimide.

The quantum dot surface modification can also be conducted by direct interaction with the biomolecule. In this case, interaction forces balance may govern the stability and the yield of the modified quantum dot. Examples are metal-affinity between polyhistidine appended proteins and Zinc atoms present on quantum dot´s structure, which leads to coordination of the biomolecule to the metallic center [38, 39] or dipole interactions between thiol groups of cysteine residues with sulfur atoms present in the surface.[40, 41] These modifications can transform quantum dots into efficient elements to immunoassays applications. Examples were presented by Puchades et al. In their review, [42] they showed that by electing a quantum dot, taking into account its size and by coating it with a variety of substances, from antibodies to silica, the quantum dot can by assigned to a specific immunoassay. For example, immunoassays which employ fluorescence spectroscopy as analytical technique had used distinct quantum dots as labels, such as quantum dots conjugated to antibodies, covered by biotin or bare quantum dots, while lanthanide-quantum dots were assigned to time-resolved fluorescence experiments and streptavidin-modified quantum dots were used in either in fluorescent or chemiluminescent experiments.

Such a variety of methods to adapt the inorganic nanoparticles to immunoassays leads to another classification, with respect to the exploited mechanism. In this sense, immunoassays are classified *as non-competitive* or *sandwich assays*, when involving an analytical path in which the antigen in the sample is bound to the antibody site and a second labeled antibody is bound to the antigen, resulting in a response that is directly proportional to the concentration of the analyte; and as *competitive immunoassays*, when involving the competition of the antigen in the sample and the labeled antigen to bind to specific antibodies, resulting in a response that is inversely related to concentration. In a brief comparison, direct assays are faster, once they employ only one antibody, eliminating the secondary antibody cross-reactivity. In contrast, indirect immunoassays are much more sensitive.

As a competitive immunoassay example, Ding et al. [43] coated a microtiter platewith ovalbumin haptenand observed the decrease of the fluorescenceat higheranalyte concentrations. This indirect immunoassay was used to determine sulfamethazine residuesin chicken-muscle tissues with alimit of detection of 1 ng/mL for the immunoassay. Compet-

itive assays also contemplate those using fluorescent probes as internal standards, such as quantitative assays.

As an example of non-competitive detection strategies is the new indirect immunoassay proposed by Li et al., [44] in which quantum dot fluorescent labels were combined to enzymaticchemiluminescentlabels. This system was used to simultaneously detect three cancer markers in human serum and thelimits of detection were the same for all markers, in the ng/mL range. Seeking for a new strategy that to be applied to the construction of a biosensor for most distinct application, they coupled the quantum dot to other two chemiluminescent enzymes, creating a hybrid multiplexed detection system for lung cancer.

2.6.1. The Surface Enhanced Resonance-Raman Scattering applied to quantum dots biosensors

It is noteworthy that quantum dots can be applied to surface-enhanced resonance Raman scattering (SERS) measurements as a powerfultool for ultrasensitive analysis, since unlike fluorescence techniques, SERS-active groups do not self-quench. An example is Han et al. work, [45] where fluorescein was used as Raman probe for a microtiter plate covered by antigen (human IgG) samples of unknown concentration. In their experiments, a solution of fluorescein-conjugated antibody was added to the sample and fluorescein SERS spectrum was recorded with a limit of detection of 0.2 ng mL^{-1} in a several-days stable device.

2.7. Labeled and labeled-free optical biosensors

Among optical biosensors that can be constructed based on this spectroscopic technique, there are those classified as labeled-fluorescent biosensor, as the above mentioned examples, but there is also a evolving rinsing class of optical label-free biosensors [46]. The most crucial distinction between these techniques is that label-free biosensors can directly evaluate the properties of the system, instead of the fluorescent response of a labeled material to the effect of its local environment. In their review, Fan et al. [46] make a clear distinction between labeled and label-free optical biosensors, with respect to techniques of detection, sample preparation, sensibility and versatility. They mentioned that although there are some great differences between fluorescence-based and label-free detection techniques, both are widely used in optical sensors construction.These distinct characteristics of the label-free devices make of these optical biosensors the most versatile among all types of sensing technologies that are only able of label-free detection, as in the case of surface acoustic wave and quartz crystal microbalance technologies. It also discusses Raman, refractive index and absorbance as detection methods. These approaches enable the optical detection to be yet more versatile, enabling the construction of a series of other biosensors, only by specifying even more de detection technique. It is not unusual that an optical label-free biosensor mixes more optical structures to enhance its sensing performance. For example, among the refractive index optical biosensors, they can be:

• Surface plasmon resonance

The first work that employed the plasmon resonance phenomenon to sensing was developed by Liedberg et al. in 1983.[47] Since then, this remarkable technique has been widely

employed in many biochemical and biotechnological fields and greatly developed. Several types of biorecognition elements are currently used, depending on the application. This technique is so versatile that have been employed in a wide range of processes, including food and environmental monitoring and clinical analyses.

In simple words, surface plasmon is a charge density wave over a metallic surface. In the case that a thin layer of a metal is deposited on glass, there must be distinct dielectric constants on both faces of the film: the one in contact with glass surface and the other in contact with air. Then, a charge density oscillation occurs at these interfaces, leading to the phenomenon occurrence. It is observed as a sharp minimum of the light reflectance when the incident angle is changed, leading to a very important sensitivity to refractive indexes variations. These systems can be excited by some methods, as the waveguide coupling, where a dielectric and a metal are positioned over the substrate, generating a waveguiding layer, which creates an interface between metal and the waveguide. Light, therefore, propagates in this waveguide through totalinternal reflection, giving rise to an evanescent field at the interface, which excites the surface plasmon wave. [48, 49] Although this is a very popular technique, there are other methods to excite the surface plasmon wave that can lead to better detection limits, such as prism coupling. Nevertheless, waveguide coupling consists of an alternative due to easily combine to other optical components.

Some advances of these techniques had been presented in the last few years. In their work, Sacarano et al.[50] presented the SPR imaging technique, or *"SPR microscopy"* as the "most attractive and powerful advancement of SPR-based optical detection", which presents the advantage of coupling the sensitivity of the SPR measurements with the spatial capabilities of imaging. In this approach, the entire biochip surface is visualizedin real time, which, as a perspective, might enable experiments based on the continuous monitoring of immobilized spot arrays, with controlled size and shape and with no need of labeling.

• Interferometry:

Based on the interferometry technique of improving analytical signals, some types of interferometer-based biosensors were developed, such as Mach-Zehnder, Young's multi-channel and Hartman, among the most commonly used. They are based on the concept that a guided wave suffers a phase change when its evanescent field interacts with the sample. This interaction produces an optical phase change that is quantitatively related to the sample. In these constructions, a sensitive biosensor must present a long interaction length between guided wave and sample. Although many are the interferometric components that can be employed in a biosensor construction, these are by far the most commonly found:

• Mach-Zehnder interferometer:

It is composed of beam splitter that divides a coherent, polarized single frequency of a laser beam into two branches. The first one is passed through a window that leads to the reference branch of the interferometer. The second one passes through the detection branch window, in which the evanescent field interacts with the sample. Both beams are kept apart by a thick coating layer. They recombine at the output, resulting in an interference pattern that is

detected at the photodetector. This type of interferometer gives rise to excellent bulk refractive index detection capability, but until now, there had not been much development of devices based on this interferometer.

- Young´s interferometer:

Similar to Mach-Zehnder interferometer, this is also based on the passage of a laser beam into a slit, reaching a splitter. The laser beam is divided into reference and sensing branches, but instead of recombining at the output, the optical output of the two branches combine to form interference fringes on a CCD detector. This improves the signal, giving information about spatial intensity distribution along the CCD.

- Hartman interferometer:

In this configuration, optical elements are placed over a planar waveguide, organized in strips. A laser beam enters the device by an input grating, reaching the optical elements composed of functionalized molecules and leaving the device by the output grating. [51] Integrated optics is positioned after the output, creating interference between pairs of functionalized strips.

- Backscattering interferometer:

The most common backscattering interferometers employ in their construction a simple optical arrangement composed of a coherent light source (ususally a low-power He-Ne or red-diode laser), a microfluidic path, and, of course, a phototransducer. The backscattering interferometry technique presents some advantages when compared to the above mentioned techniques. Since it is based on microfluidic concepts, it shows comparable performance as former interferometers, but using a much smaller sensing area,which permits a wide range of configuration. In this approach, the coherent laser beam is focused on a small sensing area, the interaction of the laser beam with the fluid-filled microchannelleads toan interference pattern that is registered in the photodetector, which is sensible to the laser reflected intensity. When a biological sample is placed over the illuminated surface, a laser of distinct intensity is detected due to a phase change caused by the light reflection over this surface.

- Photonic Technologies

The photonic technology has been object of many research fields and of a very rapid improvement, compared to other technologies. In this sense, there are many methods to employ photonicprinciples, and a wide range of scientific and technological issues to apply it, resulting in several equipment proposals. The broad range of applications of photonic devices permits to glimpse the importance of this emerging field. It is possible to incorporate different types of lasers, dielectric waveguide structures and photodetectors in a variety of possible equipments, that enables the perspective of explore from ultraviolet to far infrared, extending the fundamental research approach and the application possibilities, that can explain why photonics application, in other potential technologies, has grown in such impressive way.

In the biosensor perspective, many materials and concepts of application have been developed and a wide range of the proposed devices employ optical fiber and waveguides, as well as photonic crystals. Here some characteristics of such devices are pointed.

• Optical fiber

The two basic concepts of optical fiber based biosensors are the *Fiber Bragg´s grating* and the *long-term grating*. They differ from each other not in principle, but in construction. While the fiber Bragg´s grating concept requires the etching of the fiber (or grating) surface, followed by the physical pattern of the surface, the long-term grating is a configuration based on periodic grating of 100 μm to 1 mm, which make them much larger than the common Fiber Bragg´s gratings and confer them the advantage of an increased sensing to refractive index changes. Moreover, they are easier to build and can be customized by chemically removal of the coating. Either fiber Bragg´s or long term grating designs can lead to very high refractive index sensitivity and low detection limits, which consist of the most desired characteristics of promising biosensors.

• Optical waveguide

This elegant technique has been applied for biosensing in the last decade with a considerable success. Due to that, many structures of construction had been proposed usually directed by the analyte of interest. In this concept, some popular structures are:

• *Resonant mirror*, in which a low refractive index spacer composed of a metal or a dielectric layer, is sandwiched between a high refractive index substrate, usually a prism from where light reaches the biosensor and is refracted, and a high refractive index waveguide layer. There, the incident light at the resonant angle is coupled into the high-index waveguide layer and has a strong reflection at the output side of the system. Then, it is conducted through the waveguide, creating the evanescent field that leaves the waveguide. This approach turns the resonant angle sensitive to any change of refractive index and the signal is produced.

• *Metal coat waveguide*, which differs from the former structure by employing a low refractive index waveguide layer, separated from the high refractive index substrate by a metal layer. In this configuration, light is guided through the low refractive index and the light intensity is increased by the metal spacer, leading to an increased light-sample interaction and, therefore, to an increased sensitivity.

• Optical ring resonator

In this configuration, light suffers a total internal reflection in the boundaries of a curved interface between a high and a low refractive media. This process leads light to propagate in the circulating waveguide form or in the whispering gallery modes, as illustrated by Fan and cited by some other works therein.[46]Devices based on this technique can be constructed in a much smaller scale than those based on the former techniques with similar sensing capability, which is their great advantage. They can be constructed in a number of configurations, in which the microfabricated ring shaped, disk shaped or

microtoroid shaped resonators on a chip, the stand-alone dielectric microspheres and the so called capillary-based opto-fluidic ring resonators are the most common examples. The chip-based ring resonators present some advantages which include the capability of opto-electronic integration, but, apart from the microtoroid configuration, they usually present problems of low quality factors (Q-factor), which are designated as all intrinsic and extrinsic losses occurred in the optical resonant cavity system [52] and in this case, these problems are related to their surface roughness. These types of ring resonators are very well presented and discussed in Fan's review. [46]

- Photonic crystal

This class of biosensors is, in fact, an evolution of the optical fiber based ones. They are formed by photonic crystal microcavities, obtained by introducing a defect in periodically organized microstructured holes, usually of silica, by altering their dimensions. Some can be embedded with molecules, which are responsible for the occurrence of a change in the refractive index of the biosensor, leading to a detectable signal in the form of a spectral shift of the resonant wavelength of the photonic crystal cavity. Also, polymers can be used as a coating layer for the photonic crystal cavities, as showed by Chakravarty et al., [53] which doped the photonic crystal microcavities with a quantum dot and coated it with anion-selective polymer. With this procedure, they were able to construct a sensor with good properties, such as a very specific and accurate detection for changes of perchlorate anions and calcium cations at submicro concentrations in solution, while Lee et al. [54] presented a photonic crystal suitable for protein and single particle detection. In their experimental and theoretical work, they claimed their device achieved a sensing volume of 0.15 μm^3, and that it presented a limit of detection as small as 1 fg. They also determined its performance for particles in the size range of a variety of viruses, using latex spheres as models.

In a recent work, Aroua et al.[55] have studied, also by experimental and theoretical means, a label-free biosensor in order to determine it characteristics, the field intensity and the resonant wavelength shift when the nanocavities of the photonic crystal are filled with blood plasma, water or dried air. With this protocol, they showed that the enhancement on sensitivity is related to the photonic crystal design parameters.

2.8. Carbon nanotubes and graphene-based biosensors

Some new materials had also found a great deal of applications, especially in biosensing, such as carbon nanotubes and lately, graphene. As for single-walled carbon nanotubes, (SWNTs), they are known to exhibit unique intrinsic properties, which include a semiconductive behavior and photophysical propertiesdependent of their structure. For example, nanotubes with some chirality, band gap fluorescence is observed, as well as strong resonance Raman scattering.In this way, hybrid materials of SWCNTs and biomolecules is a way to obtain good materials for biosensing applications, since the fluorescence band-gap of SWNTs is highly sensitive to its environment and show shifts when the nanotube is in contact with other molecules.

In their work, Jin et al. [56] proposed the construction of a platform for selectively determine the hydrogen peroxide efflux from living cells, in order to biosensing human carci-

noma, in an array of fluorescent single-walled carbon nanotubes. In this biosensor, the carbon nanotubes have their fluorescence quenched when H_2O_2 is liberated by A431 human epidermal carcinoma cells, in response to the epidermal growth factor. They show that this array is able to distinguish between peroxides originated on the cell membrane from other contributions.

Also to show the versatility of carbon nanotubes, Chen et al. [57]presented a sensitive method for multiplexed protein detection by using functionalized single-walled carbon nanotubes (SWNTs) as multicolor Raman labels. They claim that this method is a good alternative for standard fluorescence-based techniques since, unlike fluorescence, Raman detection benefits from the sharp scattering peaks of SWNTs with minimal background interference. Also, it can be combined to surface-enhanced Raman scattering substrates, allowing protein detection sensitivity down to 1×10^{-15}Mol L^{-1}, which is three orders of magnitude minor then the detection limit of fluorescence-based methods. They used these modified SWNT to Raman detection of human autoantibodies against proteinase 3, a biomarker for the Wegener's granulomatosisautoimmune disease, and by conjugating different antibodies to pure (12)C and (13)C SWNT isotopes, they had demonstrated the multicolor Raman protein detection.

In their work,Morales-Narvaéz and Merkoçi[58] took advantage of graphene´sinnovative mechanical, structural (several graphenes present lattice-like nanostructures), electrical, thermal and opticalproperties. They employed graphene oxide (GO) as a biosensing platform due to its ability of nanoassemble in wire form when in presence of biomolecules, its processability in solution and due to its heterogeneous chemical and electronic structure, which confers to GO the ability to be used as insulator, semiconductor or semi-metal. Also, they presented graphene oxide as a universal highly efficient long-range quencher, with the perspective of been applied to several novel biosensing strategies.

Phan and Viet also worked on testing graphene application on biosensors by replacing carbon nanotubes for graphene ribbons in biosensors,[59] which were able to sense the transition of DNA secondary structure from the native right-handed form to the alternate left-handed form.

Although studies ongraphene´s propertiesare still preliminary, it is thought as a promising platform for biosensing. In their review, Yang et al. [60] discuss all aspects of functionality, performance, properties, fabrication, handling and challenges of these carbon-based materials as part of biosensors. Ina critical analysis, they present the great opportunities yet to come with the use of these materials and point us what is necessary to have in mind when proposing a new architecture for biosensors and new materials to be employed. Nevertheless, graphene application on bioelectronics is still controversial.

3. Construction basics

The devices architectures that have been found in the literature and in the market are as numerous as the applications that they find. As well resumed by Reardon et al., [61] optical

sensing opens a wide range of methodologies that can be based in either one of the electromagnetic wave parameters analysis, as their phase, polarization and amplitude. Analysis and detection methods applied to the amplitude parameters are the most common, due to the direct information that they can give about the system and due to the possibility of coupling this information to those obtained by polarized systems or phase optics.

There are no limits to explore regarding to optical properties or to instrumentation to be applied, alone or combined. Nevertheless, the most common optical approach is to promote the interaction of the analyte with light to produce light as response. Conventional methods permit the detection of absorption, reflection or scattering of light, through which it is possible to infer about the interaction between light and the analyte through signal changes.

Most elegant methods employ the detection (and promotion) of emitted light from the analyte, which in general relies on larger wavelengths that those used to illuminate the sample. The wavelength shift can be caused by either nonlinear interaction processes between light and analyte, as harmonic or inharmonic oscillations, such as vibrational modes that give rise to Raman shifts or by the electronic excitation of a system that result in the loss of a portion of the excitation energy as photons through luminescent phenomena that occur at shifted wavelength range. These luminescent processes include fluorescence and phosphorescence, which differ from each other with respect to the quantum levels involved in each electronic transition and that are affected by the interaction with the environment and, consequently, to analyte concentration.

There are diverse new methods of optical detection involving combined techniques to deliver real-time imaging of the sample. In this sense, fluorescence or Raman confocal microscopies are coupled to time-resolved fluorescence or to Raman spectrophotometers and images obtained can be evaluated in terms of concentration, energetic processes, charge transfers and localized events.Once detection methods and finality of the biosensor are defined, the size scale must be determined and, then, convenient materials for the construction of the biosensor must be selected and tested.

With respect to materials, it is important to consider environmental, economical and sustainable issues when choosing the ideal material combination for a biosensor. It is important to elect safe and ease of processing materials that can lead to a cheap method of fabrication and, in addition, can be recycled. Also, it needs to be chemically stable and permits a good selectivity and sensibility to the biosensor. Thus, the functional nanostructures can be composed of metals, semiconductors, magnetic materials, quantum dots, molecular or polymeric dyes and some hybrid materials, proposed to achieve any specific property that is an issue in other materials. [62]

Electrodes must be also adequately elected, with respect to work function as well as to processing. Usually, gold surface is preferred due to its possibility of modification with sulfur containing biological elements, such as SH-protein and antibodies, but also due to its optical and low potential properties, which enables refractive index measurements by several techniques, as mentioned in previous sections. Nevertheless, there are some alternative electrodes, such as metal-modified carbon electrodes, as those described by Wang et al. in which

carbon electrodes were modified with rhodium [63], ruthenium [64] and platinum [65] to achieve lower potentials. More recently, some works presented Indium Tin Oxide thin layers as alternative electrodes for optoelectrical biosensors, with transmittance similar to the glass substrate. In their work, Choi et al. [66] showed that a 100 nm thick ITO layer as electrode permits simultaneous optoelectric measurements to record optical images and micro-impedance to examine time-dependent cellular growth.

Transduction methods are responsible for the identity of the biosensor and, thus for address their applicability. Since the first step on the optical transduction in a biosensor is the chemical interaction between the analyte and the indicator phase (the recognition element) that will produce the optically detectable signal, it is critical because it will determine the biosensor stability, selectivity and sensitivity as well as the optical region in which the effect will be observed and the best detection means. Also, it is important to take into account whatever the biosensor will be used in continuous measurements or in simple detection. It will inform if strong interactions will be needed and in what conditions the biosensor can be employed. If chemical reactions are the signal origin, such it is in catalysis-based biosensors, an immobilized enzyme is preferred, once it can permits steady-state measurements to explore the sample. In this case, high selectivity can be achieved using antibodies as reagents and basing sensing on competitive binding. Also, methods to immobilize the recognition element include adsorption on solid substrates, covalent bonding to a substrate and confinement by membranes with selective permeability. [67]

In optical fiber-based biosensors, immobilized recognition elements are also needed, as well as the optical fiber to enable remote measurements. The immobilization technique and the reagent must be well selected to avoid undesired effects such attenuation on fiber´s transmission efficiency, coupling of light into the fiber and attenuation characteristics of the fiber itself. Nevertheless, this is a good choice for transduction since it enables a range of photophysical processes to be detected, including light absorption, absorption followed by luminescence, light reflection and scattering, among others. Optical instrumentation is then selected, based on the most important photophysical effect. [68]

Finally, when proposing a biosensor, it is always good to make two considerations: first, nanoscaled materials are not really small to living bodies, they can be sensed as intrudersand be attacked and second, biosensors, in some way, will be employed in a living body. Issues related to toxicity, lifetime, stability, durability, mechanical properties, body adjustment, coherence and adaptability must be evaluated by a scientific and systematic method. It is not unusual to find information that was mistakenly collected, and conflicting results can be found on the same subject, leading to confusion. For example, carbon nanotubes are said to be health safe, nevertheless it is known that their structure is very similar to that of asbestos, great villain to miner´s lungs. Actually, aspired nanosize fibers of asbestos are recognized by the lung cells as an aggressive agent, which can cause lung cancer, pleural disorders, pleural plaques, pleural thickening, and pleural effusions. Another aspect is that asbestos present some properties such as electricity insulate and is a chemically resistant material and it is quickly incorporated in many materials.

4. Applications of biosensors

Biosensors find various applications, limited only by the creator´s imagination. Since they can be constructed on unlimited configurations, based on the most diverse detection and recognition methods, employing a whole world of polymeric materials, biological elements, luminescent organic and inorganic molecules, in film form or solution, their application can be as numerous as the elements combinations on their construction.Some of them can be numbered:

- Medicine development: for example, cellular biosensors are engineered to optically report specific biological activity. Since they employ living cells in their construction, they can be used to form a "data basis"of cell behaviour when in the presence of several actives, which can be used for design new drugs and medicines.

- Drug Abuse or addiction: detection (and quantification) at nanoscale are possible due to the recent biosensors development. This feature is essential in the determination of the limits at which a person can be susceptible to addiction reactions. In this way, biosensor can limit whether a drug can be used in therapeutics, avoiding the addiction. Furthermore, DNA biosensors act as potential detection devices for investigation of DNA–drug interactions, which can be used to elucidate the addiction mechanisms.

- Clinical diagnostics: this must be the most moving topic for the biosensors development. They are capable of detecting single-nucleotide polymorphisms, which are caused by gene mutations. Faster detection methods are developed every day, aiming the early detection of breast cancer, prostate cancer, AIDS, genetic diseases, bacterial and viral infections. Although clinically relevant point mutations are detected by piezoelectrical biosensors, optical biosensors also had a large contribution, especially the FRET-based biosensors, which are able to identify minor changes related to these mutations and permit the construction of nanodevices, a feature that has been attractive due to the possibilities offered by the nanoscaled biosensors perspective.

- Genome analysis: biosensors are able to inform about the interaction nature between proteins, peptides and DNA sequences present in the gene and identify it.They can also be used in genetic-engineered products proposaland in newdrugs development.

- Food Quality control: Toxins, microbes, bacteria, fungal contamination are all detectable by optical biosensors, as presented in former sections. In this way, they provide a mechanism of controlling the quality of food and industrial processes involved, contributing with the industrial development of preventing processes. Biosensors are also useful in monitoring fermentation processes, being applied on the food and beverage fabrication processes.

- Industrial microbiology monitoring: these analyses are indispensible in cosmetic and pharmaceutical industries, among others. By using biosensors, they can be accomplished in real time, avoiding product contamination and possible destruction.

- Good fabrication practices: nanobiosensors in the strategic steps of a product fabrication are able to detect minimum defects that could lead to the final product mischaracterization.

- Environmental safety: within this focus, biosensors can be useful for determining the type and concentration of contaminants present in an environment in which non specific studies or determinations had been previously made. Also, they find application on monitoring aspects changes of the considered environment and, in this case, the objective is to track the concentration of known contaminants over time at one or more defined locations. Employing biosensors to do this task, confers them one more important advantage, which consists of using their ability to provide continuous measurements of the environment, enabling for instance, the full characterization of contaminated sites. The environmental monitoring can be extended to systems such soil, water sources, such as ground water wells, rivers, lakes, and can be extrapolated to industrial factories on monitoring water treatment stations. [61]

- Agriculture and cattle monitoring: biosensors can be used to monitor the cattle, in a way to protect it from mad cow disease or foot and mouth disease (FMD) guarantying the quality of the meat, as well as fungal contamination of vegetables, enabling prevention and the health growth of the plants. Also, it enables the correct application of pesticides and fertilizers, at controlled amounts per field size, by recognizing the effect of quantities of these compounds in different types of soil, places with distinct sun, rain or wind exposure, natural factors that cause a variation in the concentration limits or bring about new compounds to the site. In this approach, in situ biosensors would provide feedback to farmers on how to maintain their culture healthy.

- Military defense: hazards biomolecules, such as salmonella, anthrax, the H1N1 influenza virus, in the military defense point of view can represent a threat, since they can be considered powerful weapons in terrorists hands. The Salmonella bacteria fiber optic biosensor, developed by Zhou et al. [69] in the late nineties, for example, is able to detect this pathogen in food and water, with a sensitivity as low as 10^4 cell colony forming unit/ml.

- Microfluidic sensing and implantable biosensors: microfluidic technique is based on the fact that all biological molecular reaction takes place in liquid environments. The microfluidic channels work as a guide for fluids and biofluids carrying the target or the sample to be tested by the biosensor. The microfluidic technique provides a controlled flow of quantities of fluids, in microscale, containing desired compounds. As they intend to provide information on biological living systems, they usually employ as fluidic carriers buffers, aqueous suspensions of analytes (proteins or antibodies), bacterial cell suspensions, and even whole blood samples. Microfluidic devices and implantable biosensors fabrication are intimately related by the fact that both need to be fabricated using biocompatible materials. Many are the options to achieve the biocompatibility suggested and usually choices are between mimetic or inert materials. The most common inert materials employed are polydimethysyloxane, thin laminated plastic components made of polyimide or poly methylmethacrylate and conductive electroactive polymers combined with hydrogels. These are interesting due to the fact that they give rise to a class of multifunctional, bioactive polymers which are proposed aiming several biotechnological applications. As

showed by Guiseppi-Elie, [70] when polymers such as polypyrrole and polyaniline are combined to hydrogels, they generate bioactive polymers that act as bioreceptor hosting membranes of enzyme-based implantable biosensors.

• Aging research: optical biosensors based in fluorescence and bioluminescence emissions are proposed as devices for monitoring certain hormones dosage in the living body, as well as the release and the action of drugs administrated via dietary paths in these hormone levels. [71] The proposal is that an implantable biosensor could, in periods of days or even in the lifespan of an individual, inform about hormone fluctuations and valuable insights into the mode of action of the intervention could result from this monitoring. It is well known that in dietary restriction and other interventions, hormones such as insulin show profound changes. To be able to continuously monitor blood levels of these hormones, drugs or other factors by less invasive methods, could allow, for example, the adjustment of the hormone levels, retarding aging processes and preventing aging diseases.

5. Insights into the future

The first insight that is important to consider must be whether there are or not advantages in developing such a variety of biosensors prior to conventional detection systems. The most important advantage that one can think of refers to quick and in vivo early detection of severe conditions, which will lead to mature diagnostics at the earlier stages of diseases that today are challenging. With that and searching for utilizing all information on materials and techniques earned from scientific issues of other natures, such as utilizing the supramolecular chemistry to design new materials and coupled methods of detection that confer richer and reliable information, biosensor development contributed to the development of a number of research fields with some common interests. For example, more accurate information on microfluidic electronics to build sensors; new organic, hybrid and inorganic materials with interesting physical, chemical and morphological properties needed to be proposed; better methods to prepare homogeneous and thin films; new supports and electrodes are constantly demanded; new techniques for characterization and properties evaluation, which ask for improvements and new approaches to exploit all features of biosensors; new methods of modification and immobilization of biological elements, to cite some of these contributions.

Now, even greater perspectives are lying in the biosensor´s development into the nanotechnology approach. It had been demonstrated that the use of nanobiosensors can provide several advantages not yet considered for conventional systems. They can be built under the nanoscale limit, they need to use similar materials to those used in the large scale devices, but in much smaller sizes. We know for sure that nanoscaled materials provide quite distinct properties, which could generate distinct biosensors, maybe even more sensitive and selective biosensors. For example, metallic nanoparticles have their bandgap energies widen at nanoscale, which turns to be a very important optical effect, since it generates a quantum confinement expressed by the observed spectral blue-shifts; magnetic materials show super magnetic behavior at nanoscale [72, 73, 74] other semiconducting

nanoparticles present tunneling and Coulomb blockade effects [62]and all these impressive properties are not observed for the same thick materials. Some properties are clearly added to the biosensors processed at nanoscale, along with some thought advantages. The first ones that come directly to our minds are the possibility of using a reduced amount of materials in the device construction; reduced power is needed to make biosensors to work and, with it, they can be thought as portable, once they do not require a power source ratter then a small battery; they can be more stable with time; easier to recycle or dispose and, of course, since in nanoscale, we are leading with some new properties, it is expected that the nanobiosensors produced might posses new properties and so, new capabilities. With respect to detection and operation of these devices, detection processes thought to be simpler and faster, in constructions that tend to be more friendly, since it do not need bulky detection systems, conferring also the direct advantage of low cost of construction.

Nanobiosensors are thought to be as simple as possible, so, in the most recent proposed devices, the more acceptable approach is the label-free nanobiosensor. In this way, in a biosensor, the recognition element does not need to interact with other molecules or labels. There is no need to target the analyte or activate the biosensor by processes as conjugation with an enzyme, resonant energy transfer to generate the desired fluorescence, or chemical processes generated by the incorporation of molecules in order to functionalize the recognition element to produce a luminescent response, and others. In these biosensors, the probe and target-binding or substrate reactions are expected to be recorded by the transducer in the absence of any labelrequirement. Nevertheless, applications of such nanodimensioned devices are of a great variety, it is evident that the major target is the diagnostic and medical ones. Since these biosensors consist of a nanoscale detection method, they can sense target molecules in very low concentration into the body, which consist of a key factor in early detection of diseases such as breast cancer and AIDS. In fact, this is the idea behind the home method to detect AIDS, the OraQuick® HIV test presented by Orasure Technologies Inc. Their portable sensor comprises a visually read, qualitative flow immunoassay for the detection of antibodies to HIV-1 and HIV-2. In the device, HIV-1 and HIV-2 antigens are immobilized on a nitrocellulose strip and reactive antibodies are visualized by colloidal gold labeled with protein-A. The oral fluid are collected directly on the device and the positive test appears within 20 minutes as a purple line at the visor.[75]

Nevertheless, a point that needs to be made is, clearly miniaturization is the focus of many research that have been conducted in this theme, but it also can bring about some undesired properties, which need to be taken into account when engineering a biosensor. It means that not everything in nanoscale is adequate. One must have in mind which problem needs an answer, otherwise there is no scientific method, only the old fashioned "accidental discovery".

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References

[1] Clark LCJr, Lions C. Electrode Systems For Continuous Monitoring In Cardiovascular Surgery. Annals of the New York Academy of Sciences 1968; 102 29-45.

[2] Shi J, Cha TG, Claussen JC, Diggs AR, Choi JH, Porterfield DM. Microbiosensors Based on DNA Modified Single-Walled Carbon Nanotube and Pt BlackNanocomposites. Analyst 2010; 136 4916-4924.

[3] Leyden MR, Messinger RJ, SchumanC, Sharf T, Remcho VT, Squires TM, Minot ED.Increasing the Detection Speed of an All-Electronic Real-Time Biosensor. Lab Chip 2012; 12 954-959.

[4] Chen X, Xu S, Yao N, Shi Y. 1.6 V Nanogenerator for Mechanical Energy Harvesting Using PZT Nanofibers. Nano Letters 2010; 10(6) 2133-2137.

[5] Park K, Xu S, Liu Y, Hwang GT, Kang SJL, Wang ZL, Lee KJ. Piezoelectric BaTiO3 Thin Film Nanogenerator on Plastic Substrates. Nano Letters, 2010; 10 4939–4943.

[6] Urgun-Demirtas M, Stark B, Pagilla K. Use Of Genetically Engineered Microorganisms (Gems) For The Bioremediation Of Contaminants. Critical Reviews in Biotechnolog 2006; 26(3) 145– 164.

[7] D'Souza SF. Microbial Biosensors. Biosensors and Bioelectronics 2001; 16, 337–353.

[8] Ahuja T, Mir IA, Kumar D, Rajesh K. Biomolecular Immobilization On Conducting Polymers For Biosensing Applications. Biomaterials 2007; 28(5) 791–805.

[9] Malhotra BD, Chaubey A, Singh SP. Prospects of Conducting Polymers In Biosensors.AnalyticaChimicaActa 2006; 578(1) 59–74.

[10] Shulga AA, Soldatkin AP, Elskaya AV, Dzyadevich SV, Patskovsky SV, Strikha VI. Thin-Film Conductometric Biosensors for Glucose And Urea Determination. Biosensors and Bioelectronics 1994; 9 217–223.

[11] Opitz N, Lubbers DW.New Fast-Responding Optical Method to Measure PCO2 In Gases and Solutions, PflugersArchiv-European Journal of Physiology 1975;355(S) R120.

[12] Lubbers DW, Opitz N. PO2-Optode, A New Tool To Measure PO2 Of Biological Gases And Fluids By Quantitative Fluorescence Photometry.PflugersArchiv-European Journal of Physiology 1975; 359(S) R145.

[13] de Silva, A.P.; Gunaratne, H.Q.; Gunnlaugsson, T.; Huxley, A.J.; McCoy, C.P.; Rademacher, J.T.; Rice, T.E. Signaling Recognition Events with Fluorescent Sensors and Switches. Chem. Rev. 1997, 97, 1515-1566.

[14] Johnson I. Fluorescent Probes for Living Cells. Histochemistry Journal 1998; 30 123-140.

[15] Terai T, Nagano T. Fluorescent Probes for Bioimaging Applications. Current Opinion in Chemical Biology 2008; 12 515-521.

[16] Chen JH, Fang ZY, Liu J, Zeng LW. A Simple And Rapid Biosensor For Ochratoxin A Based On A Structure-Switching Signaling Aptamer, Food Control 2012; 25(2) 555-560.

[17] Zargoosh K,Chaichi MJ,Shamsipur M,Hossienkhani S,Asghari S,Qandalee M. Highly Sensitive Glucose Biosensor Based On The Effective Immobilization Of Glucose Oxidase/Carbon-Nanotube And Gold Nanoparticle In Nafion Film And Peroxyoxalate-Chemiluminescence Reaction of a New Fluorophore. Talanta 2012; 9337-43.

[18] Hu P, Zhu CZ, Jin LH, Dong SJ. An Ultrasensitive Fluorescent Aptasensor for Adenosine Detection Based on Exonuclease III Assisted Signal Amplification. Biosensors & Bioelectronics 2012; 34(1) 83-87.

[19] Reed B, Blazeck J, Alper H. Evolution of an Alkane-Inducible Biosensor for Increased Responsiveness to Short-Chain Alkanes. Journal of Biotechnology 2012; 158(3) 75-79.

[20] Pavan S,Berti F. Short Peptides As Biosensor Transducers, Analytical And Bioanalytical Chemistry 2012; 402(10) 3055-3070.

[21] Gonzalez-Andrade M, Benito-Pena E, Mata R, Moreno-Bondi MC. Biosensor For On-Line Fluorescent Detection Of Trifluoroperazine Based On Genetically Modified Calmodulin, Analytical And Bioanalytical Chemistry 2012; 402(10) 3211-3218.

[22] Hinde E,Digman MA, Welch C, Hahn KM,Gratton E. Biosensor Forster Resonance Energy Transfer Detection By The Phasor Approach To Fluorescence Lifetime Imaging Microscopy, Microscopy Research and Technique, 2012; 75(3)271-281

[23] Lakowicz JR. Principles of Fluorescence Spectroscopy; Second Edition Kluwer Academic/Plenum Publishers: NY. 1999.

[24] Giepmans BNG, Adams SR, Ellisman MH, Tsien RY. The Fluorescent Toolbox for Assessing Protein Location and Function. Science 2006; 312 217-224.

[25] Martins TD, de Souza MI, Cunha BB, Takahashi PM, Ferreira FF, Souza JA, Fileti EE, Alves WA. Influence of pH and Pyrenyl on the Structural and Morphological Control of Peptide Nanotubes. Journal of Physical Chemistry C 2011; 115 7906–7913.

[26] Parak WJ, Pellegrino T, Plank C. Labelling of Cells with Quantum Dots. Nanotech 2005; 16, R9-R25.

[27] Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Quantum Dots for Live Cells, In Vivo Imaging, and diagnostics. Science 2005; 307 538-544.

[28] Alivisatos AP, Gu W, Larabell CA. Quantum Dots as Cellular Probes. Annual Reviews on Biomedicine and Engineering 2005; 7 55-76.

[29] Cheung HC, Resonance Energy Transfer InLakowicz J.R. (ed): Topics In Fluorescence Spectroscopy: vol 2: Principles, Kluwer Academic Publishers, NY 2002.p128-176.

[30] Liu B, Bazan CG. Homogeneous Fluorescence-Based DNA Detection with Water-Soluble Conjugated Polymers. Chemistry of Materials 2004; 16(23) 4467–4476.

[31] Yildirim N, Long F, Gao C, He M, Shi HC, Gu AZ. Aptamer-Based Optical Biosensor For Rapid And Sensitive Detection Of 17β-Estradiol In Water Samples. Environmental Science and Technology 2012;46(6) 3288-3294.

[32] Lin HY, Tsai WH, Tsao YC, Sheu BC. Side-Polished Multimode Fiber Biosensor Based On Surface Plasmon Resonance With Halogen Light, Applied Optics 2007; 46 800-806.

[33] Jorgenson RC, Yee SS. A Fiber-Optic Chemical Sensor Based on Surface Plasmon Resonance, Sensors AndActuators B: Chemical 1993; 12(3) 213-220.

[34] Suter JD, White IM, Zhu H, Fan X. Thermal Characterization Of Liquid Core Optical Ring Resonator Sensors, Applied Optics 2007; 46 389-396.

[35] Goldman ER, Medintz IL, Mattoussi H. Luminescent Quantum Dots in Immunoassays. Analytical and Bioanalytical Chemistry 2006; 384 560-563.

[36] Bustos ARM, Trapiella-Alfonso L, Encinar JR, Costa-Fernandez JM, Pereiro R, Sanz-Medel A. Elemental and Molecular Detection For Quantum Dots-Based Immunoassays: A Critical Appraisal. BIosensors and Bioelectronics 2012; 33(1) 165-171.

[37] Pinwattana K, Wang J, Lin CT, Wu H, Du D, Lin Y, Chailapakul O. CdSe/ZnS Quantum Dots Based Electrochemical Immunoassay for the Detection of Phosphorylated Bovine Serum Albumin, Biosensors & Bioelectronics 2012; 26 1109-1113.

[38] Zhang P. Investigation of Novel Quantum Dots/Proteins/Cellulose Bioconjugate using NSOM and Fluorescence. Journao o. Fluorescence 2006; 16 349-353.

[39] Sandros MG, Shete V, Benson DE. Selective, Reversible, Reagentless Maltose Biosensing with Core-Shell Semiconducting Nanoparticles. The Analyst 2006; 131 229-235.

[40] Tsay JM, Doose S, Weiss S. Rotational and Translational Diffusion of Peptide-Coated CdSe/CdS/ZnSNanorods Studied by Fluorescence Correlation Spectroscopy. Journal of American Chemical Society 2006; 128 1639-1647.

[41] Tsay, JM. Doose S, Pinaud F, Weiss S. Enhancing the Photoluminescence of Peptide-Coated Nanocrystals with Shell Composition and UV Irradiation. Journal of Physical Chemistry B 2005; 109 1669-1674.

[42] Chafer-Pericas C, Maquieira A, Puchades R. Functionalized Inorganic Nanoparticles Used As Labels In Solid-Phase Immunoassays, Trends in Analytical Chemistry 2012; 31 144-156.

[43] Ding S, Chen J, Jiang H, He J, Shi W, Zhao W, Shen J. Application Of Quantum Dot-Antibody Conjugates For Detection Of Sulfamethazine Residue In Chicken Muscle Tissue, Journal Of Agricultural And Food Chemistry 2006; 54 6139-6142.

[44] Li H, Cao Z, Zhang Y, Lau C, Lu J. Combination Of Quantum Dot Fluorescence With Enzyme Chemiluminescence For Multiplexed Detection Of Lung Cancer Biomarkers , Analytical Methods 2010; 2 1236-1242.

[45] Han XX, Cai LJ, Guo J, Wang CX, Ruan WD, Han WY, Xu WQ, Zhao B, Ozaki Y. Fluorescein Isothiocyanate Linked Immunoabsorbent Assay Based On Surface-Enhanced Resonance Raman Scattering, Analytical Chemistry, 2008; 80 3020-3024.

[46] Fan X, White IM, Shopova SI, Zhu H, Suter JD, Sun Y. Sensitive Optical Biosensors For Unlabeled Targets: A Review, AnalyticaChimicaActa2008; 620 8-26.

[47] Liedberg B, Nylander C, Lunstrom I. Surface Plasmon Resonance For Gas Detection And Biosensing* Sensors & Actuators1983; 4299- 304.

[48] LiedbergB,LundstromI, Stenberg E. Principles of Biosensing with an Extended Coupling Matrixand Surface Plasmon resonance. Sensors and Actuators 1993; 11 63-72.

[49] Petryayeva E, Krull UJ. Localized Surface Plasmon Resonance: Nanostructures, Bioassays AndBiosensing—A review. AnalyticaChimicaActa 2011; 706 8– 24.

[50] Scarano S, Scuffi C, Mascini M, Minunni M. Surface Plasmon Resonance Imaging for Affinity-Based Biosensors, in P. Malcovati (eds), Sensors and Microsystems: AISEM proceedings 2009, Lecture Notes in Electrical Engineering. 2010; 54(part 5). p425-428.

[51] Schneider BH, Edwards JG, Hartman NF. Hartman Interferometer: Versatile Integrated Optic Sensor For Label-Free, Real-Time Quantification Of Nucleic Acids, Proteins, And Pathogens. Clinical Chemistry 1997; 43(9) 1757-1763.

[52] Armani AM. Single Molecule Detection Using Optical Microcavities In:, Chapter 11 (), In: Chremmos I (eds.), Photonic Microresonator Research and Applications Springer Series in Optical Sciences: Springer Science+Business Media, LLC. 2010; 156.p253-273.

[53] Chakravarty S, Topol'ancik J, Bhattacharya P, Chakrabarti S, Kang Y, Meyerhoff ME. Ion Detection with Photonic Crystal Microcavities. Optical Letters 2005; 30 2578-2580.

[54] Lee MR, Fauchet PM. NanoscaleMicrocavity Sensor For Single Particle Detection Optical Letters 2007; 32 3284-3286.

[55] Aroua W, Haxha S, AbdelMalek F. Nano-Optic Label-Free Biosensors Based On Photonic Crystal Platform With Negative Refraction Optics Communications 2012; 285 1970–1975.

[56] Jin H, Heller D A, Kalbacova M, Kim JH, Zhang JQ, Boghossian AA, Maheshri, StranetMS. Detection of single-molecule H_2O_2Signalling From Epidermal Growth Factor Receptor Using Fluorescent Single-Walled Carbon Nanotubes. Nature Nanotechnology 2010; 5 302–309.

[57] Chen Z, Tabakman SM, Goodwin AP, Protein microarrays with carbon nanotubes as multicolor Raman labels. Nature Biotechnology 2008; 26 1285–1292.

[58] Morales-Narváez E, Merkoçi A.Graphene Oxide: Graphene Oxide as an Optical Biosensing Platform. Advanced Materials, 2012; 24(25) 3298–3308.

[59] Phan AD, Viet NA. A new type of optical biosensor from DNA wrapped semiconductor graphene ribbons. Journal of Applied Physics 2012; 11, 114703 (5 pages)

[60] Yang W, Ratinac KR, Ringer SP, Thordarson P, Gooding JJ, Braet F. Carbon nanomaterials in biosensors: should you use nanotubes or graphene?, AngewandteChemie International Edition in English, 2010; 49(12) 2114-38

[61] Reardon KF, Zhong Z, LearKL. Environmental Applications of Photoluminescence-Based Biosensors. Advances in Biochemistry Engineering/Biotechnology 2009; 116 99–123.

[62] Janagama DG, Tummala RR. Nanobiosensing Electronics and Nanochemistry for Biosensor Packaging C.P. In: Wong (eds.) Nano-Bio- Electronic, Photonic and MEMS Packaging, C Springer Science+Business Media 2010. p613-663 DOI 10.1007/978-1-4419-0040-1_17

[63] Wang J, Chen Q, Pedreno M. Highly Selective BiosensingOf Lactate At Lactate Oxidase Containing Rhodium Dispersed Carbon Paste Electrodes.AnalyticaChimicaActa 1995; 304 41-46.

[64] Wang J, Fang L, Lopez D, Tobias H. Highly selective and sensitive amperometric biosensor of glucose at ruthenium-dispersed carbon paste enzyme electrodes. Analytical Letters 1993; 26(9) 1819-1830.

[65] Wang J, Naser N, Angnes L, Wu H, Chen L. Metal-dispersed carbon paste electrodes. Analytical Chemistry 1992; 64 1285-1288.

[66] Choi CK, Kihm KD, English AE. Optoelectric biosensor using indium-tin-oxide electrodes, Optics Letters 2007; 32 (11) 1405-1407.

[67] Seitz WR. Transducer mechanisms for optical biosensors. Part 1: The chemistry of transduction, Comput Methods Programs Biomedicine 1989; 30(1) 9-19.

[68] Ashworth DC, Narayanaswamy R. Transducer mechanisms for optical biosensors. Part 2: Transducer design, Computer Methods Programs in Biomedicine 1989; 30(1) 21-31.

[69] Zhou C, Pivarnik P, Rand A G, Letcher SV. Acoustic standing-wave enhancement of a fiber-optic Salmonella biosensor. Biosensors and Bioelectronics 1998; 13(5) 495–500.

[70] Guiseppi-Elie A. An Implantable Biochip to Influence Patient Outcomes Following Trauma-induced Hemorrhage, Journal Analytical and Bioanalytical Chemistry 2011, 399 403-419

[71] He Y, Wu Y, Mishra A, Acha V, Andrews T, HornsbyPJ. Biosensor technology in aging research and age-related diseases, Ageing Research Reviews 2012;11 1– 9.

[72] Woods S. I., Kirtley J. R., Sun S., Koch S. H., Direct investigation of superparamagnetism in Co nanoparticle films. Physical Reviews Letters 2001; 87(13) 137205(4 pages).

[73] Min C, Shao H, Liong M, Yoon TJ, Weissleder R, Lee H. Mechanism of Magnetic Relaxation Switching Sensing, ACS Nano, 2012, article ASAP DOI: 10.1021/nn301615b.

[74] Bogart L K, Taylor A, Cesbron Y, Murray P, Lévy R.Photothermal Microscopy of the Core of Dextran-Coated Iron Oxide Nanoparticles During Cell Uptake, ACS Nano, 2012, Article ASAP, DOI: 10.1021/nn300868z.

[75] Lee SR, Guillon G, Ferko G, DeTurk C, Hershberger M, Marshall T, Kardos K. Performance of a Rapid Point of Care Test for HIV Antibodies, OraSure Technologies, Inc. http://www.orasure.com/docs/pdfs/products/infectious/Performance-Rapid-Point-Care-Test-HIV-Antibodies.pdf (accessed 10 July 2012)