Curso

Dispositivos Biosensores: desde el diseño a la aplicación real

Biosensor devices: from design to practical implementation

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A biosensor can be defined as a compact analytical device incorporating a biological or biologically-derived sensing element either integrated within or intimately associated with a physicochemical transducer. The aim of a biosensor is to produce discrete or continuous digital electronic signals which are proportional to a single analyte or a related group of analytes. The biological element is capable of sensing the presence, activity or concentration of a substance in solution. These interactions produce a measurable change in a solution property, which the transducer converts into a quantifiable electrical signal. Therefore, biosensor is a general term for a wide range of devices that measure the presence or concentration of biological or chemical molecules by translating a biomolecular interaction at the probe surface into a quantifiable physical signal.

The development of biosensor devices has been a very active research field both in academic and in industrial laboratories during the last decades. This technology could be a serious alternative to conventional assay techniques because they can avoid expensive, complex and time-consuming procedures. A large number of applications for these devices range from clinical diagnostics, drugs research at pharmaceutical industry, online screening of water quality for both household and industrial use, control of industrial processes, veterinary field, food industry or environmental monitoring, among others. Ideally, for all these applications, it will be desirable to have a compact sensor with response time as low as possible, allowing real-time measurement and with a high sensitivity. The most widely used and well-known biosensor is the glucose one, which is used diary by thousands of people all around the world.

There are two important steps in the development of any biosensor device:

(i) The design and fabrication of a high sensitive physical transducer, that is, a device capable of transform efficiently the biomolecular reaction in a measurable signal.
(ii) The stable and reliable biofunctionalization of the physical transducer with the appropriate selective biological receptor (proteins, DNA, cells, aptamers,…)

(i) Physical transducers

There are several physical methods to obtain a transducing signal like those based on amperometric, potentiometric or acoustic systems. However, transducers that make use of optical principles offer more attractive characteristics such as the immunity to electromagnetic interference, the use in aggressive environments and, in general, a higher sensitivity. Generally, in optical transducers, the chemical or biological stimulus produces changes in the characteristics of the medium in contact with the light path, like a variation in its emission properties (luminescence), in the absorption coefficients or in the refractive index. This variation will induce a change in the propagation properties of light (wavelength, intensity, polarisation, phase velocity). Several techniques have been proposed to measure the induced

change in the propagation properties of light. One of the most successful label-free and commercially accepted optical biosensors is the Surface Plasmon Resonance (SPR) sensor. This biosensor is widely employed for determination of many types of interactions and hundreds of publications appear in the literature every year. The detection limit of actual SPR devices is below 1 pg.mm^{-2}. Other optical biosensors make use of optical waveguides as the basic element of their structure for light propagation and are based on the evanescent field sensing. For example, the interferometer device (as Mach-Zehnder or the Young interferometer) based on waveguides is able to achieve impressive sensitivity levels in a label-free and real-time scheme in the range of 0.1 pg/mm^2 (picomolar concentration) which means a detection limit of 10^{-8} in refractive index.

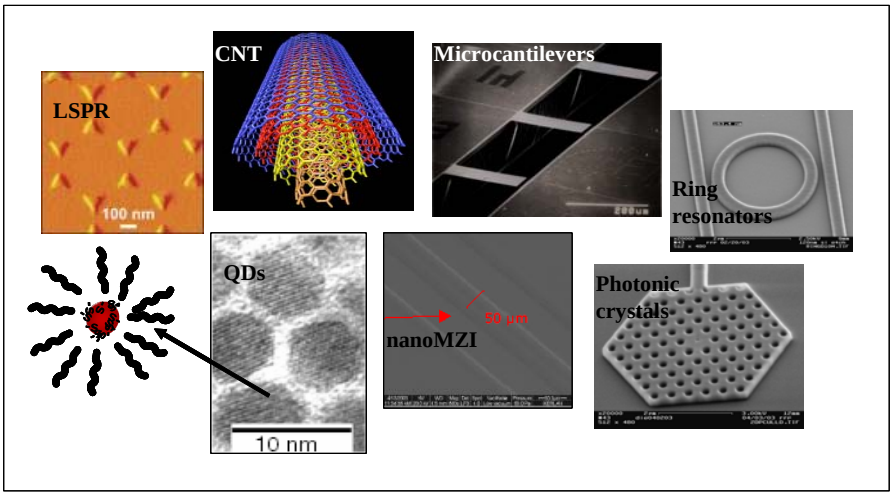

Fig.1 Schemes and photographs of some nanodevices which has been proposed for biosensing.

In recent years several interesting nano-developments (see Fig. 1) have been proposed as highly sensitive transducers for biosensing (as metal or magnetic nanoparticles, quantum dots, carbon nanotubes, photonic crystals, ring resonators, nanocatilevers, etc.,). Although all of these developments are interesting from a scientific point of view, the real implementation of much of them is still hampered by several factors. First, most of them still require the use of labels to readout the biomolecular interaction, which are undesirable for real applications (direct reading is much more precise). Second, the path to connect such nano-developments to the operation in a real world environment has not been paved and large and complicated laboratory setups are needed for signal acquisition and processing.

In contrast, micro/nanobiosensor devices based on microelectronics and related (BIO)MEMS/NEMS technologies could provide a technological solution for achieving label-free devices which could be operated in a stand-alone fashion outside a laboratory environment. This fabrication approach allows the flexible development of miniaturized compact sensing devices, microfluidics delivery systems and the possibility of fabricating multiple sensors on one chip, opening the way for high-throughput screening (see Fig. 2). Additional advantages are the robustness, reliability, potential for mass production with consequent reduction of production costs, low energy consumption and simplicity in the alignment of the individual elements.

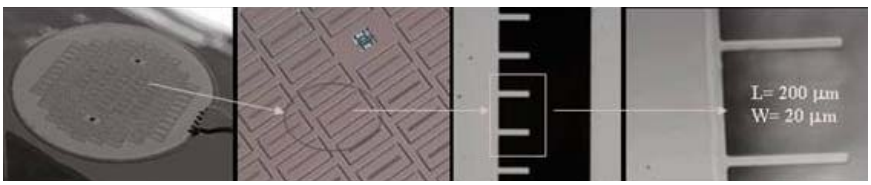

Fig. 2. Nanomechanical biosensors employed for clinical diagnosis

(ii) Biofunctonalization

The immobilisation of the receptor molecule on the sensor surface is a key point for the performance of the sensor. A selective biological coating (receptor) must be immobilized onto the sensing areas in order to make a highly selective surface. The chosen immobilisation method must retain the stability and activity of the bound biological receptor. The immobilisation methods available are divided in two general categories: (i) covalent coupling and (ii) affinity non-covalent interactions.

In the affinity bonding, a high affinity-capture ligand is non-reversible immobilised on the sensor surface: for example, streptavidin monolayers using biotinylated biomolecules for recognition. Another approach is to form a Self-Assembled Monolayers (SAM) of alkylsilanes and then the receptor can be coupled using the end of the SAM via a functional group (-NH_2, -COOH,...). The affinity binding can be performed with biotin conjugated ligands to avidin, streptavidin or neutravidin modified surfaces. The advantage of this procedure is an oriented binding ensuring equal binding sites. The functionalisation of the transducer also depends on the surface. Transducers with gold are usually modified with thiols, forming self-assembled monolayers. Oxidise surfaces can easily be modified with silane chemistry.

The Course will start with a basic introduction to biosensors, and will follow with a deep overview of the different physical transducer, with special emphasis in photonics and nanotechnology transducers. During the course, the design, fabrication, characterization, and testing of biosensor devices will be reviewed. In addition, the biofunctionalization techniques for biological receptor inclusion will be studied as well. Further integration of biosensors, microfluidics, and electronic functions on a single sensing circuit could lead to a complete ‘‘lab-on-achip’’ technological solution which could be used for performing in situ analysis. The way of the integration in ‘‘lab-on-a-chip’’ Microsystems will be also reviewed.

Bibligraphy

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