Genosensors: from small molecules to nucleic acids

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Biosensors are analytical devices that measure/detect biological or chemical substances by generating a signal proportional to the concentration of analyte. They are composed of three parts: a biorecognition layer, a transducer and a signal processor. Genosensors are biosensors whose biorecognition layer is composed of nucleic acids; these can be DNA, RNA, or an oligonucleotide mimic. Interest in this type of biosensor has increased exponentially in the last decade, mainly due to their versatility with applications in the environmental, food, health and drug discovery industries.
This talk will focus on three different transducers (field-effect transistors [1], electrochemiluminescent [2,3] and photoelectrochemical [4]) that can be used for the detection of various analytes, including small molecules and nucleic acids.
DNA hybridisation was detected using all three transducers. An electrolyte-gated field-effect transistor, with a two-dimensional channel made of a single graphene layer, was developed and was able to achieve label-free detection of DNA hybridisation down to attomolar levels [1]. Electrochemiluminescence (ECL) and photoelectrochemistry (PEC) were used to detect micro-RNAs associated with cancer [3,4]. In both assays, the excitation and readout of the signal are independent: an electrochemical stimulation followed by an optical readout for ECL, and the reverse for PEC. A 'sandwich' approach is incorporated, consisting of a biotinylated capture probe immobilized on streptavidin-functionalized surfaces and a detection probe labelled. Micro-RNAs were quantitatively measured in medical samples from prostate cancer patients by a low-cost electrochemical setup equipped using a LED and without the need for additional PCR or other amplification techniques.
Electrochemiluminescence was the approach chosen to develop a proof-of-concept assay for the detection and quantification of small molecules based on aptamer recognition [2]. Testosterone was used as the model small molecule and the assay showed selectivity and sensitivity, which opens a new avenue for the development of reliable and robust ECL biosensor assays for biochemical analysis.

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Disposable affinity electrochemical biosensing platforms: Towards reliable tools for food safety and personalized nutrition

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Food quality is nowadays a subject of great concern to society, as the incorrect labelling of food products can represent commercial fraud as well as a health concern, especially to allergen sensitized individuals who must strictly avoid consuming them. This fact represents one of the major challenges of modern clinical nutrition, whose task is the implementation of individualized nutritional recommendations. Although there are several analytical methods available for detecting food allergens, there is still an urgent need to develop alternative methods capable of meeting some features demanded by marketization including speed, simplicity, multiplexing capability, automation, miniaturization, and reduced costs. In this context, electrochemical biosensors combine the attractive advantages of electrochemical transduction strategies with those from biosensors technology. Among them, electrochemical affinity biosensors have been used in a large number of applications, focusing either on detecting allergenic proteins or genes encoding allergenic proteins or other specific DNA fragments, providing sufficient data to evaluate food quality, as well as to determine a variety of compounds at trace levels [1].

In this presentation, attractive bioelectroanalytical tools for the determination of analytes at different molecular level will be presented. In this sense, the main features of electrochemical immunoplatforms for determining allergenic proteins in fresh and processed foods at trace levels will be highlighted. Also, attractive nucleic acid-based bioplatforms for the sensitive, selective, simple, and rapid determination of relevant animal or plant-food derived nucleic acids, using genomic fragments characteristic of coding sequences of allergenic proteins will be described. Moreover, the possibility of further simplifying the methodology and/or improving the yield of gene extraction processes through isolation of genetic information containers, such as mitochondria and chloroplasts, which contain a higher number of copies per gene [2], has also been explored. All these methodologies have been implemented onto the surface of commercial magnetic microbeads and imply amperometric transduction at screen-printed electrodes, with no need for using nanomaterials and/or complex amplification strategies to make smooth their transition from the bench to the field, in addition to being easily extended to the determination of other relevant protein targets or nucleic acids regardless of their naturally occurring variety, origin and length (from very long intact nucleic acids to degraded samples, difficult to be analyzed by conventional PCR-based methodologies).

These smart biosensing platforms can be advantageously compared with other commonly used methodologies in terms of multiplexing, even at different molecular levels, simplicity, cost, assay time and portability, which make them particularly attractive analytical tools for the implementation of affordable cost and user-friendly devices for routine determinations in field to ensure food quality and consumer protection.

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