Advances in biosensor development based on integrating nanotechnology and applied to food-allergen management

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The risks associated with the presence of hidden allergens in the food chain have raised the need for fast, sensitive, and reliable methods to trace food allergens in different commodities.

We highlight advances and future trends in biosensor systems applied to food-allergen management. We discuss critical aspects of biosensor development with particular emphasis on integrating nanotechnology.

Keywords: Allergen; Biosensor; Electrochemical biosensor; Electromechanical biosensor; Electrochemical impedance spectroscopy (EIS); Food; Nanomaterial; Nanotechnology; Optical detection; Surface-plasmon resonance (SPR)

Abbreviations: Ab, Antibody; CCD, Charge-coupled device; CHOM, Chicken ovomucoid; CL-μarray, Chemiluminescence-based microarray; DNA, Deossiribonucleic acid; DPV, Differential pulse voltammetry; DVD, Digital versatile disk; EIS, Electrochemical-impedance spectroscopy; ELISA, Enzyme-linked immunosorbent assay; EU, European Union; f-EIS, Faradaic electrochemical-impedance spectroscopy; FPW-MEMS, Flexural plate-wave micro-electromechanical systems; Fluo-μarray, Fluorescence microarray; F-SLW, Front-surface long-wavelength; GCE, Glassy-carbon electrode; IgE, IgG, IgY, Immunoglobulin E, G, Y; IMS, Immunomagnetic separation; LS, Light scattering; NA, Not available; nf-EIS, Non-Faradaic electrochemical-impedance spectroscopy; NP, Nanoparticle; NB, Nile Blue; OVA, Ovalbumin; PCR, Polymerase chain reaction; P-L-Arg/MWCNT, Poly(l-arginine)/multi-walled carbon nanotube; QCM, Quartz-crystal microbalance; QD, Quantum dot; RT-CA, Real-time chronoamperometry; SERS, Surface-enhanced Raman scattering; SPR, Surface-plasmon resonance; SWV, Square-wave voltammetry

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1. Introduction

Food allergy is nowadays regarded as a problem of public-health relevance, the main concern being the unintentional exposure of allergic consumers to the offending ingredient through allergen-containing food. Even a little intake of allergen can trigger unpredictable, highly variable reactions, depending on the dose and the sensitivity of affected individual, thus compelling the allergic consumer to avoid allergen-containing food totally.

The European Community fixed labeling regulations for 14 allergenic food ingredients so it is mandatory for them to be labeled on the relevant food products [1]. Given the lack of official regulatory thresholds, allergen-labeling laws have taken a conservative approach to allergy-risk management, so that no level of the major food allergens can be deemed safe for consumers and the presence of any amount intentionally added to food must be labeled. However, these requirements only affect allergens used as ingredients, and the potential for undeclared allergens in foods is not addressed by the directive. The presence of an allergen can also be caused by cross-contamination in a shared production line or the raw-material or ingredient supply chains [2].

Both unlabeled contaminated products and labeled products with negligible contamination must be avoided, so precautionary labels should be used only where cross-contact is likely to occur and health risks are expected. In order to identify when such a situation applies, the concepts of assessing and managing the ‘‘risk’’ are applied to allergen contamination [3]. The probabilistic modeling of risk assessment,

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first described by Spanjersberg et al. [4] in 2007, is nowadays considered to be the most promising approach. This methodology allows an estimation of the allergic population percentage that may present a reaction due to the presence of a certain level of undesired allergen in a food product. This, evidently, asks the food manufacturers for a sound knowledge about the frequency and the extent of allergen cross-contamination during production and entails the critical choice of threshold-decision level. A risk of 0% would be only possible when the industry is able to produce food ‘‘free of allergens’’, but such an option is not cost effective for the consumer or the manufacturer [5]. Risk management focuses mainly on enforcement of good manufacturing practices (i.e. allergen clean-up and control measures). However, although allergen clean-up of shared processing lines has been identified as one of the critical points for effective allergen control, so far few investigations have focused on this topic. It is only recently that a certain amount of effort was put into evaluating the best practices at the industrial scale for allergen sanitization in order to reduce the undesired cross-contamination down to a threshold level at which it becomes irrelevant for public health [6,7]. Clearly, two main parallel research directions need to be followed:

- clinical investigations for the identification of relevant threshold levels; and,
- technological advances for validation and harmonization of analytical methods.

From the analytical point of view, the extreme sensitivity of allergens or their specific markers to various processing and matrix effects, and the consequent issues occurring in identifying reference materials hamper the development and the harmonization of reliable reference protocols. To fix some of these open issues, the effect of food processing on allergens is being considered more and more frequently, with particular emphasis on how such processing can modify the detection performance.

Complementary to confirmatory techniques {e.g., liquid chromatography mass spectrometry (LC-MS) [8,9]}, rapid diagnostic tools are increasingly being promoted for food companies to verify the efficiency of their management schemes for food safety [10].

As a result of a recent survey carried out within European Union (EU) networked project MoniQA [11], it was definitely established that the food industry is rapidly extending the range of rapid tests utilized, with particular interest in implementation of allergen-related test kits, ranked second in requests after microbiological tests [12]. Indeed, in food allergens, among other areas, rapid-test methods can provide a valuable tool for validating and verifying the effectiveness of sanitation practices, consequently minimizing the risk of cross-contact contamination.

The most commonly used rapid methods for routine monitoring are enzyme-linked immunosorbent assays (ELISAs) in 96-well-plate format. Even though some level of automation has been achieved in the recent years, ELISAs remain laborious, time consuming and expensive, particularly when multiple targets need to be screened.

Biosensors represent a potential alternative to ELISAs, and provide probably one of the most promising ways to solve some problems concerning simple, fast, reproducible, and cheap multi-analyte detection. A biosensor is an integrated receptor-transducer device, which converts the biological-recognition event into a measurable chemical-physical signal, which is proportional to the target concentration. The receptor can be an antibody raised against an allergen, a single-stranded DNA molecule capable of hybridizing with an allergen-specific DNA fragment, or an aptamer selected to recognize the target allergen directly.

Featuring high speed of execution, ease of use and high degree of automation, biosensors have all the potential for direct, real-time, on-line, monitoring of allergens along the production chain, and there remain important challenges (i.e. the dependence of the efficiency of allergen detection on matrixes and processing), both strictly related to the specific target and significantly affecting any biosensor performance. However, in recent years, great efforts were devoted to this innovative application field for biosensors (see Fig. 1).

In particular, taking advantage of advances in materials science, new opportunities of improvement of the current technology were experimented on, by implementing nanomaterials. The integration of the high specificity of biological receptors with the unique optical, electrical and electrochemical properties of the nanomaterials provides novel interesting alternatives to conventional platforms, by exceeding the sensitivity of existing techniques [13,14].

The present article discusses several critical aspects of biosensor development for food-allergen management. We review the literature of the past four years using a transduction-based classification (optical, electrochemical and electromechanical), which highlights the most important achievements and the new research trends, with particular emphasis on the potential for implementing nanotechnology.

2. Optical biosensors

Optical biosensors represent powerful detection tools with applications in food, healthcare, and environmental monitoring [15]. Combining the selectivity of biology with the processing power of modern microelectronics and optoelectronics, they offer great analytical potential with major applications in food safety, thanks to advantages of detecting analytes in complex matrices with minimal sample treatment. Most optical biosensors are based upon measurement of changes in the surface properties of a sensor chip when the analyte is bound to a sensing layer