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Invited critical review

Biosensors in clinical chemistry — 2011 update

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ABSTRACT

Research activity and applications of biosensors for measurement of analytes of clinical interest over the last eight years are reviewed. Nanotechnology has been applied to improve performance of biosensors using electrochemical, optical, mechanical and physical modes of transduction, and to allow arrays of biosensors to be constructed for parallel sensing. Biosensors have been proposed for measurement of cancer biomarkers, cardiac biomarkers as well as biomarkers for autoimmune disease, infectious disease and for DNA analysis. Novel applications of biosensors include measurements in alternate sample types, such as saliva. Biosensors based on immobilized whole cells have found new applications, for example to detect the presence of cancer and to monitor the response of cancer cells to chemotherapeutic agents. The number of research reports describing new biosensors for analytes of clinical interest continues to increase; however, movement of biosensors from the research laboratory to the clinical laboratory has been slow. The greatest impact of biosensors will be felt at point-of-care testing locations without laboratory support. Integration of biosensors into reliable, easy-to-use and rugged instrumentation will be required to assure success of biosensor-based systems at the point-of-care.

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1. Introduction

In an earlier report [1], biosensors were reviewed from the perspective of fundamental measurement technologies and their applications to clinical analysis, including examples of biosensors available as commercial products. Other recent reviews have covered advances in electrochemical biosensors from both the fundamental science and prospects for commercialization [2,3], progress and hurdles toward realizing implantable biosensors for clinical application [4] and biosensors for point-of-care testing, particularly those designed for home-use setting [5].

In this report, developments in technologies and applications of biosensors to clinical analysis over the past 8 years will be reviewed. A few recent examples of commercial biosensors for clinical analysis, in

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particular for point-of-care testing, will be included, but in general, commercialization of biosensors continues to lag behind research output as reflected by the numerous publications and patents appearing in the last 8 years. Many of the biosensor reports found in the literature demonstrate concepts to detect one or a few target analytes, however, biosensors with commercial promise should be based on versatile sensing technologies to support interchangeable recognition elements, the possibility for miniaturization to allow for parallel sensing [6], or biosensors based on technologies presenting performance advantages over existing methods, e.g., improved sensitivity or specificity. The latest work in the area of biosensors and their application to clinical chemistry will be reviewed in light of these requirements.

2. Biosensors based on nanotechnology

Perhaps the most notable trend in biosensor research over the past decade has been application of nanotechnology for construction of biosensors. Besides some analytical performance advantages discussed below, nanotechnology brings to biosensors the possibility for construction of biosensor arrays for high throughput parallel measurements and the possibility for integration of biosensors with microfluidics for construction of lab-on-a-chip devices. Nanotechnology has even been proposed as a method to enhance biocompatibility of biosensors and a possible solution to the problem of fouling of implantable biosensors — a problem which has hindered progress in the area of *in-vivo* sensing for years [7].

Nanotechnology is defined as the study of synthesis, properties and application of structures and materials having at least one critical dimension on the scale of <100 nm [8,9]. In relation to biosensors, a critical dimension is one directly related to the measurement function of the biosensor, such as a dimension that controls the area available for immobilization of a biorecognition element, or a dimension that controls the magnitude of a signal, such as electrode surface area in the case of electrochemical biosensors, or area available for detecting formation of complexes between recognition elements and target analytes in the case of biosensors based on mechanical transducers. Structures such as nanowires, metallic nanoparticles, magnetic nanoparticles, nanopores and carbon nanotubes offer unique electrical, optical and magnetic properties that can be exploited for chemical sensing. The large surface area to volume ratio available on nanostructures for immobilization of labels and biological recognition elements offers the potential for high signal amplification and improved measurement sensitivity. Biosensors based on nanocantilevers measure a mechanical property in response to an affinity reaction and offer the possibility of label free measurements. Recent developments in the field of DNA nanobiosensors have resulted in molecular detection and amplification schemes, capable of outperforming PCR in sensitivity and ease of use [10].

2.1. Carbon nanotubes, metallic nanoparticles and nanowires coupled to electrochemical and optical transducers

The advantages of carbon nanotubes (CNT) as an immobilization matrix for both biocatalytic and affinity biosensors, owing to high surface to volume ratio, fast electron transfer kinetics (advantageous for electrochemical biosensors) and the presence of reactive groups on the surface, have been demonstrated [11,12]. Applications of CNT to improve sensitivity, response time and other analytical performances for electrochemical biosensors for several analytes of clinical interest have recently been reviewed [13]. Enhanced electron transfer between immobilized proteins, mediators and CNT results in electrochemical biosensors capable of operation at lower overpotentials, where signals from electrochemically active interfering substances are minimized, as was recently demonstrated for an amperometric glucose biosensor [14]. A biosensor for measurement of cholesterol in blood with cholesterol oxidase immobilized on multiwall CNT in a sol–gel matrix showed marked improvements in response time and sensitivity compared to an equivalent biosensor without CNT [15]. A biosensor for measurement of uric acid in serum was realized by immobilization of uricase in a polyaniline/CNT matrix. The sensor exhibited a response time of 8 s which the authors attributed to fast electron transfer properties of CNT [16]. Immunosensors are a particular area where the improvements in sensitivity brought by CNT can be exploited. Immunosensors using field effect transistors (FET) as transducers have been widely used for proof of concept studies because of the inherent sensitivity and signal amplification offered by FET [17]. In one example using prostate specific antigen (PSA) as a model analyte, CNT were deposited on the silicon gate of a FET [18]. Antibodies to PSA were immobilized on the CNT via a succinimidyl linking molecule. In a parallel experiment, indium oxide nanowires were deposited on the gate of a FET and antibodies to PSA immobilized on the nanowire. After incubation of the device with PSA, the nanowire device showed enhanced conductance between the source and drain electrodes, while the CNT device showed reduced conductance. The difference in response can be understood because the nanowire is an n-type semiconductor, while CNT is a p-type semiconductor, leading to different mechanisms of electrical conductance. Based on the signal to noise ratio observed at a PSA concentration of 5 ng/mL, it was predicted that a lower detection limit approaching 0.25 ng/mL, where the signal to noise ratio is approximately 3, could be obtained. In a similar study, an electrochemical immunosensor for PSA was constructed by depositing CNT on a 30 microelectrode array on a silicon substrate and modifying the CNT surface with a succinimidyl linking molecule to which monoclonal antibodies to PSA could be covalently bound [19]. Upon incubation of the array with PSA, the intrinsic oxidation signal of the electroactive amino acid residues present in PSA was detected using differential pulse voltammetry. The detection limit toward PSA was 0.25 ng/mL with a signal to noise ratio of 3. Aptamers have been used in place of antibodies as recognition elements to construct immunosensors by attaching the aptamer to CNT on the gate of a FET. Claimed advantages are improved shelf life of the devices and the potential to realize reversible immunosensors, not practical using antibodies as recognition elements [20]. When used to construct electrochemical DNA biosensors, CNT allow an efficient method to amplify label-free electrochemical detection of DNA hybridization by enhancing charge transfer between surface-anchored DNA sequences and CNT [21,22]. Increases in sensitivity offered by nanomaterials, when coupled to electrochemical modes of transduction, have resulted in highly sensitive, highly specific sensors attractive for detection of small DNA sequence variations [23].

Similar to CNT, gold nanoparticles (AuNP) have been shown to result in large signal enhancements and lower limits of detection for enzyme-based electrochemical biosensors, electrochemical immunosensors for disease-related protein biomarkers, and for detecting DNA hybridization events [24,25]. AuNP may be attached to electrode surfaces using self assembled monolayers (SAM) of alkanethiol molecules and the strong S–Au bond, providing a large active surface area for immobilization of biomolecules in close proximity to an electrode surface. AuNP have been used as both immobilization platforms and labels for electrochemical immunosensors [26]. Electrodes with layers of densely packed 5 nm gold nanoparticles were used as a matrix for immobilization of horseradish peroxidase in a sandwich immunoassay for prostate specific antigen with a detection limit of 0.5 pg/mL [27]. Optical properties of AuNP have also been exploited for biosensing. It is known that well dispersed suspensions of AuNP produce a red color, while aggregated AuNP produce a blue color [28]. A simple, aptamer-based colorimetric sensing of thrombin using 13 nm AuNP probes has been described [29]. AuNP, modified with thrombin aptamers, are observed as red because the nanoparticles are in suspension. Upon addition of thrombin, the aptamer undergoes a