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ScienceDirect
Biosensors and Bioelectronics 23 (2008) 987–994
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

A novel fluorescence-based array biosensor: Principle and application to DNA hybridization assays

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Received 14 June 2007; received in revised form 1 October 2007; accepted 5 October 2007
Available online 22 October 2007

Abstract

A novel fluorescence-based array biosensor targeted for field applications, such as environmental monitoring, has been developed, and successfully applied to DNA hybridization assays. The purpose was to meet the demand for automated, portable but easy-to-maintain systems allowing continuous flow monitoring of surface reactions. The biosensor presented here can be distinguished from the existing systems by the optical method used, which provides an enhanced simplicity and robustness, and enables a simple maintenance by potentially unskilled personnel. The system is based on a conventional microscope slide which acts both as transducer and biological array sensor. The excited fluorescence is guided by total internal reflection into the slide to the detector which is directly interfaced to the slide. Each region of the sensor array is successively optically interrogated, and the detection of the corresponding fluorescent emission synchronized. A real-time three-analyte analysis is thus feasible without any mechanical scanning movement or optical imaging systems as generally used in the existing instruments. The ability of the biosensor to operate in continuous flow for several tens of hours has been demonstrated. The biosensor has been assessed in terms of stability, and slide-to-slide reproducibility, which is found to be less than 3.7%, thus far below the standard biological reproducibility. DNA hybridization assays were performed to estimate a limit of detection, which was found to be 16 mol/μm^2, and to determine the reaction kinetics associated to the DNA model used. The developed biosensor is thus shown to be able to predict reaction kinetics, and to monitor in real time surface reactions between targets and probes.

Keywords: Optical biosensor; Total internal reflection; Real-time detection; Surface reaction monitoring

1. Introduction

Array biosensors are small multisensor systems designed for biomedical analysis (Giannetti et al., 2006), environmental monitoring (Rodriguez-Mozaz et al., 2004), or for the detection of biological warfare agents (Rowe-Taitt et al., 2000). To meet the demands of the field of the environmental monitoring, the biosensor described here has been targeted for being cost-effective, compact (200 mm × 280 mm × 170 mm, 2 kg), automated, capable of continuous multi-analyte detection, robust, and compatible with a use outside the laboratory by potentially unskilled personnel. The demand of such portable identification systems has led in the recent years to the development of two major classes of integrated systems: in the first one fall the truly integrated microdevices which combine integrated circuit elements, electro-optic excitation/detection systems, and bio-receptor probes (Misiakos et al., 2004); in the second one, the systems implement conventional instrumentation (charge-coupled devices, photo-multiplier tube, laser diodes, optical components, etc.) assembled around the central element which is the sensor medium or transducer (Tschmelak et al., 2005). Fluorescence transduction schemes predominate this micro-array field since they provide a better sensitivity and specificity, as well as decreased background signals over free-label arrays (Baldini and Giannetti, 2005). The array biosensor presented here clearly falls in the latter category, differing though from the existing systems by its reduced number of elements – thus its simplicity, combined with continuous flow operation and real-time monitoring.

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0956-5663/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.bios.2007.10.006

Let us first detail the assets and drawbacks of some of the most advanced systems of the first approach. A technique involving in situ hybridization of biological probes on a sensor element was first introduced by Karpf et al. (1988), and then applied by Eggers et al. (1994) to a CCD chip with radio-active or fluorescent labelling of target molecules. Although promising, the system is far from being compatible with out of laboratory testing due to radioactive labelling which is restricted to research applications. Mallard et al. (2005) investigated the use of CMOS elements in biochip. A photoelectrical detection system compatible with decentralised testing and able to detect as low as pM target concentrations on dense DNA arrays has been developed. The system is based on the use of a low cost CMOS photodetector array as a solid support for DNA chip, coupled with revelation by enzyme-catalysed chemiluminescence. This method however prevents any operation in continuous flow, and is thus not adapted to environmental monitoring applications. Vo-dinh et al. (1999) demonstrated the usefulness and feasibility of a DNA biochip using a phototransistor integrated circuit. The system has sensors, amplifiers, discriminators and logic circuitry on board and is shown to be able to include also light-emitting diodes for fluorescence excitation. Up to now, the only sensing device having the light source monolithically integrated with the waveguide and the detector has been presented by Misiakos et al. (2004). In this sensor, the signal transducer is based on an evanescent wave excitation scheme. This approach allows measurements to be confined to the waveguide surface rather than in bulk solution, thus enabling low limits of detection and high signal-to-noise ratios. Detection limits as low as 3.8 pM of gold-labelled streptavidin and 20 fM after a silver revelation step have been reported. Real-time detection without any washing step can be performed using this sensor, thus allowing a simple fluidic and instrumental design. However, the method requires a labelled target, which is not possible with haptens.

In the second approach, efforts have been made on reducing the size of the instrument while increasing the number of analyte to be screened. The portable semi-automated fibre optic immunosensor RAPTOR (Anderson et al., 2000) enables the detection of four bacterial agents, viruses, and toxins. The parallel affinity sensor array (PASA) (Weller et al., 1999) based on chemiluminescence labels showed a high potential for multi-analyte detection and a good regenerability of the indirect assay chips. The RIANA biosensor based on total internal reflection fluorescence has been validated for simultaneous detection of three contaminants (atrazine, isoproturon, and estrone) in natural waters (Rodriguez-Mozaz et al., 2004). The novel analytical system AWACSS (Tschmelak et al., 2005; Hua et al., 2005) enables to extend the number of compounds to be screened up to 32. These instrumentations are based on an evanescent wave technology: laser or led light is coupled into a waveguide, and the surface bound labelled areas are excited in the evanescent field. An imaging system consisting of a CCD camera equipped with imaging optics (Feldstein et al., 1999; Anderson et al., 2000; Weller et al., 1999), or with a fibre-coupled array (Rodriguez-Mozaz et al., 2004; Tschmelak et al., 2005) enables the analyte identification by pattern recognition. On the contrary, the novel biosensor described in this paper uses the following transducing method: (1) the sensing areas are interrogated normally to the surface similarly as in an epi-fluorescence scheme, which allows the totality of the fluorescent molecules present at the interface of the substrate to be excited. (2) The evanescent modes of the fluorescence emission are coupled into the substrate and (3) these are guided by total internal reflection to a mono-detector positioned at one edge of the substrate. The chosen substrate is a standard microscope slide whose large dimensions compared to the sensing areas enables to collect the energy of all evanescent modes with a good tolerance to excitation misalignment. Moreover, the fluorescence collection efficiency on the detector is good enough to avoid the use of any imaging optics interfacing the detector to the substrate edge, which, thus, enhances the simplicity of the biosensor and its robustness to optical or mechanical misalignments. The principle of this signalling method has been first reported as “solar concentrator” in the 1970s for photovoltaic applications (Weber and Lambe, 1976). This principle was then used for capillary gas sensors (Kieslinger et al., 1997; Gouin et al., 1998), yet for single analyte detection only. In 1987, a novel type of optical biosensor called “Fluorescent Capillary Filled Device (FCFD)” using the low-cost technology of liquid crystal display (LCD) to produce the capillary device has been presented (Bradley et al., 1987). The fluorescence signal is collected at the edge of the device, yet through custom optics aimed at extracting the light in the angular range of interest, and at discriminating the excitation and output light. The development of an assay for human chorionic gonadotrophin (Deacon et al., 1991) demonstrated the performances of this device, which has been later improved for multi-analyte identification (Daniels et al., 1998).

The purpose of this work is to describe and characterize a novel array biosensor in which the FCFD signalling method has been adapted to glass slide devices and automated continuous flow assays. The applicability of this biosensor is demonstrated trough a run of DNA hybridization assays.

2. Materials and methods

2.1. Biosensor set-up

A schematic view of the set up is depicted in Fig. 1.

The central element is a conventional microscope slide (size 3×1 in., cat No3010 Gold Seal® Products) which acts both as the transducer and the biological array sensor. A typical device is composed of four sensing areas of 1.4 mm^2, as illustrated in Fig. 2. Each sensing area is successively orthogonally excited using focused led emission, and the detection of the corresponding fluorescent emission synchronized. This configuration makes a real-time four-analyte analysis feasible without any mechanical scanning movement. The device is mounted into a custom monolithic fluidic chamber allowing continuous flow operation. The software implemented enables the automation of the assays, as well as the real time monitoring of the measurements.

The excitation module (Fig. 1) is composed of four low-cost light emitting diodes (Luxeon Emitter III, Lumiled) delivering