Biosensors and Bioelectronics 30 (2011) 78–86

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Optical microchip array biosensor for multiplexed detection of bio-hazardous agents

D. Bhatta[a,*], A.A. Michel[a], M. Marti Villalba[a], G.D. Emmerson[a], I.J.G. Sparrow[a], E.A. Perkins[b], M.B. McDonnell[b], R.W. Ely[c], G.A. Cartwright[c]

[a] Stratophase Ltd., Unit 10a, The Quadrangle, Premier Way, Romsey SO51 9DL, United Kingdom
[b] Defence Science and Technology Laboratories, Porton Down, Salisbury, Wiltshire SP4 0JQ, United Kingdom
[c] Bristol Industrial and Research Associates Ltd., Unit 8, Harbour Road Trading Estate, Portishead, Bristol BS20 7BL, United Kingdom

ARTICLE INFO

Article history:
Received 23 June 2011
Received in revised form 18 August 2011
Accepted 24 August 2011
Available online 3 September 2011

Keywords:
Antibody–antigen
Bio-hazardous agent
Multi-analyte detection
Optical sensor
Bio-defence

ABSTRACT

An optical waveguide array biosensor suitable for rapid detection of multiple bio-hazardous agents is presented. SpectroSens™ optical microchip sensors contain multiple spatially-separated waveguide channels with integral high-precision Bragg gratings sensitive to changes in refractive-index; selective surface-functionalisation of discrete sensing channels with different antibodies as bio-recognition elements enables selective multi-analyte biological detection. Interactions between target antigens in the test sample and respective surface-immobilised antibodies result in localised changes in refractive-index; the biosensor response manifests as increases in wavelength of light reflected from specific sensing channels. Multiplexed, label-free detection of 8 different biological agents, encompassing bacterial spores, vegetative cells, viruses and proteinaceous toxins has been demonstrated in real-time. Selective detection of *Bacillus atrophaeus* (BG) spores, *Escherichia coli* cells, MS2 viruses and ovalbumin (OVA) protein (simulant bio-hazardous agents) was first demonstrated as proof-of-concept; subsequently, detection of *Bacillus anthracis* (BA) spores (UM23CL2 strain), *Franciscella tularensis* (FT) cells (live vaccine strain), Vaccinia viruses (heat-killed) and ricin toxin (bio-hazardous agents) was proven. Two optical microchip sensors, each comprising 8 sensing channels were packaged into a single disposable cartridge allowing simultaneous 16-channel data acquisition. The specific antibody deposition sequence used in this study enabled detection of either 4 simulants or 4 bio-hazardous agents using a single consumable. The final device, a culmination of the multidisciplinary convergence of the fields of biology, chemistry, optoelectronics and microfluidics, is man-portable and inherently robust. The performance characteristics of the SpectroSens™ technology platform highlight its potential for exploitation as a 'detect to warn/treat' biodetector in security and defence operations.

1. Introduction

Rapid detection of pathogenic micro-organisms and toxins is prerequisite for an escalating number of applications, including bio-security and defence, medical diagnostics, environmental analysis, bio-processing and food safety. Over the last decade, there has been particular interest in the detection of bio-hazardous agents, since the 'anthrax events' (post 11th September, 2001) substantiated the threat of future incidents of bio-terrorism in both military and civilian environments (Inglesby et al., 2002). Conventional methods for pathogen detection are laborious, time-consuming procedures confined to specialised laboratories with expensive read-out instrumentation. Optical biosensors comprising target-selective bio-recognition probes are particularly well-suited to rapid, sensitive detection of biological agents in environmental samples. These have been receiving escalated interest in recent years for applications requiring 'on-site' analyses due to their portability, simplicity of operation, multiplexing potential and relatively low cost (Fan et al., 2008; Horvath et al., 2003; Kozma et al., 2011; Lazcka et al., 2007; Luchansky et al., 2010; Ramachandran et al., 2008; Rowe-Taitt et al., 2000; Taitt et al., 2008; Sai et al., 2010; Zourob et al., 2005). Whilst these devices exhibit numerous advantages over competitive systems, very few have successfully demonstrated detection of multiple biological targets using a single sensing chip (Rowe-Taitt et al., 2000; Taitt et al., 2008).

Recently, a novel integrated optical microchip sensing system (SpectroSens™), based on technology leveraged from the telecommunications industry, has been reported in its capacity for biological agent detection (Bhatta et al., 2010a, 2010b). SpectroSens™ optical microchip sensors comprise multiple

* Corresponding author. Tel.: +44 1794 511 226; fax: +44 8704 580 754.
E-mail address: devaki.bhatta@stratophase.com (D. Bhatta).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.08.031

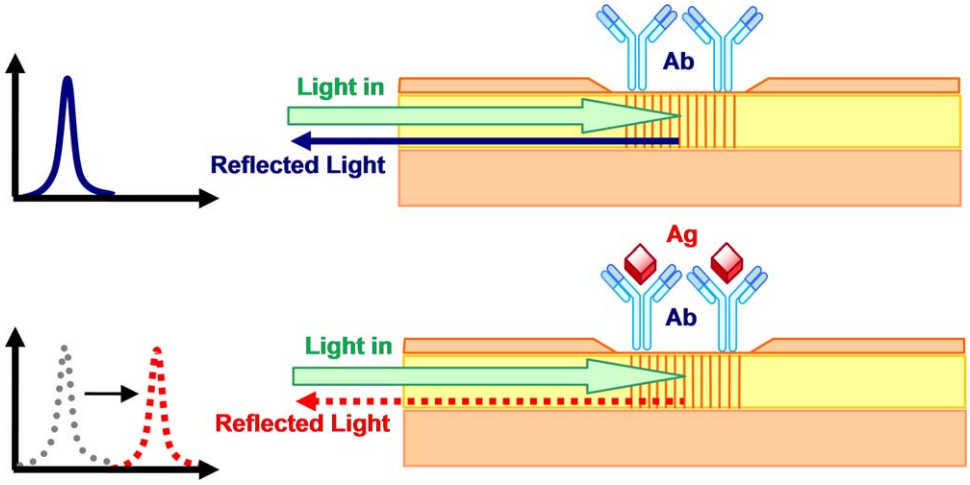

Fig. 1. Schematic illustration of the operating principle of a SpectroSens™ sensor for biological detection. Light travels through a waveguide within the microchip sensor to the Bragg grating, which reflects a precisely defined wavelength of light. Interaction of target agents with antibodies immobilised on the sensing region results in localised changes in refractive index, which manifest as changes in sensor reflected wavelength. Each chip contains multiple sensing channels operating independently enabling assay multiplexing.

high-precision Bragg gratings defined within spatially-separated waveguide channels, written into planar silica substrates using the direct UV writing technique described by Emmerson et al. (2002). Bragg gratings act as sensitive wavelength filters, reflecting light at precisely defined wavelengths as governed by the Bragg condition:

$$\lambda_{max} = 2\Lambda n_{eff}$$

where λ_{max} is the wavelength of light at which maximum reflectivity occurs, Λ defines the grating period and n_{eff} is the average refractive index of the waveguide's composite structure. Hence, changes in the immediate environment surrounding a particular grating result in measurable changes in the wavelength of reflected light from the corresponding waveguide channel. The sensor design results in a large penetration depth of the sensing light (>1 μm) into the sample liquid. SpectroSens™ sensors are designed to operate in the telecommunications window (1550 nm), hence commercial off-the-shelf sensor substrates and source/detector components are robust and ubiquitous, and remote sensing using fibre-optics is feasible. Functionalisation of independent sensing channels with different target-selective antibodies determines localised sensitivity to specific biological agents, enabling multiplexed detection using a single optical microchip sensor. The sensor response manifests as changes in reflection wavelength from specific sensing channels as a result of cognate antigen-binding to respective surface-immobilised antibodies (Fig. 1).

Real-time, label-free multi-analyte detection using the SpectroSens™ sensing platform is demonstrated in this communication through the detection of 8 different biological agents, comprising both simulant and bio-hazardous agents; these encompass all classes of biological targets, ranging from small proteinaceous toxins to larger particulate viral and bacterial (spore and vegetative cell) antigens.

2. Materials and Methods

2.1. Materials

All reagents were of analytical grade unless otherwise stated. Albumin from bovine serum (BSA) (electrophoresis grade), 3-aminopropyltriethoxysilane (APTES) (99%) and Dulbecco's phosphate buffered saline (PBS) (pH 7.4) were purchased from Sigma-Aldrich Company (Dorset, UK). Acetone (HPLC grade) and phosphate buffered saline with Tween (PBS-Tween) (×20 concentrate) were obtained from Thermo Fisher Scientific Ltd. (Leicestershire, UK). Bis(sulfosuccinimidyl)-suberate (BS^3) (2 mg microtubes) was sourced from Pierce, Thermo Fisher Scientific Ltd. (Leicestershire, UK).

Goat anti-mouse IgG polyclonal antibody (Fc-specific, affinity-purified) and goat anti-rabbit IgG polyclonal antibody (whole molecule, affinity-purified) were purchased from Sigma-Aldrich Company (Dorset, UK). Rabbit anti-sheep IgG polyclonal antibody (H+L specific) was purchased from Abcam (Cambridge, UK). Recombinant protein A/G from *Escherichia coli* (lyophilised powder) was obtained from Pierce, Thermo Fisher Scientific (Leicestershire, UK). Ovalbumin (OVA) protein (Grade V), *Bacillus atrophaeus* (BG) spores, *E. coli MRE 162*, bacteriophage virus MS2, *Bacillus anthracis* (BA) spores (UM23CL2 strain), *Franciscella tularensis* (FT) cells (live vaccine strain), Vaccinia viruses (heat-killed) and ricin toxin; rabbit anti-OVA polyclonal antibody (antigen-affinity-purified), rabbit anti-BG polyclonal antibody, rabbit anti-*E. coli* polyclonal antibody, sheep anti-MS2 polyclonal antibody, mouse anti-BA monoclonal antibody (DSTL110), mouse anti-Vaccinia monoclonal antibody (DSTL111), mouse anti-ricin monoclonal antibody (DSTL109) and rabbit anti-FT polyclonal antibody were provided by Defence Science and Technology Laboratories (Dstl) (Porton Down, Wiltshire, UK).

2.2. Instrumentation

SpectroSens™ microchip sensor outputs were monitored using a SIS:LabIIBio detector module (see Section 3.1) produced by Stratophase Ltd., UK, providing data at a refresh rate of 2 Hz, with a resolution of 1 pm. This instrument comprises the sensor light source and readout components, as well as a basic sample (liquid) delivery module. Introduction of test samples containing simulant agents was carried out using a QuadPump module (produced by Stratophase) comprising 4 computer-controlled 50 μl syringe pumps. Introduction of test samples containing bio-hazards was carried out using a standalone fluidic module (produced by BIRAL) comprising computer-controlled peristaltic pumps and inline degassers with automated purging and priming sequences (in anticipation of in-field use). Antibody deposition onto individual sensing channels within SpectroSens™ microchips was achieved using a microfluidic patterning system constituting an automated computer-controlled 8-channel peristaltic