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Multi-purpose optical biosensors for real-time detection of bacteria, viruses and toxins

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ABSTRACT

A universal optical microchip sensing platform demonstrating real-time, label-free detection of a wide range of biological agents is presented. SpectroSens™ chips containing high-precision planar Bragg gratings are exploited as low-cost, robust refractive index sensors. Sensitivity to biological agents is conferred by functionalising the sensing surface with antibodies selected against targets of interest. Several methods for immobilisation of bio-molecules on the metal oxide-coated sensing surface have been investigated. In this study, surfaces were modified with an amino-terminated silane monolayer and activated by glutaraldehyde cross-linking for covalent attachment of recombinant Protein A/G, to which agent-specific antibodies were immobilised. Binding of target antigens (introduced under flow) to the surface-immobilised antibodies results in localised changes in refractive index; upon laser-induced interrogation of the sensing region via optical fibres, these antibody–antigen interactions manifest as increases in wavelength of light reflected from the Bragg grating. Detection of biological targets including proteins (ovalbumin < 10 nm), viruses (MS2 < 100 nm), bacterial cells (*Escherichia coli* > 1 μm) and spores (*Bacillus atrophaeus* > 1 μm) in real-time has been demonstrated. The large size range of detection targets is attributed to a large penetration depth of the sensing light of >1 μm into the sample liquid using these sensors. This multi-analyte capability enables utilisation of this sensing technology in applications ranging from bio-threat detection for defence and homeland security to point-of-care clinical diagnostics.

1. Introduction

Rapid detection of pathogenic micro-organisms and toxins is critical in several areas including bio-defence, water and environmental analysis, clinical diagnostics and food safety. In particular, there has been heightened interest in the detection of biological warfare agents since the "anthrax event" post September 11th, 2001 substantiated the threat of future incidents of bio-terrorism in both military and civilian environments [1].

Conventional methods for pathogen detection rely on classical microbiological, immunological and genetic methods [2,3], which are laborious, time-consuming procedures confined to specialised laboratories with expensive read-out instrumentation. Such limitations have encouraged the development of rapid detection systems suitable for on-site monitoring.

In the last few decades, biosensor technology has emerged as a powerful and versatile alternative detection principle with the potential to overcome many of the shortcomings of traditional methods, primarily due to speed of analysis and simplicity of operation [3]. Biosensors comprise a biological recognition element necessary for specific interaction with the target analyte, interfaced with a physicochemical transducer to convert the biological interaction into a measurable signal [4]. Common signal transduction mechanisms include electrochemical detection; mainly potentiometric [5], amperometric [6] and impedance [7], piezoelectric measurement [8] and optical sensing [9], of which surface plasmon resonance (SPR) is the most extensively characterised in the context of biological detection [10].

Despite dramatic achievements in biosensing in recent years, individual techniques do not include all the desirable characteristics necessary to satisfy the most demanding criteria for point-of-care applications; this is exemplified by the relatively low number of commercially available detection systems (reviewed in [2]). Hence, alternative integrated sensing systems with high sensitivity and selectivity of detection, which are geared for portability, ease of use and low-cost, are highly sought after.

Over the past decade, activity within the field of optical biosensor development has been fast-paced and various different optical platforms have been investigated for sensitive, label-free detection; these include, but are not limited to, surface plasmon resonance

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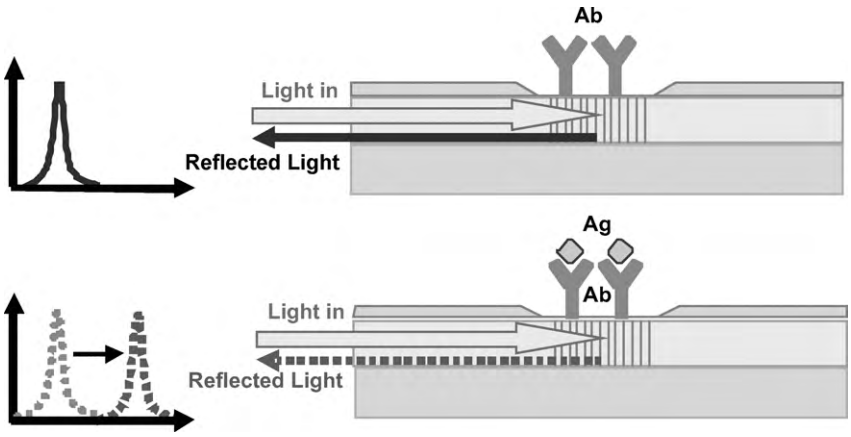

Fig. 1. Schematic Illustration of the operating principle of a SpectroSens™ sensor for biological detection. Light travels through the waveguide within the sensor chip to the Bragg grating, which reflects a precisely defined wavelength of light. Interaction of target agents with antibodies immobilised on the sensing surface results in localised changes in refractive index, which manifests as changes in the colour of reflected light.

[11,12], interferometers [13], ring-resonators [14], photonic crystals [15], fibre-optics [16] and planar optical waveguides [9,17]. Advantages of optical sensors encompass immunity to electromagnetic interference, remote sensing capability, intrinsic safety and the ability to provide multiplexed detection within a single device. One of the most significant drawbacks of commonly used optical sensing systems is the penetration depth of the evanescent field, which is typically much smaller than the average size of a bacterial cell, resulting in the inability to sense larger particulate antigens with adequate sensitivity. Many of the current systems also require specialised expensive instrumentation to deliver the signal readout.

Recently, the development of a novel sensing platform, SpectroSens™, based on optical integrated circuits leveraged from the telecommunications industry, has been reported, where high-precision Bragg gratings written into planar silica substrates are exploited as low-cost refractive index sensors [18] (and reviewed in [19]). Bragg gratings act as sensitive wavelength filters, reflecting light at precisely defined wavelengths governed by the following equation:

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

where $\lambda_{\max}$ 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 system. Hence, changes in the medium surrounding the grating associated with differences in refractive index will generate changes in the wavelength of reflected light from the sensor. Biological selectivity is conferred through the directed immobilisation of target-specific antibodies over the sensing region. The resulting biosensor response manifests as changes in the wavelength of light reflected by the sensor in response to localised changes in refractive index caused by selective antigen attachment to the antibody-functionalised sensing surface (Fig. 1).

The design of the sensor results in a large penetration depth of the sensing light of a few microns into the sample liquid, making it suitable for detection of various classes of biological targets including larger particulate antigens. The fabrication process associated with generating these sensors allows multiple discrete sensing regions to be written onto single devices rapidly, enabling straightforward multiplexing, the cost of which is dramatically reduced by economies of scale. Commercial off-the-shelf interrogation and detector units that deliver the source light and read out changes in reflected wavelength are robust and ubiquitous. The characteristics of the SpectroSens™ platform, including the self-contained nature of the sensing element, make it ideally placed for "point-of-care" and "detect-to-warn" operations.

This communication describes the development and characterisation of SpectroSens™ sensor chips for the detection of a wide range of biological targets; selective detection of bacterial spores (*Bacillus atrophaeus*), vegetative cells (*Escherichia coli*), viruses (MS2) and proteins (ovalbumin) is evaluated.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade unless otherwise stated. Albumin from bovine serum (BSA, electrophoresis grade), 3-aminopropyltriethoxysilane (APTES) (99%), Dulbecco's phosphate buffered saline (PBS), pH 7.4, glutaraldehyde solution, Grade II, 25% (v/v) and sodium cyanoborohydride were purchased from Sigma–Aldrich Company (Dorset, UK). Acetone (HPLC grade) and Decon 90 liquid detergent were purchased from Thermo Fisher Scientific Ltd. (Leicestershire, UK).

Mouse-IgG (chromatographically purified) and rabbit-IgG (chromatographically purified) were purchased from Zymed Laboratories, Invitrogen Ltd. (Paisley, UK). Recombinant protein A/G from *E. coli* (lyophilised powder) was obtained from Pierce, Thermo Fisher Scientific (Leicestershire, UK). Antigen-affinity-purified rabbit anti-ovalbumin polyclonal antibody, rabbit anti-BG polyclonal antibody, rabbit anti-*E. coli* polyclonal antibody, sheep anti-MS2 polyclonal antibody, ovalbumin protein (Grade V), and *B. atrophaeus* (historically referred to as BG) spores, *E. coli MRE 162* and bacteriophage virus MS2 were supplied by Defence Science and Technology Laboratories (DSTL) (Porton Down, UK).

2.2. Instrumentation

Spectral outputs from SpectroSens™ sensor chips were monitored using a SIS:Lab II instrument produced by Stratophase Ltd., UK, providing data at a refresh rate of 2 Hz. Sample delivery to the sensor chips was achieved by means of a fluidic module comprising 50 μl syringe pumps and PTFE flow cells of 5 μl volumes (FlowCube).

2.3. Sensor chip fabrication

SpectroSens™ chips (20 mm × 10 mm) were fabricated by simultaneous writing of Bragg gratings and waveguides into