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Surface plasmon resonance biosensing: Approaches for screening and characterising antibodies for food diagnostics

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ABSTRACT

Research in biosensing approaches as alternative techniques for food diagnostics for the detection of chemical contaminants and foodborne pathogens has increased over the last twenty years. The key component of such tests is the biorecognition element whereby polyclonal or monoclonal antibodies still dominate the market. Traditionally the screening of sera or cell culture media for the selection of polyclonal or monoclonal candidate antibodies respectively has been performed by enzyme immunoassays. For niche toxin compounds, enzyme immunoassays can be expensive and/or prohibitive methodologies for antibody production due to limitations in toxin supply for conjugate production. Automated, self-regenerating, chip-based biosensors proven in food diagnostics may be utilised as rapid screening tools for antibody candidate selection. This work describes the use of both single channel and multi-channel surface plasmon resonance (SPR) biosensors for the selection and characterisation of antibodies, and their evaluation in shellfish tissue as standard techniques for the detection of domoic acid, as a model toxin compound. The key advantages in the use of these biosensor techniques for screening hybridomas in monoclonal antibody production were the real time observation of molecular interaction and rapid turnaround time in analysis compared to enzyme immunoassays. The multichannel prototype instrument was superior with 96 analyses completed in 2 h compared to 12 h for the single channel and over 24 h for the ELISA immunoassay. Antibodies of high sensitivity, IC_{50}'s ranging from 4.8 to 6.9 ng/mL for monoclonal and 2.3–6.0 ng/mL for polyclonal, for the detection of domoic acid in a 1 min analysis time were selected. Although there is a progression for biosensor technology towards low cost, multiplexed portable diagnostics for the food industry, there remains a place for laboratory-based SPR instrumentation for antibody development for food diagnostics as shown herein.

1. Introduction

Awareness of human exposure to a wide range of contaminants through the diet, originating from anthropogenic, natural sources and fraudulent practices along the food supply chain has been heightened with increasing media attention and consumer cognizance. The food supply chain can become contaminated at various points from environment to farm to fork by bacteria, viruses or chemicals present in the environment; the improper use of agrichemicals such as antibiotics and pesticides; the illegal use of growth promoting compounds in animal production; by-products of food processing techniques; and naturally occurring toxins such as phycotoxins and mycotoxins. Food contamination can cause serious acute and chronic health effects resulting in economic and political repercussions with subsequent "food scares" and recalls of products [1,9,16,18,19,25,30,31]. In order to monitor food for contamination and to ensure that unacceptable levels do not enter the human food chain, it is imperative that products are subjected to scrutiny, in real time at critical control points in the chain, and with respect to their safety in an efficient and rapid manner.

In recent years, advances in biosensor and nanotechnology for diagnostics have offered solutions, albeit mostly as research tools in portable, multiple analyte diagnostics for small numbers of samples. Relatively expensive biosensor technology (e.g., $\geq$ $100K USD) for food analysis as a screening tool that is restricted to the laboratory setting is generally no longer deemed as cost-effective in comparison to the advances in state-of-the art confirmatory mass spectrometry methods for multiple analytes. These biosensors tend to be only suitably cost-effective when they have the ability to efficiently analyse a large number of samples for the presence of multiple chemical contaminants in food matrices with

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a relatively short turnaround time [28]. Additionally, biosensors may be used as an alternative to mass spectrometry as a way of diversifying resources and skills in a routine analytical laboratory, but employing both technologies still remains an innovative challenge.

Advances over the past two decades have led to the manufacture of a range of instrumentation employing a variety of detection principles. Modern biosensors offer the ability to be portable devices for regulatory bodies and the food industry alike to verify the safety and quality of products intended for human consumption along the food supply chain. It is expected that biosensors will have added advantages over traditional technologies, since they combine the high affinity of the biochemical interactions, resulting in high sensitivities and low limits of detection, with the possible miniaturisation, "point-of-use" portability and automation which make them interesting for in situ monitoring [3]. Importantly, as biosensors operate on the principle of measuring a biomolecular interaction of two components on a surface, the key component for food analysis still remains the biorecognition element for the interaction and detection with the target contaminant. Polyclonal antibodies, and more recently monoclonal antibodies that reduce the use of animals, still tend to be the most suitable biorecognition elements in commercial immunodiagnostics for food analysis. For the production and characterisation of biorecognition elements, the use of a laboratory based biosensing instrument that operates with a high degree of automation, user friendliness, accuracy and precision with real-time analysis is a vital tool for the robust screening and characterisation of antibody binding and matrix performance.

Surface plasmon resonance (SPR) biosensor technology has been the most successful immunosensor to date for food analysis and is primarily employed in a laboratory setting. SPR biosensors are affinity, optical sensors where the signal is based on measuring binding-induced changes in refractive index and this platform thus allows for label-free, real-time analysis (Fig. 1). Depending on SPR response to the binding of analytes to a surface, an analyte can be detected with a direct, sandwich, or inhibition assay. For small analytes (molecular weight <1000 Da), an inhibition assay is often used, where small analytes are premixed with antibodies and unbound antibody sites are captured by the small analytes immobilized on a sensor surface [43]. This approach has been used widely with SPR biosensors for the detection of aquatic toxins ([6,7,12,13,14,26,32,40,42]). Variations in SPR technology is available with different capabilities in analysis due to the utilisation of single or multiple flow channels (Fig. 1). Therefore, SPR biosensor technology enables simultaneous observations of molecular interactions on a surface containing multiple channels offering multiplexed evaluations in a single analysis [4,5,29].

In this research, a Biacore SPR high throughput prototype instrument was employed for effective multiplexed analysis for evaluation of the marine toxin, antibody interaction. The system is designed primarily for rapid, simultaneous screening of multiple ligands (up to 16) by employing a 4×4 format with four 0.6 mm^2 flow cell areas that each contain four SPR sensing spots. This flexible, multichannel format enables assays with either multi-ligand or multi-sample focuses. When a Series S CM5 chip with 16 immobilised ligands is docked, four independent parallel flow cells are formed by the sensor chip pressing against moulded channels on the integrated microfluidic cartridge (IFC), and the response is measured from four detection spots in each flow cell [4]. Variation in assay design offers alternative approaches for antibody selection, characterisation and target analysis which may be extremely beneficial when the target analyte is expensive or difficult to source ([5,41]).

Marine biotoxins are an exemplar case whereby the development of rapid methods including antibody-based assays have been restricted due to the limitations in and relatively expensive costs of many toxins. In order to investigate SPR biosensor technology for characterizing antibodies to small-molecule toxins, the cost-effective and easily obtainable domoic acid (DA) toxin is used as a model compound in this study. This neurotoxin is renowned to cause amnesic shellfish poisoning (ASP) illness in humans

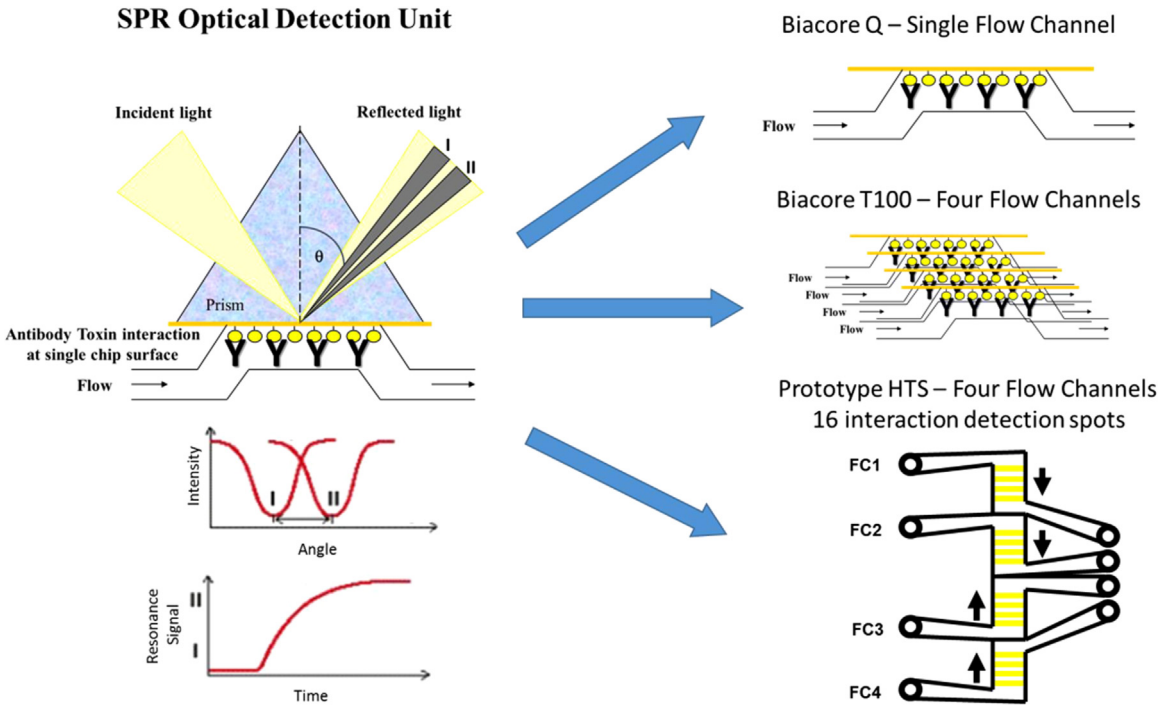

Fig. 1. Surface plasmon resonance (SPR) technology measures light reflection from the side of the chip opposite the flow channel. Upon antibody-toxin interaction at the chip surface, the change in reflected light angle (I to II) is detected. Each of the biosensors has different capacity for the simultaneous determination of toxin interaction on the surface due to the number of flow channels and detection points on each channel.