CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

A micro-fluidic device to create microarrays for multiple analyte determination by surface plasmon resonance using phycotoxin concentration analysis as a model system

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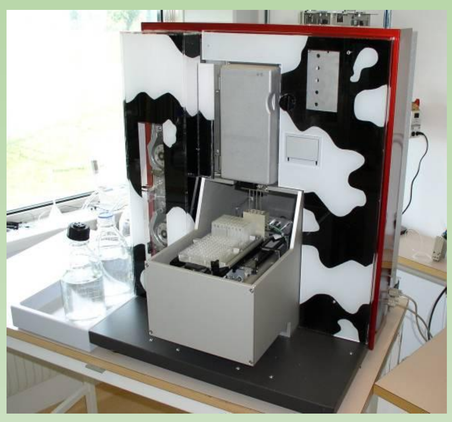
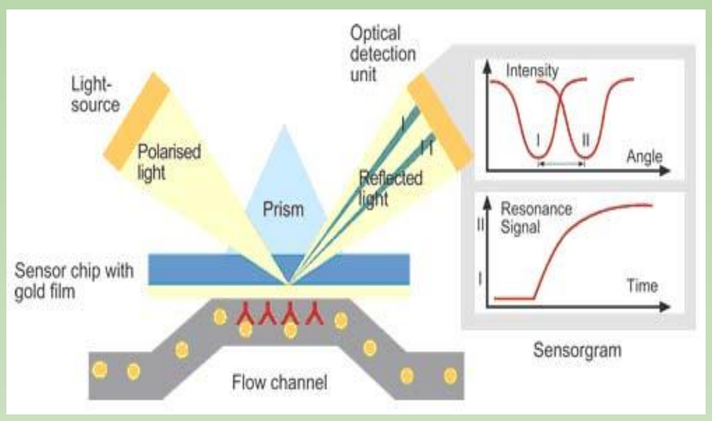
Introduction

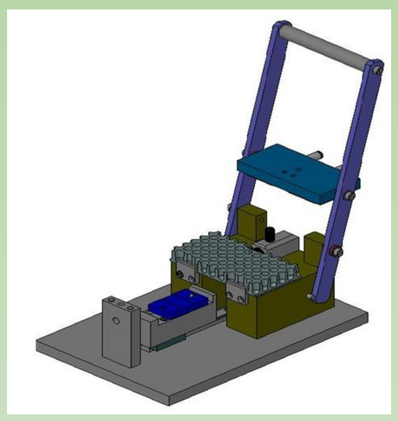
There is an increasing demand to develop biosensor monitoring devices capable of biomarker profiling for predicting animal adulteration and for detecting multiple chemical contaminants in food produce. Surface Plasmon resonance (SPR) biosensors are label free detection systems that monitor the binding of specific biomolecular recognition elements to target antigens as an alternative to classical binding assays such as ELISA. Their scope is sometimes limited by the cross-reactivity of the bio-recognition element. However, multiplexing can be achieved by the development of multi-flow cell SPR and/or by the development of multi-spots within a single SPR flow cell for different bio-recognitions. Within the scope of FP6 project BioCop, a prototype 16-plex (4 flow cells x 4 flow spots) SPR instrument was developed for the detection of protein biomarkers. This device is a molecular interaction array system that can perform simultaneous concentration analysis of multiple analytes. Essential to this technology are the production of biochips where the selected target antigens, biomarker proteins or low molecular weight contaminants, are immobilised. A compatible micro-fluidic immobilisation device to the prototype instrument allowing for the covalent attachment of 16 antigens in a linear array on a single chip using various surface chemistries was developed. Operation of the immobilisation unit, i.e. control of flow and sensor chip docking, is fully controlled by a programmable logic controller.

Prototype Multiplex SPR Optical Biosensor and Theory



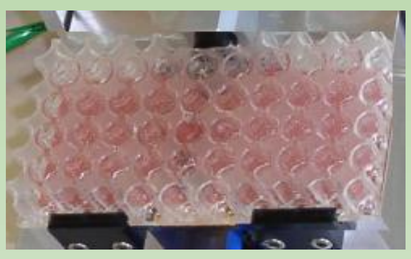


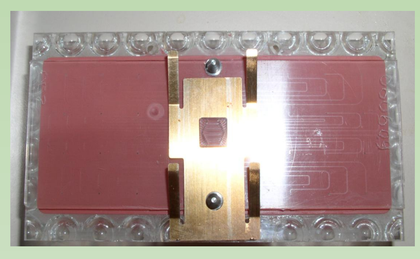
Chip Surface Immobilisation Unit



The chip surface immobilisation unit was designed with 16 isolated microfluidic channels for the immobilisation of up to 16 different antigens onto a chip surface.

When docked onto the prototype, the sensor then forms 4 microfluidic flow cells with 4 immobilised spots in each.





Aim

The aim of this research was to evaluate the immobilisation unit and multiplex SPR biosensor in their ability to be fit-for-purpose for antigen attachment of high (proteins) and low (toxins) molecular weight compounds and the concentration analysis of four different phycotoxin groups.

Methods

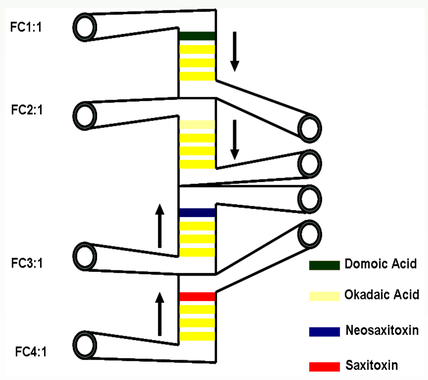
Characterisation of Sensor Chip Immobilisation

Two interaction systems, human serum albumin (HSA) and anti-HSA antibody, and myoglobin and anti-myoglobin antibody were used:

- **To evaluate the precision of immobilising specific regions of the sensor chip with various antigens. HSA antibodies were immobilised on odd numbered spots and myoglobin antibodies on even numbered spots. Immobilisation areas were inspected visually and the potential risk of cross contamination of immobilised antibodies was evaluated by measuring binding responses of HSA and myoglobin to all spots;**
- **To determine the reproducibility of immobilisations by immobilising 4 different concentrations of anti-myoglobin antibody in all flow cells on seven different sensor surfaces and evaluating by measuring the variation in immobilisation levels;**
- **To demonstrate that immobilisation levels could be controlled and that the activity of immobilised antigens was proportional to antigen immobilisation levels by varying antigen concentrations during the immobilisation procedure and determining the binding response.**

Multiplexed Phycotoxin Analysis

Within the CONffIDENCE project this technology was applied to multiplex marine biotoxin analysis. A toxin assay was incorporated into each of the four flow cells of a single biosensor chip thus allowing samples to be analyzed under different cycle conditions. These toxin assays were designed as inhibition assays with toxin immobilised on the surface.

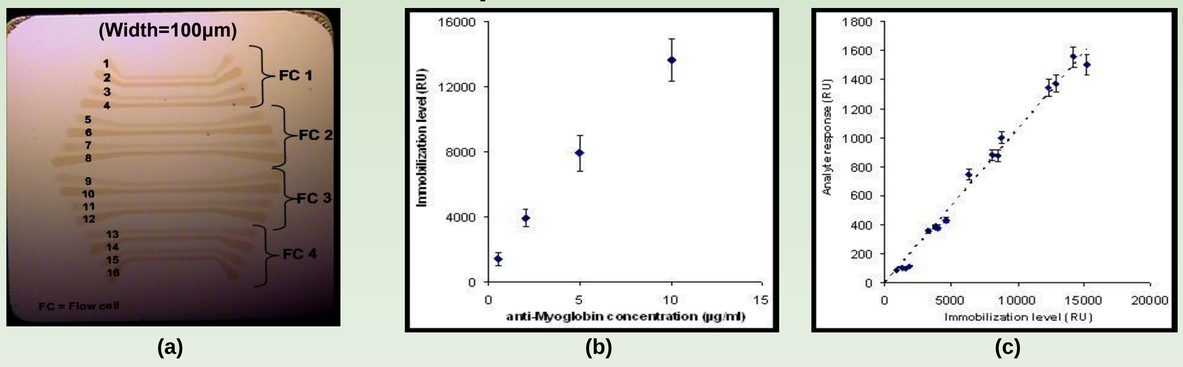


For the immobilisation of each toxin an amine surface was prepared on the appropriate spot. Domoic acid and okadaic acid were conjugated to each amine surface spot using carbodiimide chemistry, whereas neosaxitoxin and saxitoxin were conjugated using a modification of the Mannich reaction. Each flow cell was assessed using the antibodies available at QUB for each of the toxin targets.

Each antibody was mixed in a 1 to 1 ratio with toxin standards to produce calibration curves. The mix was then injected over the chip surface at a flow rate of 20µl/min with a contact time of 180s. The regeneration solutions were optimised for each antibody toxin combination. The flow rate for the regeneration solution was also set at 20µl/min and the contact time at 30s followed by a 90s stabilisation time prior to the next injection.

Results

Characterisation of Sensor Chip Immobilisation



(a) (b) (c)

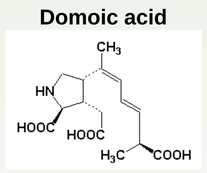
(a) Contrast microscope image of a sensor chip (7 x 7mm²) on which 16 lanes with immobilised antibodies were created using the immobilisation unit (b) immobilisation levels as function of anti-myoglobin concentration during immobilisation (c) Binding levels of myoglobin as function of anti-myoglobin immobilisation levels.

The levels of immobilisation could be controlled by varying the antigen concentration during immobilisation.

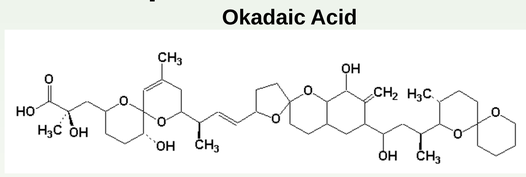
Multiplexed Phycotoxin Analysis

Calibration Curves were produced on each flow cell for the specified toxin

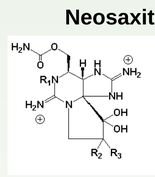
Domoic acid



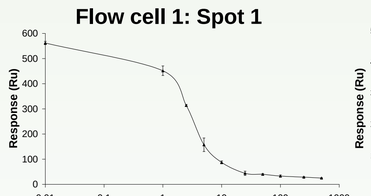
Okadaic Acid

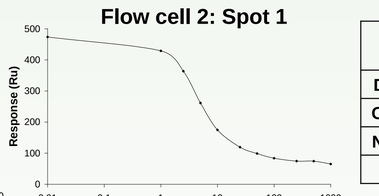


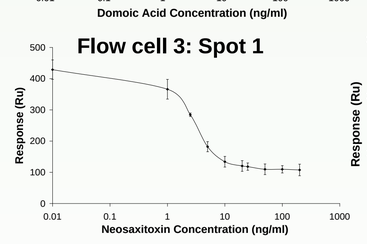
Neosaxitoxin /Saxitoxin

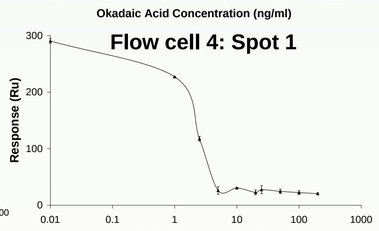


STX	R_1	R_2	R_3
STX	H	H	H
GTX-II	H	H	OSO_3^-
GTX-III	H	OSO_3^-	H
NeoSTX	OH	H	H
GTX-I	OH	H	OSO_3^-
GTX-IV	OH	OSO_3^-	H









Toxin	Antibody	Titre	IC_{50} (ng/ml)	$IC_{20} – IC_{80}$ (ng/ml)
Domoic Acid	R867	1/200	2.6	1.0 – 6.4
Okadaic Acid	R739	1/4000	4.9	1.7 – 14.4
Neosaxitoxin	NEO-Ab	1/25	2.6	1.1 – 6.0
Saxitoxin	R895	1/1000	1.9	1.0 – 3.7

Toxin	RMM	Regeneration solution
Domoic Acid	311	75mM sodium hydroxide
Okadaic Acid	805	180mM sodium hydroxide with 15% acetonitrile
Neosaxitoxin	315	100mM Hydrochloric Acid
Saxitoxin	299	50mM Hydrochloric Acid

Conclusions

The immobilisation and multi-analyte SPR detection of both high and low molecular weight compounds was successfully achieved. Calibration curves using different biorecognition elements for each toxin group were produced. This SPR biosensor prototype and immobilisation device exhibits enormous potential for multiple phycotoxin screening within a single chip format with the additional benefit of compartmentalisation of toxin groups. This model is particularly contemporary with the current drive to replace the animal based testing for phycotoxins.

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