Detection of ligand-elicited cellular responses using Surface Acoustic Wave biosensors

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Abstract *– The work presented here is a part of the iCHEM project, which aims at developing a new class of technology for biosynthetic infochemical communication by exploiting recent advances in the understanding of both pheromone biosynthesis pathways and the detection of molecules in the insect nervous system. We present the design and development of a novel surface acoustic wave (SAW) biosensor system and report preliminary results using a functionalised coating (Sf9 whole cells), to detect secondary cellular responses triggered when 12.5µM of octopamine, an invertebrate neurotransmitter, binds to the endogenously expressed receptors present within the cell plasma membrane.*

Introduction to the "iCHEM" project

The overall objective of the ***iCHEM*** project is to:

- Engineer biosynthetic components for chemical signal generation and detection based on insects' pheromone production and sensing pathways.
- Integrate these biosynthetic modules ***(Figure 1 (A))*** into a communication system comprising MEMS-based microreactors, novel biological microsensors, and artificial neuronal algorithms with VLSI implementation ***(Figure 1 (B))***.

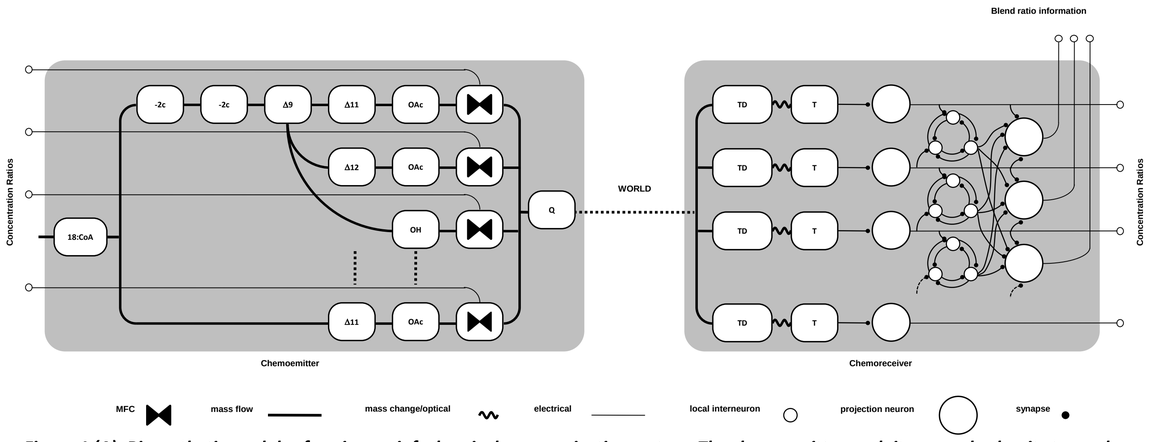

Figure 1 (A). Biosynthetic modules forming an infochemical communication system. The chemoemitter exploits several subunits to produce infochemicals based on the enzymatic activity within the exocrine system of a moth and a microevaporator or a nebulizer (Q) releases the infochemical blend. The chemoreceiver exploits transmembrane domain (TD) olfactory receptors, which are transduced (T) using binding specific changes. Infochemical binding signals are processed in a ratiometric neuronal model based on the antennal lobe of the same animal.

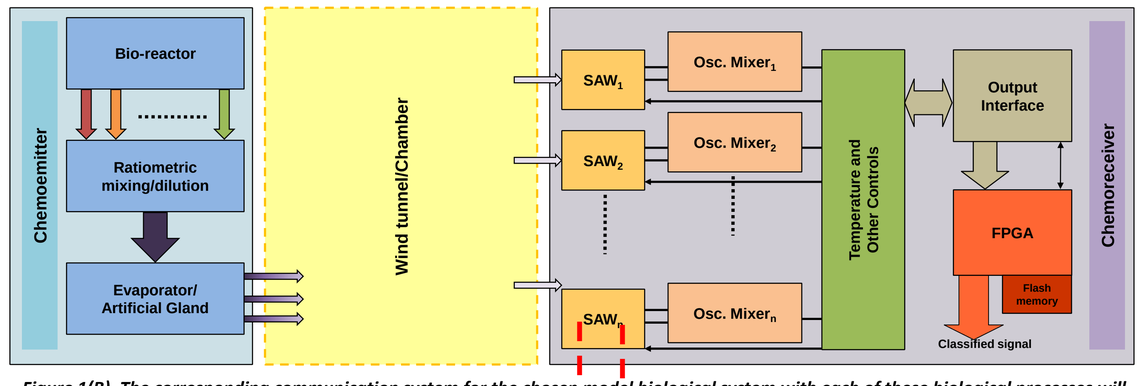

Figure 1(B). The corresponding communication system for the chosen model biological system with each of these biological processes will be characterised and deployed in MEMS-based microreactors, novel biological microsensors, and artificial neuronal algorithms with VLSI implementation.

SAW Biosensor based ligand detection

Here, we present the development of a chemoreceiver side of the iCHEM project. The chemoreceiver is based on a novel surface acoustic wave (SAW) resonant biosensor capable of detecting ligand-activated cellular responses within a functionalized insect cell. We report preliminary results from our SAW biosensor setup, acoustically measuring cellular responses which are triggered when 12.5 µM concentration of octopamine, an invertebrate neurotransmitter, binds to the endogenously expressed receptors present within the plasma membrane of Sf9 insect cells. The technique demonstrated here will be employed in the iCHEM project to monitor changes in the intrinsic properties of cells which are transfected with specific olfactory receptors (ORs) and activated with receptor-specific ligands.

- A SAW-based sensors, consists of an input interdigital transducer (IDT) which sets up an electric field in the substrate that, by means of the piezoelectric effect, generates a surface acoustic wave ***(Figure 2)*** propagating towards the output IDT which in turn converts this wave into an electrical signal. Changes in the properties of the adjacent biological layer or liquid change the propagation characteristics of the wave (i.e. attenuation, phase, frequency), thus, allowing detection.

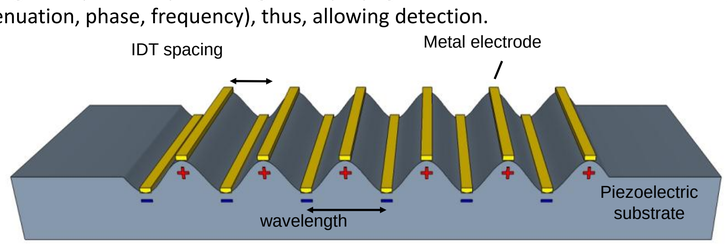

Figure 2. The basic principle of exciting surface acoustic waves by an interdigital transducer created by micro-patterned metal electrodes on a piezoelectric substrate

- A SAW microsensor was designed in a dual resonator configuration, as shown in ***Figure 3 (A)***, to allow for a differential measurement in which only one device of the pair is coated with *Sf9* cells, as shown in ***Figure 3 (B).***
- Differential measurement ameliorates environmental and other common mode variations and ensures that the measured responses are produced purely by the functionalized cells.
- The devices have been designed to operate at frequency of 60 MHz and were fabricated on lithium tantalate substrate.

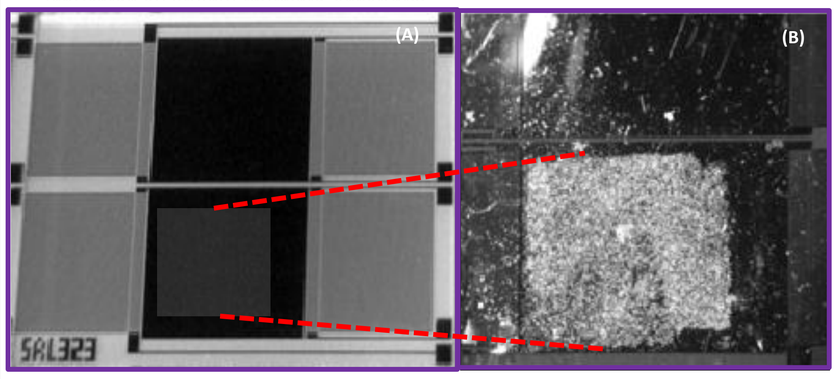

Figure 3. Optical micrograph of a metalized dual SAW biosensor (A) and the same biosensor functionalized with adherent Sf9 cells (B).

- In the present work, we have employed *Sf9* insect cell line, derived from parental colony *Spodoptera frugiperda*, as the functional layer ***(Figure 4)***.
- *Sf9* cells endogenously express octopamine receptors that are activated by the biomedical agent octopamine, an invertebrate neurotransmitter.
- Due to the ligand-receptor interaction, biochemical reactions are triggered within the cell which are detected by the SAW biosensor.

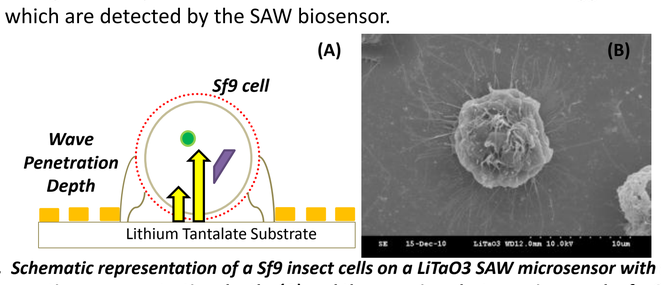

Figure 4. Schematic representation of a Sf9 insect cells on a LiTaO3 SAW microsensor with the associated acoustic wave penetration depths (A) and the scanning electron micrograph of a Sf9 cell adhered to the surface of a LiTaO3 SAW device after 1hr (B).

Experimental Setup

- A miniature RF oscillator circuitry for driving the SAW biosensors and a computer controlled microfluidic system for cell culturing and liquid phase measurements were developed in-house. ***(Figure 5)***

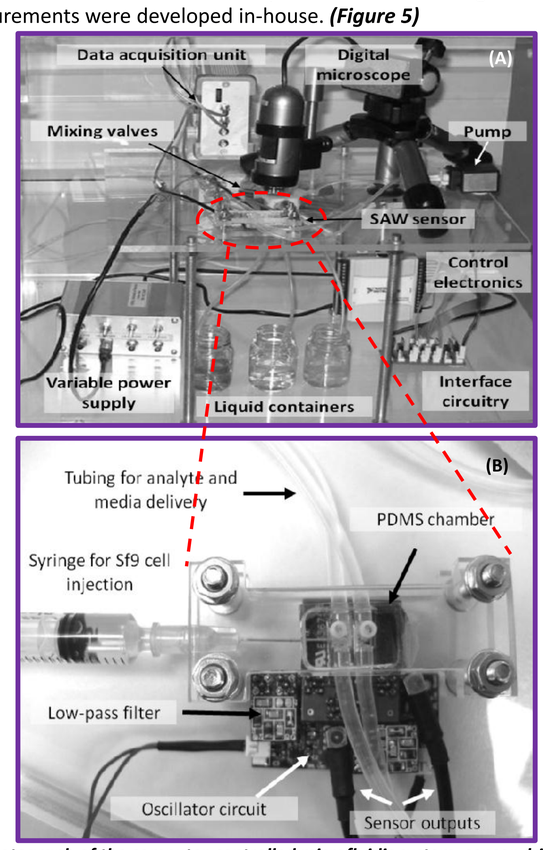

Figure 5. Photograph of the computer-controlled microfluidic system, sensor drive and interface circuitry (A) with an assembled dual SAW biosensor with its associated oscillator circuitry (B).

Results

Sf9 cell deposition and Surface Attachment Measurements

- Initial experiments were focussed on measuring acoustically the attachment of *Sf9* cell's onto the sensors surface ***(Figure 6)***. The process of cell attachment has been divided into three distinct phases:
  - an initial phase, ***Phase A,*** which depicts when the *Sf9* cells are injected onto the sensor surface.
  - an adherence phase, ***Phase B,*** which depicts the time taken by the injected cells to settle and start adhering to the sensor surface.
  - a stationary phase, ***Phase C,*** which depicts that most of the injected cells have attached successfully to the sensor surface.
- The entire process of *Sf9* attachment takes approximately 45 minutes and the shift in the resonant frequency of the sensor functionalised with *Sf9* cells with respect to the reference sensor was found to be **2840 Hz**.

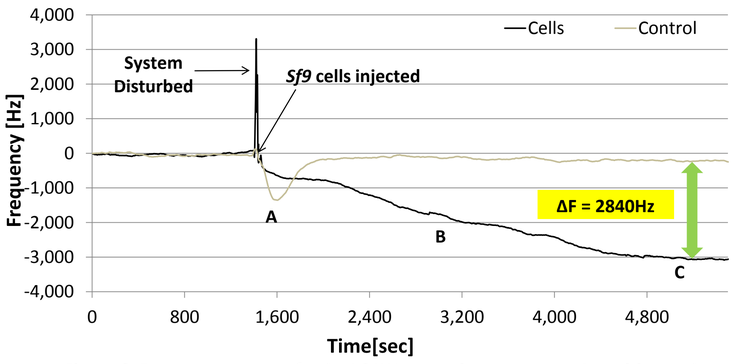

Figure 6. Frequency output of a dual SAW sensor in response to deposition and adherence of Sf9 cells on the sensing sensor surface.

Ligand Elicited Response

- Ligand-specific cellular responses are triggered when octopamine interacts with endogenously expressed octopamine receptors present on the cell membrane of *Sf9* cells. As shown in ***Figure 7***, these responses can also be categorized into 3 distinct stages.
  - An ***initial stage*** which includes ***Phase A and Phase B.***
    - ***Phase A*** depicts the time when 12.5µM of octopamine flows over the cells and the control sensor causing only the control signal to attenuate denoting a chemical response.
    - A ***Phase B***, in which we believe that, as a result of the interaction between octopamine and the endogenous receptors, downstream biochemical reaction are triggered. This happens after a time-lag of approximately 30 seconds (time taken for a measurable response to occur due to the ligand-receptor interaction) that according to Wicher et al. is the window within which the cAMP-dependent responses peak [1].
  - An ***intermediate stage*** which occurs between ***Phases B*** **and *C,*** show that there is a small transient increase in the intracellular Ca^{2+} level (in the nM region), which we believe, could be a store mediated effect or is caused by the influx of ions from the external media [2].
  - A ***final stage***, which is represented by ***Phase C***, which is when base media, for washing away octopamine, flows over the cells causing the signals to return to their respective baselines, depicted by ***Phase D***.

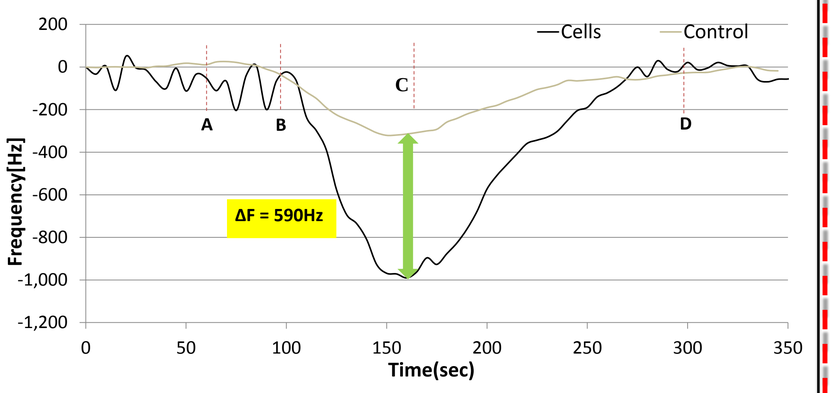

Figure 7. Frequency output of a dual SAW sensor in response to the introduction of 12.5 µM octopamine. The Sf9 cell-coated sensor's response is shown in black, the uncoated reference sensor's in grey.

Conclusions

Here, we have presented the design and the development of a *Sf9* whole-cell functionalized SAW biosensor and have reported preliminary results detecting ligand-activated cellular responses within a functionalized insect cell. This concept can be further exploited to enable the detection and quantification of cellular responses due to a wide variety of biomolecular agents. Further work will include functionalizing both the sensors surfaces (dual SAW) with whole cells, however, one of the cells will be transfected with insect ORs, thus eliminating non-specific cell response from the differential signal.

Acknowledgements

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