CELL-BASED SURFACE ACOUSTIC WAVE RESONANT MICROSENSOR FOR BIOMOLECULAR AGENT DETECTION

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ABSTRACT

This paper describes the development of a novel surface acoustic wave (SAW)-based biosensor system for liquid phase biomolecular agent detection. The functional layer of the biosensor comprises Sf9 insect cells that can be efficiently used for the expression of specific ligand receptors and is coupled to the acousto-electric transducer. We introduce the dual bio-SAW sensor concept where only one side of a device pair is functionalized while the other side serves as a reference: enabling a differential output that obviates common mode variations. The detection of cellular responses to octopamine (an invertebrate neurotransmitter) was used to demonstrate the biosensor system's efficacy. We believe that this biological sensor system can be used more generally to monitor changes in cell biochemistry and physiology when subjected to different biomolecular agents, e.g. the detection of receptor-specific ligand binding.

KEYWORDS

Surface acoustic wave, dual bio-SAW, cell-based, biosensor, Sf9, liquid phase, microfluidic system

INTRODUCTION

Chemical sensors have been employed to detect biomolecules; however, their use has only had partial success due to the limited compound specificity. The motivation for the work presented here was to improve sensor specificity (and sensitivity) by constructing a biosensor that employs a dual SAW resonator device coated with a cell-based biological functional layer.

The functional layer comprises a biological cell coating expressing specific ligand-receptors and is coupled to an acousto-electric transducer. In response to the presence of target biomolecules, cells with ligand receptors change their biochemical/ physiological properties, such as morphology or cytoplasmic Ca^{2+} concentration. The key specificity in the response of the biosensor to extracellular molecules (e.g. toxins, neurochemicals or odours) is due to the specificity of the transfected ligand-binding receptors. Recognition of a broad range of agents requires the synchronous activity of receptors such that the diversity of biomolecules can be matched. The employed SAW resonators were designed to operate at different frequencies that not only enhance sensitivity but also enable the characterization of different cell penetration depths. A dual configuration design is employed where only one device of the pair is functionalized and the other serves as a reference. The concept of dual SAW sensors is shown in Figure 1.

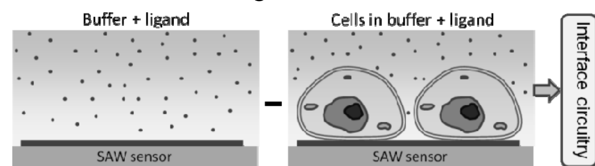

Figure 1. Dual Bio-SAW sensor concept: Sensing device is coated with cells while reference device is left uncoated. The differential output is free of common mode variations.

The non-functionalized device is placed in the same medium and subjected to the same changes as the sensing device, while the difference between the output signals is measured. This differential technique ameliorates environmental and other common mode variations and ensures that the measured responses are produced purely by the functionalized cell coating.

The changes in the velocity, $\Delta v/v$, and the attenuation, $\Delta\alpha/k$, of the propagating SAW due to the response of the Sf9 cells to ligand can be approximated by the following equations [1]:

$$\frac{\Delta v}{v} = -\frac{K^2}{2}\frac{\left(\frac{\sigma'}{\omega}\right)^2 + \varepsilon_0\left(\varepsilon_r^{'} - \varepsilon_r\right)\left(\varepsilon_r^{'}\varepsilon_0 + \varepsilon_P^T\right)}{\left(\frac{\sigma'}{\omega}\right)^2 + \left(\varepsilon_r^{'}\varepsilon_0 + \varepsilon_P^T\right)^2} \quad (1)$$

$$\frac{\Delta\alpha}{k} = -\frac{K^2}{2}\frac{\left(\frac{\sigma'}{\omega}\right)^2\left(\varepsilon_r\varepsilon_0 + \varepsilon_P^T\right)}{\left(\frac{\sigma'}{\omega}\right)^2 + \left(\varepsilon_r^{'}\varepsilon_0 + \varepsilon_P^T\right)^2} \quad (2)$$

Here, k is the wave number, ω is the angular frequency, K is the electromechanical coupling constant, ε_0 is the electrical permittivity of free space, ε^T_P is the effective permittivity of the SAW crystal, ε_r is the relative permittivity of the unperturbed biological layer, and ε'_r and σ' are the relative permittivity and conductivity of the perturbed biological layer, respectively.

MATERIALS AND METHODS

Biological microsensor design

A biological microsensor was implemented that utilizes the techniques described above and consists of a lithium tantalate-based dual SAW resonator functionalized with Sf9 insect cells. The sensor device is shown before (A) and after (B) functionalization in Figure 2.

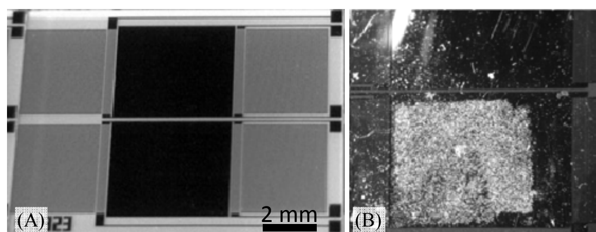

Figure 2. Optical micrograph of a metalized dual SAW biosensor (A) and the same biosensor fluorescence functionalized with adherent Sf9 cells (B).

Although the dual bio-SAW concept may be implemented using various cell types (e.g. yeast, HEK-293 [2], Sf9), several characteristics of Sf9 make these the ideal candidate for acoustic sensor applications. We have shown that Sf9 cells readily grow on different piezoelectric substrates, but more importantly, cells adhere to the surface of the SAW devices within ~45 min at the desired confluency; eliminating the need for a lengthy and infection-sensitive cell growth process. Moreover, the Sf9 cell-line grows at 28 °C and is more tolerant of variation in culture conditions. We have employed an SF9 insect cell line, derived from parental colony *Spodoptera frugiperda*, as a functional layer for the SAW biosensors.

The ability of the SAW biosensor to detect biochemical/physiological reactions within the cells due to ligand-receptor interaction depends on the SAW penetration depth which in turn is defined by the resonant frequency of the SAW sensor.

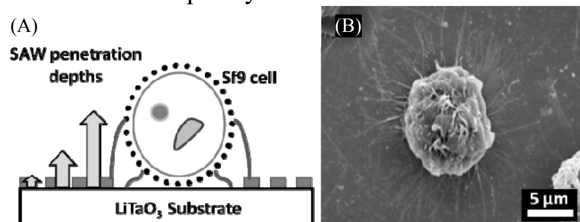

Figure 3. Schematic representation of an Sf9 insect cell on a $LiTaO_3$ SAW sensor with Au electrodes showing the different acoustic wave penetration depths (A) enabling the characterization of ligand-elicited cellular reactions, and a scanning electron micrograph of a Sf9 cell adhered to the surface of a $LiTaO_3$ SAW device (B).

A variety of SAW resonators operating at different frequencies (60 to 868 MHz) have been designed and fabricated to enable the measurement of intracellular changes occurring at different regions within the cell coating and quantifying the magnitude of the secondary cellular response with respect to the ligand-receptor interaction as shown in Figure 3.

Miniature radio frequency oscillator circuits with low-pass filters for driving the SAW biosensors and a computer controlled microfluidic system for liquid delivery during biomolecule measurement were developed (see Figure 4). A ~2 × 40 µL poly-dimethylsiloxane (PDMS) chamber is mounted on top of the sensors using a perspex holder to contain the Sf9 cells, the cell culture media and the analyte solutions. The Sf9 cells are introduced into the PDMS chamber from a syringe via a hypodermic needle.

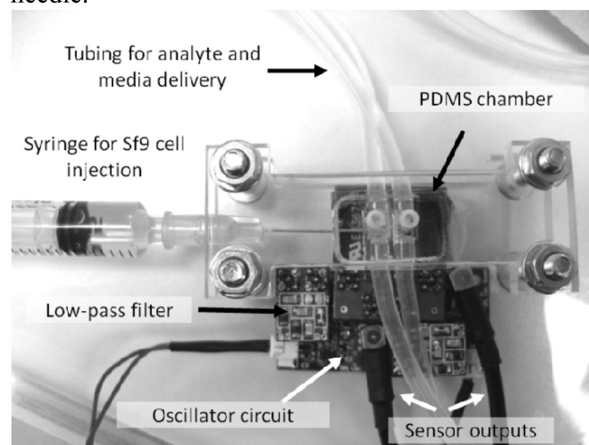

Figure 4. Photograph of the dual SAW biosensor with its associated oscillator circuitry.

Automated measurement setup

The biosensor's circuitry is powered by a 4-channel variable power supply and it is connected to a personal computer via a commercial JLM FQ4 interface (JLM Innovation GmbH, Germany). The microfluidic system comprises a diaphragm metering pump (KNF Neuberger, United Kingdom), six manifold mounted solenoid valves, C-Flex® tubing, a NI-6009 multifunction data acquisition board (National Instruments, USA), and a custom high-current interface circuit. A Dino-Lite digital microscope (Dino-Lite Europe, The Netherlands) enables the real-time visual monitoring of the Sf9 cells on the sensor surface. A bio-compatible incubator is used to ensure temperature stability. The complete set-up is shown in Figure 5.

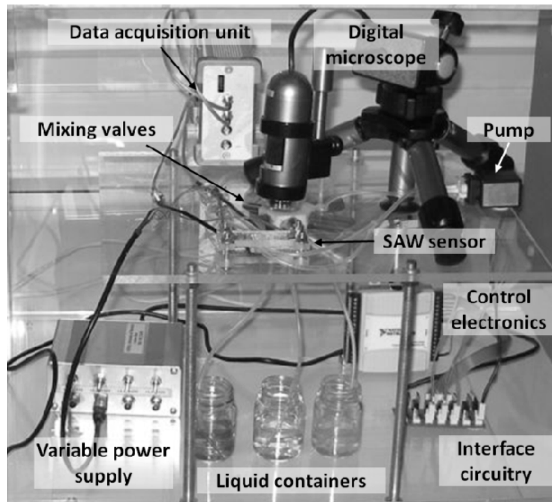

Figure 5. Photograph of the computer-controlled microfluidic system, sensor drive and interface circuitry.

RESULTS

Sf9 cell deposition on SAW biosensors

The sensing device of the SAW biosensor is functionalized by an Sf9 cell coating. A typical frequency output of the SAW biosensors in response to the deposition and adherence of ~40,000 Sf9 cells onto one sensing region of the dual device is shown in Figure 6. The frequency plot shows three distinct phases: an initial phase (A) which corresponds to the disturbance to the biosensor during the injection of Sf9 cells onto the sensor surface, an adherence phase (B) that is related to the cells adhering to the sensor surface by forming extracellular matrices (as shown in Figure 3B), and finally a stationary phase (C) which indicates that most of the injected cells have adhered to the sensor surface. An important point to note is that the control sensor (i.e. the sensor without cells), returns to its previous baseline after phase A.

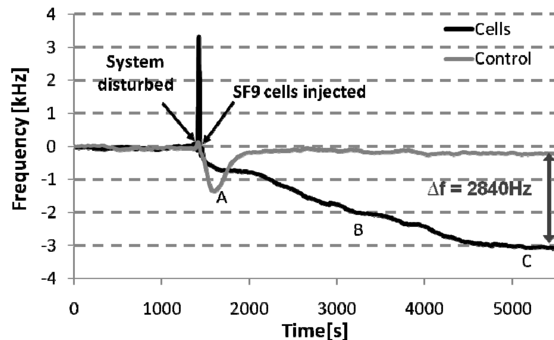

Figure 6. Frequency output of a dual SAW sensor in response to deposition and adherence of Sf9 cells on the sensing sensor surface.

The process of cell adherence takes ~45 min and the differential frequency shift is ~2.8 kHz. We believe that the frequency shift observed is associated with the difference in the mass density of the medium and the cells. Sf9 cell adherence was found to be surface independent as similar time-spans were recorded on SAW biosensors with and without the gold coating.

Biomolecular agent detection

The biomolecular agent octopamine hydrochloride was used to demonstrate the dual SAW biosensing concept, because the Sf9 cells endogenously express octopamine receptors. In order to elicit cellular responses in the Sf9 cells, 12.5 µM of octopamine hydrochloride, an invertebrate neurotransmitter, was delivered to the dual SAW biosensor using the microfluidic system. The frequency responses of both the cell-coated and the uncoated (i.e. control) SAW sensors are shown in Figure 7.

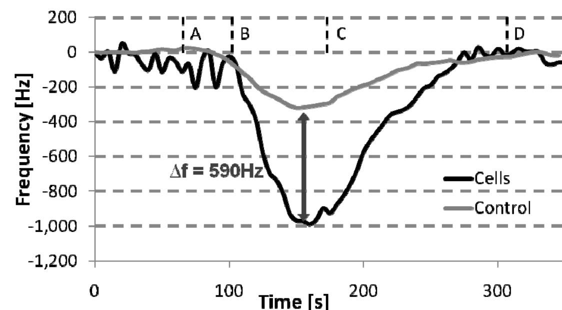

Figure 7. Frequency output of a dual SAW sensor in response to the introduction of 12.5 µM octopamine hydrochloride. The Sf9 cell-coated sensor's response is shown in black, the uncoated reference sensor's in grey.

Secondary cellular responses are triggered when octopamine interacts with endogenously expressed receptors present on the cell membrane of Sf9 cells. As shown in Figure 7, the frequency-response curve can be divided into four distinct phases. During the first phase (A) when octopamine flows over both the cell-coated and the control sensor, only the reference signal changes indicating a purely chemical response. In the second phase (B), the cell-coated sensor's signal changes indicating the start of secondary cellular responses due to ligand-receptor interactions. This response is delayed by ~30 s relative to the reference signal and is in agreement with the time-window reported by Wicher *et al.* within which the cAMP-dependent responses peak [3]. This delay may be the time needed for the activation of signal transduction within the cell. We believe that during phase B, the frequency shift associated with the introduction of the octopamine is a result of the change in the cell conductivity associated with the change in intracellular Ca^{2+}

concentration [4]. However, it is also possible that we could be observing other effects, such as a change in cell viscosity or volume.

After a two minute exposure to octopamine, the growth medium was allowed to wash away the neurotransmitter. As the octopamine-free cell growth medium reaches the reference and the cells, it starts removing the octopamine from the device surface and the receptors and, as a consequence, both signals start rising (phase C) and finally return to their respective baselines (phase D). The differential frequency shift due to 12.5 μM of octopamine was measured to be about 590 Hz (~10 ppm).

Enhanced sensitivity dual SAW sensor concept

This concept can be further exploited to enable the detection of a wide variety of biomolecular agents as shown in Figure 8(A). Now both devices have cell coatings, however, only cells on the sensing device express receptors to the target agent, thus non-specific cell response is eliminated from the differential signal. The implementation of this concept is demonstrated in Figures 8B-D, where Sf9 cells without exogenous receptors (B), with olfactory receptors (ORs) Or22a (C), or Or67d (D) are shown. The magnified confocal fluorescence micrograph (see the inset of Figure 8(D)) shows that the ORs are expressed at the plasma membrane.

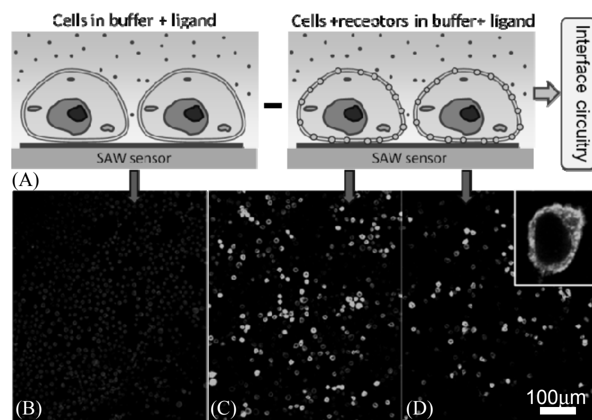

Figure 8. Enhanced specificity dual SAW sensor concept: cells with target-specific receptors on sensing device and cells without receptors on reference device. The differential output is free of both non-specific responses and common mode variations (A). Confocal fluorescence immunocytochemistry of transiently expressed ORs in Sf9 cells. Sf9 cells were transfected with a control construct pIB-empty (B) or OR constructs pIB-myc-Or22a (C) and pIB-myc-Or67d (D).The ORs are expressed on the surface of the Sf9 cells as visible in the inset micrograph.

CONCLUSIONS

We have described the development of a novel surface acoustic wave based biosensor system for the detection of biomolecular agents. The biosensing system comprises a low-cost, low-loss dual SAW device, a biological functional layer of Sf9 cells that was deposited on the SAW sensor surface, and microfluidic components for automated medium and analyte delivery. A computer controlled measurement and cell monitoring setup was designed and built that allows the simultaneous real-time electrical characterization and optical imaging of the biosensor.

We have shown that Sf9 cells adhere well to the surface of the $LiTaO_3$ SAW devices in approximately 45 minutes. This biological sensor device was used to detect successfully a biomolecular agent (octopamine hydroxide) in the micromolar range.

We believe that this dual SAW resonator-based biological sensor system can be used not only to detect receptor-specific ligand binding but also, and more generally, in order to monitor cell biochemistry (e.g. membrane potentials, intra-cellular ionic conductivity) and physiology (e.g. shape, turgidity, viability).

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