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On the acoustic wave sensor response to immortalized hypothalamic neurons at the device-liquid interface

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ABSTRACT

The response of a thickness shear mode biosensor to immortalized murine hypothalamic neurons (mHypoE-38 and -46 cells) under a variety of conditions and stimuli is discussed. Cellular studies which lead to the production of detectable neuronal responses include neuronal deposition, adhesion and proliferation, alteration in the extent of specific cell-surface interactions, actin filament and microtubule cytoskeletal disruptions, effects of cell depolarization, inhibition of the Na^+-K^+ pump via ouabain, effects of neuronal synchronization and the effects ligand-receptor interaction (glucagon). In the presence of cells, f_s shifts are largely influenced by the damping of the TSM resonator. The formation of cell-surface interactions and hence the increase in coupling and acoustic energy dissipation can be modeled as an additional resistor in the BVD model. Further sensor and cellular changes can be obtained by negating the effects of damping from f_s via the use of R_m and θ_{max}.

1. Introduction

The use of analytical techniques for the examination of cellular biophysical and biochemical phenomena has always constituted a very important area of research in many aspects of medical science, especially in the study of infectious and genetic diseases. Fundamental studies of a single, or a population of cells, in vitro, improve our basic knowledge of how such systems operate and, therefore, can act as a pillar for understanding the function of complex systems such as biological organs and life itself. A number of approaches stemming from the worlds of physics and engineering have been applied to the study of the behavior of cells: among these are microelectrode arrays [1], field effect transistors (FET) [2], light addressable potentiometric sensors (LAPS) [3], electric cell-substrate impedance sensor (ECIS) [4], patch clamp chip [5], surface plasmon resonance (SPR) [6] and scanning Kelvin nanoprobe (SKN) [7]. (A comprehensive review of specialized *electronic* techniques for this purpose is provided in Chapter 3 of reference [8]) A key issue, which is common to all these methodologies, is the necessity to attach and proliferate cells on non-biological substrates such as gold, silicon or quartz [9,10]. This process often requires the use of biologically functioning coatings or other forms of surface modification in order to mediate the surface adhesion. A further consideration is the necessity to operate a technique within the framework of the stringent conditions required to maintain the physiological conditions for cell viability and normal cell function.

The thickness shear mode (TSM) acoustic wave device offers the ability to study cells at acoustic wavelengths, non-invasively, under label-free conditions and in real-time [11].The sensor has been employed in the study of a variety of cells including endothelial cells [12], kidney cells [13], cancer cells [14], epithelial cells [15,16], fibroblasts [15,16] osteoblasts [17], and smooth muscle cells [18]. A common feature of a number of these investigations is the correlation of acoustic response with cell adhesion and spreading [19–22]. With regard to cellular behavior, studies have been conducted on the cell cytoskeleton using binding drugs to disrupt intracellular microtubules to cause hyperstabilization effects [20], cell-microparticle interactions [22], apoptosis and necrosis studies [23], toxicity [13], cell motility [24] and drug studies [7]. In addition, there have been investigations into the dynamics concerned with the processes of exocytosis and vesicle retrieval in PC12 and NG 108-15 cells [25] examination of the contributions of the extracellular matrix to the TSM signal for MDCK and 3T3 cells [26], and cellular cytoskeletal disruptions using nanomolar concentrations of nocodazole [27]. What is evident from all this research is that the TSM sensor does not behave as a simple “Sauerbrey-like” mass sensor in the presence of adhered cells and that published models are inadequate to describe what the TSM sensor actually detects.

In our own work on cells, we have concentrated on the response of the TSM device to hypothalamic neurons prepared from mouse embryo tissue at a confluence in the range of 80–100%. The cell lines were developed to provide a homogenous single cell model for studying molecular and biochemical investigations of the neuroendocrine hypothalamus. In addition to being clonal and providing a homogenous cell population as a monolayer for studies, the neurons are easy to maintain and culture onto the TSM substrate, with no required pre-coating of the sensor surface. In early work we studied the interaction of neurons with drugs such as forskolin and cerebrolysin [7]. Changes were observed with respect to acoustic parameters but no firm connection with alteration of

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neuronal behavior was feasible. In experiments with electrolytes, both series resonance frequency, *fs*, and motional resistance, *Rm*, were measured in a number of experiments involving the injection of KCl and NaCl into the flow injection- sensor-neuron system [28]. The various responses to these electrolytes were interpreted in terms of changes in cellular structure associated with the depolarization process. We have also examined in detail the interfacial behavior of neurons under various conditions [29]. These studies involved monitoring responses to solution flow, the absence of serum proteins, the effect of reducing specific cell -surface interactions and the disruption of the neuronal cytoskeleton components. For the adhesion and deposition of neurons, *fs* and *Rm* shifts are positively correlated to the amount of adhered neurons on the sensor surface, whereas non-adhered neurons do not produce any significant change in the monitored parameters. In the absence of serum proteins, initial cell adhesion is followed by subsequent cell death and removal from the sensor surface. The presence of the peptide, GRGDS is observed to significantly reduce cell-surface specific interactions compared to the control of SDGRG and this produces *fs* and *Rm* responses that are opposite in direction to that observable for cell adhesion. Finally, we have investigated the synchronization of the circadian rhythm generator and the effects of glucagon on hypothalamic neurons [30]. The process of partial and full synchronization of the cells resulted in different responses. For full synchronization, the addition of the serum bolus triggered resonant frequency and motional resistance shifts of +75 Hz and +18.5 U respectively, which decayed back to baseline levels after 30 min. The duration of this decay closely matched the time required for full synchronization in a separate study. The changes observed for partial synchronization were significantly different from full synchronization as the baseline levels in both resonant frequency and motional resistance were not re-achieved.

In the present paper, we collectively evaluate the capability of acoustic wave measurement with specific regard to the information that can be generated regarding cellular perturbations. In essence, this work constitutes an extension of our previous ideas presented with regard to biological macromolecule chemistry and conformational shifts occurring at the device-liquid interface [31].

2. Experimental

The details of a number of the experiments discussed in this article have been published as described above [28–30], accordingly, only a concise summary is provided here in order to assist in appreciation of discussion of the results of this work.

2.1. Cell lines

Hypothalami were harvested and dissected from mice at embryonic day 15, 17 and 18 and grown in primary culture. These cultures were infected with a murine, replication-deficient retrovirus containing a cDNA construct of the SV40 T-antigen and a neomycin resistance cassette. After infection, the cells were treated with geneticin (G418) at an initial concentration of 400–600 μg mL^{-1} to select for antibiotic resistance. The mixed hypothalamic cultures were then sub-cloned using serial dilutions into a 96-well plate to provide individual clonal populations.

2.2. TSM sensor configuration

9 MHz AT-cut quartz crystals (13.4 mm diameter) with symmetric gold electrodes (4.9 mm diameter) were used in the experiments. Subsequent to thorough cleaning, immortalized hypothalamic mouse neurons were cultured onto cleaned quartz crystals with Dublecco's Modified Eagle Medium (referred to as DMEM) supplemented with 5% Fetal bovine serum, FBS, cells were grown to 80–100% confluence and subsequently placed into either a home-made growth chamber or into a typical flow-through TSM chamber (Fig. 1), for measurements and solution introduction. In both configurations, the sensor with cells attached was placed horizontally into the assemblies.

The flow through configuration used a flow rate of 5–80 μl min^{-1} to deliver solution to the sensor surface via a syringe pump. For the growth chamber, a sterile syringe or micropipette was used deliver the solutions and the cell suspensions to the sensor surface. Both assemblies were placed in a hybridization oven to maintain the temperature at 37 °C, and 5% CO_2/air was maintained for the growth chamber assembly. For data collection, acoustic parameters, the series resonant frequency *fs*, the motional resistance *Rm* and other parameters were measured with a network analyser.

2.3. Procedures

For depolarization experiments, all solutions were warmed to 37 °C prior to use and maintained throughout the experiment. The mHypoE-38 immortalized hypothalamic mouse neurons were exposed to varying concentrations of potassium chloride to trigger cellular depolarization. The process was monitored with the TSM sensor and the two parameters specified above were recorded every 10 s. To better examine the process of depolarization of the neurons, the cells were exposed to varying concentrations of sodium chloride (NaCl) and to varying concentrations of potassium chloride (KCl). The study was also repeated on the mHypoE-46 cell line. The removal

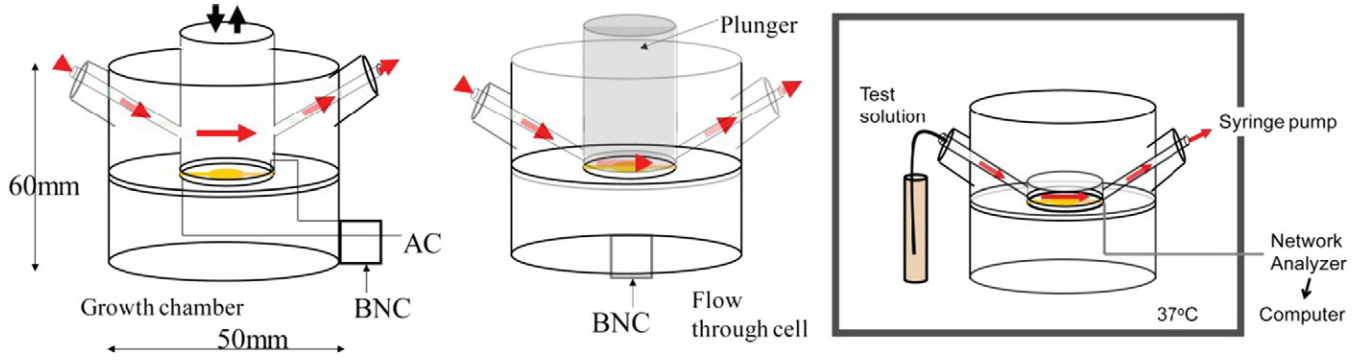

Fig. 1. Left: Static TSM chamber used for neuronal deposition experiments. Middle: Flow TSM chamber used for neuronal studies that require solution flow. Right: Experimental setup enabling temperature control via a hybridization oven set at 37 °C.