A Novel Love Wave Biosensor for Rapid and Sensitive Detection of Marine Toxins *

Xi Zhang, Jiaru Fang, Yingchang Zou, Ling Zou, Ning Hu, Ping Wang

Abstract—**Marine toxins are produced by plankton and do a great harm to human through food chain by accumulating in shellfishes and fishes. It is highly required and favorable to develop novel methods for the rapid and efficient detection of marine toxins to avoid the poisoning cases that have occurred frequently in many countries. This study presents a real-time Love Wave biosensor for the rapid detection of okadaic acid (OA), which used HepG2 cell lines as the sensing elements. The results indicate that this cell-based biosensor can provide real-time information of cellular activities induced by okadaic acid and has a higher sensitivity than the conventional cell-based assay. It is suggested that this cell-based biosensor can be used as a convenient and efficient method for marine toxin detection, which has a great potential to contribute to avoid the harmful effects of marine toxins on the human health.**

I. Introduction

Marin toxins, which are produced by algae, are easily accumulated to a high level in filter-feeding fishes or shellfishes. People get poisoned commonly by eating the contaminated seafood [1, 2]. Among all of the marine toxins, diarrheic shellfish poisoning (DSP) toxin is one of the most widely-distributed toxins in the world [3]. And okadaic acid (OA) and its acidic derivatives are important components of DSP toxin. OA could destroy some intracellular processes and structure such as metabolism, signal transduction, cytoskeleton and even cause cell death [4-7]. Therefore, the OA toxicity detection is extremely necessary. At present, the most commonly used method for marine toxin detection is the mouse bioassay in many countries. However, this method has many disadvantages, such as low sensitivity and ethical problem and has been replace by other techniques [8, 9]. Considering this, a Love Wave mode surface acoustic wave sensor is proposed here to achieve real-time cellular growth detection and toxicity detection. The most significant advantage of the Love Wave sensor is that the detection is noninvasive and harmless to cells [10, 11].

II. Methods

A. Love Wave Sensors

In the present study, the Love Wave sensor (Fig. 1) is consisted of a piezoelectric quartz substrate (ST cut) [12].

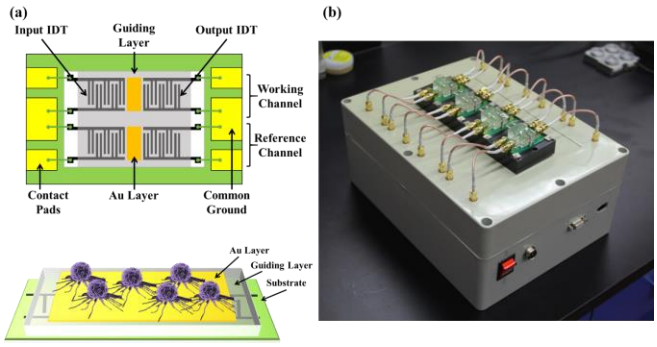

Figure 1. (a) View of Love Wave biosensor. (b) Picture of the detection system.

20/200 nm thick Ti/Au interdigitated transducers (IDTs) are deposited onto the quartz substrate in order to generate pure shear horizontal acoustic waves, which would propagate perpendicular to the X crystallographic axis. The input and output IDTs electrodes are comprised of 50 split-finger pairs [13] with a wavelength λ = 28 μm, which determines the center frequency of the sensor to be around 160 MHz. The spacing center to center between IDTs is 200λ and the acoustic aperture is 75λ. Afterwards, the IDTs patterned substrate is guided with a 3 μm SiO_2 film deposited by plasma enhanced chemical vapor deposition (PECVD), which confines the acoustic wave energy near the sensing surface and realizes the Love Wave mode [14]. For the purpose of improving the cell adherence to the sensor surface, 200 nm Au layer is deposited on top of guiding layer and the location is right between the input and output IDTs.

B. Microfluidic Chip

To implement the cell growth on the sensor surface, a polydimethylsiloxane (PDMS) chip is designed and used as the cell culturing chamber. Fig. 2 illustrates the scheme of the Love Wave sensor assembled with the PDMS chip. The chip is composed of four air cavities and two large liquid storage cavities. These air cavities could protect the IDTs and eliminate the electric influence of the liquid efficiently [15]. The liquid storage cavity is right in the middle of the air cavities, the height and volume of this cavity are about 12 mm and 150 μL, respectively. Cells and culture medium are injected in and removed from these liquid storage cavities using a micropipette. Before each experiment, the PDMS chip is mounted on the sensor surface tightly by a plexiglass plate and two screws (Fig. 2(b)), so that liquids could only stay in the large storage cavity without any leakage to the air cavities.

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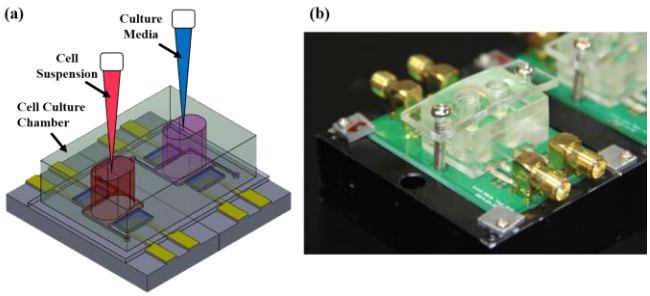

Figure 2. (a) PDMS microfluidic chip coupled with Love Wave sensor. (b) Picture of the fixation of the PDMS chip.

C. Circuit Design

The general schematic of the circuit for Love Wave sensor measurement is presented in Fig. 3. This circuit is designed in an open loop configuration. The operation principle of the circuit is as follows. The circuit consists of two parallel branches for obtaining a differential structure [16-19]. Two branches are the sensor branch and the reference branch, respectively. In order to excite the sensor, the excitation signal with a fixed frequency, which equals to the fundamental frequency of Love Wave sensor, is generated by the direct digital synthesizer (DDS). After that, the differences of the insertion loss and phase between two branches are measured. Since the signal of reference branch is constant, the variations of signal differences could be considered as the perturbation on the sensor surface. In this circuit, four wideband operation amplifiers (OPA656) are used as emitter followers in both branches. And the phase and insertion loss differences are measured by a phase detector which has been integrated in IC chip like AD8302. The AD8302 IC chip, which contains dual demodulating logarithmic amplifiers and a phase detector, is a fully integrated chip for measuring gain/loss and phase shift of two input signals simultaneously. To realize the multi-channel detection and reduce the IC chip usage, a wideband analog 4:1 multiplexer (ADG904) is utilized to isolate each channel. With this chip, only two phase detector chips are needed to meet requirement for 8-channel detection.

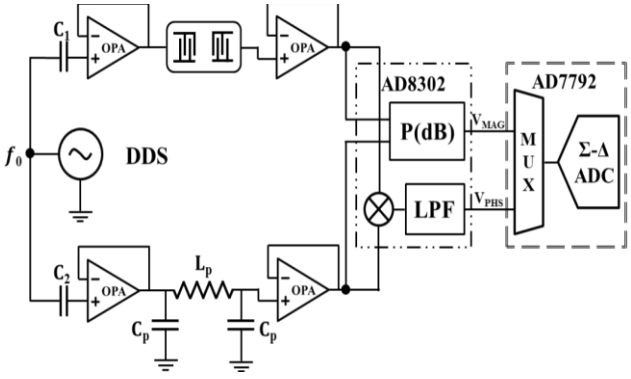

Figure 3. Schematic of the circuit for Love Wave sensor measurement.

D. Measurement System

To establish the multi-channel Love Wave sensor detection system, a portable and miniaturized instrument is employed here instead of the bulky network analyzer (Fig. 1(b)). The basic detection principle and circuit design of the instrument have been described above. By means of this circuit, both the amplitude and phase signals of sensors can be measured. The Love Wave sensor array assembled with PDMS chips is mounted on a custom-made metal holder, which is located on top of the instrument, and the sensor array is connected to the detection interface of the instrument through shielded cables. Then sensor signals are processed by the circuit and the detection results are converted via a multi-channel 16-bit ADC chip (AD7792) with high precision. After this electrical outputs converting procedure, information about both the amplitude and phase of sensors can be obtained by the MCU. Ultimately, data stored in the MCU are sent to the PC through serial communication and recorded by the LabVIEW software which was programmed specifically for this experiment. With this measurement system, responses of 8-channel Love Wave sensor array could be recorded in real-time and continuously.

E. Cell Culture

HepG2 cell line, kindly provided by Dr. Wu (School of Medicine, Zhejiang University, Hangzhou, China) were cultured in Roswell Park Memorial Institute (RPMI)-DMEM medium with 10% heat inactivated FBS and 0.5% antibiotic solution (10 mg/mL streptomycin and 1000 U/mL penicillin) at 37 °C in humidified air with 5.0% CO_2 in an incubator (Thermo Fisher Scientific, USA). The medium was changed every 1-2 days, and cells were detached from the culture flask by using 0.25% Trypsin-EDTA solution after reached the confluence of 80%.

III. Results and Discussions

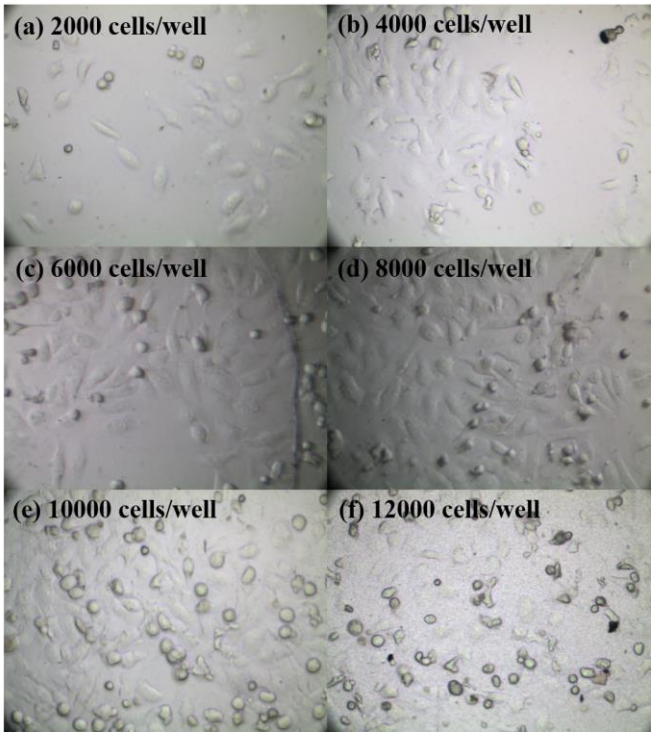

Figure 4. Cells growth situation observed by microcopy at 24h after cultured in the CO_2 incubator.

The number of initial seeding cells has a great effect on the output of sensor signals [20]. In order to establish a sensitive cell-based biosensor, it is important to optimize the number of initial seeding cells. So HepG2 cells were detached from culture flask and inoculated onto the sensor chips at 2000,

4000, 6000, 8000, 10000 and 12000 cells/well. After 24h, sensors were taken from the CO_2 incubator and observed with a microscopy. Fig. 4 shows the cell growth situation on the sensor chip. And with the increase of the initial seeding cell number, the number of cells on the sensor chip also increases except the last chip which is cultured with 12000 cells. The reason for this might be that the number of cells on the last chip is too large, and the culture media could not feed so many cells after 24h. So the cell apoptosis phenomena on the last chip appears much more quickly. Based on the obtained results and analysis, 10000 is the suitable seeding number to obtain a better cell-based biosensor.

Then Love Wave biosensors cultured with HepG2 cells were used for rapid and sensitive detection of marine toxins. In this experiment, 6 different concentrations of OA (10, 20, 40, 60, 100, 200 μg/L) were tested by our Love Wave sensor system. And tests for each concentration of OA were repeated for four times. Before adding the OA, the Love Wave sensor array cultured with cells and cell culture media was placed into the CO_2 incubator for 24 h. During this period, cell adhesion process lead to the amplitude value decrease. As we can see from Fig. 5, in the first 3 to 4 hours, the amplitude signal of sensor decreased greatly and the amplitude shift could reach about 4 dBm. The reason for this is that cells on the sensor surface started to deposit and attach to the sensor surface after cells were added to the cell culture chamber. And since the Love Wave sensor is very sensitive to the mass loading or the viscosity change on its surface, the amplitude signal of sensor quickly damped once cells attached to the sensor surface.

After cells were cultured for 24 h, different concentrations of OA were added to the cell culture chamber. And the sensor responses were measured for another 6 h by the detection system. OA is a powerful inhibitor of phosphatases type 1 and 2A, which affect phosphorylation and dephosphorylation of protein imbalance. The continuous phosphorylation of cytoskeletal protein will lead to the cytoskeleton damage and result in abnormal cell morphology or even cell death with OA concentration dependence. From Fig. 5, the amplitude of sensor starts to rise after adding OA. This shows that OA in the solution can influence the normal cell growth process and even cause cell death with high concentration. Dead cells will leave away from the sensor surface, which induces the amplitude increasing. According to the sensor responses, we find that the ultimate amplitude shift of sensors could distinguish the OA solution with different concentrations. Fig. 6 showed the correlation curve and equation for concentrations and amplitude shift at 12 h after OA treatment. A linear relation was found between the concentrations of OA and amplitude shift values ($R^2 = 0.9698$).

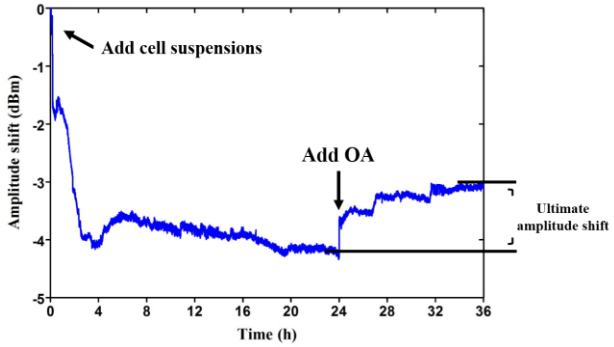

Figure 5. 36 h real-time detection curve of Love Wave biosensor to 200 μg/L OA.

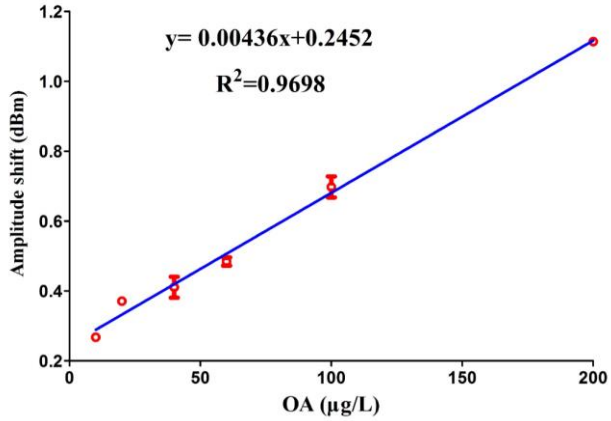

Figure 6. The relationship between the concentrations and amplitude shift at 12 h after OA added. All the data are presented in the form of means±SD (standard deviation), n=4.

IV. Conclusion

In this study, we described a Love Wave biosensor which is a label-free, real-time method to monitor cell activities induced by OA. Cell activities are dynamic in presence of toxicants, so conventional end-point assays miss much valuable information. Unlike the end-point assay, the Love Wave sensor provided a dynamic method to monitor cell activities caused by OA. For the further investigation, other cell lines and more toxins with various concentrations will be tested in order to establish a versatile cell-based biosensor. Cell-based biosensor developed in this study will be a helpful and important detection method in marine application of food analysis and environmental monitoring.

Acknowledgment

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