Detecting Drug screen by Acoustic Wave Sensor

Ching-Jui Shih [*], **Wen-Wang Ke** [*] and **Chien-Hui Lieu** [**]

[*] Electronic Research & Service Organization, Industrial Technology Research Institute, Tainan 709, Taiwan, R.O.C.

[**] Institute of Biotechnology in Medicine, National Yang-Ming University, Taipei 112, Taiwan, R.O.C.

ABSTRACT

The Severe Acute Respiratory Syndrome (SARS) is induced to the patients pervasively pneumonia and respired exhaustion. In previously study [2], the nucleic acid 318 and 510 of S protein was the crucial fragments to bind angiotensin-converting enzyme 2 (ACE2), which was very recently identified as a functional receptor for the SARS virus. For developing anti-SARS drug in traditional way, it's usually found out the chemical compound with anti-SARS capability to test tissue or animal with infective SARS virus. In this study, we develop a new method to rapidly and simply screen drugs, which can interfere with the interaction between ACE2 and S protein.

Electrostatic attraction and molecular recognition, S protein and hACE2, were employed to the functionalized Flexural Plate Wave (FPW) biosensor via Ni^{2+} chemical reactions. In our research, FPW sensor was used to distinguish the biomaterial recognizable property between adsorption of biomaterial and drugs precursor. We try to detect an anti-SARS drug by the measurement of frequency shift of FPW sensor due to the variation of weight. In our system, an anti-SARS drug transmit into sensor system with immobilization by S-hACE2 hybrid protein in Fig.1, it would destroy interaction between hACE2 and S protein by blocking S protein activity site. Furthermore, the results obtained by FPW measurements would be compared with the traditional methods such as gene engineering, PCR and western blot. Transfering gene and cloning S protein or hACE2 into Sf-9 insect cell and confirming protein activity were shown as in Fig.2, Fig.3 and Fig.4.

Keywords: SARS, Flexural Plate Wave (FPW), angiotensin-converting enzyme 2 (ACE2), S protein, Drug screen

1 INTRODUCTION

We report an on-line and quantitative Drug-screening system using a flexural plate wave (FPW) throughout the entire drug lead-compounds detection procedure and provides information on the surface coverage. Compared to nowadays drug screening systems the FPW system offers advanges of short response times, obviates the need for additive labeling reagents, and permits direct conversion of a frequency signal into mass acculation. The FPW technique is based on the measurement of mass change cause by the specific binding of a leadcompound to a specific protein immobilized on a resonating ALN fimes. With the addition of a small amount of mass to the surface due to binding of the specific-protein, the resonating frequency is proportionally decreased in accordance with the basic gravimetric sensing expression of below equation which is for a delay-line-oscillator FPW sensor as

$$\Delta f/f=S_m \Delta m \qquad (1)$$

In this equation f is the fundametal frequency of the FPW sensor, Δf and Δm are the changes of the frequency and mass, respectively, and S_m is the sensitivity of the sensor. Here, all the determination of the experiments follow this equation which is detected by transmission spectrum, group velocity delay, phase velocity shift, amplitude, and etc. Fig.1 shows the structures of the FPW sensor and the line width of IDT is 100um and the thin film of the FPW that is constituted by multiple layers is about 70% of the all areas. It's suggested that the well-defined IDT finger patterns can be fabricated at a good high frequency [3].

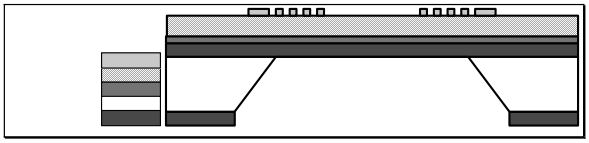

Fig.1 the structure of the FPW sensor

In the cell biology wold, enveloped viruses enter cells by binding their envelope glycoproteins to cell surface receptors followed by conformational changes leading to membrance fusion and delivery of the genome in the cytoplasm [4]. The recently, the angiotensin-converting enzyme 2(ACE2) is identified as a functional receptor for the SARS coronavirus (SARS-CoV) [5] and its binding site on the SARS-CoVS glycoprotein was localized between amino acid residues 303 and 537 [6]. In the previous studies, spike (S) proteins of coronaviruses, including the coronavirus that cause severe acute respiratory syndrome (SARS), associate with cellular receptors to mediate infection of their target cells. In general, the drug design is based on three ways, in transcription, translation, and protein-protein interaction livel. When the genome of virus ,in transcription level, is amplified by the host cell transcription system, or a lot of virus proteins, in translation

level, are expressed, or S proteins of the SARS contact with target cells, in protein-protein interaction level, all of these moments will be fousced and designed the new compounds. At the same time, we find that it is based on contacting live and infective virus for drug screen methods and do experiment with infectiving cells so that it is risky to do these kinds of screening methods. Here, we propose the new application to on-line and quantitative flexural plate wave sensors for drug-screening systems.

2 EXPERIMENT PROCEDURES

2.1 Sensor system setup and immobilization process

Because we don't direct contact with live and infective SARS coronaviruses, the gene of the S proteins are cloned in pGEM-T vector in E.coli in Fig.4. Fig.2 shows that it's the measurements of the experiment equipment and the immobilization process diagram.

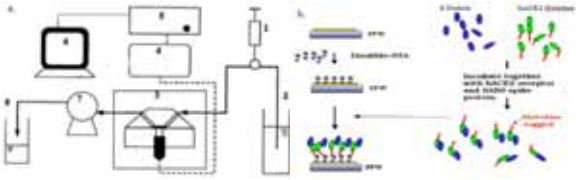

Fig.2 Sensor system setup and immobilization process diagram

In Fig.2a, the object 1 is the sample injection , the object 2 is the sample buffer , the object 3 is the flow cell holder with constant temperature and flexural plate wave sensor (FPW), the object 4 is the oscillator, the object 5 is the universal counter (Hewlett-Packard,Model 53131A), the object 6 is the computer with PCI GBIP Card for Windows OS, the object 7 is the Peristalsis pump, and the object 8 is the waste buffer and the immobilization process diagram is shown in Fig.2b. First, the Au film and the Cr film are depositioned about 2000Å and 150Å, respectively, on the sensing areas of the FPW sensors and then the crosslinker 3,3'Dithiolbi [N-(5-amino-5-carboxypentyl)-propionamide-N',N'-diacetic acid] dihydrochloride (disulfide-NTA) are patterned for 15mins on the sensing area by PECVD, washed by DI-water for two-times, and then immersed in the 0.1 M $NiSO_4$ for 10mins. The well-defind FPW sensors are stored in the buffer solution. On the other hand, S proteins and hACEs-Histidine are incubated together to form the effective affinity via protein-protein interaction at 4^oC, 5% CO_2 for 1h in the incubater. All experiments are performed at 4^oC, room temperture.

2.2 Gene recombination by PCR

Because it is stored for S proteins of SARS-CoV for a long times, there are four fragements of the gene of the S protein cloned in the pGEM-T vector in *E.coli.* In this study, the whole fragments of the genes of the S protein will be recombinated by PCR technique.

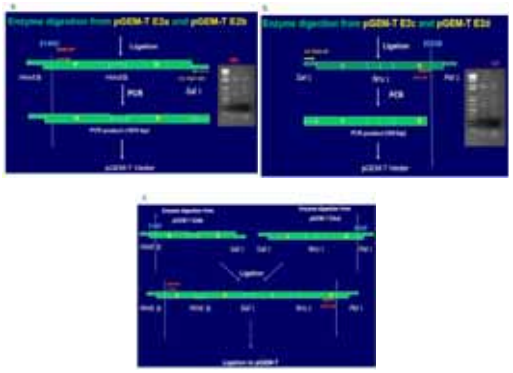

Fig.3 S protein gene recombination by PCR

In Fig.3a, the fragment E2a and the E2b are first ligated by PCR and the result is confirmed by DNA electrophoresis, the fragment E2c and E2d are ligated by PCR in Fig.3b. And then the fragment E2a-E2b and E2c-E2d are finially ligated to form whole the genome of the S protein in Fig.3c, and in Fig.4a and 4b, the whole genome fragments are cloned and confirmed in *E.coli* by DNA electrophoresis. Fig.4b shows the result of the whole genome of S protein of the enzyme digestion by *Sal I, Hind III, EcoR I, Nru I.*

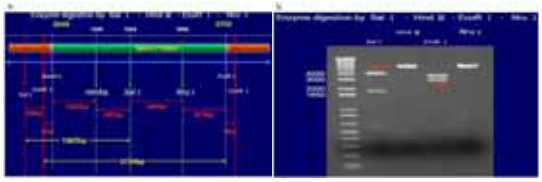

Fig.4 Clone and confirm S protein gene in pGEM-T Vector and in *E.coli.* by PCR

2.3 Clone S protein and expression in Sf-9 cell by PCR and western blot

In constant, it is lacked normal function for the gene products due to incomplete folding. Therefore, we tranfer gene to insect cell, Sf-9 cell, for produce functional protein. In Fig.5a, lane 1 is the markers, lane 2~6 are the whole genome of S protein by PCR, and then red color number 1~5 are consistant with Fig.4a which is diffent restriction enzymes cutting site. For detecting effective transfection, we count the viability of Sf-9 cells in Fig.5b (top column). The viability of living cells is maintain at 96%~97% during three days and it means that our transfection process is successfully. In Fig.5b (down column), the S proteins presented on the Sf-9 cell lysate are compared with right column by western blot.

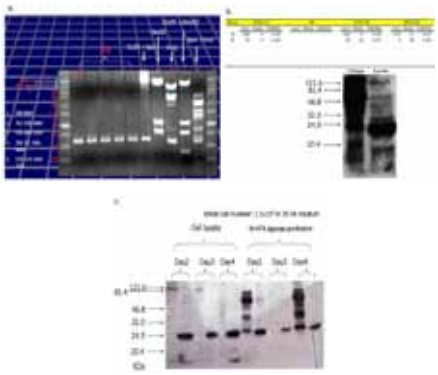

Fig.5 Clone S protein and expression in Sf-9 cell by PCR and western blot

For observing the S proteins expressed for a long time, we are compared with the Sf-9 cell lysate and NI-NTA agrose purification during four days. In cell lysate section, S proteins are expressed with the time to decrease as well as the NI-NTA agrose purification section. We seed with 1.5 x 10^8 cell in 30ml medium in both.

3 RESULTS AND DISCUSSION

The concept of this propose is focused on the drug screening system. We suggest that the researchers don't spend much time to design drugs and contact living and infectiving virus or tissues on our devices. There isn't with infective or pathological changes proteins at all the experiments process, and the theory of the devices is base on the protein-protein interaction level which is one of the drugs design process. Although we successfully transfect gene into Sf-9 cells and there are present normal functional protein, S proteins are still unstable present with time and there isn't good yield for the quantity in Fig.5. Scince these factors also change according to the protein patterning, the affinity between S protein and hACE2, the density of the disulfide-NTA on the surface area, chemical property of solid surface, immobilization of biomolecular material, etc., it's not easy to identify specific factors playing major roles for the sensitivity of the devices. One recent interesting finding is that the drug design process, incorporation acoustic wave sensor and keeping effectively immobilzing pattern appear to be key structural elements in making protein surfaces. This paper offers the unique possibility to explore not only the decreasing research times of drugs design but also without contacting infective tissues and virus.

REFERENCES

[1] P.A. Rota etc., "Characterization of a novel coronavirus associated with severe acute respiratory syndrome", *Science 300 (2003), 1394–1399.*

[2] S.K. Wong etc., "A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2", *J. Biol Chem, 279 (2004), 3197–3201.*

[3] D. S. Ballantine, Jr., R. M. White etc., "Acoustic wave sensors Theory, Design, and Physico-Chemical Applications", *ACADEMIC PRESS.*

[4] Ponraj Prabakaran, Xiaodong Xiao etc., "A model of the ACE2 structure and function as a SARS-CoV receptor", *Biochemical and Biophysical Research Communications 314 (2004) 235-241.*

[5] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K Wong, etc., "Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus", *Nature 426(2003) 450-454.*