RESEARCH ARTICLE

Rapid Detection of Ovarian Cancer from Immunized Serum Using a Quartz Crystal Microbalance Immunosensor

Yan Chen[1]*, Xian-He Huang[1], Hua-Shan Shi[2], Bo Mu[3], Qun Lv[4]

Abstract

Background: The objective of this study was to measure the antibody content of NuTu-19 ovarian cancer cells in serum samples using a quartz crystal microbalance (QCM) immunosensor. Materials and Methods: NuTu-19 cells were first cultured onto the electrode surfaces of crystals in Dulbecco's modified Eagle medium, and then specified amounts of immunized serum samples of immunized rabbit were also added. The change in mass caused by specific adsorbtion of antibodies of NuTu-19 to the surfaces of the crystals was detected. Results: The change in resonance frequency of crystals caused by immobilization of NuTu-19 cells was from 83 to 429Hz. The antibody content of NuTu-19 detected was 341ng/ul. The frequency shifts were linearly dependent on the amount of antibody mass in the range of 69 to 340ng. The positive detection rate and the negative detection rate were 80% and 100%, respectively. Conclusion: This immunoassay provides a viable alternative to other early ovarian cancer detection methods and is particularly suited for health screening of the general population.

Keywords: Ovariabn cancer - detection -quartz crystal microbalance - immunosensor - NuTu-19

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Introduction

Ovarian cancer, the leading cause of death from gynecologic cancers, is seriously imperiling the health of women. The majority of women have already in an advanced stage while diagnosing because the signs and symptoms of ovarian cancer are frequently absent early on and when they exist they may be subtle (Goff et al., 2000), and would be easily confused with other illnesses (Johannes, 2010), making prognosis poor. This has led to problem that the 5-year relative survival rate is less than 30% in advanced-stage disease (Jemal et al., 2009). Fortunately, 5-year relative survival rate would increase to approximately 90% at an early stage (Clarke, 2009), and furthermore, early detection will have no effect on fertility. Therefore, early detection of ovarian cancer plays an important role in successful treatment.

Now, bimanual pelvic examination, cancer antigen (CA) 125, and transvaginal ultrasound are the main methods for early ovarian cancer detection (Jelovac et al., 2011). However, they suffer the disadvantages of generally lacking sensitivity and specificity and being expensive. Therefore, a novel and cost-effective method with high sensitivity and specificity for ovarian cancer detection which we are currently investigating is urgently needed. Quartz crystal microbalance (QCM) immunosensor is an ultra-sensitive mass biosensor at the nanogram level. The core of the QCM immunosensor is the piezoelectric AT-cut quartz crystal sandwiched between a pair of electrodes. In QCM immunosensor, the target molecule was recognized by its homologous antibodies/antigens which are immobilized on the surface of a piezoelectric crystal. The resulting very small mass change is then transformed into the change in frequency of crystal resonator which is an easy measurable quantity. Here, the QCM immunosensor was used to evaluate the antibody content of the NuTu-19 cell in serum samples obtained from immunized New Zealand white rabbit. The frequency caused by specific adsorption on the surface of crystal significantly changed and can be easily measured by a frequency counter. The adsorption schematic is shown in Figure 1. In addition, we employed the negative control to verify that the research has significance for early ovarian cancer detection.

Materials and Methods

Animals and Cell line

New Zealand white rabbit were purchased from the West China Experimental Animal Center (Chengdu, China). NuTu-19, a rat ovarian cancer cell line were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured according to the supplier's protocols. Cells were cultured onto the crystal surface in Dulbecco's modified Eagle medium (DMEM)

[1]School of Automation & Engineering, University of Electronic Science and Technology of China, [2]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Clinical Medicine School, Sichuan University, [4]Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, [3]North Sichuan Medical College, Nanchong, China
**For correspondence: yanchencd@163.com*

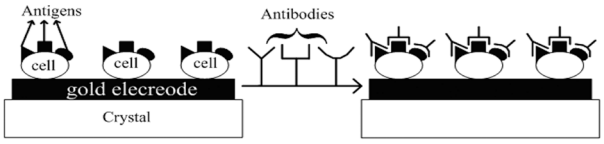

Figure 1. Schematic of Adsorption on the Surface of Crystal

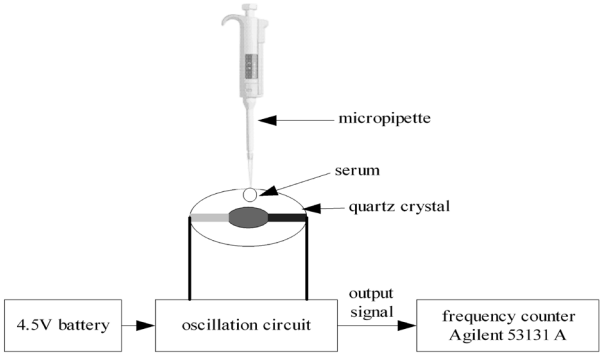

Figure 2. Test Diagram of the Whole Detecting System

(Gibco BRL, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, N.Y.) and 100 ug/ml Amikacin. Cells were maintained in humidified chamber at 37 °C in 5% CO_2 atmosphere.

Animal inoculations and antibody preparation

New Zealand white rabbits were immunized with 1×10^7 NuTu-19 cells in complete Freund's adjuvant (CFA) (Sigma) at day 0 and followed by two boosts with 1×10^7 NuTu-19 cells in incomplete Freund's adjuvant (IFA) (Sigma) at day 14 and 28. To get the antibody of NuTu-19, rabbit heart blood were collected under light anesthesia a week after the last injection. Then serum IgG was separated using a protein separation column, and non-immune rabbit serum was used as a negative control.

Equipment and apparatus

The 3rd overtone AT-cut QCM quartz crystals (10MHz, optically polished surface, 5 mm gold electrodes on both sides) were obtained from Tangshan JingYuan YuFeng Electronics co., Ltd (Hebei, China). A high resolution frequency counter (Agilent 53131A) from Agilent Technologies (US) was employed to monitor the oscillation frequency. A self-made gate crystal oscillator with a 74HC04 integrated chip and 4.5 v dc power supply was used. A 0.1 ul-5 ul variable volume micropipette obtained from KeXiao co., Ltd (Hang Zhou, China) was used for better volume control of the liquids. The test diagram is shown in Figure 2.

The relationship between the change in mass on the crystal surface and the corresponding change in the oscillation frequency has been shown by the well-known Sauerbrey equation as follows (Sauerbrey, 1959; Hlavay et al., 1977; Granstaff et al., 1994):

$$\triangle F = -2.26 \times 10^{-6} nF_0^2 \triangle m/A \quad (1)$$

where $\triangle m$ is the changed mass on the crystal surface, in g, $\triangle F$ is the change in resonance frequency of the coated crystal, in Hz, F_0 is the fundamental resonance frequency of the crystal, in Hz, n is the overtone number; A is the area of electrode surface, in cm^2.The mass sensitivity of the quartz crystal used in this study is 0.376 Hz/ng.

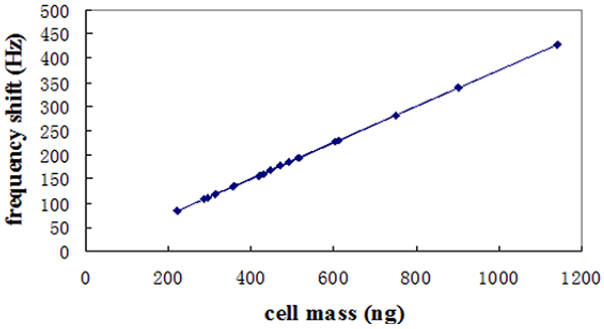

Figure 3. Relationship Between the Change in Mass of NuTu-19 cell and the Corresponding Change in Oscillation Frequency

Measurement procedure

The resonance frequency F_1 of all uncoated crystals were first measured by frequency counter at 25 °C.After culturing NuTu-19 cells on the crystal surface, the crystal was dipped in 2% paraformaldehydy-phosphate buffered solution for 10 min, then washed with phosphate buffer solution (PBS, PH 7.4) and deionized water (DW) twice to wash away all interfering substances and dried in air at 25 °C. Then the frequency F_2 of crystals with coated cells was subsequently counted. At last, the gold electrode surfaces of crystals were covered with NuTu-19–associated serum by micropipette and kept at 25 °C for 2 min, then washed with PBS and DW and air-dried, counting frequency F_3. The serum free anti-NuTu-19 IgG was measured under the same condition as reference.

Results

Cell immobilization efficiency

The immobilization of antigen on the surface of quartz crystal plays an important role not only in detecting accuracy and specificity but also in shortening the reaction time. In this study, the NuTu-19 ovarian cancer cells were separately cultured on twenty crystals so as to make NuTu-19 antigens firmly immobilize on the crystal surface. Figure 3 shows the effect of varying the mass of the NuTu-19 cells on the electrode surface of quartz crystal. Since the added mass of cells did not exceed the load limit of the crystal (0.0048 g), the added mass of NuTu-19 cells is linearly proportional to the frequency changes of the quartz crystal. Therefore, the immobilization range for NuTu-19 cell was 0.22-1.14 ug.

Stability of QCM

Figure 4 shows the frequency stability of the QCM during the test. Since the stability of QCM is a key factor to influence the detection accuracy, the QCM was placed in a thermostatic chamber at 25 °C to prevent the frequency stability from affecting by ambient temperature fluctuations and all the measurement equipments were started for 5 min in advance so as to make the equipments stable enough. The 3rd overtone AT-cut model of QCM is of excellent accuracy with ±1 Hz at 25 °C, and the results were highly reproducible.

Specificity and sensitivity of QCM

After culturing NuTu-19 cell on the surface of the

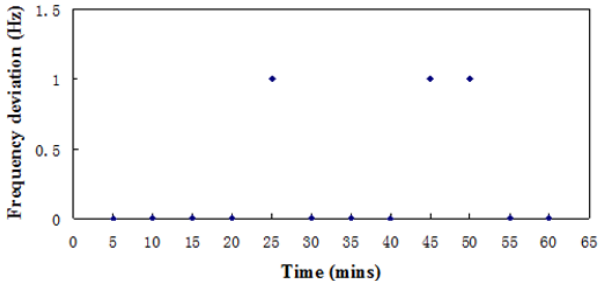

Figure 4. Example of Measured Frequency Stability

Table 1. Response of Sensors with Antigens to NuTu-19-associated Antibodies Immunized Serum (370 ng IgG/ul) and Negative Control Serum at 25 ℃

Crystal number	ΔF2 (Hz)	ΔF3 (Hz)
1	122	0
2	108	1
3	2	0
4	154	2
5	197	0
6	81	1
7	112	0
8	1	0
9	150	1
10	101	0

Table 2. Comparing Actual Change in Mass to Obtained Change in Mass

Amount of Immunized serum added (ul)	Actual Δm (ng)	obtained ΔF (Hz)	Obtained Δm (ng)
0.2	74	26	69
0.4	148	51	136
0.6	222	77	205
0.8	296	102	271
1	370	128	340

quartz crystal, the frequency shifts increased sharply from 83 to 429 Hz, corresponding to the mass change of 0.22 and 1.14 ug, respectively. When added 1ul immunized serum droplet onto the centre of the crystal electrode surface, the frequency shifts ($\triangle F_1$) increased significantly from 71 to 197 Hz, however, no significant changes in frequency shifts were observed when added on the same amount of the non-immune rabbit serum.

QCM immunosensor detection of the antibody of NuTu-19 from crude serum of immunized New Zealand white rabbit

QCM immunosensors exhibited significant increase in frequency shifts upon addition of crude serum containing the target antibody when compared with the negative control group. The frequency response to anti-NuTu-19 serum samples ($\triangle F_2$) and negative control group ($\triangle F_3$) were measured. The results were shown in Table 1. Since other interference factors may have a slight influence on frequency shifts, the frequency shifts which were below 5 Hz measured in the negative control group can be approximately equal to 0 Hz in the experiment. So there were eight out of ten crystals exhibited significant increase in frequency shifts. The average mass change in the experiment was 341 ng. This obtained mass calculated from Sauerbrey equation using $\triangle F_2$ was very close to the actual amount of mass (370 ng) added on the electrode of the crystal which was obtained using the protein quantification method.

In order to investigate the amount of frequency drop with respect to the added immunized serum droplet, the volume of serum added was from 0.2 μl to 1 μl, each time with a 0.2 μl serum droplet increase. The average of four measurements was shown in Table 2.

Discussion

In this paper, a novel method for indirect rapid detection of early ovarian cancer was proposed. From the meaningful results discussed above, it is clear that the difference between the serum samples with the antibody of NuTu-19 and without the antibody of NuTu-19 is so obvious that QCM immunosensor can be used to detect the antibody content of NuTu-19 from immunized serum. The novel antigen immobilization method not only eliminated non-specific adsorption but also shortened the response time since low sensitivity may be got when delayed the response time (König & Grätzel, 1993; Babacan et al., 2009). The 3rd overtone AT-cut model of QCM immunosensor used in this study increased the oscillation stability, ensuring the stability of whole test system. Furthermore, the sensitivity of this detection method was also ensured by this resonator. Although a difference between the obtained mass calculated from Sauerbrey equation and the actual amount of mass was existed, this work holds good for the quantitative detection of Nutu-19 in serum sample of ovarian cancer rabbits.

Besides high sensitivity and specificity, the proposed method in this study provides potential advantages, such as short analyzing time (less than 2 min), low cost (the heart of this detection system costs less than 1 dollar), and security (will not cause radiation damage for patients). Our study demonstrated that this novel detection method has no false-positive problem that it is particularly suited for health screening for the general population. This technique provides a viable alternative to early ovarian cancer detection methods. It also paves the way for its potential applicability in clinical practice. The only flaw that it can not identify the specific ovarian cancer-associated antibodies in serum samples makes our further investigation will focus on identifying the specific ovarian cancer- associated antibodies in serum.

Acknowledgements

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References

Babacan S, Pivarnik P, Letcher S, et al (2009). Evaluation of antibody immobilization methods for piezoelectric biosensor application. Biosens. *Bioelectron,* **15**, 615-21.

Clarke-Pearson DL (2009). Screening for ovarian cancer. *N Engl J Med*, **361**, 170-7.

Goff BA, Mandel L, Muntz HG, Melancon CH (2000). Ovarian

carcinoma diagnosis. *Cancer*, **89**, 2068-75.
Granstaff VE, Martin SJ (1994). Characterization of a thickness-Shear mode quartz resonator with multiple nonpiezoelectric layers. *J Appl Phys*, **75**, 1319-29.
Hlavay J, Gulbault GG (1977). Applications of the piezoelectric crystal detector in analytical chemistry. *Anal Chem*, **49**, 1890-8.
Jelovac D, Armstrong DK (2011). Recent progress in the diagnosis and treatment of ovarian cancer. CA *Cancer J Clin*, **6**, 183-203.
Jemal A, Siegel R, Ward E, et al (2009). Cancer statistics. *CA Cancer J Clin*, **59**, 225-49.
Johannes L (2010). Test to help determine if ovarian masses are cancer. *The Wall Street Journal*.
König B, Grätzel M (1993). Detection of human T-lymphocytes with a piezoelectric immunosensor. *Anal Chim Acta,* **281**, 13-8.
Sauerbrey G (1959). The use of quartz oscillators for weighing layers and for micro-weighing. *Zeitschrift for Physik A Hadrons and Nuclei*, **155**, 206-22.