Application of QCM DNA biosensor to detect a marine derived pathogenic virus VHSV

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Abstract

Each year infectious diseases cause substantial economic losses for public and commercial aquaculture ventures. VHS is one of most serious viral diseases caused by VHSV and damaging both fresh and marine fish species (Skall et al., 2005). Detecting VHSV has been dependent on the conventional methods which are time consuming and need highly skilled techniques (Skall et al., 2005). Thus this study aims to apply a sensitive QCM biosensor for detecting VHSV infection in fish to reduce detection time. Quartz micro balance (QCM) biosensors are working by measuring mass changes on sensor chip surfaces (Clark et al., 1987). Biological pathogens can be attached to the receptors immobilized surface of the biosensor chip and form an absorbed rigid thin film. This absorbed rigid films leads to the mass change of the electrodes and this mass changes leads to the change in the resonant frequency of the biosensor chip material. The receptors to detect pathogens can be any substances specifically binding ligands. Normally antibodies can be used as a receptor to detect pathogens but it has limitations as a protein which is unstable in ambient environment and in regeneration process. Recently researchers are seeking more stable receptors for example peptides, DNA, PNA, aptamers, and so on (Minunni et al., 2003, Tombelli et al., 2006). In this study we tried to develop a QCM DNA sensor to detect VHSV genome consisted by single strand RNA. We targeted to detect a main gene in VHSV genome i.e. G protein and constructed a probe specific for VHSV G protein RNA. We also attempted three different methods to immobilize probe DNA on quartz surface coated with gold: immobilization of thiol labelled probe DNA on naked gold surface (Tombelli et al., 2006), immobilization of amino labelled probe DNA on gold surface prepared as carboxyl chip using MPA followed by EDC/NHS activation, and immobilization of biotin labelled probe DNA on gold surface after immobilising avidin on carboxyl chip prior to biotin (Aung et al., 2008). As a result, immobilization method using avidin-biotin interaction was most efficient to detect target DNA. The QCM biosensor chip was outstanding for regeneration and sensitivity.

Keywords: VHSV, QCM, DNA sensor

1. QCM SYSTEM

A 9MHz AT-cut piezoelectric wafer layered with two gold electrodes of 5mm diameter had a reproducibility of ±0.1 Hz in frequency response and was used as the transducer of the QCM biosensor. It was mounted on a well holder made with acryl and connected to a home made oscillator module. The analogue frequency signals from the oscillator were converted to the digital ones in a frequency counter (Daga electronics, Korea). The in situ signal was stored in personal computer and performed data analysis using Microsoft excel program.

2. PREPARATION OF DNA PROBE AND TARGET DNA

Probe DNA was designed from the sequence of VHSV G protein and the length of probe DNA was 18 bp. Probe DNAs were designed to have biotin, thiol, or amine label at the 5‘- end. Target DNA was designed as complementary to probe DNA.

	DNA sequence
Probe	3’-X-CTGGTGACTGATAGCGGGT
Target	3'-TCCCGCTATCAGTCACCAG-5

Table 1 Sequences of probe and target DNA (X: thiol, amine, biotin)

3. Immobilization of probe DNA

We attempted three different methods to immobilize probe DNA on quartz surface coated with gold:

3.1 Immobilization of thiol labelled probe DNA Thiol labelled probe DNA (5μM) was crosslinked on the cleaned gold surface by interaction between thiol and Au and uncrosslinked Au residues were blocked by incubation with 10 mM of MPA (3-mercaptopropionic acid).

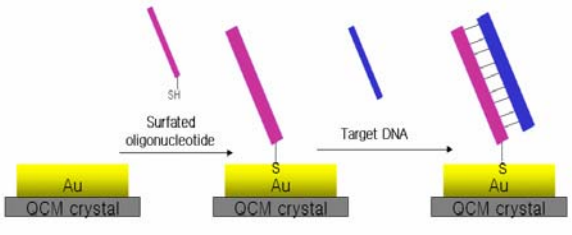

Fig. 1 Immobilization of thiol labelled probe DNA and hybridization of target DNA

3.2. Immobilization of amino labelled probe DNA Self assembled monolayer (SAM) was conformed by treating the quartz crystal with MPA and then activated with EDC(N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide)/NHS(N-hydro xysulfosuccinimide) to produce carboxyl chip. Amine labelled probe DNA (5μM) was immobilised on the prepared carboxyl chip and uncross-linked residues were blocked by ethanolamine-HCl.

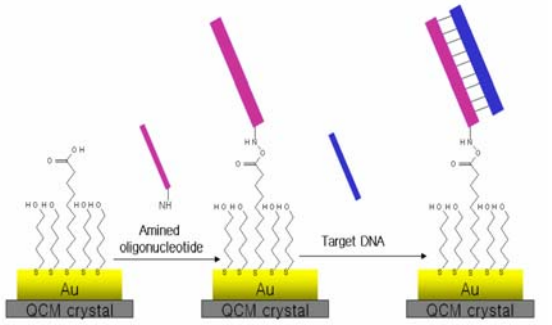

Fig. 2 Immobilization of amine labelled probe DNA and hybridization of target DNA

3.3. Immobilization of biotin labelled probe DNA Avidin chip was prepared by cross-linking avidin on the activated carboxyl chip and uncross-linked residues were blocked by ethanolamine-HCl. Biotin labelled probe DNA (5μM) was immobilised to the prepared avidin chip.

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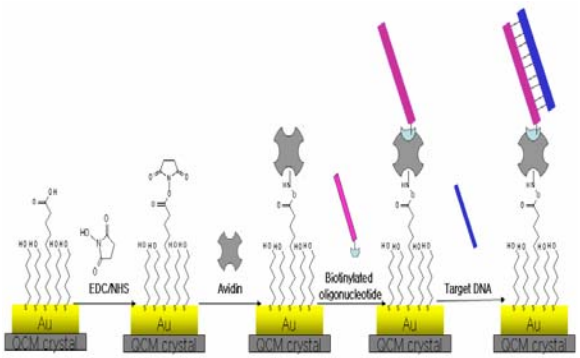

Fig. 3 Immobilization of biotin labelled probe DNA and hybridization of target DNA

3.4. Effects of immobilisation method on probe DNA immobilization

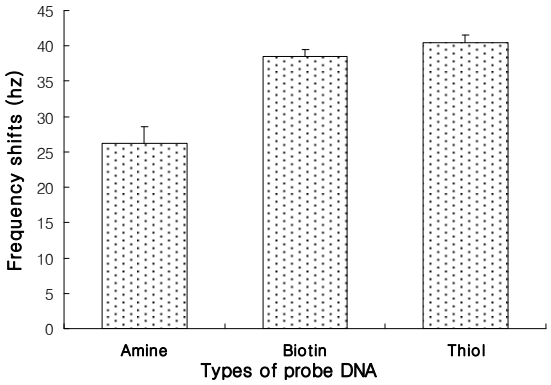

Fig. 4 Effects of immobilisation methods on probe DNA immobilisation

4. DETECTION OF TARGET DNA

4.1 Effects of immobilisation method on detecting target DNA To test sensitivity of three immobilisation methods, target DNA (T1) was added onto the sensor chip at the concentration of 1μM and the frequency shifts were calculated.

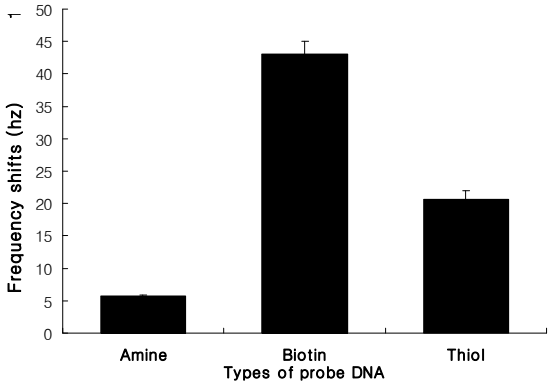

Fig. 5 Effects of immobilisation methods on detecting target DNA

4.2 Dose response of QCM system Serially diluted target DNA (T1) was added onto the sensor chip prepared by immobilising biotin labelled DNA probe on avidin chip at the concentrations of 1, 0.2, 0.04, 0.008, 0.0016 μM.

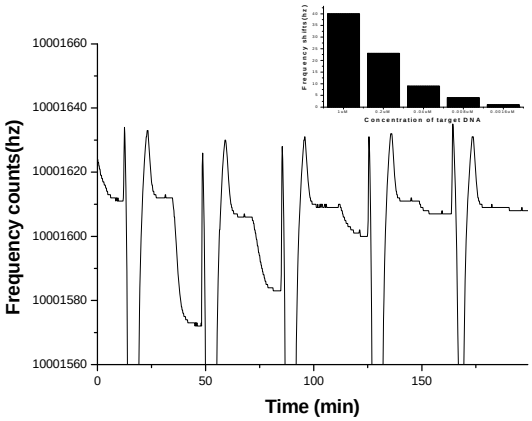

Fig. 6 Dose response

4.3 Specificity test The specificity of the prepared sensor chip was tested using a mismatched DNA. Complementary DNA (CT1) and mismatched DNA (MT1) were applied on to the sensor chip prepared by biotin labelled DNA probe at the same concentration, 1μM.

Mismatched DNA sequence:

3'-GAG CCT CCC CAC CTG ATG A-5'

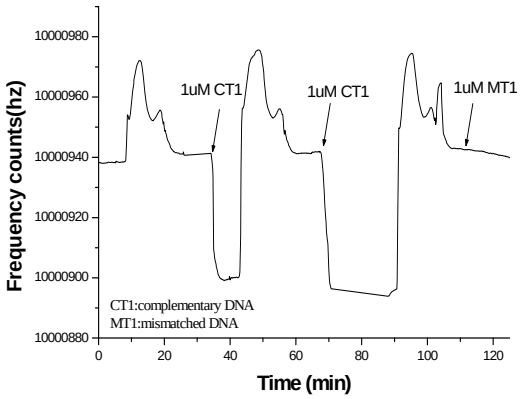

Fig. 7 Specificity test

4.4 Regeneration of sensor system The prepared sensor chip was regenerated after using by a dissociation buffer containing 0.8 M Tris-glycine, pH 2.3. Two hundred μl of target DNA (T1) was added through injection valve onto the sensor chip at the concentration of 0.2μM. For regeneration, 200μl of dissociation buffer was added, and washed out DNA by PBS. The same procedure was performed for 10 times on one sensor.

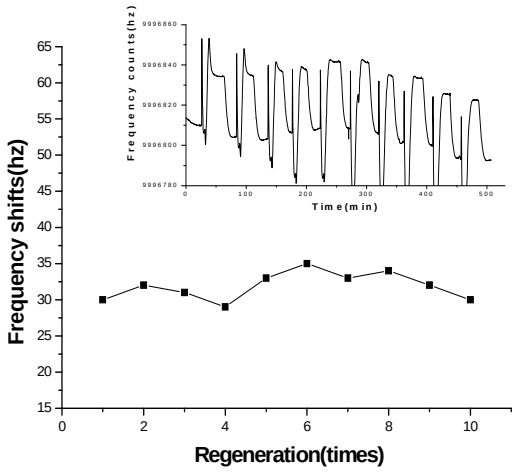

Fig. 8 Regeneration test

5. CONCLUSIONS

Among three immobilization schemes using thiol, amine, or biotin labelled DNA probe, the immobilization method using biotin labelled probe DNA was the most efficient to detect target DNA and the attachment of target DNA labelled with TAM was visualised under fluorescent microscope. The prepared sensor system was able to detect short (T1) and long (T2) target DNA as well as RNA samples from VHSV infected fish in dose dependent manner. The dose limit of the sensor system to detect T1 target DNA was less than 0.0016 μM. This QCM system showed an affinity to complementary target DNA but not to mismatched DNA. Also this QCM system was able to withstand regeneration procedure until more than 10 times uses without any significant reductions of signal. Thus QCM biosensor chip was outstanding for sensitivity, regeneration, and specificity.

REFERENCES

[1] DJ Clark, BC Blake-Coleman, and MR Ca lder “Principles and potential of piezoele ctric transducers and acoustical techniqu es, in Biosensors:fundamentals and Appl ication” Oxford University press, 551-57 1, 1987.

[2] HF Skall, NJ Olesen and S Mellergaard, J. Fish Diseases, 28, 509-529, 2005

[3] KMM Aung, X Ho, X Su, Sensors and actuators B, 131, 371-378, 2008

[4] M Minunni, S Tombelli, R Sielz, I Mannelli, m Mascini and C Gaudiano, Anal. Chim. Acta, 481, 55-64, 2003

[5] S Tombelli, M Minnuni, A Sautucci, MM Spiriti, M Mascini, Talanta, 68, 806-812, 2006