Label-Free Detection of AR-V7 mRNA in Prostate Cancer Using Yb$_2$Ti$_2$O$_7$ –Based Electrolyte-Insulator-Semiconductor Biosensors

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We developed an Yb$_2$Ti$_2$O$_7$-electrolyte-insulator-semiconductor (EIS) biosensor that can rapidly discriminate clinical statuses of prostate cancer (PCa) by detecting the existence of androgen-receptor splice variant 7 (AR-V7) messenger RNA. The structural feature of YbTi$_2$O$_5$ sensing membranes anneal to Si substrates through reactive cosputtering with different annealing temperatures investigated. Results showed annealed at 900 °C exhibited higher sensitivity of 61.11 mV/pH, lower hysteresis voltage of 1.6 mV, and smaller drift rate of 0.16 mV/hr. This attributed to the well-crystallized Yb$_2$Ti$_2$O$_7$ structure, detected using X-ray diffraction and X-ray photoelectron spectroscopy. We then compared two different methods, encapsulating or covalently bonding of the probe, to detect existence of AR-V7 in PCa. After hybridization of the probe with the target cDNA sequence, the covalent binding of AR-V7 probe method exhibited a larger $V_{REF}$ shift in the capacitance-voltage curves for the detection of AR-V7. Furthermore, highly reliable and stable results (>95% repetition rate) also could be observed when compared to that encapsulated probe on the EIS.

Our data demonstrated this AR-V7 probe-based Yb$_2$Ti$_2$O$_7$-EIS biosensor is a rapid, simple, label-free, economical, and accurate diagnostic device, and provide fast identification of resistant statuses in circulating tumor cells of PCa, essential for clinicians to make defining decisions.

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The discovery of variations in a gene pool may help distinguish genes of the mutant type that can contribute to diseases, and this is commonly accepted as a biomarker. Detecting the tumor-derived DNA or RNA is one of possible ways to perform regular surveillance of cancer statuses, for example, the stage of tumor development, tumor metastasis, recurrence or drug resistance in cancer cells etc.1,2 Thus the clinicians accord the information to select suitable treatment strategy for patients, this is considered as a meaningful approach and essential for the development of cancer precision medicine.3 In some advanced prostate cancers (PCa), for example, castration-resistant prostate cancer (CRPC). The disease is often resistant to anti-androgen hormone drugs, enzalutamide or abiraterone. The mechanism is known usually a result of androgen-receptor isoform encoded by splice variant 7 (AR-V7) messenger RNA (mRNA).4,5 Patients who’s cancer progresses into metastatic status usually have a poor prognosis, and are expected to survive less than 19 months.6,7 The AR gene is located on chromosome Xq11–12, and spans approximately 180 kb of DNA with eight known exons, whereas the AR-V7 mRNA lacks the ligand-binding domain that is the target for both enzalutamide and abiraterone. In addition, it remains constitutively active as a transcription factor,4 therefore, AR-V7 is considered very promising to serve as a biomarker for PCa, and a good prognosticator of the efficacy of these endocrine drugs in CRPC. As soon as CRPC patients have been identified as AR-V7 splicing positive, they are regarded as hormone drug resistant.8–11 Fortunately, the expression of AR-V7 can be observed in circulating tumor cells (CTCs) of PCa patients.12,13 However, there is a demand for rapid and accurate nucleotide analytical techniques to detect drug resistance in CRPC to improve management of such patients. Indeed, there are several ways nowadays to detect nucleotide alterations. For example, fluorescent in situ hybridization (FISH),14 and flow cytometry analysis,15 these labeling methods are commonly used for medical diagnostic purposes. However, these mentioned techniques are expensive, time consuming and laborsome, as they usually required technical expertise to generate results, and more often than not, it is unreliable due to dye decay, all of these present substantial restrictions in clinical application.

In recent years, semiconductor-based biosensors, e.g. ion-sensitive field-effect transistor (ISFET), field-effect transistor (FET), and electrolyte-insulator-semiconductor (EIS), have been widely used to help clinical diagnosis of cancers by measuring levels of gene expression, monitoring the occurrence of drug resistance, and detection of genetic variations16–20 etc. These biosensors have attracted much attention due to their compact dimensions, real-time response, high sensitivity, and label-free method of detection. ISFET was first introduced by Bergveld in 1970,21 the surface potential of an ISFET device is modulated due to the variation in charges at the surface-electrolyte interface. Such an ISFET sensor has been employed to estimate the hydrogen ion concentration in the solution. Moreover, it is a suitable candidate for biosensor applications, e.g. enzyme, urea, protein, DNA hybridization, and DNA methylation detection biosensors.22–26 Compared with the ISFET device, the main advantage of electrolyte-insulator-semiconductor (EIS) device is a simple structure and fabrication process. Silicon nitride (Si$_3$N$_4$) sensing membrane is the most popular material of choice for EIS (or ISFET)-based pH sensing due to the ease of fabrication, and the high compatibility for complementary metal-oxide-semiconductor (CMOS) integration.27 Although Si$_3$N$_4$ film is a chemically-stable dielectric material that shows low leakage current and surface hydration, EIS sensors based on such sensing membrane have unstable properties on threshold voltage. This primarily arose from the conversion of Si$_3$N$_4$ to a hydrated SiO$_2$ or an oxynitride layer in an aqueous solution.28 Recently, it has been reported that rare-earth (RE) oxide films (e.g. Y$_2$O$_3$, Pr$_2$O$_3$, Er$_2$O$_3$, Yb$_2$O$_3$, Lu$_2$Ti$_2$O$_7$ and Lu$_2$O$_3$)29–33 are promising substitutes for Si$_3$N$_4$ because of their high dielectric (high-$κ$) constants, large conduction band offsets and good thermal stability with a silicon substrate. Nevertheless, the main problems of using RE oxide are a silicate layer and high hygroscopicity,24 which has an adverse effect on sensing performances. These problems can be overcome through the incorporation of TiO$_2$ or Ti into RE oxides to improve the structural and electrical properties such as lower reactivity to water, thinner interfacial layer, higher capacitance value, and lower leakage current.35,36

Recent interest in the immobilization of biomolecules onto FET sensors has been directed toward the development of high-sensitivity and long term-stable biosensors.37 The entrapment method of biological molecules on the sensor is generally believed to provide a relatively milder condition to possibly minimize the alteration or the loss of biological activity during a gelatinization process. This method has been widely employed for immobilization of biological components.38–40 However, the repeated operations of biosensing might lead to cross contamination, and hence they cause detection biases. Organic monolayers have the ability to self-assemble onto the sensor surfaces;41 the monolayer modified sensor is suitable as the template for orderly immobilization of biomolecules. The immobilization of biomolecules onto a sensor surface is mostly preceded by the silanization process.23 This process yields chemically reactive amine (-NH$_2$) groups on the
surface. Glutaraldehyde is a bifunctional reagent able to react with two primary amine groups. These glutaraldehyde groups provide cross-linking chains between the biomolecule and sensor surface under a broad range of reaction conditions. In this study, we followed our colleague’s previous study to improve the accuracy of the sensor by modifying its membrane, and to change the way in which nucleotide probes are immobilized with two new methods. Most importantly, the probe was designed specifically for detecting CRPC resistance. We successfully developed an Yb\textsubscript{2}Ti\textsubscript{2}O\textsubscript{7} EIS biosensor to discriminate the clinical statuses of prostate cancer, which detects the presence of AR-V7 mRNA. We used X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) to characterize the film structures and compositions, respectively, of the Yb\textsubscript{2}Ti\textsubscript{2}O\textsubscript{7} sensing films after annealing at various temperatures. Furthermore, we compared the immobilization of oligonucleotide probe combined with cDNA sample on an EIS sensor by entrapment or covalent attachment.

**Methods**

**Fabrication of an EIS sensor.**—The EIS structures features an Yb\textsubscript{2}Ti\textsubscript{2}O\textsubscript{7} sensing membrane were fabricated on 4-in. p-type Si (100) wafers. Prior to the deposition of Yb\textsubscript{2}Ti\textsubscript{2}O\textsubscript{7} films, the wafers were cleaned using a standard RCA process and then treated with 1% HF to remove the native oxide. An Yb\textsubscript{2}Ti\textsubscript{2}O\textsubscript{7} film (∼70 nm) was deposited on the Si substrate by means of cosputtering from both ytterbium and silicone gel (S181) acted as an isolating layer. Yb\textsubscript{2}Ti\textsubscript{2}O\textsubscript{7} EIS devices defined by using a robotic dispensing system, in which an adhesive was used to encapsulate the EIS device and the Cu line. The pH buffer solutions (Merck Inc.) were then measured through an LCR meter controlled by personal computer. All of the measurements were carried out in a dark condition to avoid light and noise obstruction.

**Design of oligonucleotide probes contained with or without AR-V7 sequences.**—The RT-PCR products were presented on gel electrophoresis showed the nearly 300 bps bands. The exact sequence of mRNA AR-V7 was then further read by sequencing analysis. Besides, both probes were also double confirmed the sequence by sequencing analysis after synthesis.

The mRNA sequences with the splicing variant expressing product of AR gene at exon 3/CE3 were denominated as “AR-V7 probe”, and formed a 26 mers sequence 5’-CAAGCATCTCAAATGACCAGACCT-3’. The other control probe without the AR-V7 sequence were denominated as “Ctr. probe”, and synthesized as exact same length of sequence 5’-GTTGGAGCTGGTGGCGTAGGCAAGAG-3’ for parallel exams. An acetyl functional group was added to 3’-end to prevent the further elongation of sequence; and an amine- group was modified at 5’-end of sequences. (Protech Technology Ltd, Taiwan).

**Testing samples dsDNA preparations.**—To test the specificity of AR-V7 probe, the positive mRNA fragments from human CWR22R cell line was diagnosed as prostate cancer, in which the AR-V7 various was confirmed by sequencing; whereas the control sample derived from the colorectal tumor cells, as the cells without harboring a AR-V7 various, therefore used a random sequence. The mRNA single strand template was converted into a complementary DNA (cDNA) by reverse transcription polymerase chain reaction (RT-PCR). Firstly, the cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Semented with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin and streptomycin at 37°C in a humidified atmosphere with 5% CO\textsubscript{2} for 72 h. Cell pellet was prepared when cells confluence reached 70–85%, then extract the total RNA by using Trizol reagent (Life technologies Ltd. Taiwan). To perform cDNA synthesis, a total of 500 ng RNA was mixed with Moloney murine leukemia virus reverse transcription kit (Promega Technologies Ltd, Taiwan), then mixed with GoTaqGreen Master Mix, and each 10 μL of forward and reverse primers (F5’-GCTGAAGGGAAACAGAAGTACCTG-3’, and R5’-AGTCTGTGTTAGGGACCAAAAC-3’) were used for preparation of AR-V7 cDNA: F5’-TAAGCCTGTTGAGGACCAAAAC-3’ and R5’-TGTTGCGCTGGAGGACCAAAAC-3’ were used to prepared control dsDNA were added. PCR was carried out under optimized conditions.
conditions by PCR machine (Applied Biosystems Veriti 96-Well Thermal Cycler), 40 cycles, at 95°C for 60 s, 54°C for 40 s, 72°C for 60 s, and final products would check on 1% agarose gel should generate total 290 bps for the AR-V7 sample. The products were further using Sanger’s sequencing method to confirm the accurate sequences (Genomics Ltd. Taiwan).

**Entrapment attachment of oligonucleotide probe onto an EIS sensor.**—Before entrapping the oligonucleotide probe on the sensing area, the C-V curve of an EIS device was firstly measured in a pH 7 buffer solution and used as a standard curve. The hybridization detection was then performed using a mixture of AR-V7 (or non-AR-V7) probe and target cDNA sample; both were immobilized by entrapment in 5 μl 1% of agarose gel on the surface of an EIS sensor (Figure 4a). The results were then compared to the standard curve (Figures 5a and 5b).

**Covalent attachment of oligonucleotide probes onto the EIS surface modified with APTES followed by glutaraldehyde.**—To steadily attach probes onto the Yb2Ti2O7 sensing membrane EIS surface by covalent bonding. The Yb2Ti2O7 membrane surface was modified with 2% APTES and then treated with 2.5% glutaraldehyde (Sigma-Aldrich) at 70°C, both conducted at N2 ambient for 10 minutes in order to form a functional amino group (–NH2) and aldehyde group (–COOH) on each end (see atomic force microscopy images. In order to the alignment the elements of APTES and glutaraldehyde,43 a uniform electric field was performed in the parallel plate capacitor (1 mm) by applying a positive voltage (0.5 V) on a metal plate while the EIS surface functionalized with APTES and glutaraldehyde was grounded. The membranes were rinsed with deionized water and oscillated for 10 minutes to remove the unbounded molecules. 5′-amino-modified probes were immersed on an aldehyde group-modified EIS surface for 1 hour at room temperature. The functional amino groups of oligonucleotide probe were also subjected to a constant electrical field. Before hybridizing with cDNA samples, double strand (dsDNA) samples were denatured at 82.5°C for 3 min and dipped on the Yb2Ti2O7 surface, then rinsed by deionized water an hour to remove unbinding cDNA samples (Figure 4b).

**Figure 2.** (a) XRD patterns of YbTi2O7 sensing films annealed at different temperatures. XPS spectra of (b) Yb 4d, (c) Ti 2p and (d) O 1s for YbTi2O7 sensing films annealed at different temperatures.
Results and Discussion

Structural properties of YbTixOy thin films.—The crystalline structures of the Yb2Ti2O7 sensing membranes subjected to different annealing temperatures were analyzed using XRD, as shown in Fig. 2a. For the sample annealed at 300 °C, no peak was found in the 2θ diagram, indicating an amorphous structure. Conversely, the crystalline structure of Yb2Ti2O7 was observed in the sample above 500 °C, showing one stronger Yb2Ti2O7 (400) peak. Moreover, it was also noticed that the sample annealed at 700 °C became from Yb2Ti3O7 (400) to Yb2Ti2O7 (222) peak in the 2θ plot. The peak intensity of the Yb2Ti3O7 (222) for the film annealed at 900 °C exhibited higher than that at 700 °C, indicative of a better Yb2Ti2O7 structure.

In addition, the compositional properties of the Yb2Ti2O7 membranes treated with different annealing conditions were examined using XPS. Figure 2b showed the Yb 4d XPS spectra for the Yb2Ti2O7 membranes after annealing at different temperatures. Each fitting peak is assumed to follow the general shape of the Lorentzian–Gaussian function. The binding energy of Yb 4d5/2 peaks at 185.8 eV (the solid lines) can be assigned to the Yb2Ti2O7 composition, whereas they are located at 185.1 eV for the reference Yb2O3 (the dashed lines)(Figure 2b).41 The chemical shift of the above Yb 4d5/2 peak moving toward higher binding energy indicates the difference between the Yb-O-Ti bonding in Yb2Ti3O7 and that in Yb2O3. The shift of the Yb 4d5/2 peak to a higher binding energy increases with increasing the annealing temperature within the experimental conditions was investigated. For example, the binding energy of the Yb 4d5/2 peak shifted to a higher level of 185.8 eV, for the case of thermal annealing at 900 °C. This finding can be explained by the formation of a better crystal structure during high temperature annealing, which can also be observed in Figure 2b. For Ti 2p XPS spectra for the Yb2Ti2O7 membrane annealed at different temperatures, the results were shown in Figure 2c. It can be noticed that the binding energy of the Ti 2p1/2 and 2p3/2 peaks of sample annealed at 300 °C and located at 464.3 and 458.6 eV, respectively, suggesting a poor crystal structure incorporating Ti in the form of TiOx. For the sensing membrane annealed at 900 °C, the two Ti 2p peaks shifted to high binding energy (Ti 2p1/2 and Ti 2p3/2 at 464.8 and 459.1 eV, respectively). This shift was attributed to Ti in Yb2Ti2O7 compound. Moreover, Fig. 2d shows the O 1s spectra with appropriate curve-fitting of peaks for the Yb2Ti2O7 sensing membranes explored. In the four sets of spectra, the O 1s peaks at 529.6, 530.2, 531, and 532.2 eV represent the Yb-O (Yb2O3), Yb-O-Ti (Yb2Ti2O7), Ti-O (TiOx), and Yb-OH (Yb(OH)x) bonds,39,40,41 respectively. The intensity of the film after annealing at 300 °C exhibited four Yb(OH)x, TiOx, Yb2Ti2O7, and Yb2O3 peaks, suggesting a poorly crystalline structure. It can be observed that the O 1s peak intensity with respect to Yb2O3, TiOx, and Yb(OH)x decreased with the increase of annealing temperature, whereas the O 1s peak intensity corresponding to Yb2Ti2O7 increased accordingly. This phenomenon could be due to the reaction of O and Yb atoms with Ti atoms, forming a well-crystallized Yb2Ti2O7 membrane.

Sensing characteristics of YbTixOy EIS sensors.—In this study, Yb2Ti2O7 sensing film could serve as an ion-sensitive membrane through the binding of biological and chemical molecules onto a sensing membrane to detect analytes. The flatband voltage (VFB) is the most important measurable parameter of an ISFET or EIS device, which can be defined in the following equation.42

\[
V_{FB} = E_{Ref} - \phi_0 + \chi_{sol} - \frac{Q_{OX} + Q_{SS}}{S_C} \frac{\chi_{sol}}{\phi_1} + \frac{Q_{OX}}{S_C} \frac{\chi_{sol}}{\phi_1} [1]
\]

where ERef is the potential of reference electrode, \(\chi_{sol}\) is the potential of the electrolyte solution, \(\phi_0\) is the silicon work function, \(Q_{SS}\) is the interface state between surface and silicon surface, \(Q_{OX}\) is the charge of oxide, \(C_{OX}\) is the insulator capacitance, and \(\Psi_0\) is the sensing surface potential, because all terms are constant except of \(\Psi_0\), which leads to the EIS sensor sensitive the pH solution. Fig. 3a demonstrates that the VREF shifts were observed in the C-V curves due to the ionization variation on the Yb2Ti2O7 sensing film. The VREF of an EIS sensor was evaluated at the normalized capacitance of 0.4. The inset of Fig. 3a depicts that the EIS sensor after RTA at 900 °C exhibited a high pH sensitivity of 61.11 mV/pH and a good linear pH response. To evaluate the sensing performance of the YbTixOy EIS sensors after RTA at different temperatures, we recorded a set of C–V curves at the pH ranging from pH 2 to 12. Fig. 3b demonstrates that the pH sensitivity of the YbTixOy EIS sensors as a function of the RTA.
Figure 4. Scheme of the two methods for entrapment and covalent bonding of biological components on an Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub>-EIS sensor. (a) The entrapment of oligonucleotide probe mixed with cDNA sample on the Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> surface. (b) The Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> surface was modified using APTES and glutaraldehyde, then the 5'-end amino-functionalized AR-V7 probes were covalently bound on the Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> surface modified with aldehyde groups. Whole the procedures were under electric field affect.

temperature. The sensitivity of Yb<sub>Ti</sub>O<sub>3</sub> EIS sensor enhances with the increase of RTA temperature. This behavior suggests that high-temperature annealing can reduce the crystal or chemical defects in the Yb<sub>Ti</sub>O<sub>3</sub> film. In addition to the detection sensitivity issue, the hysteresis and drift are also two critical performances for a successful sensing device. In this study, the hysteresis effect of the Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> EIS sensors annealed at different temperatures were evaluated by directly immersing in each pH standard solution for up to 1,500 sec in a set cycle of pH 7→4→7→10→7, as shown in Fig. 3c. The hysteresis voltage herein is defined as the reference voltage difference between the initial and terminal voltages measured in the above cycle. Results revealed that the Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> EIS sensor annealed at 900 °C had the lowest hysteresis voltage of 1.6 mV among the cases studied. This finding can be explained by the formation of a well-crystallized Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> structure at the higher annealing temperature of 900 °C. This accordingly results in a lower number of crystal defects in the sensing membrane, and thus a less hysteresis phenomenon. Conversely, the sample treated with 300 °C showed a higher hysteresis voltage (64 mV) over the other, suggesting a high density of crystal defects on the Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> sensing membrane. Furthermore, the signal drift of pH sensing was evaluated by measuring the change in the reference voltage after submerging the device in a pH 7 standard buffer solution for up to 12 hr. The change in the reference voltage can be described as ΔV<sub>REF</sub> = V<sub>REF</sub>(t) − V<sub>REF</sub>(0). Fig. 3d revealed the signal drift of the EIS-based sensor with Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> sensing membranes annealed at different temperatures explored, in which the slope of the voltage deviation reflects the stability of an EIS sensor. It was found that the EIS with the membrane annealed at 900 °C exhibited a better long-term stability (slope = 0.16 mV/hr) in comparison with the other cases studied (300 °C, 500 °C, and 700 °C-samples: 0.9, 0.81, and 0.25 mV/hr, respectively). The lower signal drift rate occurred at the membrane annealed at a higher temperature of 900 °C might be due to its lower level of crystal defects that leads to lower ions mobility. The current study showed a great improvement of sensitivity, small hysteresis voltage, and lower drift rate than our previous report, which were 60.04 mV/pH, 3 mV, and 0.62 mV/hr for Lu<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> sensing membrane, respectively.33

Detection of AR-V7 mRNA in prostate cancer using Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> EIS biosensors.—In this study, we aimed to create a rapid and label-free detecting system, which specifically target on the PCa that harbor the genetic variation of AR-V7 mRNA. We bonded a homemade AR-V7 probe onto an Yb<sub>2</sub>Ti<sub>2</sub>O<sub>7</sub> sensing membrane with modified APTES/glutaraldehyde that cover on a EIS biosensor, and under an
denaturation. Figs. 5a and 5c depict that a mixture of AR-V7 probe cDNA sample binding to oligonucleotide probe was carried out after (Fig. 4a). Based on the characteristics of DNA itself, the detection of surface due to a 289 bp sequence in the double stranded cDNA sample presence of a large number of negatively charged phosphates at the EIS sensing surface. The Yb$_2$Ti$_2$O$_7$ EIS device presented here can be expected when negative (positive) charges are introduced on its sorption of charged biomolecules onto the Yb$_2$Ti$_2$O$_7$ surface should variation at the electrolyte–insulator interface. In summary, the ab-

considered as a type of FET, so it should be sensitive to the charge-alter its surface potential, thus leading to the V$_{\text{REF}}$ shift of an EIS de-

vice. According to Eq. 1, the reduction of surface potential as a result of absorption of negatively charged biomolecules causes the decrease of the V$_{\text{REF}}$ value.

For an EIS sensor, a decrease (increase) of the V$_{\text{REF}}$ value will be expected when negative (positive) charges are introduced on its sensing surface. The Yb$_2$Ti$_2$O$_7$ EIS device presented here can be considered as a type of FET, so it should be sensitive to the charge variation at the electrolyte–insulator interface. In summary, the absorption of charged biomolecules onto the Yb$_2$Ti$_2$O$_7$ surface should alter its surface potential, thus leading to the V$_{\text{REF}}$ shift of an EIS device. According to Eq. 1, the reduction of surface potential as a result of absorption of negatively charged biomolecules causes the decrease of the V$_{\text{REF}}$ value.

Indeed, the ways of immobilization of biomolecules on the sensor surface play a very important role for analyte detection. Chu et al. reported that nanowire-based FET sensor after applying electric field could significantly align the self-assembled probing molecular as monolayers and guide to the direction of the applied electrical field to improve the sensing capability of a FET sensor. Herein, we compared before and after the electric field alignment procedures, physically provide more spaces enough to probes and sample sequences interact, the similar data has proven previously report (Figure 4b). To improve the detection efficiency of hybridization of oligonucleotide probe with target cDNA sample producing negatively charged phosphates at the EIS surface. On the contrary, the entrapment of non-AR-V7 probe mixed with cDNA sample on the EIS biosensor is almost no different to V$_{\text{REF}}$, the reason from no complement occurred between non-AR-V7 probe and target CWR22R cell sample (Figures 5b and 5d). The entrapment method of oligonucleotide probe was modified according to our previous report. We here mixed with target cDNA sample on the EIS sensor can easily make the loading and removal of a biological molecules/agarose gel mixture on its surface. However, this method could result in barriers to the diffusion of substrate, thus slowing the reaction. Furthermore, the combination rate of probe hybridizing and cDNA target sample might affect the V$_{\text{REF}}$ value in the C-V curve, so the results repetition rate is $<50\%$.

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bonded onto the surface of APTES-modified EIS. Because all measurements were conducted in pH 7 solution, the effect of either pH variation or ionic strength change may be neglected. When the 5′-end amino-functionalized oligonucleotide probes was attached on the EIS surface immobilized with APTES and glutaraldehyde, the V\text{REF} shifted in the negative direction (Figures 6c and 6d) since oligonucleotide probes have a negative charge that originates from deprotonated phosphate esters in aqueous solution. These results demonstrate that the V\text{REF} of the AR-V7 probe-immobilized EIS biosensor to the buffer solution at pH 7 was to identify the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7, a small hysteresis voltage (1.6 mV in the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7). The value of AR-V7 probe is to identify good sensing characteristics, including high sensitivity (61.11 mV/pH units), high accuracy (90% for detection of manual labor, time and cost. Therefore, the AR-V7 probe-immobilized EIS sensor is expected to have a higher potential for higher sensitivity and selectivity detection of PCa cells by using an \text{Yb}_2\text{Ti}_2\text{O}_7 EIS biosensor. Based on XRD and XPS data, the optimal annealing temperature in this membrane with a stoichiometric \text{Yb}_2\text{Ti}_2\text{O}_7 film can be performed at the 900 °C. The \text{Yb}_2\text{Ti}_2\text{O}_7 EIS device annealed at 900 °C exhibited good sensing characteristics, including high sensitivity (61.11 mV/pH units) in solutions from pH 2 to 12, a small hysteresis voltage (1.6 mV in the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7), and a low drift rate (0.16 mV/h in the buffer solution at pH 7). The value of AR-V7 probe is to identify drug-resistant status to hormone treatments in PCa cells, as this is bounded onto the surface of APTES-modified EIS. Because all measurements were conducted in pH 7 solution, the effect of either pH variation or ionic strength change may be neglected. When the 5′-end amino-functionalized oligonucleotide probes was attached on the EIS surface immobilized with APTES and glutaraldehyde, the V\text{REF} shifted in the negative direction (Figures 6c and 6d) since oligonucleotide probes have a negative charge that originates from deprotonated phosphate esters in aqueous solution. These results demonstrate that the V\text{REF} of the AR-V7 probe-immobilized EIS biosensor to the buffer solution at pH 7 was to identify the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7, a small hysteresis voltage (1.6 mV in the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7). The value of AR-V7 probe is to identify good sensing characteristics, including high sensitivity (61.11 mV/pH units), high accuracy (90% for detection of manual labor, time and cost. Therefore, the AR-V7 probe-immobilized EIS sensor is expected to have a higher potential for higher sensitivity and selectivity detection of PCa cells by using an \text{Yb}_2\text{Ti}_2\text{O}_7 EIS biosensor. Based on XRD and XPS data, the optimal annealing temperature in this membrane with a stoichiometric \text{Yb}_2\text{Ti}_2\text{O}_7 film can be performed at the 900 °C. The \text{Yb}_2\text{Ti}_2\text{O}_7 EIS device annealed at 900 °C exhibited good sensing characteristics, including high sensitivity (61.11 mV/pH units) in solutions from pH 2 to 12, a small hysteresis voltage (1.6 mV in the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7), and a low drift rate (0.16 mV/h in the buffer solution at pH 7). The value of AR-V7 probe is to identify drug-resistant status to hormone treatments in PCa cells, as this is

Conclusions

In conclusion, we are the first to report and successfully demonstrate a rapid, label-free detection of genetic splice variation AR-V7-specific probe for PCa cells by using an \text{Yb}_2\text{Ti}_2\text{O}_7 EIS biosensor. Based on XRD and XPS data, the optimal annealing temperature in this membrane with a stoichiometric \text{Yb}_2\text{Ti}_2\text{O}_7 film can be performed at the 900 °C. The \text{Yb}_2\text{Ti}_2\text{O}_7 EIS device annealed at 900 °C exhibited good sensing characteristics, including high sensitivity (61.11 mV/pH units) in solutions from pH 2 to 12, a small hysteresis voltage (1.6 mV in the pH loop 7 \rightarrow pH 4 \rightarrow pH 7 \rightarrow pH 10 \rightarrow pH 7), and a low drift rate (0.16 mV/h in the buffer solution at pH 7). The value of AR-V7 probe is to identify drug-resistant status to hormone treatments in PCa cells, as this is
necessary for clinicians to determine appropriate treatment for their patients. The AR-V7 probe immobilized on the EIS functionalized with APTES and glutaraldehyde exhibits a larger negative VREF shift of −33 mV in the C-V curves for the detection of AR-V7, compared to the agarose gel entrapment. The more reliable and stable results could be observed (>95% of results repetition rate) in the AR-V7 probe immobilized on EIS modified with APTEs and glutaraldehyde. This AR-V7 Yb2Ti2O7 EIS biosensor is cost effective, simple, rapid, and obtains extensive properties for further confirmation of sequence specific nucleotide recognition. The EIS biosensor is also much easier to operate and requires no in depth knowledge of molecular biology and genetics, or technique (e.g. skill, expertise, proficiency, mastery etc.) It can be carried out with training for only a short period of time. Hence, this biosensor allows for real-time detection with particularly high sensitivity, and requires no further procession of other instruments to display the results. Thus, the current developed biosensor can have the potential to serve as a diagnostic tool for cancer liquid biopsy in the future.

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