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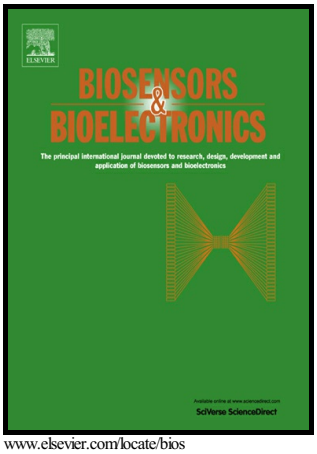

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Polymerase chain reaction-free detection of hepatitis B virus DNA using a nanostructured impedance biosensor

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Abstract

A polymerase chain reaction (PCR)-free technique for the effective detection of genomic length hepatitis B virus (HBV) DNA is described in this study. The honeycomb-like barrier layer of an anodic aluminum oxide (AAO) film having a uniform nanohemisphere array was used as the substrate of the sensing electrode. A

30-nm gold film was sputtered onto the AAO barrier layer surface as the electrode, followed by electrochemical deposition of gold nanoparticles (GNPs) on the hemisphere surface. A specially designed single-strand 96-mer gene fragment of the target genomic DNA of HBV based on the genome sequences of HBV was immobilized on the nanostructured electrode as the capture probe. Target HBV DNA obtained from clinical samples were hybridized to the sensing probes. Detection results illustrate two dynamic linear ranges, 10^2–10^3 and 10^3–$10^{5.1}$ copies/mL, having R^2 values of 0.801 and 0.996 could be obtained, respectively. The detection limit of the proposed sending scheme was measured to be 111 copies/mL. The total of 45 target samples, including 20 samples with HBV concentration being lower than 10^2 copies/mL and 25 samples with HBV concentration being in the range of 10^3–$10^{5.1}$ copies/mL, were used for real test. The concentration of these 45 HBV DNA samples was measured by the COBAS Ampliprep system. Comparing the measured results of the COBAS Ampliprep and our system, it was illustrated that the HBV DNA concentrations measured by the proposed method in this study had a high linear correlation with the COBAS Ampliprep, having R^2 values of 0.983. The proposed sensing scheme is highly feasible for future clinical applications.

Keywords: HBV DNA, PCR-free detection, nanostructured impedance biosensor

1. Introduction

Chronic hepatitis B virus (HBV) infection is an important health burden because of its worldwide prevalence and potential adverse outcomes, including liver cirrhosis and hepatocellular carcinoma (HCC) (Chu, 2000). Although vaccination has significantly decreased its prevalence in the younger generations, there are still more than 350 million people infected with HBV (Liaw and Chu, 2009). Once a patient is infected with HBV, the life cycle of the virus includes an immune tolerant phase, immune clearance phase, and inactive phase (Liaw, 2011). During these phases, the levels of HBV DNA may fluctuate significantly. HBV viral load is an independent factor associated with the development and recurrence of HCC (Chen et al., 2006; Wu, 2009). Antiviral therapy to decrease HBV viral load, effectively decreases the risk of HCC development (Liaw, 2004). In our previous studies, we observed that antiviral therapy was associated with a reduced risk of HCC recurrence among HBV-infected patients after surgical resection (Wu, 2014). In addition to the risk of HCC, we also observed that HBV DNA titers could be stratified according to mortality risk in HBV patients with severe acute exacerbation (Hsu, 2013).

Although the level of HBV DNA in the serum is an important biomarker for patient outcomes, it is not regularly monitored in clinical practice because of its high cost and requirement of intensive labor. Real-time reverse-transcription polymerase chain reaction (RT-PCR) is the most used technique in clinical practices for the measurement of HBV concentration in sera (Heid et al., 1996). An RT-PCR approach with a detection limit of 56 IU/mL has been developed by Tania et al. (2006). At least two primers and probes are required for its effective amplification and detection, respectively, to overcome the issue of sequence heterogeneity in HBV (Gardner, 2003). However, high cost and a relatively long detection time limit its application (Mohamed, 2004). At present, the most accurate HBV detection technique probably is

the COBAS® AmpliPrep automated DNA amplification system (Roche Molecular Systems Inc.) (Weiss et al., 2004), the widespread application of which may still be restricted by the relatively expensive equipment and long detection time. Therefore, there is a need to develop a highly sensitive and cost-effective technique for the detection of HBV in blood.

Recent advancements in micro/nanotechnologies have enabled the development of highly sensitive and cost-effective biosensing schemes for HBV detection. In 1998, a microfabricated, disposable-type sensor for the detection of HBV genomic DNA based on the reaction with the Hoechst 33258-labeled HBV-DNA-inserted plasmid was developed by Koji et al. (1998), offering a detection limit of 10,000 copies/mL. Shun et al. (2013) electrochemically deposited gold nanoparticles (GNPs) on a single-walled carbon nanotube (SWCNTs) array as the electrode, followed by the immobilization of a single-stranded DNA (ssDNA) probe on the GNPs/SWCNTs electrode for successive hybridization of a 21-mer short sequence complementary ssDNA of HBV. A detection limit of 600 copies of HBV in a 1.0-mL sample could be achieved. Li et al. (2007) reported a glassy carbon electrode-based HVB sensor. The 21-mer ssDNA probes were then immobilized through the 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide)/N-hydroxysuccinimide (EDC/NHS) self-assembled monolayer approach for the detection of HBV through cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A chitin ionic solution-modified carbon electrode immobilized with 20-mer ssDNA probes was used for HVB DNA detection using electrochemical impedance analysis (EIS) and DPV (Erdem et al., 2013). A silica nanoparticle-enhanced microcantilever biosensor for the detection of a 243-mer HBV target DNA was proposed by Cha et al. (2009). HBV target DNAs of 23.1 fM to 2.31 nM could be detected. An electrochemical biosensor based on the sequential immobilization of thioglycolic acid (TGA) and ssDNA probe on the gold electrode

surface was developed by Ye et al. (2003) for the detection of sequence-known short-stranded HBV DNA fragment (181 bp) obtained by PCR. An HBV detection technique using nonfaradic EIS was proposed by Hassen et al. (2008). Streptavidin-modified magnetic nanoparticles immobilized with biotin-modified ssDNA were directly immobilized on a bare gold electrode for hybridization with specific complementary DNA targets. A detection limit of 50 pmol of HBV DNA using nonfaradic impedance spectroscopy was obtained. A rolling-circle amplification (RCA)-based quartz crystal microbalance (QCM) biosensor was created by Yao et al. (2013) for the direct detection of HBV genomic DNA from clinical samples. A detection limit of 10,000 copies/mL was reported.

The abovementioned HBV DNA biosensors generally require a sequential process of DNA purification, sequencing, and PCR amplification. In this study, a PCR-free technique for the detection of HBV DNA directly obtained from clinical samples using a nanostructured impedance biosensor was developed. The barrier layer of an anodic aluminum oxide (AAO) membrane having a uniform nanohemisphere array was used as the substrate for the biosensor. A thin gold film was sputtered onto the AAO barrier layer surface as the electrode, followed by uniform deposition of GNPs on each hemisphere surface. A specially designed single-stranded 96-mer gene fragment of the target genomic HBV DNA was then immobilized onto the nanostructured electrode as the probe. Target HBV DNA (3,020–3,320-mer) (Kay and Zoulim, 2007) directly obtained from clinical samples were hybridized to the sensing probes. EIS analysis was conducted to measure the concentration of the target HBV.

2. Materials and Methods

2.1 Nanostructured sensor fabrication

Figure S1 is the schematic of the sensor structure, which has been reported in our

previous work (Chin et al., 2014) and the fabrication process is briefly summarized as follows. An AAO film was fabricated using a conventional anodization process (0.3 M oxalic acid solution, applied voltage of 50 V, temperature of 0 ± 1°C, and processing time of 5.5 h). The unoxidized aluminum was eliminated by immersing the AAO film into a $CuCl_2$-HCl solution (13.45 g of $CuCl_2$, 35 wt% in 100 mL of HCl) to obtain a barrier-layer of a honeycomb-like surface. The shape of the convex honeycombs was further modified using a 30 wt% phosphoric acid. A 30-nm gold electrode was sputtered onto the modified barrier layer surface by radio-frequency (RF) magnetron sputtering. To ensure consistency of the sensing area of each biosensor, a ϕ = 6 mm hole was punched into the center of a 2.5 × 2.5 cm^2 Parafilm square. The punched Parafilm was glued onto the barrier-layer surface of the AAO film using AB glue. GNPs were electrochemically deposited onto each hemisphere surface by applying a voltage of −0.7 V at 25°C for 180 s using an SP-150 electrochemical analyzer (Bio-Logic, USA). The electrolyte for the GNP deposition was prepared by mixing 1 mL of 0.02 M $HAuCl_4$ (Aldrich Inc.) solution in 39 mL of deionized water.

2.2 Capture probe preparation

Three kinds of ssDNA probe with nucleotide length of 96, 100, and 103 mer, respectively, were developed using the HBV genome sequences deposited at the National Center for Biotechnology Information (NCBI) database. After comparing different genotypes, the sequences that could distinguish various genotypes were selected for the primer design. PCR was conducted for 35 cycles to produce the specific DNA segment. The synthesized segment was sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit and the automated ABI PRISM® 3100 (Applied Biosystems, CA, USA) to validate the sequences. A single-strand

synthesized primer that was synthesized according to the confirmed sequences was then used as the capture probe.

2.3 Target HBV DNA purification

Four mL of RBC lysis buffer (RBC Bioscience) was added to a microcentrifuge tube that containing 1 mL of buffy coat, followed by uniform mixing. The tube was incubated at room temperature for 10 min, and inverted every 3 min. The tube was centrifuged at 3,000 rpm for 5 min, then the supernatant was completely discarded. The leukocyte pellet was resuspended in 1 mL of RBC lysis buffer. The tube was centrifuged at 3,000 rpm for 2 min and then the supernatant was completely discarded. The leukocyte pellet was again resuspended in 3 mL of RBC lysis buffer. After incubating at 37°C for 30 min, 6 µL of RNase A (10 mg/mL) (Amresco, OH, USA) was added to the clear lysate and subjected to vigorous shaking (150 rpm). The tube was incubated at 37°C for 30 min. Approximately 1 mL of protein precipitation solution (RBC Bioscience) was added to the tube and vigorously shaken, followed by centrifugation at 3,000 rpm for 5 min at room temperature. The supernatant was transferred to a clean 15-mL microcentrifuge tube. Approximately 3 mL of isopropanol was added and then mixed by inversion. The tube was centrifuged at 3,000 rpm for 5 min, then the supernatant was completely discarded. The DNA was transferred to a clean 1.5-mL microcentrifuge tube. One mL of 70% ethanol was added to the tube and the solution was mixed by inverting the tube several times. The tube was centrifuged at 3,000 rpm for 5 min and the supernatant was completely discarded. After complete volatilization of ethanol, DNA hydration buffer was then added to dissolve the DNA. A COBAS Ampliprep real-time PCR system (Roche Molecular Systems, Branchburg, NJ) was used to measure the concentration of the HBV DNA in the samples.

2.4 Capture probe immobilization and target HBV DNA hybridization

The sequential process for the capture of immobilized ssDNA is as follows: the probe–ssDNA solution (10 μM) was diluted with phosphate-buffered saline (PBS), followed by vigorous shaking. Approximately 40 μL of the diluted probe–ssDNA solution was added onto the electrode surface of the biosensor and incubated at room temperature for 1 h, followed by three washes with deionized water.

The hybridization process for the complementary target HBV DNA included the following steps: double-stranded target DNAs prepared from clinical samples were denatured at 95°C for 10 min to produce single-strand DNA; the solution was cooled to 50°C, followed by vigorous shaking; 40 μL of the diluted target DNA solution was added onto the biosensor and incubate at 50°C for 30 min, followed by two washes with deionized water.

2.5 Electrochemical analysis

EIS analysis using an SP-150 potentiostat (Bio-Logic, USA) was conducted to measure the concentration of HBV DNA in the samples. The nanostructured biosensor was placed at the working electrode, while a Pt film and an Ag/AgCl liquid-junction were adopted as the counter electrode and reference electrode, respectively. A mixture of 5 mM $Fe(CN)_6^{4-}$, 5 mM $Fe(CN)_6^{3-}$, and 0.1 M KCl in 100 mM of 2-(N-morpholino) ethanesulfonic acid (MES) (pH 6.0) was used as buffer solution. The scanning AC frequency was between 0.01 Hz and 100 kHz under an applied AC power of 10 mV.

3. Results and Discussion

3.1 Sensor fabrication results

An SEM image of the hemisphere array surface electrochemically deposited with

uniformly distributed GNPs is illustrated in Figure 1. The diameter of the Au thin film-coated hemispheres of the AAO barrier layer was estimated to be about 100 nm, whereas the diameter of the deposited GNPs was less than 10 nm. To achieve an effective PCR-free detection of the HBV DNA, the biosensor requires a relatively high sensitivity. The small GNPs uniformly deposited on the nanoscale hemispheres as shown in Figure 1 facilitates binding of more capture probes onto the sensor electrode, hence enhancing the sensitivity of the biosensor. The diameter of each nano hemisphere was measured about 100 nm as shown in Figure 1. The sensing area is about 28.27 mm^2 (ϕ = 6 mm), which contains approximate 36×10^8 nano hemispheres. Defect on small amount of nano hemispheres will have no significant influence on the sensing performance.

<Figure 1>

3.2 Characterization of the capture ssDNA probe immobilization

Cyclic voltammetry (CV) was employed to examine the variation of the electrode surface capacitance after the immobilization of the capture ssDNAs. The cyclic voltammogram shown in Figure 2(a) indicates that both the anodic peak current (I_{pa}) and the cathode peak current (I_{pc}) of all three kinds of capture ssDNA probe immobilized electrode were lower than that of the bare electrode. The electrical repulsion between the ferrocyanate molecules in the buffer solution and the capture ssDNAs contained phosphate groups led to a decrease in electrode surface capacitance. The CV results also indicate that the probe (I) exhibited the smallest I_{pa} and the cathode peak current I_{pc}, implying it was the most completely immobilized on the nanostructured electrode. Therefore, the probe (I) as the sequences shown below was selected for the succeeding experiments.

ssDNA probe (I): 5‘−CTCTCTTTACGCGGGACTCCCGTCTGTGCCTTCCATCTG CCGGACCGTGTGCACTTCGCTTCACCTCTGCACGTCGCAAGGAGACCACC GTGAAC-3'

Gel electrophoresis was further employed to confirm the immobilization of the type (I) ssDNA probe. Immobilized ssDNA probes were sonicatedly detached from the nanostructured electrode and magnified by a 30-cycle PCR process. The gel electrophoresis illustrated in Figure 2(b) indicates that an evident band at 100-mer, which is close to the 96-mer ssDNA probe, can be observed. The immobilization of the ssDNA probe can be further assured.

<Figure 2>

For an effective detection, the electrode surface should be covered by the capture ssDNA as completely as possible. However, the capture ssDNA consumption for each trial affects the cost of the diagnosis. In this study, we used EIS analysis to determine the suitable amount of the capture ssDNA. The original 10 μM capture ssDNA solution was diluted 10-, 20-, 100-, and 1000-fold. An equivalent circuit model that faithfully models the electric properties of the GNPs deposited hemisphere array electrode as depicted in the inset of Figure 3 was selected (Chin et al., 2013). R_s denotes the solution resistance; C is the double-layer capacitance; R_{ct} is the charge transfer resistance; Q models the constant phase element; W is the Warburg element; and R_n denotes the nanostructure resistance. The concentration of the capture ssDNA was represented in terms of the charge transfer resistance variation (ΔR_{ctc}) of the electrode after and before the capture ssDNAs immobilization. The relationships between the ΔR_{ctc} values and their corresponding capture ssDNA concentrations are illustrated in Figure 3. The ΔR_{ctc} values increase with the concentration of the capture

ssDNA. It can be observed that the ΔR_{ctc} values of the 500 nM and 1 μM capture ssDNA were very close, implying that capture ssDNA with concentration of 500 nM could be further implemented for the target HBV DNA detection. The original EIS data is tabulated in Table S1. The average of the charge transfer resistance (R_{ct}) of 12 bare electrodes as indicated in Table S1 is 16.26 ±3.41 Ω. These results can at certain degree illustrate the reproducibility and repeatability of the biosensor.

<Figure 3>

3.3 Characterization of the complementary target HBV DNA hybridization

In this study, the 96-mer capture ssDNA was used for the detection of the 3,020–3,320-nucleotide long HBV DNA directly obtained from clinical samples. Successful hybridization of the complementary target HBV DNAs is crucial for effective detections of the HVB DNA. Nyquist plots of EIS, which were fitted by the equivalent circuit shown in the inset of Figure 3, as illustrated in Figure 4 were used for the characterization of the complementary target HBV DNA hybridization. These six curves in Figure 4 represent the EIS results of a bare electrode, capture ssDNA immobilized electrode, complementary target HBV DNA hybridized electrode, respectively. The diameter of individual semicircular of each Nyquist curve represents the charge transfer resistance of the electrode with regard to this specific EIS analysis. Figure 4 shows that the charge transfer resistance of the electrode increased when the capture ssDNAs were immobilized. Hybridization of the complementary target HBV DNAs further increased the charge transfer resistance of the electrode. Complementary target HBV DNA with higher concentrations resulted in a larger charge transfer resistance. The EIS results confirmed the successful hybridization of the complementary target HBV DNAs.

<Figure 4>

3.4 HBV DNA detection

With approval from the Institutional Review Board of Taichung Veterans General Hospital, Taichung, Taiwan, thirteen HBV DNA clinical samples with concentrations having already been measured by COBAS® AmpliPrep (Roche) were used for the HBV DNA detection experiments. The concentration of the target HBV DNA (copies/mL) was characterized in accordance with the charge transfer resistance variation (ΔR_{ctt}) of the electrode between the target HBV DNA hybridization and the capture ssDNAs immobilization.

Figure 5(a) shows ΔR_{ctt} as a function of the logarithmic concentration of the target HBV DNA. For each concentration, five trials were conducted with standard deviations for different concentrations depicted by the error bars. The PBS in the concentration axis denotes the condition of no target HBV DNA added. The ΔR_{ctt} values of the $10^{5.1}$ and 10^{6} copies/mL target HBV DNA were similar, implying that the target HBV DNA with concentration up to $10^{5.1}$ copies/mL could hybridize to the 500 nM capture ssDNA immobilized on the nanostructured electrode. Two linear dynamic ranges, 10^{2}–10^{3} and 10^{3}–$10^{5.1}$ copies/mL, having R^2 values of 0.801 and 0.996, respectively, could be achieved. The detection limit (3σ) was estimated to be about 111 copies/mL.

Forty-five additional HVB DNA samples from different patients, including 20 samples with HBV concentration being lower than 10^{2} copies/mL and 25 samples with HBV concentration being in the range of 10^{3}–$10^{5.1}$ copies/mL, were further adopted to verify the calibration curves shown in Figure 5(a). The concentrations of

these forty-five HVB DNA samples were measured by the COBAS Ampliprep real-time PCR system (Roche Molecular Systems, Branchburg, NJ). Figure 5(b) displays the concentrations of these forty-five samples using the calibration curve. Comparing the measured results of the COBAS Ampliprep and our system, it was illustrated that the HBV DNA concentrations measured by the proposed method in this study had a high linear correlation with the COBAS Ampliprep, having R^2 values of 0.983.

<Figure 5>

To illustrate the specificity of the ssDNA probe to the HBV DNA target, four normal samples without HBV and a HBV DNA containing sample (1 ng/mL) were hybridized to the ssDNA probes, respectively. The EIS results shown in Figure 6 demonstrate that the ΔR_{ctt} of the HBV DNA-containing sample is much larger than that of the normal samples. The specificity of the ssDNA probe can be confirmed.

<Figure 6>

3.5 Discussion

HCC is one of the most frequently occurring tumors. The mortality rate of HCC is the third largest among the cancer death, after lung and gastric cancer. Approximately, 15~40% of HBV infected people turn into hepatic carcinoma gradually, starting from chronic hepatitis, then fiver failure (Lok, 2002; Szabó et al., 2004; Anthony, 2001; Okuda, 2000). Hence, early clinical diagnosis of HBV followed by suitable treatments is crucial for the prevention of HCC.

The levels of glutamic oxalacetic transaminase (GOT) and glutamic pyruvic

transaminase (GPT) in serum have been the commonly used indexes for HBV diagnosis. Several useful methods such as fluorescence and UV absorbance, colorimetric, spectrophotometric, chromatography, chemiluminescence, and electrochemical techniques have been employed for GOT and GPT detection (Huang et al., 2006). However, some clinical cases have shown that a patient who had normal GOT and GPT levels was diagnosed suffering from liver tumor or hepatic carcinoma in the end. It is because that the levels of GOT and GPT can only indicate whether the patient's liver is in the inflamed condition. Yet it may have been fibrosis, cirrhosis and even cancerous. Furthermore, the GOT and GPT levels are not necessarily increased in the early stage of hepatic carcinoma because that only those oppressed and invaded cells around the hepatoma cells will necrosis. Hence, it is likely to be ignored, then leading to a late treatment of HCC.

In addition to GOT and GPT, biomarkers such as the HBV surface e antigen (HBeAg), HBV surface antibody (Anti-HBsAb), and HBV core antigen (anti-HBcAg) can also be used for the diagnosis of HBV. Among them, the HBeAg can be used as the index for the replication and activity of HBV. A positive reaction of the HBeAg in blood implies that strong reproductions of HBV are proceeding in the liver cells. A high concentration of HBV in blood can be detected, leading to a high risk of HBV. However, false negative or positive reaction of the antibody-antigen based detection may result in a false diagnosis (Bottero et al., 2013), especially for the condition of a low amount of virus or biomarkers. Therefore, direct detection of the HBV concentration in blood can be a more feasible approach to reduce the risk of HCC (Pawlotsky, 2003).

A PCR-free biosensor based on a nanostructured electrode for effective detection of genomic length HBV DNA directly obtained from clinical samples was proposed in this study. A specially designed single-strand 96-mer gene fragment of the target

genomic DNA of HBV based on the genome sequences of HBV was used as the probe. In general, a successful immobilization of the capture probes on the sensing electrode can lead to an effective detection of the target DNAs. Several feasible methods such as using a thiolated-probe (Liu and Lu, 2003; Ryu et al., 2010), implementing DNA/ mercaptohexanol (MCH) monolayers (Zhang et al., 2007; Hong et al. 2012), with an applied electrode field (Malic et al., 2009; Estrela et al., 2005), and via a bifunctional molecule of *p*-aminobenzoic acid (ABA) (Liu, et al., 2011) have been proposed to enhance the immobilization of the ssDNA probe. The larger nanohemispheres and small nanoparticles hybrid electrode used in this study requires no additional effort to successfully immobilize the 96 mer capture ssDNA on it. It can be conjectured that the uniformly distributed GNPs enabled uniformly and dispersedly immobilization of the ssDNA probes. Furthermore, the HBV DNA samples can be directly prepared from clinical samples without any PCR process and only 40 μL of the target DNA solution is required for an effective detection. Hence cost-effective clinical detections can be achieved.

4. Conclusion

We have proposed a PCR-free technique for effective detection of genomic length HBV DNA directly obtained from clinical samples. The proposed sensing technique is based on larger nanohemispheres and small nanoparticles hybrid electrode. A single-strand 96-mer gene fragment of the target genomic DNA of HBV, which was specially designed according to the genome sequences of HBV from the NCBI, could be successfully immobilized on the nanostructured electrode as probe. Complementary target of genomic HBV DNA (3,020–3,320 mer) directly obtained from clinical samples were hybridized to the sensing probes. Detection results through EIS illustrate that two dynamic linear ranges, 10^2–10^3 and 10^3–$10^{5.1}$ copies/mL, with

R^2 values of 0.801 and 0.996, respectively, could be obtained. The detection limit was estimated to be 111 copies/mL. Testing experiments using additional HVB DNA samples from different patients revealed that the HBV DNA concentrations measured by the proposed method are close to that of the COBAS Ampliprep. Further compared to the reported micro/nanotechnology- based HBV DNA detection schemes (Wang, 2013; Yao et al., 2013), the detection method presented in this study can provide a much better detection of low concentration samples.

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Figure legends

Figure 1. SEM image of the larger nanohemispheres and small nanoparticles hybrid electrode used for the detection of genomic length HBV DNA detection, the GNPs were uniformly deposited on the nanohemisphere array

Figure 2. Verification of the capture ssDNA probe immobilization; (a) Cyclic voltammetry for the bare electrode and the capture ssDNA immobilized electrode, (b) Gel electrophoresis for the capture ssDNA (I)

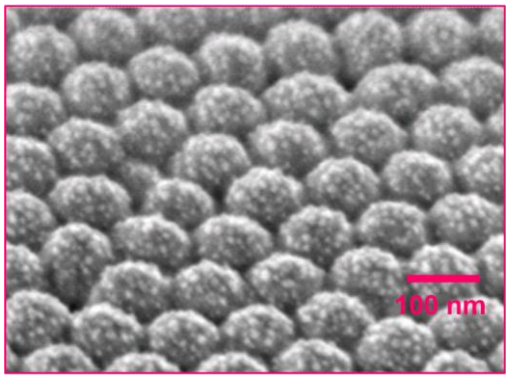
Figure 3. Optimization of capture ssDNA concentration; inset: equivalent circuit model for the GNPs deposited hemisphere array electrode

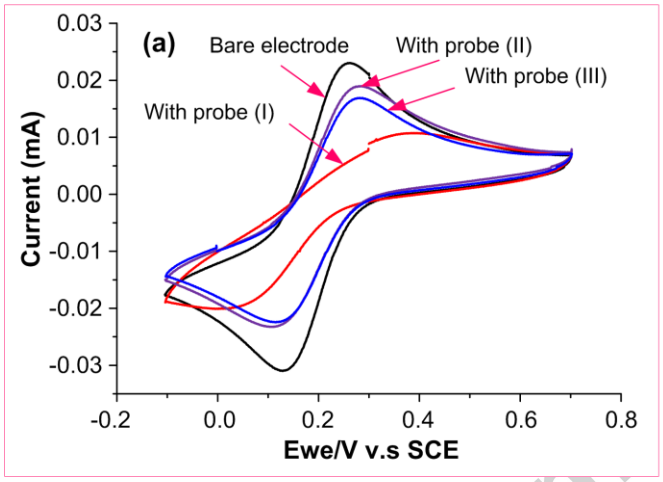
Figure 4. Hybridization verification of the complementary target HBV DNA, the charge transfer resistance (R_{ct}), which is represented by the diameter of the semicircle, increases with the increase of the concentration of the target HBV DNA.

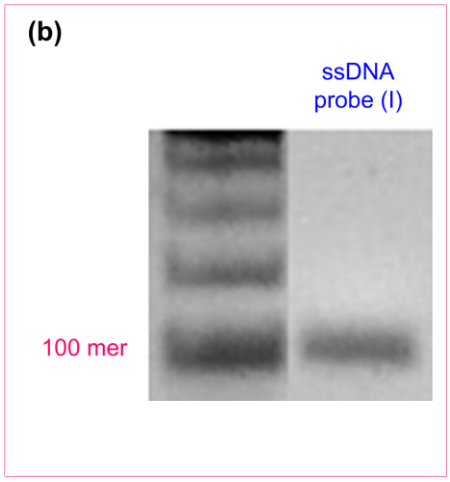
Figure 5. HBV DNA detection results; (a) the charge transfer resistance variation (ΔR_{ctt}) of the electrode between the target HBV DNA hybridization and the capture ssDNAs immobilization as a function of the logarithmic concentration of the target HBV DNA (b) verification of the calibration curves in (a) using forty-five additional HVB DNA samples from different patients

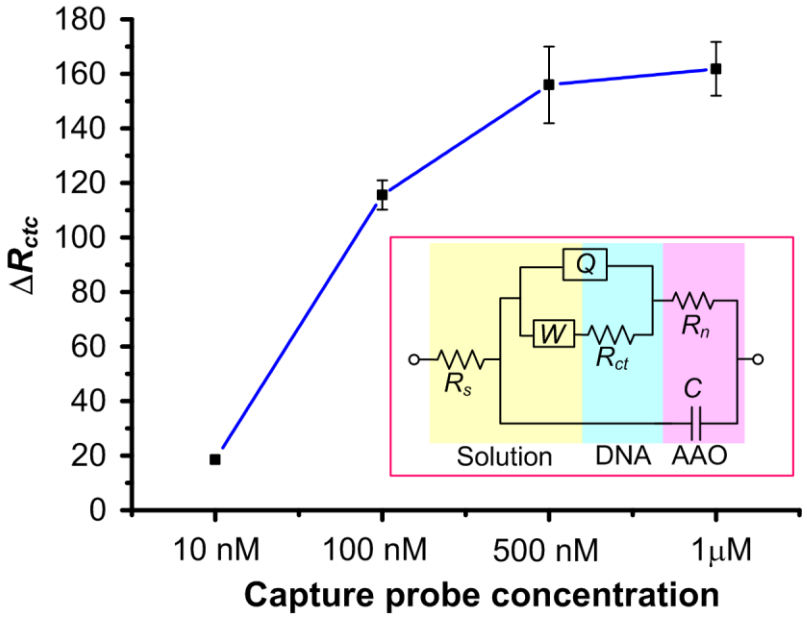
Figure 6. Specificity of the ssDNA probe to the HBV DNA target, the samples from

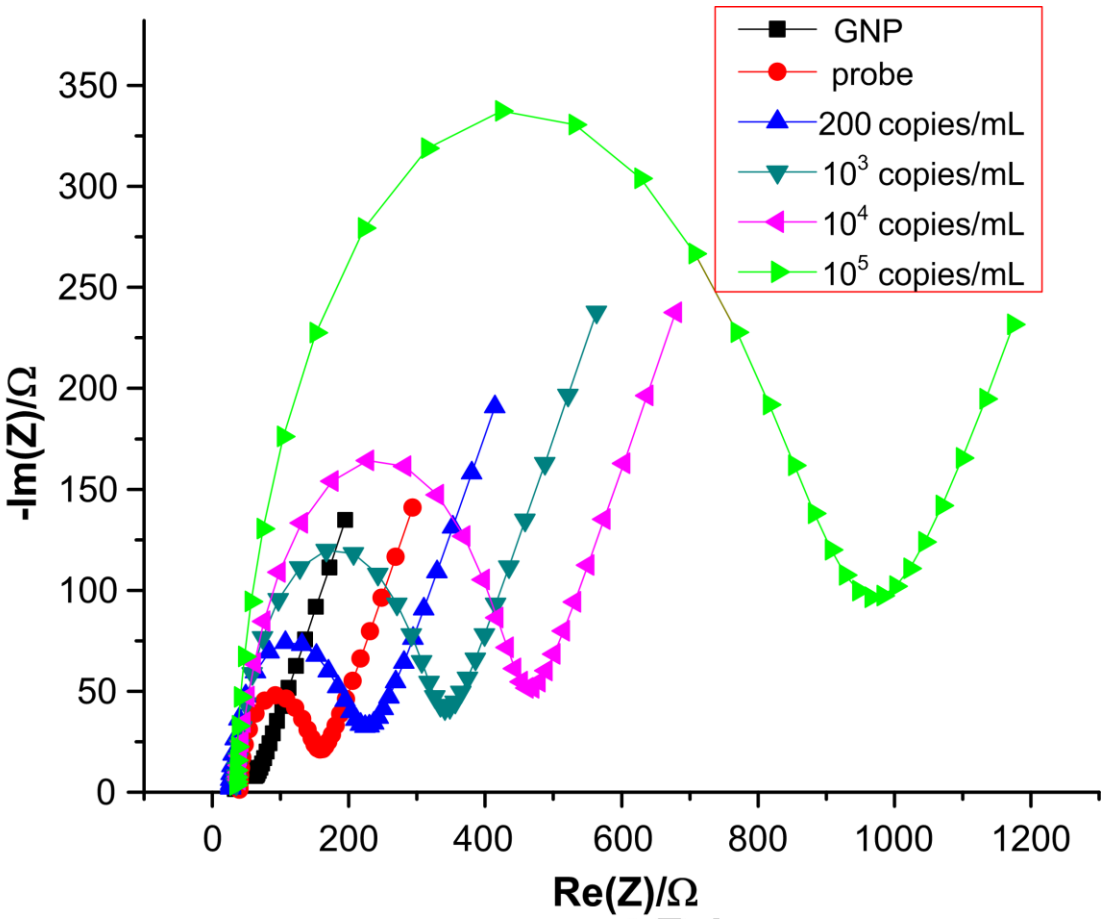
normal patients and the HBV DNA containing sample could be clearly distinguished.

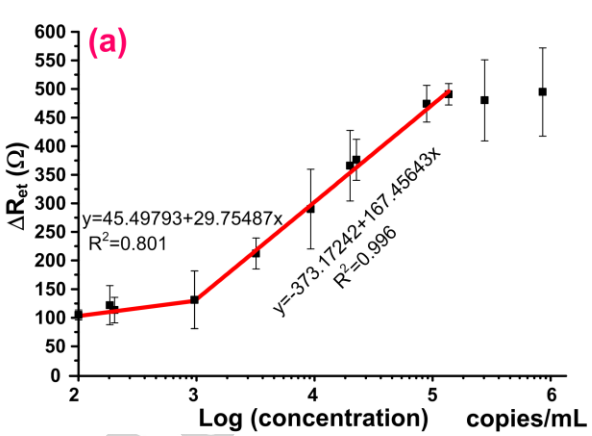


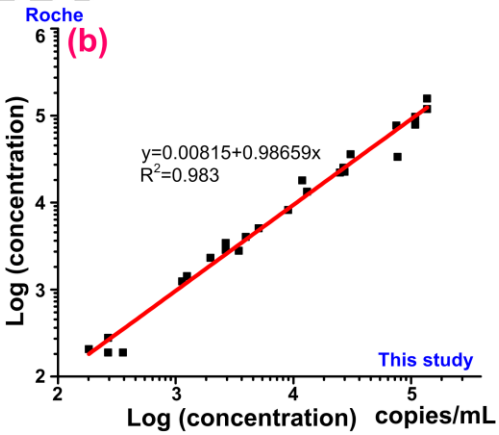


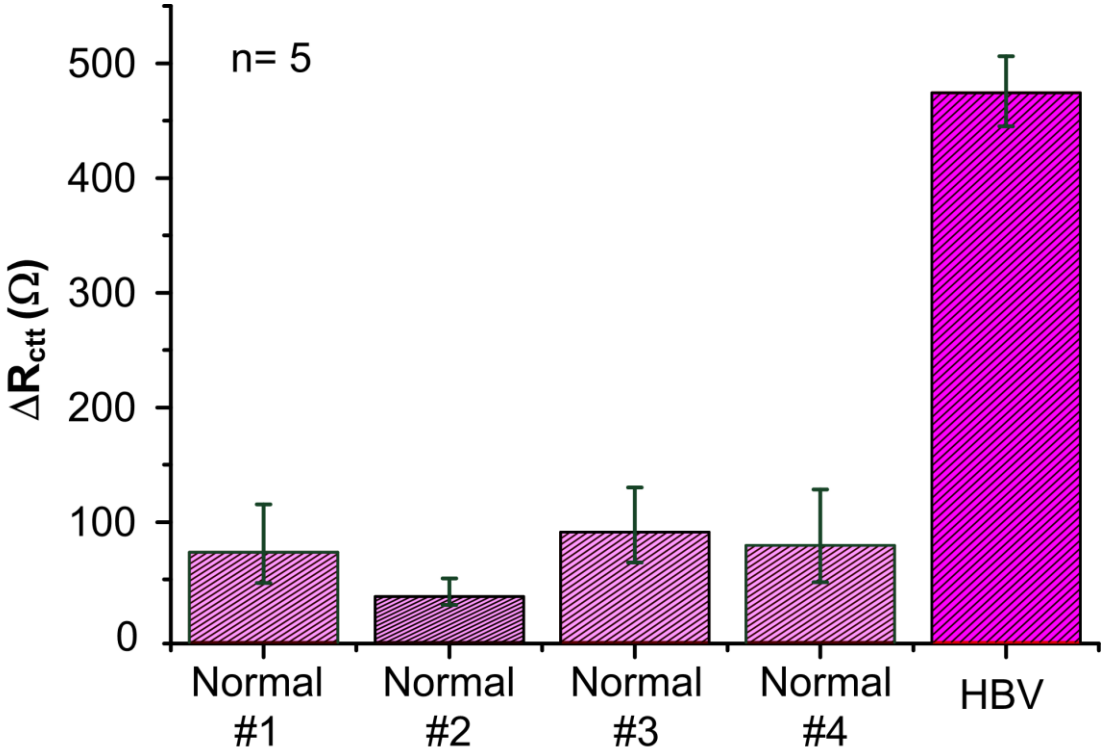












- We report a polymerase chain reaction (PCR)-free technique for the effective detection of genomic length hepatitis B virus (HBV) DNA.
- A single-strand 96-mer gene fragment of the target genomic DNA of HBV based on the genome sequences of HBV was specially designed.
- The detection limit of the proposed sending scheme was measured to be 111 copies/mL.
- The HBV DNA concentrations measured of 45 patients by the proposed method had a high linear correlation with the COBAS Ampliprep (Roche Molecular Systems Inc.), having R^2 values of 0.983.