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Highly sensitive and specific electrochemical biosensor for direct detection of hepatitis C virus RNA in clinical samples using DNA strand displacement

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Hepatitis C virus (HCV) is a common blood-borne infection that can lead to long-term illnesses such as hepatocellular cancer and liver cirrhosis. Early diagnosis is crucial for effective management, as no vaccine is available for preventing HCV infection. However, the high cost and complexity of current molecular diagnostic tools hinder efforts to achieve early diagnosis and prevent transmission, particularly in resource-limited settings. We developed a novel electrochemical biosensor for point-of-care testing (POCT) of HCV RNA. The sensor utilizes a strand displacement method, where the target RNA displaces a gold nanoparticle-labeled reporter probe (AuRP) from a pre-hybridized duplex with a magnetic nanoparticle (MNP)-labeled capture probe. The amount of displaced AuRP, detected using differential pulse anodic stripping voltammetry (DPASV), is directly proportional to the target RNA concentration. The biosensor exhibited excellent analytical performance, with a detection limit of 4 fM for synthetic targets and 43 ng/µL for RT-PCR products. Importantly, it successfully detected HCV RNA directly in clinical plasma samples without the need for RNA extraction or amplification. The sensor was used to analyze 30 RNA samples from HCV-positive patients, 20 cDNA samples from viral RNA, 30 HCV-positive plasma samples, and 22 HCV-negative plasma samples. The sensor results show good concordance with the RT-PCR results, demonstrating the sensor's potential for detecting HCV in clinical samples.

Keywords Hepatitis C virus, Gold nanoparticles, Strand displacement, Electrochemical sensors, Isothermal

Hepatitis C virus (HCV) infection presents a global public health challenge, affecting approximately 150 million individuals worldwide, with an estimated 58 million people living with chronic HCV infection and approximately 1.5 million new infections occurring annually[1]. In Thailand, the incidence of HCV infection is 1–2% of the population[2]. Liver cancer has emerged as the most common malignancy among males and the third most common among females, ranking as the third leading cause of death in Thailand[3]. Approximately 75% of patients progress from acute to chronic HCV infection, which can result in long-term liver complications such as liver fibrosis, cirrhosis, and hepatocellular carcinoma[4]. The causative agent responsible for this is an enveloped plus-strand RNA virus belonging to the Flaviviridae family[5].

Acute hepatitis manifests as jaundice in only 20% of patients and is rarely severe. In 85% of infected individuals, the development of chronic infection typically occurs without noticeable symptoms, making it challenging

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to detect the infection due to its asymptomatic nature and the marginalization of at-risk populations[4,6]. Consequently, the World Health Organization (WHO) has called for increased screening efforts and aims to reduce new hepatitis infections by 90% and viral hepatitis-related deaths by 65% by 2030[1].

HCV is present in blood and secretions and can be transmitted through blood transfusions or organ transplantation. Thailand, in particular, faces a significant issue with transfusion-transmitted HCV infection, emphasizing the importance of screening blood donors to prevent and minimize the risk of infection[7,8]. In various regions of Thailand, including Phetchabun province, there has been a notable increase in the prevalence of HCV infection and liver cancer[9]. Among all HCV infections, subtype 3a (38.5%) is the most prevalent[10–13]. Therefore, proactive measures, such as enhancing diagnostic screening and expanding treatment coverage, are essential in reducing the incidence of hepatitis C infection.

The current methods for detecting HCV include an antibody test that detects the presence of antibodies against the HCV in blood samples and reverse transcription PCR (RT-PCR) that detects the viral RNA. HCV RT-PCR tests such as Abbott RealTime HCV Assay, Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, and Qiagen Artus HCV RG PCR Kit are highly sensitive and specific. However, the RT-PCR test requires specialized laboratory equipment and trained personnel.

This study aims to develop a rapid and accurate HCV screening tool based on electrochemical sensor technology that does not require complex or costly equipment. The sensor operates on the principle of strand displacement of a gold nanoparticle labelled reporter probe (AuRP) by the target DNA or RNA in the sample[14]. Upon displacement of the gold nanoparticle-reporter probe, the target will hybridize into a capture probe with magnetic particles. The electrochemical signal of the displaced AuRP is proportional to the concentration of the target (Fig. 1). We also demonstrated the ability of the sensor to detect HCV from clinical samples.

Experimental

Chemicals and reagents

All solutions were prepared with ultrapure water (18.2 ΩM) from Milli-Q® Integral Water Purification System (Merck, Germany). Dynabeads™ MyOne™ Streptavidin T1 was purchased from Thermo Fisher Scientific, USA. Acetic acid, Potassium chloride, Potassium dihydrogen phosphate, Sodium dodecyl sulphate (SDS), Sodium chloride, Sodium phosphate dibasic, Sucrose, Tris (2-carboxyethyl) phosphine (TCEP), and Tween 20 were purchased from Sigma-Aldrich, USA. hydrobromic acid (HBr) and bromine solution (Br_2) were purchased from R&M Chemicals, UK and 40 nm gold nanoparticles were purchased from DCN Diagnostics, USA.

The primer and probe sequences were designed based on the genomic sequence of Hepatitis C virus available from GenBank (accession number M62321)[12] and all oligonucleotides used in this work were supplied by Integrated DNA Technologies, USA. The capture and biotin blocking probes are modified with biotin at the 5'

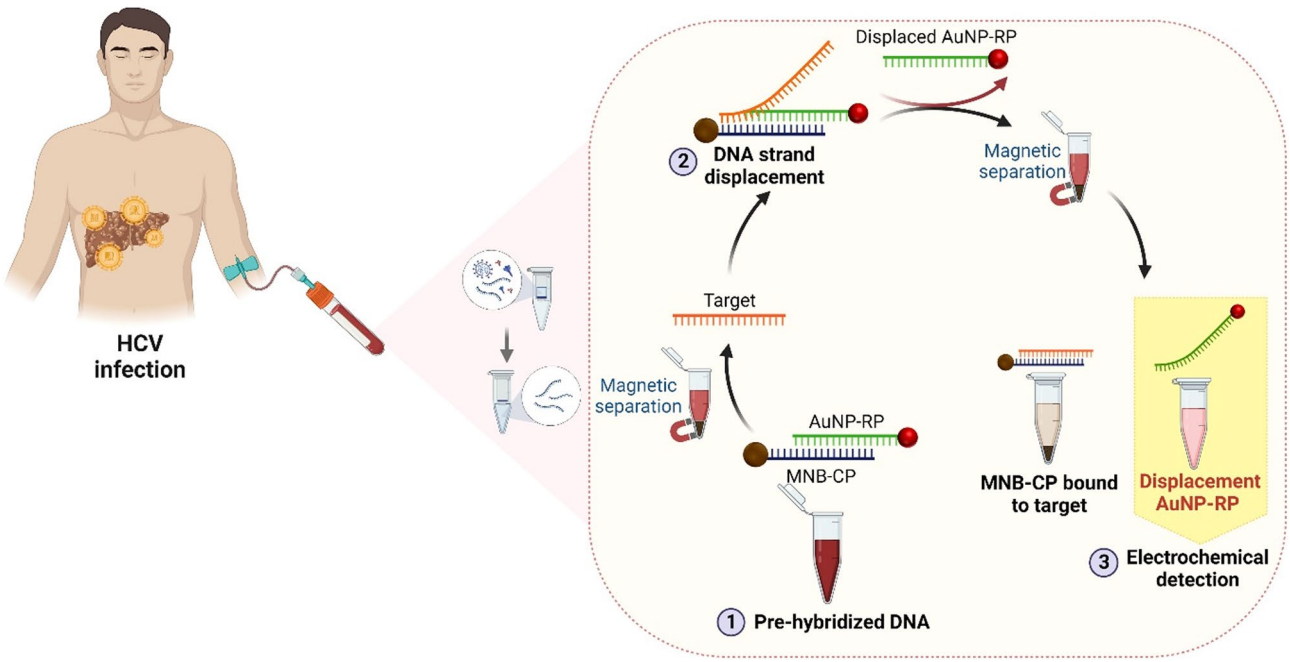

Figure 1. Detection of Hepatitis C virus RNA or synthetic DNA target using the electrochemical sensor platform. (1) First, the Capture probe with magnetic particle (MB-CP) is pre-hybridized to gold nanoparticle-reporter probe (AuNP-RP); (2) The target RNA extracted from the patient sample or synthetic linear DNA target is added to the pre-hybridized strands. Since the target is complementary to the CP, it will displace the AuNP-RP. The displaced AuNP-RP is separated from the MB-CP bound to the target using magnetic separation; (3) The signal of AuNP is detected using an electrochemical technique. The signal is proportional to the concentration of the target.

ends, while the reporter and thiol blocking probes are modified with the thiol group at the 5' ends. The details of the oligonucleotide sequences are listed in Supplementary Table 1.

Instruments

Electrochemical measurements were carried out using Palmsens4 computer-controlled potentiostat with PSTrace version 5.8 software obtained from Palmsens, The Netherlands. Nucleic acid concentration was measured using the NanoDrop™ One Microvolume UV-Vis Spectrophotometer obtained from Thermo Fisher Scientific, USA. Disposable electrochemical screen-printed carbon electrodes (SPCE) were obtained from Quasense Co. Ltd., Thailand, which consisted of two carbon tracks as working electrodes, reference electrodes, and counter electrodes in DPASV.

Functionalization of AuNPs conjugate with reporter probe (AuRP)

Preparation of AuNPs-reporter probe conjugate was performed using salt aging method as described by Ngamdee et al[14]. Firstly, 10 µL of 100 µM reporter probe DNA and 30 µL of 100 µM blocking probe ($PolyT_{10}$) thiolated DNA were activated with 10 mM TCEP. Then, the thiol-activated DNA was added into 1 mL of 4 nm AuNPs solution and allowed to incubate for 16 h at room temperature. After incubation, 10 µL of 500 mM Tris-acetate (pH 8.2) and 100 µL of 1 M NaCl were added to the mixture. The reaction was incubated for at least 24 h. The excess probes and AuNPS were removed by centrifugation at 14,000 rpm for 30 min, followed by washing with 25 mM Tris-acetate pH7.4 for 3 times. Lastly, the pellet containing AuNPs-reporter probe conjugate was resuspended with 500 µL of 25 mM Tris-acetate containing 8% sucrose, pH7.4 (w/v). The gold reporter probe (AuRP) solution was kept at 4 °C until use.

Immobilization of capture probe on magnetic bead particles (MB-CP)

The immobilization of biotinylated capture probe (CP) on Dynabeads™ MyOne™ Streptavidin T1 (MB) was performed according to the manufacturer's instructions. 100 µL of MB (10 µg μL^{-1}) was washed 3 times with 200 µL of 20 mM PBS/0.05% tween and 20 mM PBS, pH7.4. Next, 4 µL of 100 µM of biotin capture probe and 12 µL of 100 µM $PolyT_{10}$-Biotin blocking probe were added into 184 µL of 20 mM PBS pH7.4. The mixture was incubated for 40 min on a rotary platform at room temperature. After that washing was performed 3 times using 20 mM PBS pH7.4 with the aid of magnetic separation to remove excess probes. The pellet was resuspended with 100 µL of 20 mM PBS pH7.4 to obtain the final concentration of 4 µM capture probe DNA. The magnetic bead-conjugated capture probe DNA (MB-CP) was kept at 4 °C until use.

Preparation of pre-displacement duplex strand

The pre-displacement duplex strand containing MB-CP and AuRP was prepared by adding 2 µL of MB-CP and 10 µL of AuRP into a tube containing 18 µL of 20 mM PBS/0.1% SDS pH7.4. The mixture was incubated for 30 min at 60 °C in a waterbath. Next, washing step aided by magnetic separation was performed using 100 µL of 20 mM PBS/0.05% Tween pH 7.4 for 5 times, followed by 20 mM PBS pH7.4 for 3 times. The supernatant was discarded, and the pellet of the pre-displacement duplex strand (MB-CP bound to AuRP) was resuspended with the target sample.

Target hybridization and strand displacement reaction

The target hybridization was performed using 30 µL of sample, which was added to the pre-displacement duplex strand and incubated at 60 °C for 30 min. Due to competitive binding, the target RNA that is complementary to the MB-CP will displace the AuRP strand. Magnetic separation was used to separate the target bound to MB-CP from the unbound (displaced) AuRP. 5 µL of the supernatant containing the displaced AuRP was used for signal detection.

Electrochemical detection

5 µL of the supernatant containing the displaced AuNP-RP was pipetted onto the working electrode surface of a 2-electrode screen printed carbon electrode or SPCE (Quasense, Thailand). This was followed by the addition of 50 µL of 1 M HBr/0.1 mM Br_2. Pre-treatment step was performed using the deposition potential of -0.75 V for 100 s. Differential pulse anodic stripping voltammetry (DPASV) technique was used with applied potential of 0.35–0.75 V, step potential of 0.01 V, pulse potential of 0.05 V, pulse time of 0.05 s, and scan rate of 0.1 V/s.

Evaluation of plasma samples

Protocols and use of leftover plasma samples were approved by the Institutional Review Board of the Faculty of Medicine of Chulalongkorn University (IRB Number 028/63) and Mahidol University (MU-CIRB 2023/065.1702) and all subjects provided signed informed consent. All the experiments were performed in accordance with relevant guidelines and regulations. A total of 52 plasma samples (30 HCV-positive and 22 HCV-negative) were used to evaluate the assay. The samples were obtained as leftover plasma from a previous HCV diagnostic screening project of Thai residents in Phetchabun province[15].

The plasma samples were mixed with a high salt lysis buffer to lyse the HCV. Viral RNA was extracted from HCV-positive plasma samples using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and was reverse transcribed using random hexa-primer and ImProm-II Reverse Transcription System (Promega, Madison, WI), according to the manufacturer's protocol[16]. Amplification of the 5'UTR region of the HCV RNA was performed using RT-PCR with 2X Perfect Taq PlusMasterMix (5 PRIME, Gaithersburg, MD)[17].

Target hybridization and strand displacement were performed using 30 µL of either plasma sample, RNA, cDNA, or RT-PCR product. The sample was added to the pre-displacement duplex strand and incubated at

60 °C for 30 min. After performing magnetic separation, 5 µL of the supernatant was used for electrochemical measurement.

Results and discussion

Functionalization of AuNP with thiolated DNA reporter probes

Gold nanoparticle (AuNPs) and thiolated-DNA conjugation were achieved by using the salt aging method. The molar ratio of AuNPs and DNA probe (i.e. the DNA density on the AuNPs surface) is considered one of the main factors that affect colloidal stability and DNA recognition. The adsorption of small, thiol-containing molecules on a gold surface is a spontaneous reaction, this reaction is used to attach the thiolated DNA to the AuNP surface[18]. During the salt-aging process, the thiol group gradually displaces the DNA bases adsorbed on AuNPs[19]. Unlike bulk gold surfaces, the colloidal stability of AuNPs must be maintained during the conjugation process. The repulsion between the DNA on AuNPs is reduced by the addition of NaCl, so that a high density of DNA on the AuNP surface can be achieved. After the conjugation process, the supernatant was taken to measure the excess DNA to determine the percentage of DNA loading. The amount of DNA attached to one AuNP was estimated to be ~ 13,000 molecules, which had similar results as Ngamdee et al. described previously[14].

Optimization of the assay conditions

The AuNPs-reporter probe conjugate was resuspended with 25 mM Tris-acetate containing 8% sucrose which acted as a stabilizing agent (Fig. 2a). The sucrose capped AuNPs-reporter probe conjugate showed monodispersity in an aqueous solution because the sucrose molecules protect them from aggregation[20]. The assay conditions of pre-treatment deposition potential step before measurement, the amount of capture probe-magnetic bead (CP-MB), and hybridization temperature were optimized using 1 pM of complementary target DNA (HCV-LT) and 1 pM of non-complementary target (mismatch 1 bp) to achieve optimal target hybridization and strand

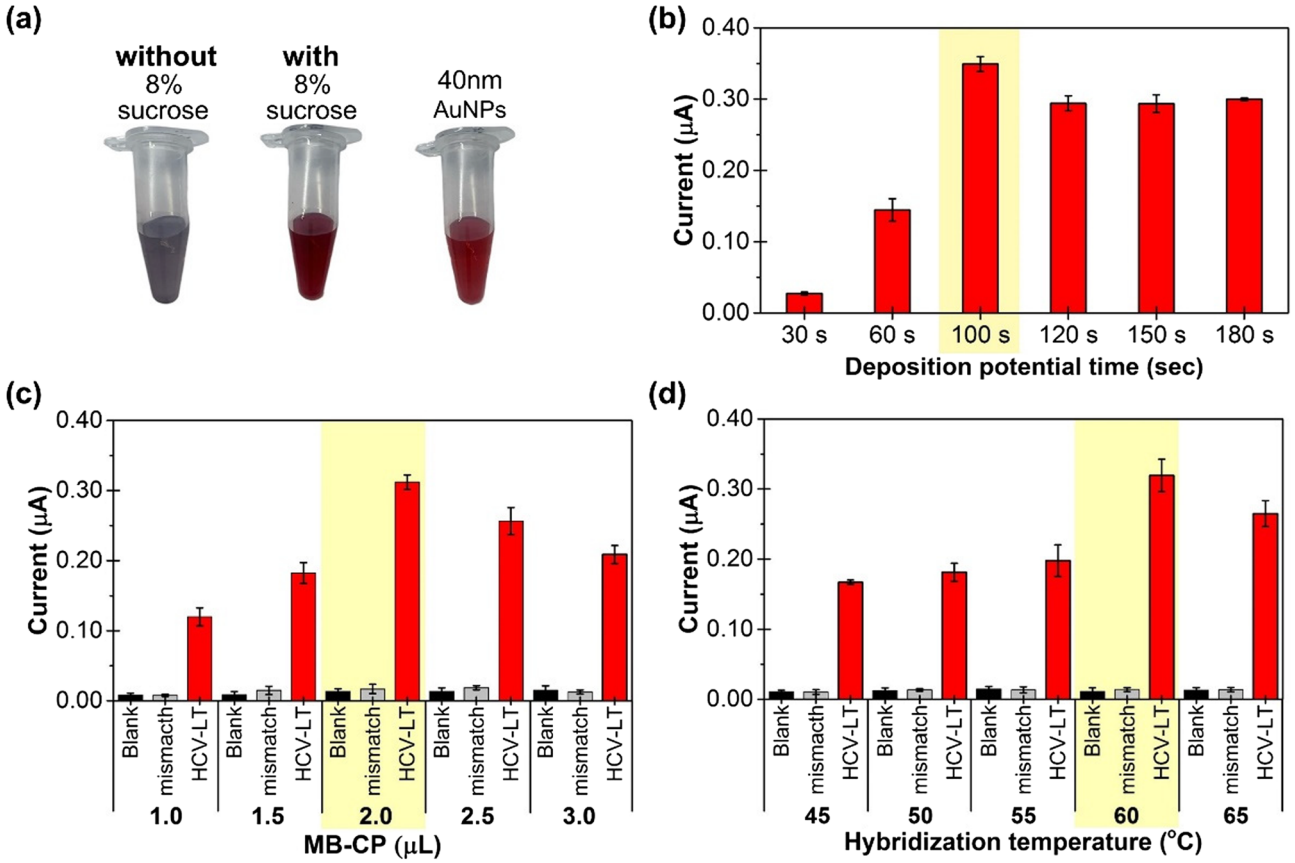

Figure 2. Optimization of the assay conditions. (**a**) AuNPs conjugate with reporter probe using salt aging method with the effect of sucrose addition; all samples contained 25 mM Tris-acetate pH 7.4. The first tube was without 8% sucrose (w/v), the next tube with 8% sucrose (w/v), and the last tube was 40 nm AuNPs for control, respectively. (**b**) The pre-treatment of deposition potential time when applying the deposition potential − 0.75 V before measurement. 100s was best deposition time, when increased the time. (**c**) The effect of varying the amount of MB-CP. The amount of MB-CP was tested from 1.0, 1.5, 2.0, 2.5, and 3.0 µL, while all the other parameters were kept constant. The best signal was achieved at 2.0 µL of MB-CP. (**d**) Hybridization temperature optimization using 1 pM HCV-LT. The optimal hybridization temperature was 60 °C. The conditions that provided the best signals are highlighted in yellow. All current responses are presented as mean values ± SD, $n = 5$.

displacements (Fig. 2b-d). The optimal condition of pre-treatment deposition step before measurement at -0.75 V was 100 s. The highest signal for 1 pM HCV-LT was achieved with 2.0 µL of MB-CP. The best sensor performance in hybridization was achieved at 60 °C. Other conditions were consistent with the observations of Ngamdee et al[14].

The sensor works based on competitive hybridization between the target DNA / RNA in the sample with the AuRP in the pre-displacement duplex strand (Fig. 3a). The concentration of the target is proportional to the electrochemical signal of the displaced AuRP. The electrochemical signals for blank, 1 pM mismatch DNA, 1 pM HCV-LT and 1 µM HCV-LT are shown in Fig. 3b. The results revealed that this method could distinguish a single base mismatch in the sequence. The mismatch target has a 1-base mismatch (A → T) at the center of the 18-base sequences. When the target and the CP-MB sequences are not perfectly complementary, the affinity of the two strands is reduced, causing a decrease in the duplex thermal stability. The ability of this method to distinguish single base mismatch proves the useful in detecting point mutations and single nucleotide polymorphisms.

Assay sensitivity and specificity

Detection of HCV-LT and RT-PCR product at different concentrations was performed to determine the sensitivity of the assay. The result showed a positive linear correlation between the logarithmic concentrations of the linear target DNA and the current response ($R^2 = 0.9846$) (Fig. 4a). The limit of detection (LOD) for the analytical range was determined as 4 fM. In addition to linear target DNA, a positive linear correlation was observed in the electrochemical signal when the concentration of the PCR product increased (Fig. 4b). The calibration curve was obtained with a correlation coefficient, R^2 of 0.9986 and the limit of detection for the HCV RT-PCR products was 43 ng/µL. The results of electrochemical detection and visual color of released AuRP for both linear target and PCR product are illustrated in Supplementary Fig. 1.

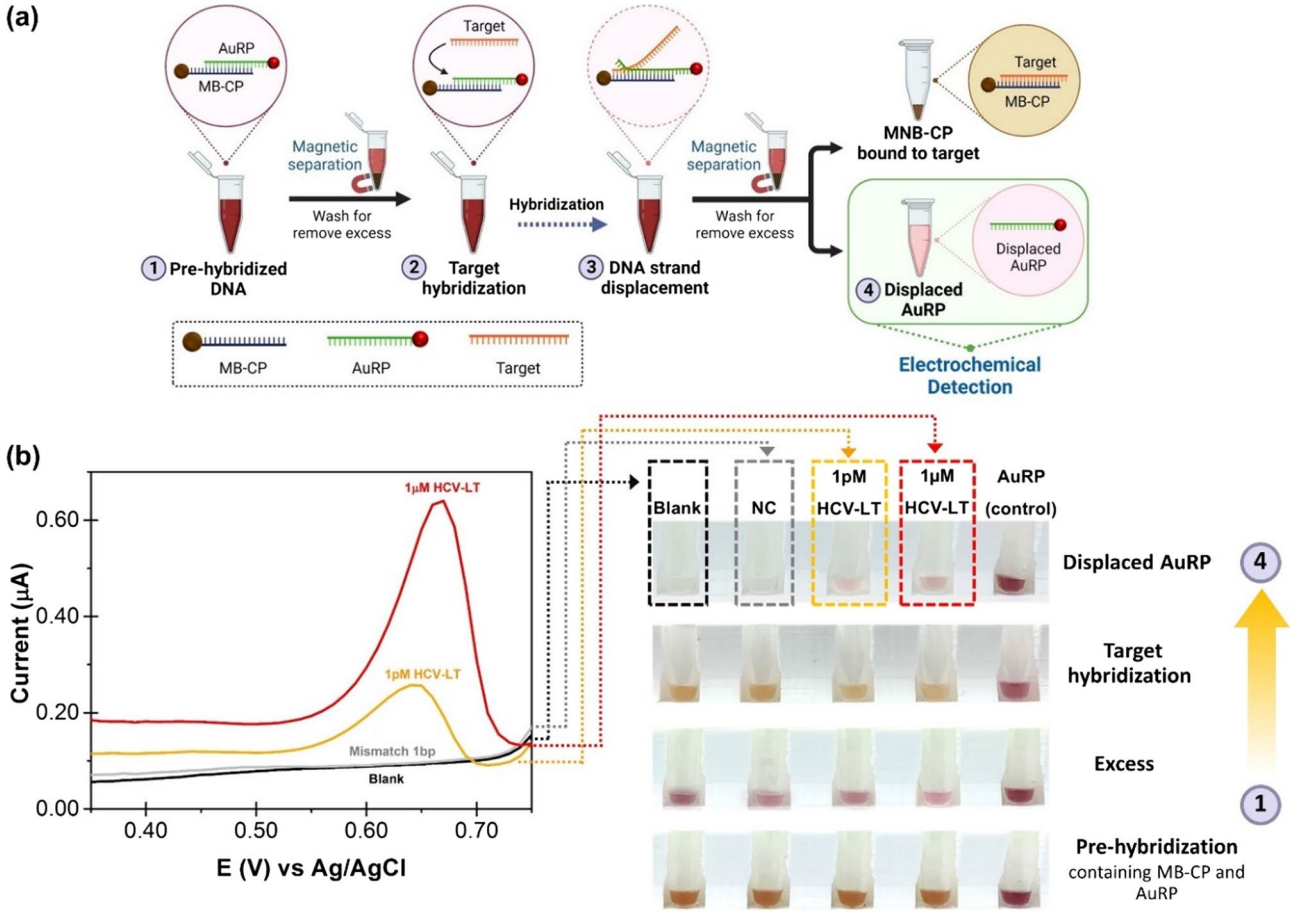

Figure 3. Detection of linear targets. (**a**) Step-wise procedure for performing the assay. (1) Prepare pre-hybridized duplex strand consisting of MB-CP and AuRP. (2) & (3) Target hybridizes with MB-CP and displaces the pre-hybridized AuRP strand. (4) Displaced AuRP after the target hybridization and the magnetic separation. The electrochemical signal of the gold from released AuRP is proportional to the concentration of target. (**b**) Differential pulse anodic stripping voltammetry (DPASV) shows the current response with 1 µM HCV-LT, 1 pM HCV-LT, mismatch 1 bp target (1pM) and blank. There is a peak current signal at 0.65 V for 1 pM and 1 µM HCV-LT, while no peaks for blank and mismatch target. Visual detection of displaced AuRP, and control color of AuRP are shown. All current responses are presented as mean values ± SD, $n = 5$.

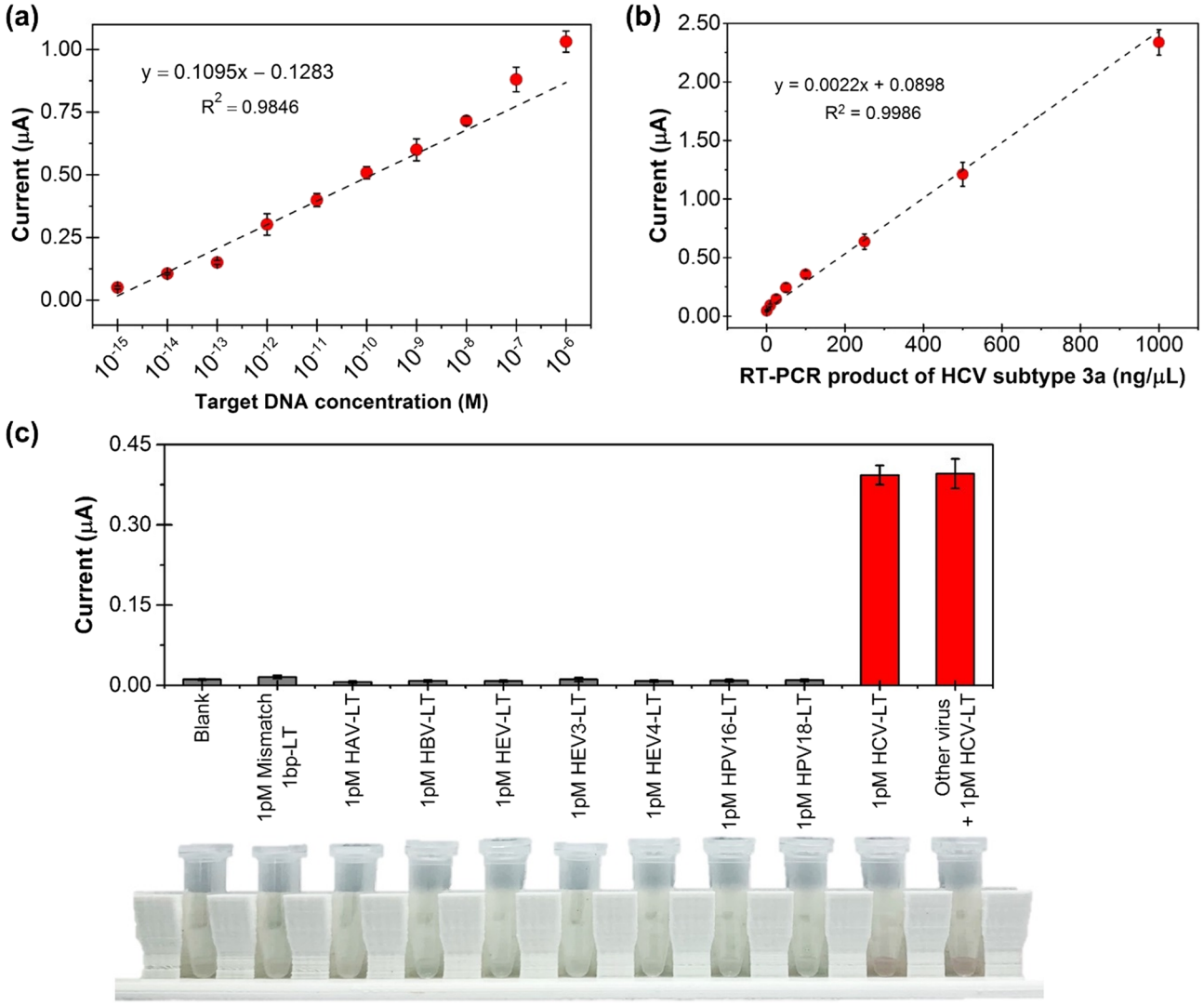

Figure 4. Sensitivity and specificity of the assay. (**a**) Calibration curve of linear target DNA, the concentration ranges from 1 fM to 1 μM. The sensitivity of the assay shows a positive linear correlation between current response and log DNA concentration. (**b**) Sensitivity of the assay using RT-PCR product of HCV subtype 3a (1ng/μL – 1000ng/μL). The current signal increases when increasing the concentration of PCR product. (**c**) The specificity of the assay was evaluated using 1 pM of the other viruses (HAV-LT, HBV-LT, HEV-LT, HEV3-LT, HEV4-LT, HEV-LT, HPV16-LT, and HPV18-LT) and target (HCV-LT and other viruses + HCV-LT). In addition, the Visual red color of AuRP is shown. All current responses are presented as mean values ± SD, $n = 5$.

The specificity of the assay indicated that only the samples containing HCV-LT yielded positive signals (Fig. 4c). The cut-off value was calculated from the mean of blank signal (background) + 3 standard deviations (3 SD). The current signal above the cut-off value was considered positive. Moreover, this sensor could be used to distinguish HCV infection from other types of hepatitis viral infections.

Besides that, the positive signal was obtained when HCV-LT is present with the DNA of other viruses (Fig. 4c). The result shows that it could also be used to detect co-infection of HCV with other viruses. Co-infection of HCV with human immunodeficiency virus (HIV)[21] or Hepatitis B virus (HBV)[22] are common among people at risk, and identification of a co-infection would guide the treatment and management of the diseases.

The analytical performance of the developed electrochemical biosensor was compared with previously published biosensors for HCV as shown in Table 1. Our biosensor showed comparable results of limit of detection (LOD) when compared to other studies. Notably, the studies by El-Sheikh et al. (2021) and Sheta et al. (2020) reported lower LOD than this study. Both studies used label-free electrochemical biosensor based on metal–organic framework (MOF) platform. The MOF detection platform is complex to develop as well as having longer turnaround time. In the case of Sheikh et al. (2021), the high sensitivity could be attributed to the synergistic effect of silver and zinc in the bimetallic MOF to catalyze glucose to gluconolactone. However, the presence of other reducing agents or compounds in the blood could potentially lead to false positive results.

Sensor platform	Technique	sample	Detection time	Detection limit	Ref.
Silver/zinc bimetallic metal-organic framework (Ag/Zn-MOF) for enzymatic catalyzation on glassy carbon electrode (GCE)	DPV	HCV-RNA	50 min	0.64 fM	23
Polyaniline@nickel metal-organic framework (Ni-MOF) on glassy carbon electrode (GCE)	EIS	HCV-RNA	60 min	0.75 fM	24
Magnetic reduced graphene oxide-copper nanocomposite (mrGO-CuNCs) on gold electrode	DPV	HCV DNA	18 min	405.0 pM	25
Screen printed electrode (SPE) modified with E2-MIP for direct detection of HCV via E2 envelope protein	DPV	HCV E2 protein	15 min	8.52 fmol/L	26
Thiol-peptide nucleic acid immobilized on gold nanodots modified indium tin oxide substrate (PNA-SH/Au/ITO)	CV and SWV	HCV-RNA	60 min	101.5 IU/mL	27
Screen printed graphene electrode (SPGE) in microfluidic platform modified with acpcPNA probes specific to HCV	CA	HCV-cDNA	30 min	1.20 nM	28
Gold electrodes modified with graphene oxide functionalized with ethylenediamine (GO-ETD)	DPV	HCV-RNA	not specified	1.36 nmol/L	29
Gold aggregating gold: A novel nanoparticle biosensor approach for the direct quantification of hepatitis C virus RNA in clinical samples	Colorimetric/spectrophotometric	HCV-RNA	30 min	4.57 IU/µl	30
Detection of unamplified HCV RNA in serum using a novel two metallic nanoparticle platform	Colorimetric/spectrophotometric	HCV-RNA	30 min	15 IU/mL	31
Electrochemical signal of AuNP modified reporter probes from strand displacement	DPASV	HCV-RNA Human serum	30 min	43 ng/µL	This study

Table 1. Comparison of our platform with various HCV biosensors. DPV, Differential pulse voltammetry method; EIS, Electrochemical impedance spectroscopy; CV, Cyclic voltammetric method; SWV, Square wave voltammetric method; CA, Chronoamperometry; DPASV, Differential pulse anodic stripping voltammetry.

Moreover, several advantages were encountered in our platform in terms of hybridization time, specificity assay, discriminating single base pair mismatch, visual detection, and application of real sample. First, hybridization time of target with capture probe was shorter (30 min) and we detected linear targets from different infectious diseases to show the ability of our platform to detect co-infection as well as differentiate other infections. Discriminating single base pair mismatch added more value for high specificity. In addition to electrochemical detection, visual detection of released AuRP (red color) gave an advantage of first qualitative decision before quantitative measurement. Therefore, our sensor is best for real application to detect HCV-RNA from plasma compared to previous studies.

Detection of HCV from clinical samples

A total of 52 plasma samples (30 HCV positive and 22 HCV negative) were used to detect HCV RNA without any extraction. Then, RNA was extracted from 30 HCV-positive samples and cDNA was synthesized from 20 HCV-positive samples (from extracted RNA). All the samples were checked for HCV RNA individually with electrochemical detection. Figure 5a. Box-and-whisker plot shows the mean current values of the differential pulse anodic stripping voltammetry (DPASV) detection of HCV in 22 negative plasma samples, 30 positive plasma samples, 20 cDNA samples, and 30 RNA samples. The current responses from DPASV were compared with the Ct values of RT-qPCR. The results are illustrated in Fig. 5b-d. Moreover, cut-off value was determined to differentiate HCV positive and negative in clinical samples based on the mean current value of the 22 negative plasma samples + 3 standard deviations (SD) (mean = 0.194, SD = 0.077). The current signal that was equal to or greater than 0.426 µA was considered as positive. The current signals of all the 30 HCV-positive plasma, 30 HCV-positive RNA, and 20 HCV-positive cDNA samples were above the cut-off value, indicating positive results. Further, results of DPASV graph and visual color of released AuRP of clinical samples are illustrated in Supplementary Fig. 2 and the current values of electrochemical detection, Ct values, and viral load (IU/mL) are listed in Supplementary Table 2.

In our study, we obtained the leftover plasma samples and extracted RNA and cDNA from a previous study that quantitatively measured the HCV RNA viral load among Phetchabun residents in Thailand using RT-PCR on the Cobas 4800 System[15]. The study found that an overwhelming majority of the Petchabun residents screened had HCV viral load ≥ 5,000 IU/mL. The leftover plasma samples, extracted RNA, and cDNA were used for electrochemical detection. To the best of our knowledge, this work is the first to report the detection of HCV RNA directly from plasma samples without any RNA extraction or amplification. The plasma samples were mixed with a high salt lysis buffer to lyse the virus. The lysed plasma samples were then directly applied onto the electrode for hybridization and strand displacement, followed by electrochemical detection. Previous studies as listed in Table 1had reported the direct detection of HCV RNA from blood, serum, or plasma samples. However, those studies involved multiple steps of RNA extraction and may involve an amplification step before detection with the biosensor. The RNA extraction step often relies on silica-membrane-based method or magnetic-beads-based method. A study by Hongjaysee et al[32]. compared the efficacy of four extraction methods (a silica-membrane-based method, a magnetic-beads-based method, boiling with diethyl pyrocarbonate (DEPC)-treated distilled water, and using a commercial lysis buffer) for RNA extraction from plasma with varying HCV viral load. The study found that boiling plasma samples with a commercial lysis buffer provided the highest yield of RNA, while the purity was highest with the silica-membrane-based extraction method.

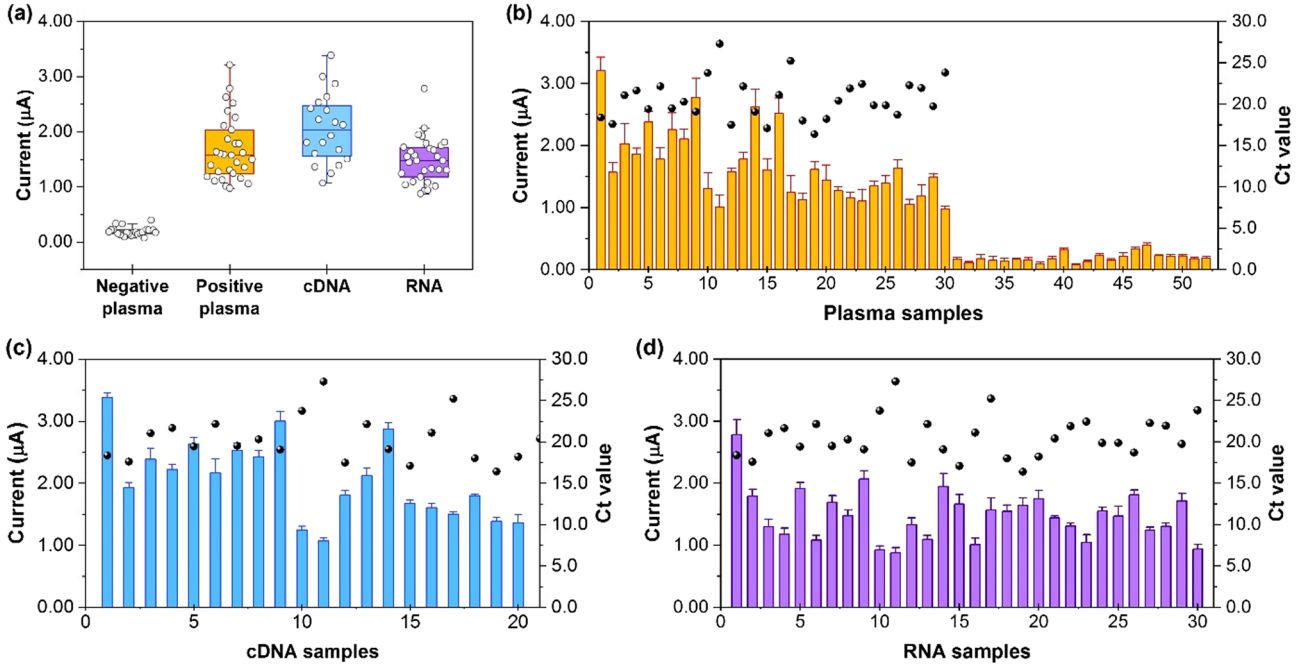

Figure 5. HCV with electrochemical detection in clinical samples. (**a**) Box-and-whisker plot shows the mean current values of the differential pulse anodic stripping voltammetry (DPASV) detection of HCV in 22 negative plasma samples, 30 positive plasma samples, 20 cDNA samples, and 30 RNA samples. The circles represent the mean current responses of five independent replicates. The boxes represent the interquartile range. Whiskers represent the maximum and minimum values of each set. The top and bottom of the whiskers and boxes represent the maximum and minimum values, and 75% and 25% quartiles respectively and the lines inside the boxes represent the median values. There was no statistical significance in pairwise comparisons between different forms of samples (plasma, RNA, and cDNA) where the electrochemical results of positive samples are statistically significant when compared with negative samples. (**b – d**) Comparison of Electrochemical current values with Ct values of (**b**) 52 HCV plasma samples, (**c**) 20 cDNA samples, and (**d**) 30 RNA samples. Ct values are indicated in black dots for all positive samples. Low current signals were obtained for samples with high Ct value and vice versa. All current responses are presented as mean values standard deviation (±SD), $n = 5$.

In our study, we used a simple lysis step which involved the lysis of viral form and release of its RNA in plasma. This shows that the highly sensitive biosensor could detect the HCV RNA without the need for purified RNA or an amplification step such as real-time reverse transcription polymerase chain reaction (RT-PCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP). Studies have also identified HCV RNA from saliva samples of patients[33,34], therefore, this biosensor could potentially be used with saliva samples as well.

Conclusion

We developed a highly sensitive and specific electrochemical biosensor using the principle of target hybridization and strand displacement for the detection of HCV. The biosensor could detect as little as 4 fM of linear targets and 43 ng/μL of RT-PCR product. It could also be used to detect viral nucleic acid directly in plasma samples, as well as extracted HCV RNA and cDNA. With the growing burden of HCV globally, the short turnaround time and isothermal assay condition make this sensor an excellent choice for "point-of-care testing" (POCT) of HCV.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper, (and its supplementary information file), and from the corresponding author upon reasonable request. Sequence information used in this study was from the National Center for Biotechnology Information (NCBI) with GenBank accessions of M62321 Source data are provided with this paper.

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Author contributions

B.L. contributed to designing the study. T.C., L.S.Y., R.W. and S.L., performed the experiments. B.L, S.K., R.W., S.L., T.N. and T.C. analyzed data and prepared figures. B.L. and Y.P. conceived and supervised the study. B.L., R.W., S.L. and Y.P. obtained ethical approval for this study. T.C and T.N drafted the manuscript. All authors participated in manuscript editing and approved the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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