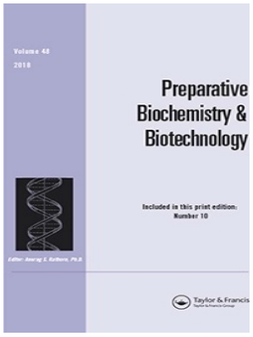

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Electrochemical nucleic acid hybridization biosensor based on poly(L-Aspartic acid)-modified electrode for the detection of short oligonucleotide sequences related to hepatitis C virus 1a

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ABSTRACT

This work describes, for the first time, the fabrication of poly(L-aspartic acid) (PAA) film modified pencil graphite electrode (PGE) for the detection of hepatitis C Virus 1a (HCV1a). The presence of PAA on the electrode surface can provide free carboxyl groups for covalent binding of biomolecules. The PGE surface was first coated with PAA via electropolymerization of the L-aspartic acid, and avidin was subsequently attached to the PAA modified electrode by covalent attachment. Biotinylated HCV1a probes were immobilized on avidin/PAA/PGE via avidin-biotin interaction. The morphology of PAA/PGE was examined using a scanning electron microscope. The hybridization events were monitored with square wave voltammetry using Meldola's blue (MDB). Compared to non-complementary oligonucleotide sequences, when hybridization was carried out between the probe and its synthetic targets or the synthetic polymerase chain reaction analog of HCV1a, the highest MDB signal was observed. The linear range of the biosensor was 12.5 to 100 nM and limit of detection was calculated as 8.7 nM. The biosensor exhibited favorable stability over relatively long-term storage. All these results suggest that PAA-modified electrode can be used to nucleic acid biosensor application and electropolymerization of L-aspartic acid can be considered as a good candidate for the immobilization of biomolecules.

KEYWORDS

Nucleic acid biosensor; Poly (L-aspartic acid); HCV 1a

Introduction

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV). HCV has a global distribution; worldwide, about 71 million people have chronic hepatitis C infection and approximately 399 000 people die each year from hepatitis C.[1] HCV isolates are classified into seven major genotypes (from 1 to 7) and a large number of subtypes (a,b,c, etc.). HCV genotype 1 is the most prevalent worldwide. In addition, subtype 1a is one of the most common types.[2] Enzyme-linked immunosorbent assays (ELISA), chemiluminescent methods, and nucleic acid tests such as polymerase chain reaction (PCR) tests are the most widely used detection techniques for HCV.[3] However, these methods are time consuming, are often expensive and require qualified personnel to conduct them.[4]

The ability to detect specific nucleic acid sequences is in great demand within clinical diagnostics, environmental analysis, food safety and drug screening.[5,8] In recent years, a number of novel nucleic acid-based electrochemical biosensors have been fabricated that provide a simple, inexpensive, reliable, sensitive and selective platform for the detection of clinical pathogenic microorganisms and genetic disorders.[9,10] These biosensors combine short nucleic acid sequences with an electrochemical transducer, which is an electrode that nucleic acid sequences immobilize onto the electrode as the biorecognition species. The sensitivity and lifetime of nucleic acid sensors depends on the immobilization of DNA probes onto electrode surfaces. There are various methods available for immobilizing DNA onto electrodes, such as entrapment in a gel or polymer, covalent binding, physical adsorption, cross-linking, avidin-biotin interaction, and electrochemical polymerization.[11]

Polymeric material coating on the electrode surface provides wide flexibility to the electrode because polymers include several functional groups. This feature allows relatively large quantities of the compound to be attached to the polymer film–coated electrode.[12,13]

The use of polyamino acid films such as those of poly(L-glutamic acid), poly(L-alanine), and poly(L-lysine) has been reported in the literature to construct voltammetric sensors.[14,16] These types of films offer advantages such as stability, reproducibility, rapid preparation, and the presence of some functional groups.[17] L-aspartic acid has one amino group ($-NH_2$) and two carboxyl groups (–COOH), which can be easily electropolymerized on an electrode surface to form poly(L-aspartic) acid. Although the structure of PAA can be highly relevant as a biopolymer model, it is rarely used to construct electrochemical sensors.[18] To the best of

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our knowledge, the use of a PAA-modified electrode for the fabrication of a nucleic acid sensor has not been reported.

Here, we report, for the first time, an electrochemical nucleic biosensor for the detection of specific DNA sequence related to HCV1a using PAA-modified pencil graphite electrode (PAA/PGE). We used MDB and avidin-biotin interaction as the indicator agent and immobilization method, respectively. PAA/PGE showed efficient catalytic activity in the electrochemical detection of HCV1a sequences. The biosensor also successfully discriminated noncomplementary DNA sequences. Therefore, PAA-modified electrode can be considered a good nucleic acid biosensor for the detection of HCV1a sequence.

Experimental

Reagents and chemicals

A CHI-1020 potentiostat with a three-electrode cell was used for voltammetric measurements. PGE (as a working electrode), Pt wire (as an auxiliary electrode), and Ag/AgCl (as a reference electrode) were connected through a potentiostat to construct a DNA biosensor. The brand of pencil graphite lead used was Tombow (type HB), obtained from a local bookstore. The diameter of the lead was 0.3 mm, and the length of the lead was 50 mm. During each measurement, a total of 5 mm of the pencil was dipped in the solution. A copper drill chuck was used as the working electrode holder. A fresh PGE surface was used for each measurement. The pH values of the prepared buffer solutions were measured using a pH meter (Orion Model 720 A). Temperature control of the solution was carried out using a Grant W14 thermostat.

All the oligonucleotides were purchased from Ella Biotech Company (Germany) (Table 1), and stock solutions (500 µg mL^{-1}) of the oligonucleotides were prepared by using ultra-pure water and were stored frozen. The stock solutions of the probe were prepared by diluting the solutions with 0.5 M of acetate buffer containing 20 mM NaCl (pH 4.80); and the stock solutions of targets were diluted with 2X Saline Sodium Citrate (SSC) buffer solution (pH 7.0). L-Aspartic acid, N-hydroxysulphosuccinimide (NHS), avidin and N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide (EDC) were obtained from Sigma. All other chemicals used were of an analytical purity grade, and ultrapure water (18 MΩ cm^{-1}) was used in the preparation of solutions.

Preparation of Avidin-Modified PAA/PGE

PAA electrodes were prepared using by cyclic voltammetry (CV). PGE was vertically immersed into aqueous solution containing 0.02 M L-aspartic acid in 0.1 M phosphate buffer solution (PBS) of pH 8.0 and cycled 20 times between −0.8 and +2.0 V at a scan rate of 100 mV s^{-1}. After the electropolymerization of the PAA, unreacted aspartic acid monomers were removed by rinsing with deionized water. An aqueous solution containing 5 mM EDC and 8 mM NHS was used to activate the terminal carboxylic acid groups of the PAA/PGE (1 h at room temperature). After rinsing of the PAA/PGE with 0.1 M PBS, it was immersed into PBS containing 1000 µg mL^{-1} avidin for 2 h and then cleaned with 0.1 M PBS. The resulting electrode is denoted as avidin/PAA/PGE

Immobilization of the biotinylated DNA probe onto the surfaces of avidin/PAA/PGE

The biotinylated probe DNA was immobilized onto the avidin/PAA/PGE via avidin-biotin interaction. The avidin/PAA/PGE was dipped into 0.1 M PBS (pH 8.0) containing the biotinylated probe DNA for several minutes at room temperature. Then, the biosensor was washed with 1X SSC buffer for 5 min to remove the unbound DNA probe. A concentration of biotinylated probe DNA ranging from 0.1 to 10 µM was selected to optimize probe concentration. After the optimum probe concentration had been determined, the optimal time of the probe immobilization was examined by reviewing the probe immobilization at 10, 20, 30, 60, and 90 min.

Hybridization with target DNA

Hybridization was performed by dipping the probe-immobilized biosensor in a portion of 1.0 µM (unless otherwise stated)target DNA (complementary or non-complementary) containing 2X SSC buffer solution at room temperature. To remove any weakly bound or any unbound oligonucleotides, the nucleic acid biosensor was washed with 2X SSC+ 0.1% SDS buffer for 5 min.

Additionally, to achieve the optimum analytical performance of the biosensor, different hybridization times (between 10 and 90 min) were tested.

Table 1. Oligonucleotides sequences used in the study.

Oligonucleotide name	Length	Sequence
Probe DNA (pHCV1a)	20-mer	5′biotin-CCCACCCGCAGCCCTCATTA-3′
Complementary Target DNA (cHCV1a)	20-mer	5′-TAATGAGGGCTGCGGGTGGG-3′
Non-complementary target DNA-1 (ncHCV3a)	17-mer	5′-GTGGGTGATATGTGTGG-3′
Non-complementary target DNA-2 (nc2-MTb)	20-mer	5′-CAATGAGGGCGGCGGGTGGA-3′
Three-base mismatch of target HCV1a (3mm-HCV1a)	20-mer	5′-CAATGAGGGCGGCGGGTGGA-3′
Synthetic HCV1a PCR analog (RS-cHCV1a)	50-mer	5′TAATGAGGGCTGCGGGTGGGCGGGATGGCTCCTGTCCCCCCGTGGCTCTC-3′
Synthetic HCV3a PCR analog (RS-ncHCV3a)	50-mer	5′GTGGGTGATATGTGTGGGGCCGTCTTCCTCGTGGGACAAGCCTTCACGTT-3′
Synthetic MTb PCR analog (RS-ncMTb)	50-mer	5′-TCTCGGGGTTTTGGGTCTGACGACTTGAACACGCCG ATACCTATTTGGTC-3′

Interaction with the intercalator molecule and measurement of MDB signal by square wave voltammery (SWV)

Following hybridization, the biosensor was dipped into tris buffer solution (TBS) (20 mM, pH 7.0) containing 20 mM MDB for different time durations ranging from 30 to 600 second. Then, the biosensor was rinsed with washing buffer (1X SSC+ 0.1% SDS, pH 7.0) and placed in a measurement cell containing TBS (pH 7.0).

SWV was performed by scanning from +0.4 V to −0.5 V to evaluate the reduction signal of MDB for probe DNA and its hybrid form with a complementary sequence. The result was given as Δi (μM), indicating the difference in MDB signal between the hybrid and the prob. The experimental conditions were as follows: an amplitude of 0.025 V, a step potential of 0.01 V, and a frequency of 15 Hz. The electrochemical detection protocol is showed in Figure 1.

Effect of hybridization temperature

The fabricated biosensor was immersed in 2X SSC buffer solution (pH 7.0) containing 2.5 μM cHCV1a at different temperatures (25, 30, 40, 50 and 70 °C) for 30 min in order to optimize the hybridization temperature. Then, SWV was performed by scanning from +0.4 V to −0.5 V to evaluate the reduction signal of MDB. The experimental conditions were as follows: an amplitude of 0.025 V, a step potential of 0.01 V, and a frequency of 15 Hz.

Shelf life of the biosensor

For the long-term dry storage stability studies, a certain amount of biosensor was prepared. Each fabricated biosensor was immersed in buffer solutions (1X SSC containing 2.5% [w/v] of glucose) for 5 min, and dried under at room temperature. The glucose in the buffer solution was used as

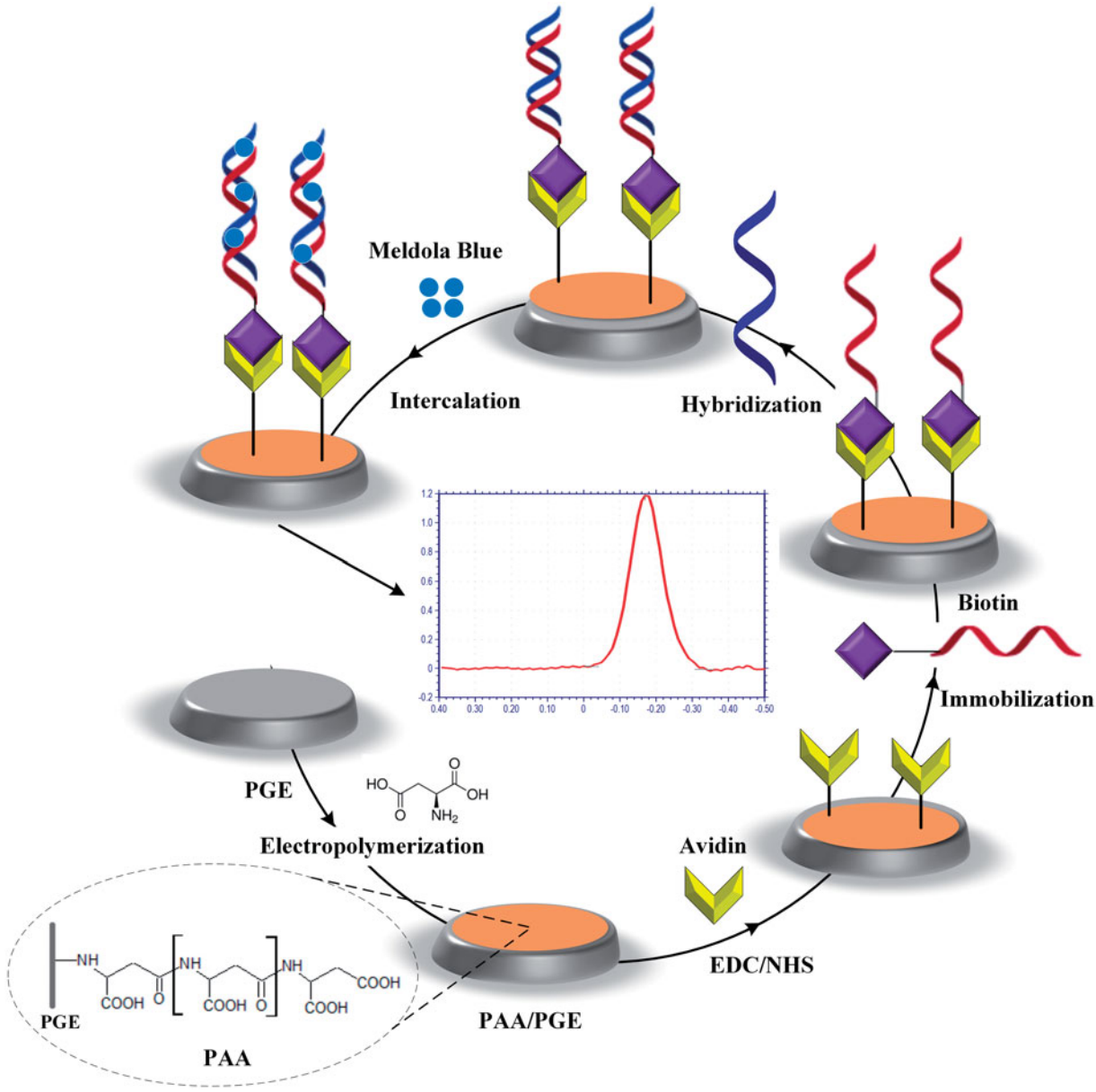

Figure 1. Schematic illustration of different steps for the fabrication of DNA biosensor.

the stabilizing agent.[19] After being stored at +4 °C for 1, 2, 3, 4, 5, 15, 30, and 60 days, the biosensors were washed with deionized water and incubated in 1X SSC for 30 min; then, they were hybridized with 2.5 µM cHCV1a sequence. Following to hybridization, the intercalation of MDB was carried out for 5 min, and SWV was performed by scanning from +0.4 V to −0.5 V to evaluate the reduction signal of MDB. Results were stated as the percentage of signal decreases in accordance with freshly prepared biosensor response.

Results and discussion

Electropolymerization of L-aspartic acid onto PGE

PAA film was formed onto PGE via the CV technique. During the first scan, an oxidation peak arose at about +1.70 V, which can be related to a peak in free radicals (Figure 2). The peak that was observed at +1.70 V disappeared in the second scan, but a new wide peak arose at about +1.78 V. It is possible that all the anodic peak currents increased gradually, showing the formation and growth of a PAA layer on the PGE surface. In addition, a gray-colored film was observed on the PGE surface, indicating the formation of a PAA acid film on the PGE surface.

The possible mechanism for this can be explained as follows: the oxidation of the amino group could convert to its corresponding cation radical, and carbon nitrogen links on the PGE surface may occur due to this cation radical.[20]

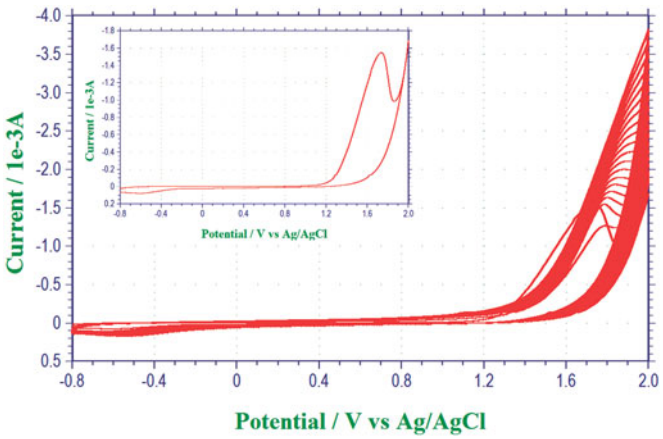

Figure 2. The electropolymerization of L-aspartic acid on the PGE surface was carried out CV technique between −0.8–2.0 V for 20 cycles (scan rate: 100 mv/s) in 0.1 M PBS (pH: 8.0) containing 0.02 M L-aspartic acid.

Scanning electron microscopy (SEM) image of poly(L-aspartic acid) film

The surface morphologies of PGE and PAA/PGE were characterized by SEM and the images are shown in Figure 3. The surface of bare PGE was almost smooth (Figure 3(a)). When aspartic acid was electrodeposited onto PGE, the layered PAA particle can be seen clearly (Figure 3(b)).

Optimization of conditions

Some experimental factors influencing biosensor performance, such as probe concentration, probe immobilization and hybridization time, hybridization temperature, and MDB intercalation time were studied.

A low probe concentration on the electrode surface may cause a small number of biorecognition sites, whereas a higher coverage of the surface can cause electrostatic and steric hindrance between probes and target oligonucleotides.[21] The effect of probe concentration was investigated to find the optimum surface coverage, and the results of this investigation are shown in Figure 4. pHCV1a concentration was increased from 0.1 to 10 µM, while the cHCV1a and ncHCV3a concentrations were kept constant, at 2.0 µM. Because the highest discrimination ratio (cHCV1a/ncHCV3a) was obtained at a probe concentration of 2.5 µM, it was chosen as the optimum probe concentration.

The detection ability of the nucleic acid biosensor can be improved with immobilization time, which increases the amount of immobilized probe on the electrode surface.[22]

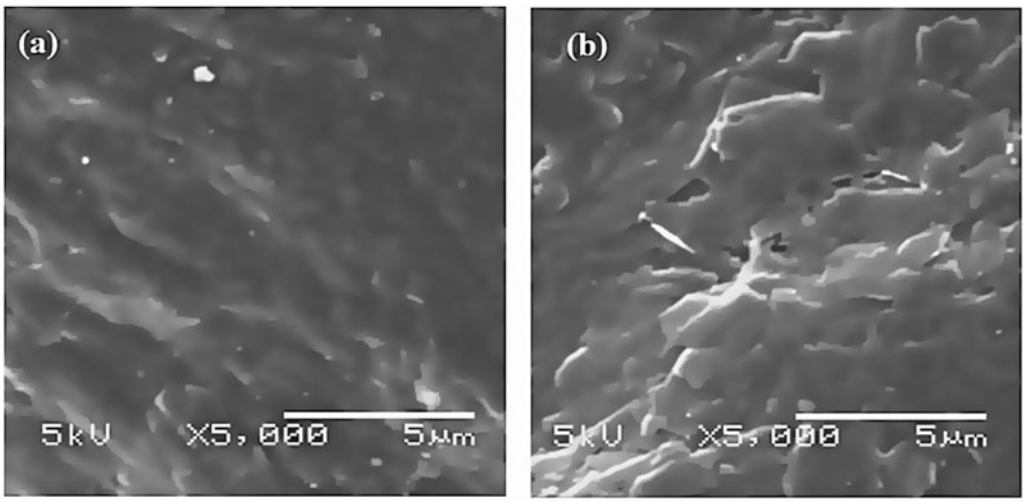

Figure 3. SEM images of (a) the bare PGE and (b) poly (L-aspartic acid) coated PGE.

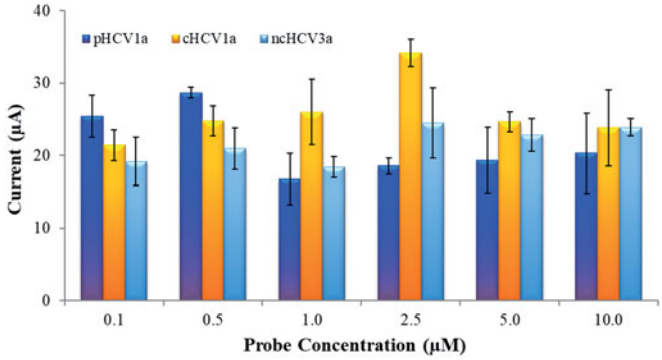

Figure 4. Current peaks of the MDB before and after hybridization of target DNA with different concentrations of the probe. Experimental conditions were as follows: probe immobilization time was 30 min.; hybridization time was 30 min with 2 μM cHCV1a and ncHCV3a; MDB binding time was 5 min., and voltammetric technique was SWV. Error bars represent the standard deviation of three independent measurements.

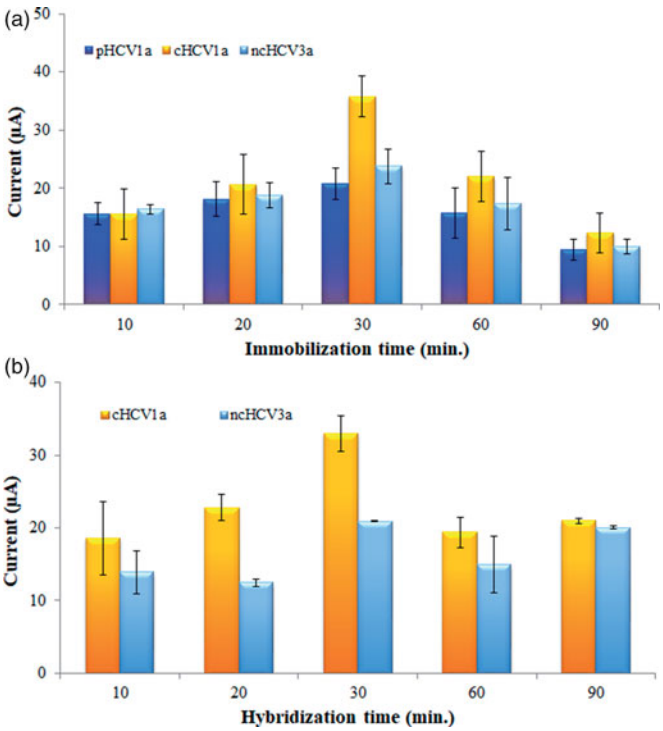

Figure 5. (a) Effect of probe immobilization time on reduction signal of MDB. b) Effect of hybridization time on reduction signal of MDB. Experimental conditions were as follows: probe immobilization time was 30 min in PBS with 2.5 μM pHCV1a; hybridization time was 30 min with 2.5 μM cHCV1a and ncHCV3a in 2X SSC, MDB intercalation time was 5 min in TBS containing 20 mM MDB, and voltammetric technique was SWV. Error bars represent the standard deviation of three independent measurements.

Therefore, the immobilization time was investigated in the range of 10–90 minutes (probe concentration 2.5 μM and target concentration 2.0 μM). As shown in Figure 5(a), the best discrimination rate (cHCV1a/ncHCV3a) was observed during immobilization for 30 minutes. Thus, a 30 min immobilization time was used for the research.

The influence of MDB accumulation time into the double-stranded DNA (dsDNA) and the hybridization time between pHCV1a and cHCV1a/ncHCV1a were also explored, as they affect the sensitivity of the biosensor. A

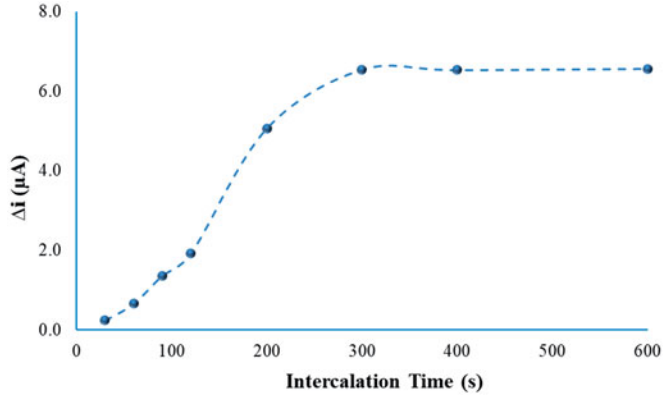

Figure 6. Effect of intercalation time on peak current of MDB. Δi represents the differences between the current peak of pHCV1a and cHCV1a sequences.

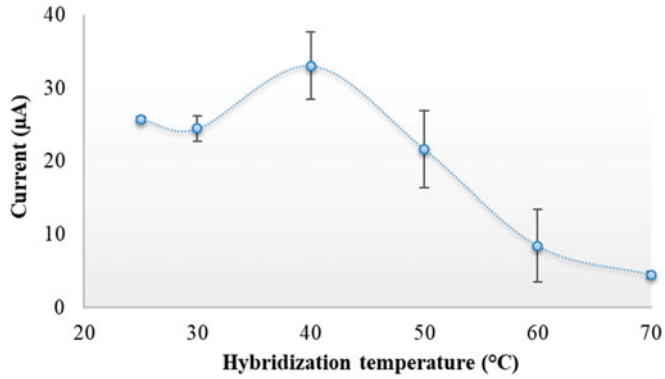

Figure 7. The effect of hybridization temperature on the biosensor. Error bars represent the standard deviation of three independent measurements.

short intercalation and hybridization time may result in low sensitivity.[23] As shown in Figure 5(b), when hybridization time was increased from 10 to 90 min, the best discrimination rate (cHCV1a/ncHCV3a) was reached after 30 min. As such, we chose 30 min to complete the hybridization between pHCV1a and cHCV1a. In addition, with the increase of the intercalation time between the formed dsDNA and MDB, SWV response enlarged rapidly, finally reaching a plateau at 300 s, indicating that the dsDNA formed on the electrode surface was saturated by MDB molecules at 300 s (Figure 6). Therefore, 300 s was the proper intercalation time in the following experiment to achieve the appropriate signal.

Hybridization temperature is an important parameter for nucleic acid biosensors.[24] Herein, a series of hybridization temperatures, ranging from 25 °C to 70 °C, were investigated (Figure 7). The results indicated that by increasing the temperature up to 40 °C, MDB signal increased, and the hybridization was at its maximum at 40 °C. When the hybridization temperature continued to increase, MDB signal decreased. This is because of DNA destabilization and denaturation.[14,25] Although the maximum efficiency of hybridization was obtained at 40 °C, 25 °C (room temperature) was preferred in the following research because MDB signal was sufficient for further studies.

Selectivity of the fabricated biosensor

The selectivity of the developed DNA biosensor was carried using the fabricated electrode to hybridize different kinds of DNA sequences (Table 1).

The SWV peak of MDB on the probe-immobilized electrode was 12.37 ± 0.46 µA. After hybridization with cHCV1a, ncHCV3a, nc2-MTb and 3 mm-HCV1a sequences, it was 24.53 ± 0.86 µA, 12.07 ± 0.62 µA, 10.90 ± 0.38 µA, and 16.33 ± 0.72 µA, respectively (Figure 8). The ncHCV3a, nc2-MTb, and 3 mm-HCV1a sequences had almost the same current values when compared to the pHCV1a because they were not fully hybridized with pHCV1a. However, when the fabricated biosensor hybridized with cHCV1a, the increase in the biosensor response was almost two-fold that of non-complementary sequences because the duplex form allowed the intercalation of MDB. This indicates that the fabricated biosensor readily discriminates between perfectly matched and mismatched or 3 mismatched oligonucleotides.

Synthetic PCR analogs related to the HCV1a gene regions were used to determine the selectivity of the fabricated biosensor. The fabricated biosensor was, to a good level of satisfaction, differentiating synthetic PCR analogs related to HCV1a and HCV3a or the *Mycobacterium tuberculosis* genome. The current of the SWV of MDB for RS-cHCV1a, RS-ncHCV3a and RS-ncMTb was 26.69 ± 0.82 µA, 16.53 ± 1.00 µA, and 12.20 ± 0.22 µA, respectively (Figure 9).

Shelf life of the biosensor

The storage life of the biosensor was tested for 60 days with a stabilizing agent used to enhance the dry storage life of the biosensor.[19,25] After 24 h, it was observed that the biosensor lost 12% of its initial current. The biosensor maintained almost 65% of the initial current after 60 days of storage (Figure 10). This result showed that the fabricated biosensor could be used for long-term application.

Quantificational detection of the target DNA

We examined the sensitivity of the prepared biosensor upon the addition of different concentrations of the target DNA (cHCV1a) in the best optimal experimental conditions. As shown in Figure 11, the SWV peak of MDB increased with

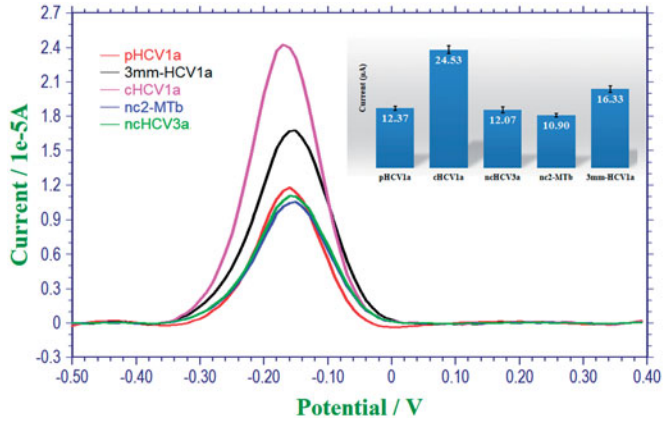

Figure 8. Square Wave voltammograms and histograms with error bars (inset graph) of MDB with synthetic oligonucleotides on PAA/PGE with pHCV1a after hybridization with complementary or non-complementary targets. Error bars represent the standard deviation of three independent measurements.

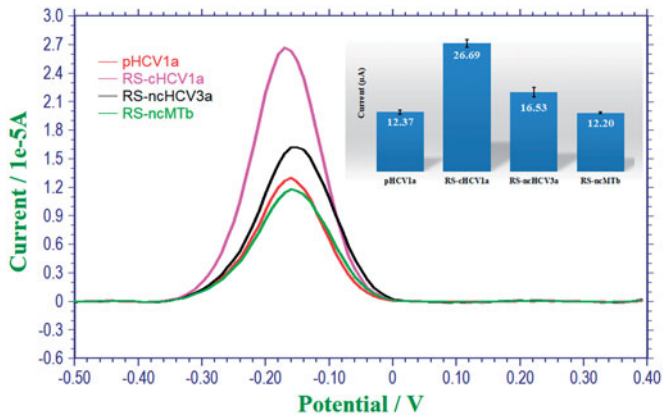

Figure 9. Square Wave voltammograms and histograms with error bars (inset graph) of MDB with synthetic PCR oligonucleotides on PAA/PGE with pHCV1a after hybridization with complementary or non-complementary targets. Error bars represent the standard deviation of three independent measurements.

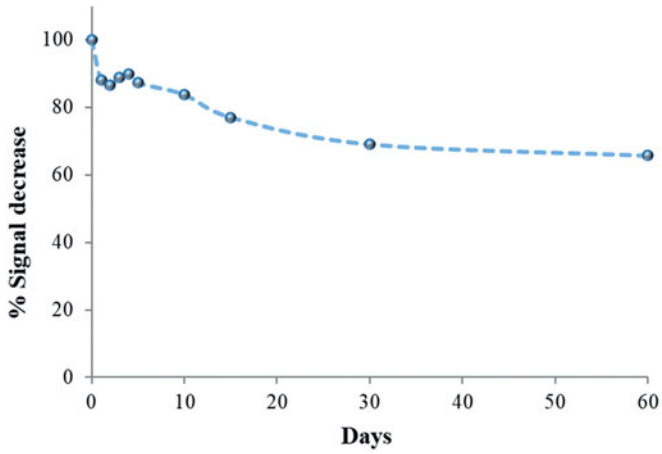

Figure 10. Shelf-life of the fabricated nucleic acid biosensor. The sensor response is plotted against the number of stored days.

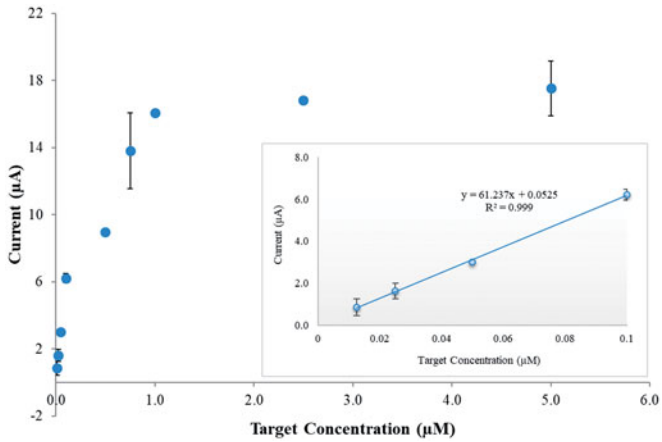

Figure 11. The SWV signal of the biosensor in the presence of different concentrations of target DNA (cHCV1a) and calibration plot for cHCV1a in the range of 12.5 nm to 100 nm (Inset graph). Error bars represent the standard deviation of three independent measurements.

Table 2. Comparison of some different nucleic acid sensor in the literature.

Sensing substrate	Probe	Detection method	Label/Indicator	Modified layer	LOD (nM)	Reference
NGE	PNA	EIS	None	None	10	[27]
BBDE	DNA	EIS	None	Polyaniline/Polyacrylate	20	[28]
NGE	DNA	SWV	Methylene blue	None	10	[29]
PGE	DNA	SWV	Meldola's blue	Poly(L)aspartic acid	8.7	This work

NGE: Nanoporous gold electrode, PNA: peptide nucleic acid; EIS: Electrochemical impedance spectroscopy; BBDE: Boron-doped diamond electrode; SWV: square wave voltammetry; PGE: Pencil graphite Electrode.

an increase in the concentration of the target DNA, ranging from 0.0125–5.0 µM. The calibration plots showed a good linear dependence between the SWV peak of MDB and the target concentrations in the range of 12.5–100 nm (inset of Figure 11); the regression equation is $y(\Delta I) = 61.237 + 0.0525c$ (µM), with a correlation coefficient of 0.999. The limit of detection was determined by using the equation yLOD (I) = yB + 3Sy/x, where yB is signal of the blank (in this study, the intercept of the calibration graph) and Sy/x is the standard deviation of the blank (in this study, the standard deviation of the calibration graph).[26] The detection limit was calculated to be 8.7 nM. The stability of the biosensor was evaluated by the successive detection of 0.1 µM of target DNA three times, and the relative standard deviation (RSD) was found to be 2.76%. Compared with other DNA-sensing platforms, this biosensor has a relatively low detection limit (Table 2).

Conclusions

The identification and detection of specific nucleic acid sequences is important in clinical diagnostics, food safety analysis, and environmental analysis. In this context, we fabricated a novel electrochemical nucleic biosensor via the electropolymerization of L-aspartic acid onto PGE for the detection of HCV1a sequence. The use of PAA film in the nucleic acid biosensor eliminates the complex immobilization process of biomolecules because of the free carboxyl group of PAA. This suggest that PAA can be considered as a good candidate for the immobilization of biomolecules. The prepared nucleic acid biosensor has a number of advantages including being cost-effective (because the PGE is used), being stable in dry storage, requiring only one-step modification, and being time-efficient; however, this biosensor does still require PCR amplification.

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Reference

[1] World Health Organization, Hepatitis C Key Facts. 2018. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c (accessed July 18, 2018).

[2] Bukh, J. The History of Hepatitis C Virus (HCV): Basic Research Reveals Unique Features in Phylogeny, Evolution and the Viral Life Cycle with New Perspectives for Epidemic Control. *J. Hepatol.* **2016**, *65*, S2–S21. DOI: 10.1016/j.jhep.2016.07.035.

[3] Freiman, J. M.; Tran, T. M.; Schumacher, S. G.; White, L. F.; Ongarello, S.; Cohn, J.; Easterbrook, P. J.; Linas, B. P.; Denkinger, C. M. Hepatitis C Core Antigen Testing for Diagnosis of Hepatitis C Virus Infection: A Systematic Review and Meta-Analysis. *Ann. Intern. Med.* **2016**, *165*, 345–355. DOI: 10.7326/M16-0065.

[4] dos Santos Riccardi, C.; Kranz, C.; Kowalik, J.; Yamanaka, H.; Mizaikoff, B.; Josowicz, M. Label-Free DNA Detection of Hepatitis C Virus Based on Modified Conducting Polypyrrole Films at Microelectrodes and Atomic Force Microscopy Tip-Integrated Electrodes. *Anal. Chem.* **2008**, *80*, 237–245. DOI: 10.1021/ac701613t.

[5] Diculescu, V. C.; Oliveira-Brett, A. M. In Situ Electrochemical Evaluation of dsDNA Interaction with the Anticancer Drug Danusertib Nitrenium Radical Product Using the DNA-Electrochemical Biosensor. *Bioelectrochemistry.* **2016**, *107*, 50–57. DOI: 10.1016/j.bioelechem.2015.10.004.

[6] Brasil de Oliveira Marques, P. R.; Lermo, A.; Campoy, S.; Yamanaka, H.; Barbé, J.; Alegret, S.; Pividori, M. I. Double-Tagging Polymerase Chain Reaction with a Thiolated Primer and Electrochemical Genosensing Based on Gold Nanocomposite Sensor for Food Safety. *Anal. Chem.* **2009**, *81*, 1332–1339. DOI: 10.1021/ac801736b.

[7] Gan, X.; Zhao, H.; Chen, S.; Quan, X. Electrochemical DNA Sensor for Specific Detection of Picomolar Hg(ii) Based on Exonuclease III-Assisted Recycling Signal Amplification. *Analyst.* **2015**, *140*, 2029–2036. DOI: 10.1039/C5AN00082C.

[8] Cui, M.; Wang, Y.; Wang, H.; Wu, Y.; Luo, X. A Label-Free Electrochemical DNA Biosensor for Breast Cancer Marker BRCA1 Based on Self-Assembled Antifouling Peptide Monolayer. *Sens. Actuators B Chem.* **2017**, *244*, 742–749. DOI: 10.1016/j.snb.2017.01.060.

[9] Jampasa, S.; Wonsawat, W.; Rodthongkum, N.; Siangproh, W.; Yanatatsaneejit, P.; Vilaivan, T.; Chailapakul, O. Electrochemical Detection of Human Papillomavirus DNA Type 16 Using a Pyrrolidinyl Peptide Nucleic Acid Probe Immobilized on Screen-Printed Carbon Electrodes. *Biosens. Bioelectron.* **2014**, *54*, 428–434. DOI: 10.1016/j.bios.2013.11.023.

[10] Chen, P. Q.; Liang, Q. N.; Huang, T. S.; Liu, T. C.; Li, M. A Simple, Rapid, and Highly Sensitive Electrochemical DNA Sensor for the Detection of ot Alpha- and beta-Thalassemia in China. *J. Clin. Lab. Anal.* **2016**, *30*, 719–726. DOI: 10.1002/jcla.21927.

[11] Zhang, Y.; Zhang, K.; Ma, H. Electrochemical DNA Biosensors Based on Gold Nanoparticles/Cysteamine/Poly (Glutamic Acid) Modified Electrode. *Am. J. Biomed. Sci.* **2009**, *1*, 115–125. DOI: 10.5099/aj090200115.

[12] Geno, P. W.; Ravichandran, K.; Baldwin, R. P. Chemically Modified Carbon Paste Electrodes. *J Electroanalytical Chem. Interfacial Electrochem.* **1985**, *183*, 155–166. DOI: 10.1016/0368-1874(85)85488-5.

[13] Chitravathi, S.; Swamy, B. E. K.; Mamatha, G. P.; Sherigara, B. S. Simultaneous Electrochemical Determination of Dopamine and Ascorbic Acid Using Poly (l-Serine) Modified Carbon Paste Electrode. *J. Mol. Liq.* **2011**, *160*, 193–199. DOI: 10.1016/j.molliq.2011.03.019.

[14] Donmez, S.; Arslan, F.; Arslan, H. A Nucleic Acid Biosensor for Detection of Hepatitis C Virus Genotype 1a Using Poly(L-

Glutamic Acid)-Modified Electrode. *Appl. Biochem. Biotechnol.* **2015**, *176*, 1431–1444. DOI: 10.1007/s12010-015-1655-6.

[15] Gilbert, O.; Chandra, U.; Char, M. P.; Swamy, B. E. K.; Char, M. P.; Nagaraj, C.; Sherigard, B. S. Poly (Alanine) Modified Carbon Paste Electrode for Simultaneous Detection of Dopamine and Ascorbic Acid. *Int. J. Electrochem. Sci.* **2008**, *3*, 1186–1195.

[16] Sun, W.; Zhang, Y.; Ju, X.; Li, G.; Gao, H.; Sun, Z. Electrochemical Deoxyribonucleic Acid Biosensor Based on Carboxyl Functionalized Graphene Oxide and poly-L-Lysine Modified Electrode for the Detection of Tlh Gene Sequence Related to vibrio parahaemolyticus. *Anal. Chim. Acta.* **2012**, *752*, 39–44. DOI: 10.1016/j.aca.2012.09.009.

[17] Santos, D. P.; Zanoni, M. V. B.; Bergamini, M. F.; Chiorcea-Paquim, A. M.; Diculescu, V. C.; Brett, A. M. O. Poly(Glutamic Acid) Nanofibre Modified Glassy Carbon Electrode: Characterization by Atomic Force Microscopy, Voltammetry and Electrochemical Impedance. *Electrochim. Acta.* **2008**, *53*, 3991–4000. DOI: 10.1016/j.electacta.2007.08.072.

[18] Zhang-Yu, Y. U.; Xiao-Chun, L. I.; Xue-Liang, W.; Jinjin, L.; Ke-Wei, C. A. O. Studies on the Electrochemical Behaviors of Epinephrine at a Poly(l-Aspartic Acid) Modified Glassy Carbon Electrode and Its Analytical Application. *Int. J. Electrochem. Sci.* **2011**, *6*, 3890–3890.

[19] Lai, R. Y.; Seferos, D. S.; Heeger, A. J.; Bazan, G. C.; Plaxco, K. W. Comparison of the Signaling and Stability of Electrochemical DNA Sensors Fabricated from 6- or 11-Carbon Self-Assembled Monolayers. *Langmuir.* **2006**, *22*, 10796–10800. DOI: 10.1021/la0611817.

[20] Li, J.; Zhang, X. Fabrication of Poly(Aspartic Acid)-Nanogold Modified Electrode and Its Application for Simultaneous Determination of Dopamine, Ascorbic Acid, and Uric Acid. *AJAC.* **2012**, *3*, 195. DOI: 10.4236/ajac.2012.33028.

[21] Farabullini, F.; Lucarelli, F.; Palchetti, I.; Marrazza, G.; Mascini, M. Disposable Electrochemical Genosensor for the Simultaneous Analysis of Different Bacterial Food Contaminants. *Biosens. Bioelectron.* **2007**, *22*, 1544–1549. DOI: 10.1016/j.bios.2006.06.001.

[22] Benvidi, A.; Firouzabadi, A. D.; Moshtaghiun, S. M.; Mazloum-Ardakani, M.; Tezerjani, M. D. Ultrasensitive DNA Sensor Based on Gold Nanoparticles/Reduced Graphene Oxide/Glassy Carbon Electrode. *Anal. Biochem.* **2015**, *484*, 24–30. DOI: 10.1016/j.ab.2015.05.009.

[23] Ni, J.; Yang, W.; Wang, Q.; Luo, F.; Guo, L.; Qiu, B.; Lin, Z.; Yang, H. Homogeneous and Label-Free Electrochemiluminescence Aptasensor Based on the Difference of Electrostatic Interaction and Exonuclease-Assisted Target Recycling Amplification. *Biosens. Bioelectron.* **2018**, *105*, 182–187. DOI: 10.1016/j.bios.2018.01.043.

[24] Benvidi, A.; Firouzabadi, A. D.; Tezerjani, M. D.; Moshtaghiun, S. M.; Mazloum-Ardakani, M.; Ansarin, A. A Highly Sensitive and Selective Electrochemical DNA Biosensor to Diagnose Breast Cancer. *Electroanal. Chem.* **2015**, *750*, 57–64. DOI: 10.1016/j.jelechem.2015.05.002.

[25] Yesil, M.; Donmez, S.; Arslan, F. Development of an Electrochemical DNA Biosensor for Detection of Specific Mycobacterium tuberculosis Sequence Based on Poly(L-Glutamic Acid) Modified Electrode. *J. Chem. Sci.* **2016**, *128*, 1823–1829. DOI: 10.1007/s12039-016-1159-0.

[26] Pournaghi-Azar, M. H.; Ahour, F.; Hejazi, M. S. Direct Detection and Discrimination of Double-Stranded Oligonucleotide Corresponding to Hepatitis C Virus Genotype 3a Using an Electrochemical DNA Biosensor Based on Peptide Nucleic Acid and Double-Stranded DNA Hybridization. *Anal. Bioanal. Chem.* **2010**, *397*, 3581–3587. DOI: 10.1007/s00216-010-3875-5.

[27] Tao, W.; Lin, P.; Liu, H.; Ke, S.; Zeng, X. A Rapid, Label-Free and Impedimetric DNA Sensor Based on PNA-Modified Nanoporous Gold Electrode. *Int. J. Electrochem. Sci.* **2017**, *12*, 10511–10523. DOI: 10.20964/2017.11.38.

[28] Gu, H.; Su, X. d.; Loh, K. P. Electrochemical Impedance Sensing of DNA Hybridization on Conducting Polymer Film-Modified Diamond. *J. Phys. Chem. B.* **2005**, *109*, 13611–13618. DOI: 10.1021/jp050625p.

[29] Daggumati, P.; Matharu, Z.; Seker, E. Effect of Nanoporous Gold Thin Film Morphology on Electrochemical DNA Sensing. *Anal. Chem.* **2015**, *87*, 8149–8156. DOI: 10.1021/acs.analchem.5b00846.