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Label-free electrochemical aptasensor for GPC3 detection based on RGO-CS-Fc/Pt NPs

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Abstract: Glypican-3 (GPC3) is a promising biomarker of early detection of hepatocellular carcinoma (HCC). Herein, an label-free electrochemical aptasensor for the detection of GPC3 was developed based on reduced graphene oxide-chitosan-ferrocene /platinum nanoparticles (RGO-CS-Fc/Pt NPs). GPC3 aptamer (GPC3-Apt) was immobilized onto the surface of the modified screen-printed carbon electrode (SPE) by RGO-CS-Fc/Pt NPs. When the GPC3 was added to the platforms of aptasensor, the specific recognition reaction between the GPC3-Apt and GPC3 produced aptamer-antigen complex and arranged on the electrode surface, which made the change of electrochemical signals of Fc obtained by differential pulse voltammetry (DPV). Hence, the calibration graph (peak current vs GPC3 concentration) can be used for quantification of GPC3. The measured peak current and GPC3 concentration have a good linearity in the range of 0.05-10.0 $\mu\text{g/mL}$, a correlation coefficient of 0.9805 and a low detection limit of 0.018 $\mu\text{g/mL}$ ($S/N=3$). Moreover, the designed aptasensor showed good performance with excellent selectivity, acceptable reproducibility and stability. Thus, this label-free electrochemical aptasensor has promising applications for the early detection of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers with a high mortality rate, especially in China. Therefore, potential biomarkers are urgently required for the early detection of HCC and the development of novel therapeutic methods [1]. Glypican-3 (GPC3) is a membrane-bound heparin sulfate proteoglycan and highly expressed in HCC tissues, while GPC3 is negative in normal liver tissue or benign hepatocellular nodules. Hence, GPC3 has been used as a biomarker for molecular imaging detection and therapeutic intervention in HCC [2, 3]. At present, an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay is commonly used to detect the level of GPC3 in serum and tissues of HCC [4, 5]. These methods rely on anti-GPC3 antibodies. However, the clinical application of the GPC3 antibodies is still limited because of its high immunogenicity, high production cost, and low stability [6]. Therefore, it is urgent to find a new type of recognition molecule with high specificity to bind GPC3 and to develop methods for detection of serum GPC3.

Aptamers are low molecular weight (6-30 kDa) single-stranded RNA or DNA oligonucleotides, and are bind to the target molecule with specificity and efficiency. Aptamers can recognizes and binds to the related target in a specific and high binding affinity via an adaptive recognition manner [7]. Therefore, aptamers that specifically binds to HCC cells in tissue samples or cell lines may facilitate the establishing novel approaches for HCC early diagnosis. Zhang et al successfully screened a batch of GPC3 aptamers and established a fluorescence analysis for the diagnosis of HCC [8]. To date, the aptamer-based electrochemical biosensor (aptasensor) had been considered to be the simple and selective tool for the



detection of target molecule [9, 10]. Recently, we constructed a label-free electrochemical aptasensor for the detection of AFP using AFP-aptamer as the recognition molecule and thionin/reduced graphene oxide/gold nanoparticles (TH/RGO/Au NPs) as the sensor platform. The aptasensor provided a promising application of aptamer with low cost, high selectivity in clinic [11].

At present, nanomaterials and nanostructures have been widely used in electrochemical biosensing systems to achieve better sensitivity [12]. Many different nanomaterials such as graphene, gold nanoparticles, platinum nanoparticles, carbon nanotubes have been used to improve the performance of aptasensors [13]. Herein, we have prepared the well-dispersed reduced graphene oxide-chitosan-ferrocene (RGO-CS-Fc) nanocomposites and used them to modify the surface of screen-printed carbon electrode (SPE). Pt NPs with good electronic conductivity were electrodeposited onto the surface of modified SPE. Then, GPC3 aptamer (GPC3-Apt) was immobilized onto the surface of the RGO-CS-Fc/Pt NPs/SPE. Thus, a highly sensitive electrochemical aptasensor has been constructed for detection GPC3 protein based on RGO-CS-Fc/Pt NPs. The analytical performance in terms of working curve, linear range, specificity, reproducibility and stability of the proposed GPC3 aptasensor were discussed.

2. Experimental

2.1 Chemicals and reagents

Graphene oxide (GO) was obtained from Timenano Company (Chengdu, China). Ferrocene formic acid, glutaraldehyde, chloroplatinic acid (H_2PtCl_6) and hydrazine hydrate were supported by Sinopharm chemical Reagent Co., Ltd (Beijing, China). N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS), N-(3-dimethylaminopropyl)-N-ethyl-carbodiimide hydrochloride (EDC), chitosan and ascorbic acid were purchased from Sigma (St. Louis, MO, USA). All solutions were prepared with ultrapure water of $18 \text{ M}\Omega\cdot\text{cm}$ purified from Milli-Q purification system (Milli-Pore, Bedford, MA, USA). The GPC3 aptamer (GPC3-Apt) [8] with the following sequences (5'-NH₂-TAACGCTGACCTTAGCTGCATGGCTTTACATGTTCCA-3') was purchased from Sangon biotech Co., Ltd (Shanghai, China).

Electrochemical measurements were carried out on a CHI-660E electrochemical workstation (Shanghai Chenhua Instruments Co., China) using a conventional three screen-printed carbon electrode system. In this system, one carbon paste electrode or modified carbon paste electrode is used as the working electrode, another carbon electrode is used as the auxiliary electrode, and pseudo-reference Ag/AgCl electrode is used as the reference electrode.

2.2 Preparation of the RGO-CS-Fc nanocomposite

RGO-CS-Fc nanocomposite has been prepared using hydrazine hydrate as the reducing agent following our previous method [14]. Briefly, 1.0 mg GO was dispersed to 10 mL ultrapure water and ultrasonic agitation for 2 h to obtain GO solution, then 10 mg ascorbic acid was slowly added and stirred for 12 h to attain RGO. Then, 0.2 mg chitosan was dispersed into 10.0 mL acetic acid solution (1.0%) to form chitosan solution. Next, 2.0 mg ferrocene formic acid, 1.0 mL 10 mM EDC and 4.0 mL 10 mM sulfo-NHS solution were added to the chitosan solution and stirred for 4 h. Thirdly, 10.0 mL 1.0 mg/mL RGO solution was added the above solution and stirred for another 12 h. Lastly, the mixed solution was centrifuged for 30 min at 20000 rpm to remove the supernatant. The precipitate (RGO-CS-Fc nanocomposite) was washed twice with ultrapure water, and re-dispersed in 10.0 mL ultrapure water to form a 1.0 mg/mL solution and stored in refrigerator (4°C) for future use.

2.3 Fabrication of the electrochemical GPC3 aptasensor

Firstly, the SPE was immersed in 0.5 M H_2SO_4 solution and subjected to electrochemical cyclic voltammetry (CV) scan for 10 cycles. The voltage was set from -0.2 V to 1.0 V and the speed was 100 mV/s. Secondly, Pt NPs were electrodeposited onto the activated SPE in 0.01% H_2PtCl_6 solution under the potential of -0.2 V for 120 s while the magnetic stirring. Thirdly, 8.0 μL 1.0 mg/mL RGO-CS-Fc solution was added dropwise onto the surface of SPE/ Pt NPs, and incubated for 30 minutes at 25°C .

Fourthly, SPE/ Pt NPs /RGO-CS-Fc was immersed in 2.5% glutaraldehyde solution for 15 minutes and cleaned with PBS solution three times. After drying, 2 μL 0.1 μM GPC3-Apt was added dropwise to the surface of SPE/ Pt NPs /RGO-CS-Fc and incubated at 25 $^{\circ}\text{C}$ for 2 h. Finally, 0.5% BSA solution was added to the surface of SPE/ Pt NPs /RGO-CS-Fc/GPC3-Apt to block nonspecific site. Thus, the label-free electrochemical aptasensor (SPE/ Pt NPs /RGO-CS-Fc/GPC3-Apt) has been come into being and stored in refrigerator (4 $^{\circ}\text{C}$) for future use.

2.4 Detection of GPC3 with the electrochemical aptasensor

The detection of GPC3 was performed by differential pulse voltammetry (DPV) method with SPE/ Pt NPs /RGO-CS-Fc/GPC3-Apt as the working electrode and PBS as the supporting electrolyte. In a typical process, 2 μL of GPC3 (different concentration) standard solution were added dropwise to the surface of SPE/ Pt NPs /RGO-CS-Fc/GPC3-Apt, and incubated for 30 min at 25 $^{\circ}\text{C}$. Subsequently, the modified electrode (SPE/ Pt NPs /RGO-CS-Fc/GPC3-Apt/GPC3) was immersed vertically in the center of the glass bottle filled with 5.0 mL PBS solution (0.2 M, pH=6.0), and record the electrochemical signals using the DPV method for quantitative analysis. Three times was detected for each sample, and the results were calculated as mean \pm RSD.

3. Results and discussion

3.1 Analytical principle of electrochemical detection of GPC3 based on Pt NPs /RGO-CS-Fc modified SPE

The analytical principle of the electrochemical detection of GPC3 based on Pt NPs /RGO-CS-Fc modified SPE was shown in Figure 1. In this assay, the bare SPE was activated by H_2SO_4 , and electrodeposited of Pt NPs onto the surface of SPE through the isostatic potentiometric deposition. RGO-CS-Fc nanocomposite was immobilized onto the surface of SPE/ Pt NPs by electrostatic adsorption. After that, the GPC3-Apt was captured by means of physical and chemical adsorption on the surface of SPE/ Pt NPs /RGO-CS-Fc. When the GPC3 solution was dropped onto the surface of SPE/ Pt NPs /RGO-CS-Fc/ GPC3-Apt, the specific recognition reaction between the GPC3-Apt and GPC3 produced aptamer-antigen complex and arranged on the electrode surface, which made the change of electrochemical signals of Fc. The value of electrochemical signal obtained by DPV decreased with the increase of the GPC3 concentration. Thus, through measuring the change of electrochemical signals of Fc, GPC3 can be detected with high sensitivity. Herein, RGO-CS-Fc was employed as the nanocarrier to load GPC3-Apt. The decorated Fc acted not only as a bridging molecule to effectively capture GPC3-Apt, but also as the electron transfer mediator to provide the electrochemical signal. Under the synergistic effect of Pt NPs with good electroconductibility and RGO-CS-Fc with high specific surface area and good biocompatibility, the electrochemical signal of Fc could be amplified to indicate the GPC3 level.

3.2 The feasibility of the electrochemical aptasensor for the detection of GPC3

The feasibility of the developed aptasensor for GPC3 detection was illustrated in Figure 2A with the DPV method. Seen from Figure 2A, there was a remarkable current response (103.60 μA) at -0.096 mV without GPC3 (curve a). While a mild current response (73.26 μA) appeared at -0.088 mV with 5.0 $\mu\text{g}/\text{mL}$ GPC3 (curve b) and a small of current response (56.17 μA) revealed at -0.093 mV in with 10.0 $\mu\text{g}/\text{mL}$ GPC3 (curve c). Hence, the aptasensor showed the analyte-dependent current, which provided a measurement for determining the GPC3 protein.

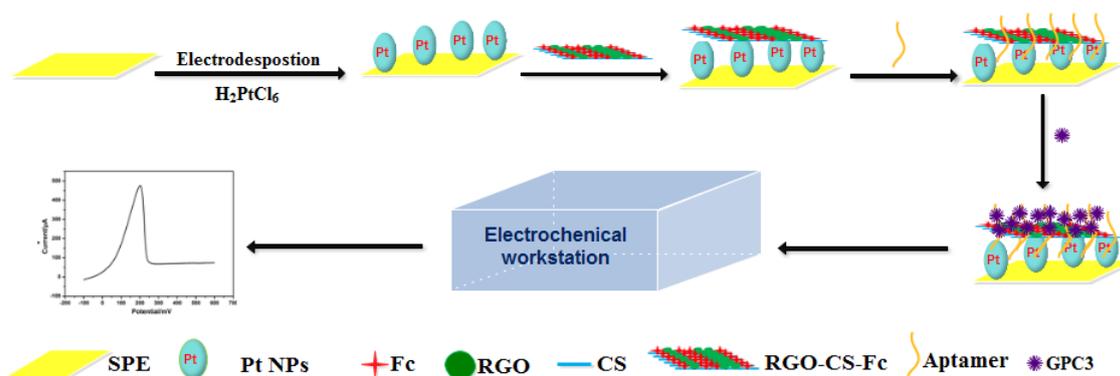


Figure 1 Analytical principle of electrochemical detection of GPC3 based on Pt NPs /RGO-CS-Fc modified SPE

3.3 Analytical performance of the electrochemical aptasensor for the detection of GPC3

Furthermore, the standard GPC3 solutions with different concentrations were tested using the proposed aptasensor with the DPV method, and the results were shown in Figure 2B. The current response of the aptasensor decreased along with the increasing concentration of GPC3. As the concentration of GPC3 increasing, more amount of GPC3 was specifically recognized by GPC3-aptamer and more aptamer-antigen complexes formed, which brought about the gradually decreasing of electrochemical signal response. In the range of 0.05-10.0 $\mu\text{g/mL}$, the concentration of GPC3 has a good linear relationship with the corresponding current of the aptasensor. The linear equation was $Y = -3.4478X + 90.0089$ with a correlation coefficient of 0.9805. The limit of detection (LOD) was calculated to be 0.018 $\mu\text{g/mL}$ at a signal-to-noise ratio of 3 ($S/N = 3$).

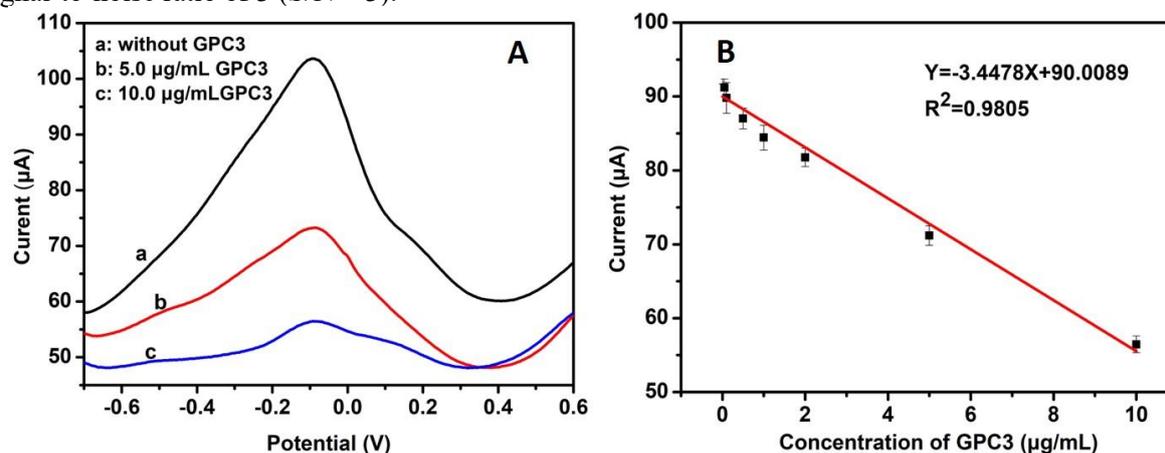


Figure 2 (A) the feasibility of the developed aptasensor for GPC3 detection using the DPV method. (B) Working curve of GPC3 by the developed aptasensor

3.4 Specificity, reproducibility, and stability of the GPC3 aptasensor

Investigating the specificity of the electrochemical aptamer was very important. The aptasensor was incubated with 10.0 $\mu\text{g/mL}$ interfering substances, including alpha-fetoprotein (AFP), bovine serum albumin (BSA), immunoglobulin G (IgG), human serum albumin (HSA), immunoglobulin E (IgE) and recorded the current response by DPV method. The aptasensor's current response value were 95.79 μA for AFP, 92.78 μA for BSA, 91.89 μA for IgG, 94.68 μA for HSA, 90.93 μA for IgE, respectively, while the current response value for CPC3 was 55.72 μA , which revealed the designed aptasensor had favorable specificity.

Moreover, the reproducibility of the aptasensor was evaluated using five freshly prepared modified electrodes to detect the same concentration of GPC3 (10.0 $\mu\text{g/mL}$). All five electrodes had similar current response value (54.96 μA , 55.72 μA , 53.93 μA , 56.55 μA , 59.86 μA) with the relative standard deviation (RSD) of 4.02%, indicating that the proposed aptasensor had well reproducibility for GPC3 detection.

Additionally, the stability of the aptasensor was investigated by keeping the same electrode at 4 $^{\circ}\text{C}$ and being testing the current value per 4 days from 1 to 16 days. The maximum and the minimum current response were maintained at 94.38% and 86.05% of the original response, which demonstrated the aptasensor had excellent operational stability.

4. Conclusions

In summary, a label-free electrochemical aptasensor based on Pt NPs /RGO-CS-Fc modified SPE has been successfully developed for the measurement of GPC3. The GPC3-Apt was immobilized on the surface of SPE/ Pt NPs /RGO-CS-Fc. In the presence of GPC3, the specific recognition reaction between the GPC3-Apt and GPC3 produced aptamer-antigen complex and arranged on the electrode surface, which made the change of electrochemical signals of Fc record by DPV method. The linear relationship between the current response and the GPC3 concentration was very well over the range from 0.05 to 10.0 $\mu\text{g/mL}$ with a readily achievable LOD of 0.018 $\mu\text{g/mL}$ ($S/N = 3$). Furthermore, the developed label-free electrochemical aptasensor had favorable specificity, well reproducibility and acceptable stability. Therefore, this ultrasensitive electrochemical aptasensor offers great application promises in detection of plasma GPC3 for HCC early diagnosis.

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References

- [1] M.S. Grandhi, A.K. Kim, S.M. Ronnekleiv-Kelly, I.R. Kamel, M.A. Ghasebeh, T.M. Pawlik, Hepatocellular carcinoma: From diagnosis to treatment, *Surg Oncol* 25(2) (2016) 74-85.
- [2] F.B. Zhou, W.T. Shang, X.L. Yu, J. Tian, Glypican-3: A promising biomarker for hepatocellular carcinoma diagnosis and treatment, *Med Res Rev* 38(2) (2018) 741-767.
- [3] M. Montalbano, C. Rastellini, J.T. McGuire, J. Prajapati, A. Shirafkan, R. Vento, L. Cicalese, Role of Glypican-3 in the growth, migration and invasion of primary hepatocytes isolated from patients with hepatocellular carcinoma, *Cell Oncol (Dordr)* 41(2) (2018) 169-184.
- [4] H.H. Han, Y.J. Qiu, Y.Y. Shi, W. Wen, X.P. He, L.W. Dong, Y.X. Tan, Y.T. Long, H. Tian, H.Y. Wang, Glypican-3-targeted precision diagnosis of hepatocellular carcinoma on clinical sections with a supramolecular 2D imaging probe, *Theranostics* 8(12) (2018) 3268-3274.
- [5] W. Yuanyuan, Z. Chenjie, L. Jing, Z. Ling, L. Mingsong, X. Bing, Value of detection of serum glypican-3 level in diagnosis and therapeutic effect evaluation of primary hepatocellular carcinoma, *J South Med Univ* 37(8) (2017) 1060-1065.
- [6] Y. Fu, D.J. Urban, R.R. Nani, Y.F. Zhang, N. Li, H. Fu, H. Shah, A.P. Gorka, R. Guha, L. Chen, M.D. Hall, M.J. Schnermann, M. Ho, Glypican-3 Specific Antibody Drug Conjugates Targeting Hepatocellular Carcinoma, *Hepatology* (2018).
- [7] R.B. Ladju, D. Pascut, M.N. Massi, C. Tiribelli, C.H.C. Sukowati, Aptamer: a potential oligonucleotide nanomedicine in the diagnosis and treatment of hepatocellular carcinoma, *Oncotarget* 9(2) (2018) 2951-2961.
- [8] M. Zhao, L. Dong, Z. Liu, S. Yang, W. Wu, J. Lin, In vivo fluorescence imaging of hepatocellular carcinoma using a novel GPC3-specific aptamer probe, *Quant Imaging Med Surg* 8(2) (2018) 151-160.
- [9] M. Yousefi, S. Dehghani, R. Nosrati, H. Zare, M. Evazalipour, J. Mosafer, B.S. Tehrani, A. Pasdar, A. Mokhtarzadeh, M. Ramezani, Aptasensors as a new sensing technology developed for the

- detection of MUC1 mucin: A review, *Biosensors and Bioelectronics* 130 (2019) 1-19.
- [10] S. Dehghani, R. Nosrati, M. Yousefi, A. Nezami, F. Soltani, S.M. Taghdisi, K. Abnous, M. Alibolandi, M. Ramezani, Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): A review, *Biosensors and Bioelectronics* 110 (2018) 23-37.
- [11] G. Li, S. Li, Z. Wang, Y. Xue, C. Dong, J. Zeng, Y. Huang, J. Liang, Z. Zhou, Label-free electrochemical aptasensor for detection of alpha-fetoprotein based on AFP-aptamer and thionin/reduced graphene oxide/gold nanoparticles, *Analytical biochemistry* 547 (2018) 37-44.
- [12] S. Yang, F. Zhang, Z. Wang, Q. Liang, A graphene oxide-based label-free electrochemical aptasensor for the detection of alpha-fetoprotein, *Biosens Bioelectron* 112 (2018) 186-192.
- [13] Y. Zhang, J. Xia, F. Zhang, Z. Wang, Q. Liu, A dual-channel homogeneous aptasensor combining colorimetric with electrochemical strategy for thrombin, *Biosens Bioelectron* 120 (2018) 15-21.
- [14] G. Li, J. Zeng, L. Zhao, Z. Wang, C. Dong, J. Liang, Z. Zhou, Y. Huang, Amperometric cholesterol biosensor based on reduction graphene oxide-chitosan-ferrocene/platinum nanoparticles modified screen-printed electrode, *J Nanopart Res* 21(7) (2019) 162-177.