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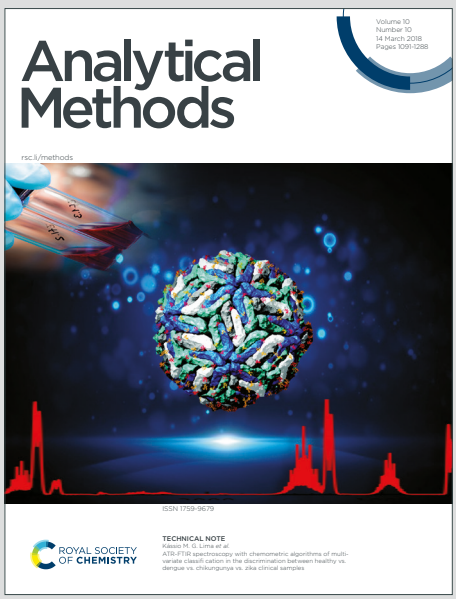




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# A simple unlabeled human chorionic gonadotropin biosensor based on peptide aptamer

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# A simple unlabeled human chorionic gonadotropin biosensor based on peptide aptamer

**Abstract**

As an essential biochemical indicator in the field of pregnancy and oncology, human chorionic gonadotropin (HCG) can be evaluated by colloidal gold immunochromatographic paper and quantified by biochemical analyzer in the principle of antibody sandwich method. In view of the inaccuracy of the former and the complication of the latter, this study constructed an accurate, sensitive and simple unlabeled biosensor based on peptide aptamer CGGGPPLRINRHILTR for HCG detection. Molecular Operating Environment (MOE) was used to simulate the aptamer and protein docking, and Western Blot (WB) was used to verify the binding effect and ratio. The peptide aptamer was characterized and was then reduced by tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP). After electrochemical deposition of chloroauric acid on the screen-printed electrode (SPE), the aptamer was self-assembled on the electrode surface under optimal conditions. The active site of the electrode surface was blocked with 6-Mercapto-1-hexanol (MCH) and BSA. Electrochemical impedance spectrum (EIS) was used to quantify HCG in the matrix. Showed a good linear relationship in the range of 5~1500mIU/mL, with a detection limit of 1mIU/mL, the biosensor remained stable at room temperature for 14 days. Because of its small size, stability, sensitivity and accuracy, this biosensor has great potential to become a portable diagnostic device for HCG.

**Keyword:** biosensor; peptide aptamer; human chorionic gonadotropin; self-assemble

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## *1. Introduction*

Human Chorionic Gonadotropin (HCG) is a glycoprotein hormone mainly secreted by trophoblastic cells of placental chorionic vesicles [1]. The serum of pregnant women contains intact molecules of HCG, whose concentration increases exponentially in the early stages of pregnancy and can reach a maximum of 21, 000 mIU/mL at 12 weeks of gestation, which plays an important role in maintaining pregnancy [2, 3]. The irregular change of HCG at a certain time indicates abnormal pregnancy. Moreover, HCG is also an important serum and urine tumor marker. Elevated HCG unrelated to pregnancy can also be seen in patients with germ cell, ovary, bladder, pancreas, stomach, lung, and liver tumors [4, 5].

As an essential biochemical indicator in the field of pregnancy and oncology, HCG can be evaluated by colloidal gold immunochromatographic paper and quantified by biochemical analyzer based on the principle of antibody sandwich method. In the analysis of HCG test paper, there will be a “high concentration hook effect” when the concentration of HCG in urine is close to the peak [6]. With the increase of HCG concentration, the color of the test line will weaken, presenting a false negative result because of the excessive antigen concentration. In addition, the correlation between HCG concentration in blood and urine is poor. Currently, Roche's electrochemical chemiluminescence analyzer is used to detect serum HCG clinically, which is unable to be widely used due to its disadvantages such as poor stability of antigens and antibodies, complex instrument, professional operation, long detection time and high requirements on samples[7].

Aptamer has played an important role in promoting the development of electrochemical biosensors recent years. Usually composed of 5~20 amino acid residues, peptide aptamers are short peptides that can specifically bind to target with high affinity [8]. They have controllable quality, simple structure but better stability than antibodies, thus are widely used in the development of biosensors. Ding et al. screened out the peptide PPLRINRHILTR via phage display technology in 2013[8], which has a high affinity for HCG.

Computer Molecular Simulation (CMS) uses theoretical methods and computational techniques to simulate the microscopic behavior of molecular action [10]. Molecular Operating Environment (MOE) is a molecular simulation software that can be used to simulate the binding of proteins. Docked with aptamer virtually by MOE, the binding site and binding affinity of HCG are

determined to explain the mechanism through the simulation, thus laying the theoretical foundation for the experiment, then Western Blot (WB) is used to verify the binding effect and ratio of HCG and the aptamer coherently.

Screen-printed electrode (SPE) is a kind of electrode prepared by screen printing technology, which can be easily, quickly, and efficiently mass-produced with high reproducibility and low cost. SPE can be used as a miniature reaction tank, which promotes the miniaturization and improvement of sensors, and creates good conditions for biochemical warfare agent detection [11, 12]. At the same time, due to the low cost, the electrode can be used only once, thus avoiding the background interference caused by the glassy carbon electrode.

In this paper, a more suitable peptide aptamer CGGGPPLRINRHILTR was designed according to the results of MOE and WB and was fixed by self-assembly on gold nanoparticles (AuNPs)-modified working electrode of SPE. With the high affinity peptide aptamer, an accurate, sensitive and stable electrochemical biosensor was developed for HCG detection afterwards.

## *2. Materials and Methods*

### *2.1 . Chemicals and reagents*

HCG (5000IU/mL) and monoclonal antibody of HCG-α (1.2mg/mL) were purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH) were purchased from Solarbio Co., Ltd. (Beijing, China). The synthetic peptide CGGGPPLRINRHILTR (≧98.0% purity) was purchased from Science Peptide Biological Technology Co., Ltd. (Shanghai, China). Potassium ferrocyanide, potassium ferricyanide, phosphate buffered saline (PBS) and chloroauric acid ($HAuCl4{\cdot}4H_2O$) were purchased from Sinopharm Group (Beijing, China). Acetic acid (99.9%, AR), ethyl alcohol (99.8%, AR), 6-Mercapto-1-hexanol (MCH) and tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Western blot (WB) kit was purchased from Bio-Rad Laboratories, Inc. (California, USA). Triton X-114, bovine serum albumin (BSA) and fetal bovine serum (FBS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was purified and deionized with a Milli-Q system manufactured by Qiqin Environmental Protection Technology Co. Ltd. (Hunan, China). Screen-printed electrode (SPE) was made and activated in the laboratory.

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### *2.2 . Protein and peptide docking by MOE*

The complete crystal structure of human HCG was obtained from PDB database (http://www.wwpdb.org/), and the highest resolution was selected to reduce model bias. Then the structure was imported into MOE software, and was prepared by hydrogenation, dehydrating and energy minimization.

After searching, the crystal structure of peptide aptamer was not found in the database, so we constructed the 3D structure of peptide aptamer through MOE, and then carried out conformational search on the structure. LowModeMD was used to search the conformation of the peptide, and the actual conformation with the lowest energy and the most stable under the same amino acid sequence was selected.

The optimal conformational peptide was used to conduct two-step docking with the critical region of HCG, with each docking at least 100 times, and select the best 10 times for output. London dG and GBVI/WSA dG were used as peptides to score the affinity of HCG. The final affinity of the interaction can be scored in terms of the bonding of the interaction and the free energy of the binding (the S value from docking).

### *2.3. Characterization of peptide aptamers*

After biosynthesized, the aptamer was characterized by reversion phase high performance liquid chromatography (RP-HPLC) and mass spectrometer (MS). The UPLC system was equipped with reversed phase column (Kromasil 100-5C18, 4.6mm * 150mm, 5μm). With both the column and sample temperature 40.0℃, the mobile phase was consisted of solvent A (acetonitrile with 0.1% trifluoroacetic acid) and solvent B (water with 0.1% trifluoroacetic acid). A linear gradient with a flow rate of 0.5mL/min was applied in the following way: 10% A (0-10min), 10% - 70% A (10-20min) and 70% A (20-25min). The detection wavelength was 220 nm and the peptide was quantified by integrating its peak area and expressed as a percentage of the total integrated peak area. The injection volume was 10μL. In the MS analysis, the parent ion types of aptamer were $[M+H]^{2+}$ and $[M+H]^{3+}$, and the corresponding mass/charge ratios (m/z) were 881.25 and 587.8. The crushing voltage was 15 V and the collision energy was 21 eV.

### *2.4. Verification of binding effect and binding ratio by WB*

HCG of the same volume was added to each centrifugal tube. Except for one centrifugal tube, aptamers of different concentrations were added to the other centrifugal tubes respectively to make

the ratio of protein and aptamer different, then the loading buffer was added to make the solution volume of each centrifugal tube consistent. Subsequently, 40μL of protein or conjugates were processed as follows: denatured, electrophoresed, transferred to nitrocellulose membrane, stained with ponceau red, sealed with 5% BSA, and incubated with root peroxidase HRP-labeled anti-HCG (1:1000) at 4℃ overnight. Whereafter, the ELC chemiluminescence kit was auto-developed in a dark room. The experiment used HCG with the same concentration as a reference and the BandScan 5.0 software was used to measure the gray value of the specific band. The ratio of the gray value of the target band to the gray value of band with only HCG indicated the amount of protein or conjugates.

***2.5. Preparation of detective electrode***

Three-electrode SPE sized 34mm*12mm was printed with conductive carbon ink and insulating ink. The ink was dried at 120℃ for 30min after each printing, and then the inter-batch and intra-batch differences of the electrodes were investigated. After dropping 90 μL of 0.01M PBS on the electrode area of SPE, the working electrode was activated 180s under the condition of -1.7V.

Before deposition, the 1mM chloroauric acid solution was permeated with high purity nitrogen for 20 min to remove oxygen. Then the AuNPs were deposited on the surface of the working electrode by cyclic voltammetry (CV) under the following conditions: 0-1.4V, scanning for 15 cycles at a scanning rate of 0.1V/s. The electrode was taken out and dried at room temperature. Then electrochemical properties of the electrode after electrodeposition were observed in 5mM $[Fe(CN)_6]^{3-/4-}$ and the stability of the electrode at room temperature was investigated.

20μL of 50 μM peptide aptamer solution reduced by TCEP (the reduction ratio was 1:5) was applied evenly on the surface of the working electrode of the SPE and self-assembled at 25℃ for 20h. The active sites on the electrode surface were sealed at 25℃ for 30min with 20μL of 1mM MCH and 5% BSA, respectively. Clean the electrode surface three times with PBS and dry with nitrogen. Electrochemical properties of the electrode were characterized in PBS containing 5mM $[Fe(CN)_6]^{3-/4-}$ by electrochemical impedance spectroscopy (EIS) .

All electrochemical data were obtained on the Electrochemical Workstation (VeraSTAT 3F, Princeton Instruments, America), and Nyquist diagrams were fitted using ZSimpWin software

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installed into the instrument.

### *2.6. Procedure for HCG detection with electrode*

HCG standard solution with a concentration gradient of 1~2000mIU/ mL was prepared by diluting HCG standard with FBS. 20μL of the standard solution was uniformly coated on the working electrode and incubated at 25℃ for 1h. The electrode surface was rinsed with PBS three times to remove non-specifically bound HCG and prepared for EIS measurement in PBS containing 5mM $[Fe(CN)_6]^{3-/4-}$. The changes of electrode resistance before and after HCG incubation were recorded. The unknown serum HCG sample was prepared by HCG standard and FSA.

### *2.7. Measurement of HCG by electrochemiluminescence (ECL)*

A sandwiched complex was formed by the reaction of a 10μL sample with a biotinylated HCG-specific monoclonal antibody and a ruthenium complex labeled HCG-specific monoclonal antibody. After adding magnetic beads coated with streptomyces affinity, the complex binds to the solid through the interaction of biotin with streptomyces affinity. The reaction mixture was sucked into the measuring cell and the particles are adsorbed on the electrode surface by electromagnetic action. The material not bound to the magnetic bead was removed by Procell. Voltage was applied to the electrode to make the compound chemiluminescence, and the luminescence intensity was measured by a photomultiplier system (MODULAR ANALYTICS E170, Roche, Switzerland).

## *3. Results and discussion*

### *3.1. Design and characterization of the aptamer*

In the single-phage binding test, six high-affinity oligopeptides-MKLKPMRIMINP, MHLMRMKPLLLT, PPLRINRHILTR, MHPRKMLQLMLN, STRLRRRSRRQT, and MKSRMLPLNRRL were obtained [9]. It was observed that there were more positively charged amino acids (29.2%) and non-polar hydrophobic amino acids (27.5%), and there were no negative charged residues, this may be because HCG (pI=2.95) was negatively charged in PBST buffer (pH=7.5) during the screening process, indicating that the binding force between HCG and oligopeptides may include electrostatic and hydrophobic effects. Thus, three polar glycine (Gly,G) were added to the oligopeptide PPLRINRHILTR with the highest affinity to boost hydrophilicity and water solubility, increase the distance from the electrode surface, and reduce steric hindrance.

In order to self-assemble the aptamer with gold nanoparticles (Au-SH), cysteine (Cys, C) was used to modify the $NH_2$-terminus of the peptide to provide an anchor point to the electrode[13], and the whole aptamer sequence CGGGPPLRINRHILTR was acquired. The hydrophilicity of the peptide aptamer was calculated by peptide calculator PepCalc, and it was found that the peptide aptamer had good water solubility, which met the experimental requirements.

After the sequence was determined, the peptide was then synthesized. In order to determine the purity and quality of the peptide aptamer, the sequence was characterized by HPLC and MS. The results of mass spectrometric identification of the sequence were shown in the Fig. S1. The results of MS showed that the excimer ion information of the peptide was consistent with the corresponding amino acid sequences with a purity of 98.01%.

### *3.2. Protein and aptamer binding verification*

Due to the limitation of the precision of crystal resolution, hydrogen atoms were not included in the crystal structure data. However, in the calculation, the protonated state was very important in molecular docking and analysis of active sites, so it was necessary to hydrodehydrate the protein. Amber99 was used when the energy was minimized and the protein was hydrodehydrated, which was a common support force field for biomacromolecules such as proteins and nucleic acids. The conformation with the minimum S value (-7.9256kcal/mol) was selected as the intermolecular interaction model between peptide aptamer and HCG protein.

Through the analysis of stereo diagram and the plane diagram (Fig. 1) between HCG and the aptamer, it was shown that the N on the leucine amide bond at site 14 of the peptide aptamer acts as a hydrogen donor and forms hydrogen bonds with the O of phenylalanine 18 and serine 19 of the HCG protein at distances of 2.87 Å and 2.83 Å, respectively. As A hydrogen donor, the N in alanine 23 of HCG protein forms a hydrogen bond with the O in the carbonyl group of proline 6 of the peptide aptamer, with a distance of 2.86 Å. In addition, the N atom of cysteine 1, as a hydrogen donor, interacts with the O of alanine 23 of HCG protein at a distance of 2.91 Å. The proline 21 and serine 19 of HCG form covalent bonds with the carbonyl C atom on the proline 6 of the peptide aptamer and the amino N atom in the cysteine 1, respectively. The protein formed more bonds with peptide aptamer, suggesting a strong affinity between them, and the main action sites were leucine and proline, which confirmed that the leucine-rich motif reported in literature

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might be the reason for the interaction between HCG and peptide aptamer [31].

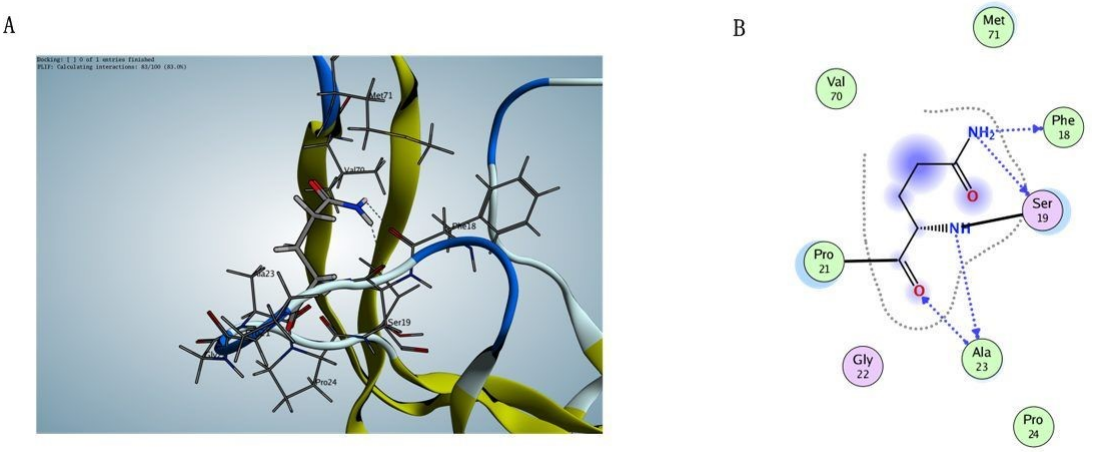


**Fig. 1.** Stereo and plane diagram of the interaction of HCG and the aptamer.
Stereo diagram (A), plane diagram (B)

Instead of two antibodies as usually used for WB, one antibody was used in this study, which was a monoclonal antibody of α side chain of HCG labeled with HRP. The antibody was diluted 1000 times before employment. After specific binding, HRP catalyzed ECL to emit light, which was simple and time-saving.

Since the protein was pure and there was no protein expressed by housekeeping genes, we used consistent level of HCG as internal standard. According to the ratio of the gray value of the target band to the gray value of the band with only HCG, the amount of protein or conjugates was obtained. The grayscale of HCG band with a molecular weight (MW) of 36kDa became lighter with the increase of the binding ratio (Fig. S2), indicating that the protein bound to the peptide into a complex and the molecular weight became larger, thus disappearing from the band of the HCG protein. The absence of bands of conjugates in this experiment may be due to the fact that the peptide aptamer interacted with HCG at the α chain and competed with the HCG-α monoclonal antibody for the action site, which could not be visualized, or it may be due to the concentration of the gel that could not separate protein with larger MW. The binding ratio of HCG to peptide aptamer was about 1:303 (Table S1), which provided a reference for subsequent electrode development.

### *3.3. Preparation and activation of SPE*

According to the needs of the three-electrode system, SPE with the size of 34mm*12mm was designed (Fig. 2). Screen plate content size was 40cm*50cm and each screen plate can print up to

84 electrodes. Parameters of carbon ink mesh plate: tension 25 N, 150 mesh, film thickness 14μm. Insulating ink mesh parameters: tension 20 N, 250 mesh, film thickness 30μm.

The larger the reticulation of the mesh plate, the less ink printed on the substrate surface, the greater the resistance of the electrode, but the flatness improved. The smaller the reticulation, the more ink permeates, and the easier it was to print a fuzzy pattern [14]. Resistance can also be controlled by the thickness of the plate. The thicker the mesh plate, the greater the tension, the more inclined the scraper, the lower the resistance value, and vice versa. In printing, the angle formed by the scraper and the plate was controlled between 60°and 80°.

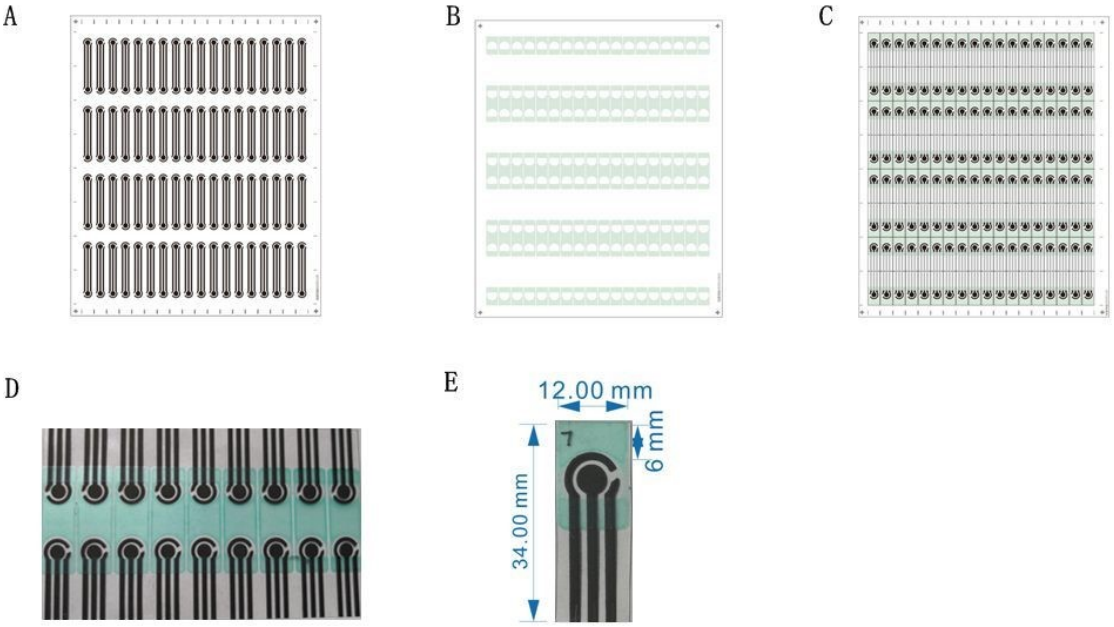


**Fig. 2.** SPE images.
Carbon ink mesh drawings (A), insulating ink mesh drawing (B) and two finished mesh printing drawing (C), printed SPE physical drawing (D) and individual SPE diagram (E).

The SPE was activated before modification, which could increase the electrochemical activity of the electrode surface and promote the heterophase electron transfer rate of commercial carbon ink. The activation conditions were as follows: the electrode was placed in 0.05M PBS and scanned 180s at -1.7V [15, 16]. Then the electrochemical behavior of the SPE in potassium ferricyanide solution was studied by CV with the scanning speed of 50mV/s. The relative standard deviation (RSD) of the resistance of 6 intra-batch SPEs was calculated below 3.4%.

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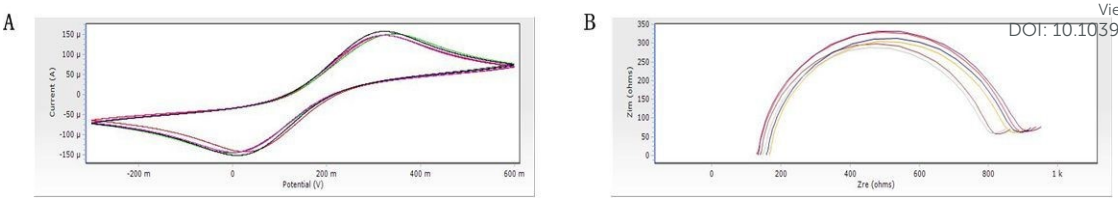



**Fig. 3.** CV (A) and EIS (B) images of 6 SPEs in 5mM $[Fe(CN)_6]^{3-/4-}$ within the same batch.

### *3.4. Electrode modification*

Different concentrations of auric chloride solution were prepared, and the electrodeposition effect of auric chloride electrode by CV and chronoamperometry method was investigated under optimal conditions [17]. It was confirmed that the electron transfer efficiency of the AuNPs modified electrode was the highest by CV electrodeposition of 1mM auric chloride with 15 cycles at 0~-1.4V ( Fig . 6). The EIS (A) was tested in frequency range of 0.1 to 10 kHz with amplitude of 5 mV. The electrochemical behavior of CV(B) was studied by cyclic voltammetry (CV) technique at 50 mV/s scan rate in PBS containing $[Fe(CN)_6]^{3-/4-}$ in a potential range −0.3V~0.6V. The resistance of the electrode was significantly reduced and the electron transfer rate on the electrode surface was increased.

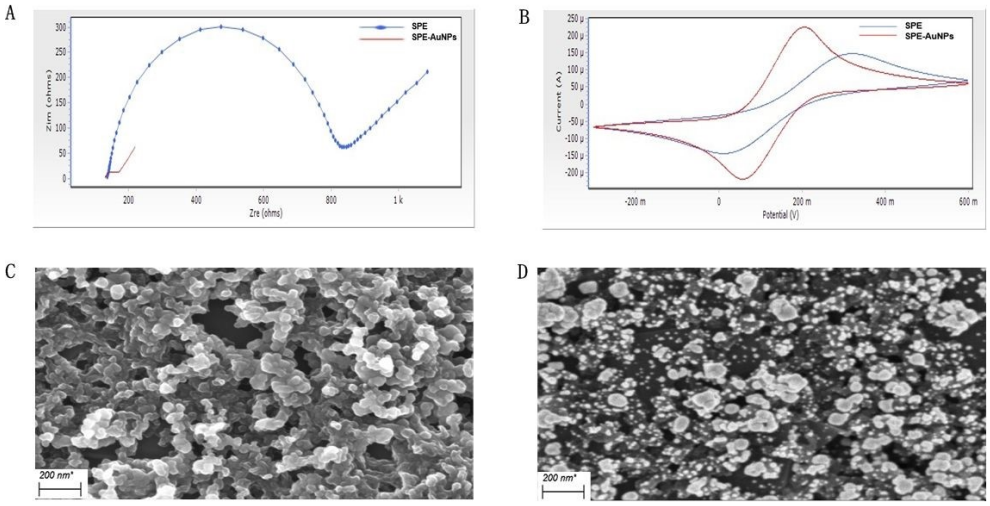


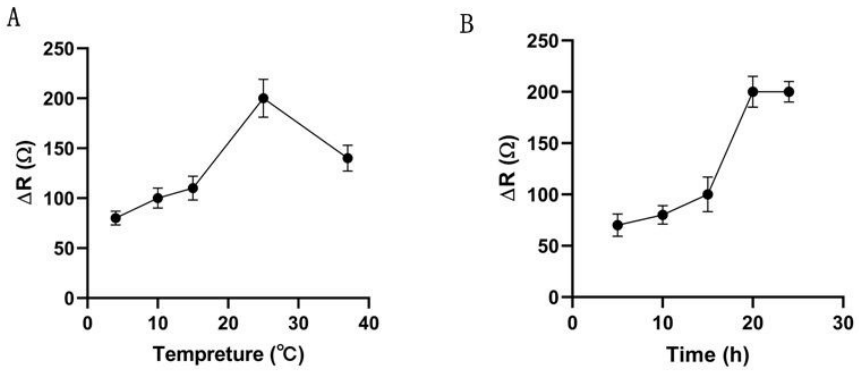

**Fig. 4. Electrochemical characterization of SPE-AuNPs**.; EIS (A) CV (B); Physical morphology characterization of SPE-AuNPs under scanning electron microscope (C); bare electrode surface after electrodeposition (D).

According to the binding ratio obtained by WB, the concentration range of the peptide aptamer was roughly determined, and aptamer solutions of different concentrations were prepared to modify the electrode surface. It was found that when the concentration of the aptamer was greater than 50 μM, the resistance no longer increased with the increase in concentration,

indicating that the binding sites on the electrode surface were saturated and no more aptamers could be bound, or the material exchange between the electrode surface and the solution has reached equilibrium [18, 19]. On the other hand, when there were more aptamers on the surface of the electrode, the higher the background resistance is, and the less obvious resistance change after combining with low concentration of HCG, which reduced the lower limit of quantification. Therefore, this experiment determined that the optimal modification concentration of the peptide aptamer was 50 μM.

In addition, since the aptamer contained cysteine which was easily oxidized, it should be reduced before use. When determining the ratio of reducing agent to sample, the number of free sulfhydryl groups on the sample and the concentration of the sample need to be considered. The more the free sulfhydryl groups and the higher the sample concentration, the greater the amount of reducing agent used [20]. TCEP was the most effective among trivalent phosphorus derivatives and the reducing power was stronger than dithiothreitol (DTT), it is sensitive for TCEP to reduce disulfide bond. TECP hardly react with any amino acids except cysteine, and its effect can be maintained for 2 to 3 weeks. After investigating the reduction ratios of 1:1, 1：3, 1:5, 1:6, and 1:8, we found that the aptamer after 1:5 reductions had the best stability.

In order to obtain the optimal conditions for aptamer self-assembly, single-factor analysis was used to investigate the self-assembly effects at different temperatures for 24h and at 25℃ for different times, as shown in the Fig.5. Therefore, self-assembly of the peptide was carried out by incubation at 25℃ for 20h, and the reaction time between HCG and peptide aptamer is 60 minutes.



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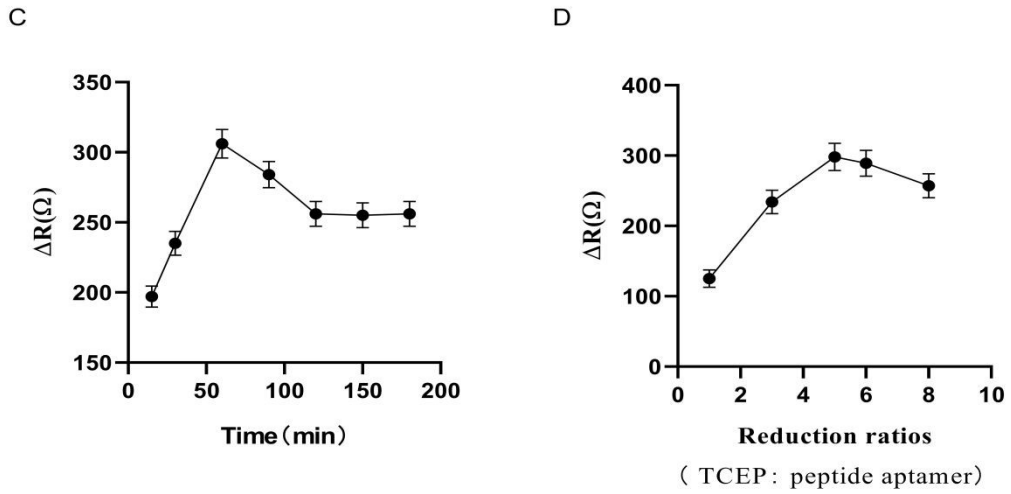


**Fig. 5.** Optimization of experimental parameters: influence of peptide aptamer self-assembly temperature (A); peptide aptamer self-assembly time (B); the reaction time between HCG and peptide aptamer (C); the peptide aptamer was reduced with different ratios of TCEP (D).

MCH and BSA were used to seal the electrode surface so as to avoid non-specific adsorption on the electrode surface, and the resistance after sealing was used as the initial resistance.

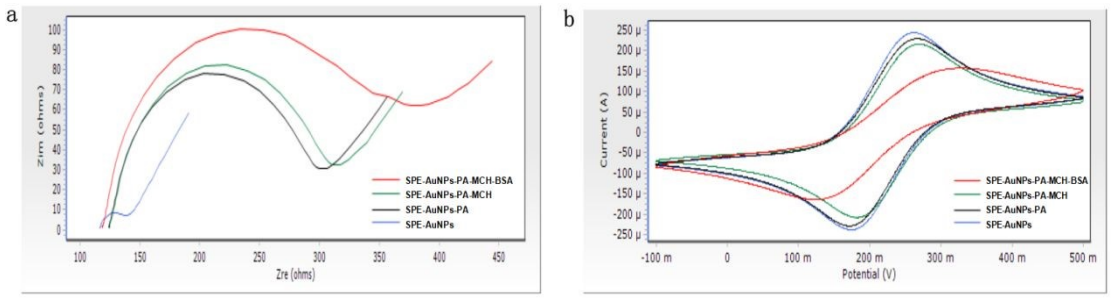


Fig.6. Electrochemical characterization of PA, MCG and BSA modified electrodes

### *3.5. Evaluation of electrode performance*

There was a positive linear correlation between peptide concentration and the analytical signal for the entire validation range (5mIU/mL~1500mIU/mL for HCG). As observed from the Nyquist plot in Fig. 7a, with an increase in concentration, the value of Rct increases. It exhibited a linear range from 5 mIU/mL to 1500 mIU/mL as illustrated in Fig. 7b, the correlation coefficient ($R^2$) for the calibration curve ranged between 0.978 and 0.992 (weight: $1/X^2$). The LOQ of our analytical assay for HCG was 5mIU/mL, which could meet the demand for clinical detection [21-23]. When HCG concentration exceeded 1500mIU/mL, the resistance change did not increase, probably due to the binding saturation of the peptide aptamer and HCG on the electrode surface,

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which determined the quantitative upper limit of the biosensor.

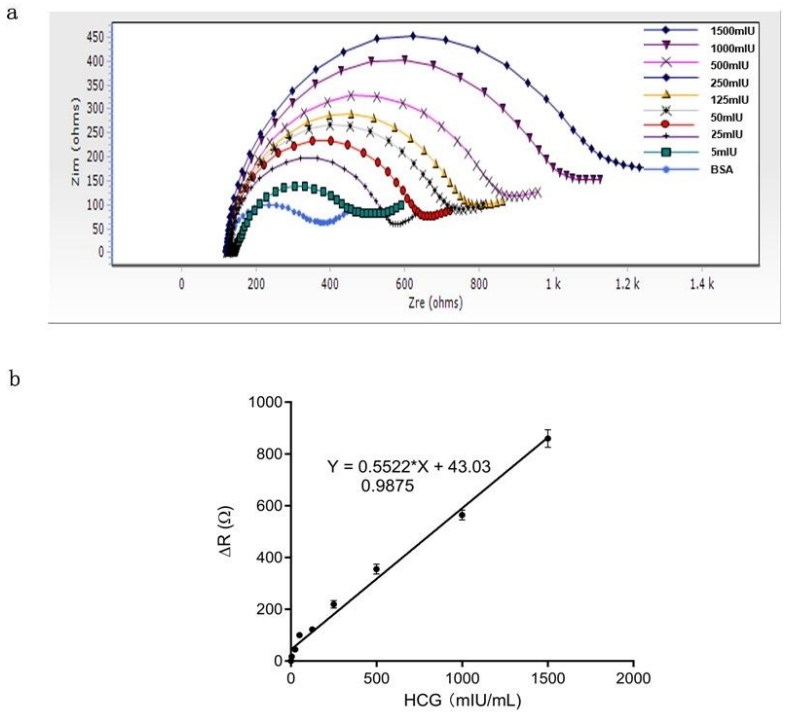


**Fig. 7.** EIS (a) and linear equation diagram (b)

The precision and accuracy of the biosensor were assessed by observing the response of three different concentrations of HCG of the quality control (QC) samples low QC (5mIU/mL, LQC), mid-QC (125mIU/mL, MQC), high-QC (1500mIU/mL, MQC). We used EIS to obtain these dates to keep it consistent with linearity. The accuracy was obtained by comparing the average calculated concentrationsto their nominal values (%RE). The precision was reflected by the percent coefficient of variation (%RSD). Compared with theoretical concentration, the measured concentration of the three were in the range of 96% ~ 105% and the coefficient of variation were within 15%, which met the demand of biological samples test.

**Table 1.** Accuracy and precision of the electrode.

| QC Level | LQC | | | MQC | | | HQC | | |
|---|---|---|---|---|---|---|---|---|---|
| Theoretical concentration | 5mIU/mL | | | 125mIU/mL | | | 1500mIU/mL | | |
| Batch | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 4.55 | 5.25 | 4.94 | 121.63 | 124.63 | 121.13 | 1515.09 | 1480.41 | 1515.37 |
| RE (%) | -9.00 | 5.00 | -1.20 | -2.70 | -0.30 | -3.10 | 1.01 | -1.31 | 1.02 |
| RSD (%) | | 7.14 | | | 1.55 | | | 1.34 | |

Under the same conditions, six modified electrodes were prepared according to the method of *2.5* to detect the same concentration of HCG (125mIU/mL). The percent coefficient of variation was 7.1%, less than 15%, which indicated that the biosensor production process was consistent.

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### *3.6. Selectivity*

Thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were used as interference substances to investigate the selectivity of the peptide aptamer biosensor. The high concentrations of each interference substance and HCG (LQC) mixture, as well as the mixture of four substances, were measured on the electrode. The response results were compared with the response of the electrode to separate HCG (LQC). The gradient concentration of interfering substances was shown in Table 2.

**Table 2.** Gradient concentrations of interfering substances.

| Interfering substances | Normal reference value | High concentration |
|---|---|---|
| TSH | 0.35~5.5μIU/mL | 25.0μIU/mL |
| FSH | 2.49~16.4mIU/mL | 50mIU/mL |
| LH | 2.75~49.7mIU/mL | 60mIU/mL |

The results showed that the response of the biosensor to each concentration of the three interfering substances was less than 5% of the response of the LQC of HCG, indicating that the biosensor constructed had high specificity for HCG. The response of interfering substances on the sensor may be caused by non-specific adsorption. The higher the concentration, the higher the response. The presence of TSH in the matrix was low, which was difficult to interfere with HCG detection. Since the B chain of LH and HCG had 80% homology in 115 amino acids at the amino end, this explains that the effect of LH on HCG was greater than that of FSH[24, 25], but on the whole, it did not affect the quantification of HCG by this biosensor.

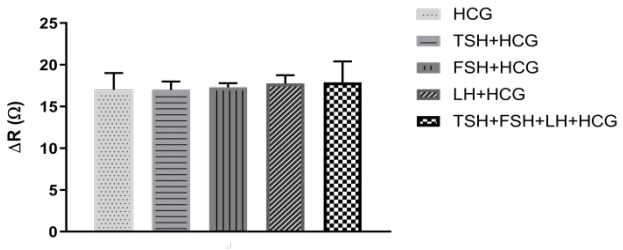


**Fig. 8.** The response of the electrode to HCG (LQC) and mixture of interfering substances (n=5), *p<0.05 (n=5).

### *3.7. Stability*

Six modified electrodes were prepared by the same method. Three of them were measured at 5mIU/mL, 125mIU/mL and 1500mIU/mL standard solutions. The other three were taken out at 4℃ for 14 days, and the standard solutions with low, medium and high quality control concentrations were measured respectively, the result was shown in Table 3.

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**Table 3.** Stability of 6 electrodes.

| QC Level | LQC | MQC | HQC |
|---|---|---|---|
| | 5 mIU/mL | 125 mIU/mL | 1500 mIU/mL |
| Before（mIU/mL） | 5.30 | 125.19 | 1498.84 |
| After 14d（mIU/mL） | 4.87 | 119.73 | 1456.00 |
| RE% | -8.11 | -4.36 | -2.86 |

### *3.8. Comparison of quantitative results between the biosensor and ECL*

Three QC samples were determined by biosensor and ECL simultaneously. It can be found that there was no significant difference between the low, middle and high HCG concentrations measured by the two methods, indicating that the detection results of the electrochemical biosensor were reliable and had the potential for product development.

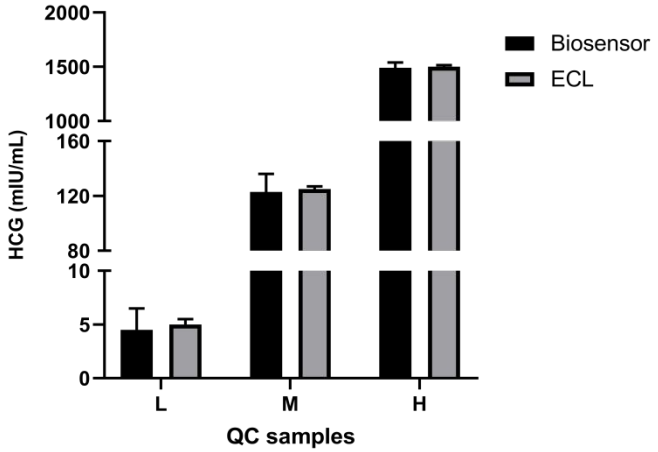


**Fig. 9.** Comparative analysis of results of HCG concentration detected by biosensor and ECL.

### 3.9. *Comparison between the current and previous aptamer-based biosensor*

There were several studies on the detection of HCG based on peptide aptamer, shown in Table 4. While exhibiting improved selectivity and sensitivity compared with biosensor based on antibody, there were some limitations with the reported methods. One of the limitations is that those previous studies lacked precision and accuracy data, and the reproducibility of electrode preparation process was uncertain. Another limitation is that a wider range of tests or more practical dilution methods may still be needed to determine HCG expression at 8 to 18 weeks of gestation [26].

**Table 4.** A comparison between this and previous aptamer-based methods for quantification of HCG.

| References | Surrogate peptide | Linear range | Correlation coefficient ($R^2$) | LOQ |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| This study | GGGPPLRINRHILT | 5mIU/mL ~ 1500mIU/mL | 0.978 - 0.992 | 5mIU/mL |
| Xiaokang Ding[9] | PPLRINRHILTR | 31.7 mIU/mL~ 200 mIU/mL | - | 25 mIU/mL |
| Chia-Chen Chang[27] | PPLRINRHILTR | 15mIU/mL ~ 1000mIU/mL | - | 15mIU/mL |
| Ning Xia[28] | FITC-PPLRINRHILTR | 0.05IU/mL~20 IU/mL | - | 20 mIU/mL |
| Aihui Liang[29] | PPLRINRHILTR | 0.2ng/mL~20 ng/mL | - | 70 pg/mL |
| Lin Li[20] | PPLRINRHILTR | 1 mIU/mL~ 0.2 IU/mL | - | 0.4 mIU/mL |
| Nan-Fu Chiu[30] | PPLRINRHILTR | 1.15 pM~2100pM | - | 1.15 pM |

### *4. Conclusions*

In our study, monoclonal antibody WB and CMS were used to verify the binding of peptide aptamer and HCG for the first time to provide theoretical support for experiments, thereby providing new ideas for similar research. The HCG detection SPE based on peptide aptamer can accurately quantify the amount of HCG in the serum. SPE based on peptide aptamer is stable and can accurately quantify HCG in fetal bovine serum. It is sensitive, non-labeled, low-cost, and simple to operate, which can be mass-produced. SPE based on peptide aptamer has great potential to be a portable HCG diagnostic device for rapid home detection.

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