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ARTICLE

High sensitivity prostate-specific antigen SERS detection platform based on laser resonance nanoparticles

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Accurate quantitative analysis of cancer-related specific biomarkers in clinical serum is very important for the early diagnosis and treatment of cancer. Hospitals often use serum prostate-specific antigen (PSA) as a biomarker associated with prostate cancer diagnosis and prognosis, and prostate cancer cells often produce more PSA than benign cells, leading to elevated PSA levels in the blood. In this study, an immunoassay based on surface-enhanced Raman scattering (SERS) was established for the detection of PSA employing magnetic beads along with SERS nanotags. The hospital now takes two hours to test the results, and the equipment price is high, the detection price is high, and the penetration rate of township hospitals in China is low. SERS has super-sensitive and fast detection ability, and the detection result in 10 minutes significantly reduces the waiting time. Besides, the detection method is simple, cheap and portable, providing a reference for the popularity of township health centers. To evaluate the clinical applicability of this method, 75 male clinical serum samples were tested, most of which were in the gray area of 4.0 - 10.0 ng/mL. The experimental results show that our detection method has a good agreement with the results measured by the electrochemical luminescence (ECL) system in the hospital clinical laboratory. Our detection limit for actual samples from patients can reach 0.029 ng/mL. Therefore, our clinical serum PSA marker detection method based on sers has a great market in towns and villages.

Introduction

Cancer is one of the major social public health security and economic issues concerning in the 21st century. Prostate cancer (PCa) is the most prevalent cancer among men and ranks as one of the top causes of cancer-related deaths.^{1,2} The most common test for prostate cancer is the detection of prostate-specific antigen (PSA) levels in serum, which can mitigate the harm of overdiagnosis caused by targeting molecular markers and, magnetic resonance imaging biopsies. Serum PSA testing can greatly avoid these problems. PSA is a Kallikrein-like serine protease that has received approval from the U.S. Food and Drug Administration (FDA) as a biomarker associated with prostate cancer diagnosis and prognosis.³ When prostatitis, benign prostatic hyperplasia (BPH), or PCa occur, serum PSA levels in patients are elevated. In particular, PSA levels in older men also rise to abnormal levels as they age. The identification of prostate cancer, prostate hyperplasia and normal samples

has aroused the interest of many researchers.^{4,5} Before the patient performs a prostate biopsy, the doctor usually performs an initial screening for PCa by testing the patient's serum PSA. The amount of PSA is used by doctors as a reference in the general diagnosis of prostate cancer. Serum PSA above 10.0 ng/mL indicates a serious potential for prostate cancer. In contrast, when PSA is below 4.0 ng/mL, the likelihood of prostate cancer is lower.⁶ The existing detection technology in hospital takes a long time and the detection efficiency is not high. Therefore, it is necessary to be able to quickly and accurately detect human PSA levels in order to accurately screen patients for PCa as an early diagnosis. So far, various detection methods have been used for direct detection of PSA, such as fluorescence immunoassay,⁷⁻⁹ electrochemical immunosensor method,^{10,11} electrochemical luminescence method,¹² mass spectrometry¹³ and so on. However, the disadvantages of the present method are long time consuming and complicated operation method. In China, due to the large equipment used for testing, high price and long time, the testing work is mainly completed in large hospitals, and the penetration rate of townships is low.

Surface-enhanced Raman scattering (SERS) is a surface-sensitive technique that improves the Raman scattering efficiency of molecules adsorbates to rough nanostructures, typically silver or gold nanomaterials.¹⁴ It can increase the sensitivity of analytical methods by several orders of magnitude (ranging from 10^4 to 10^{11}).^{15,16} SERS detection has emerged as a promising and powerful spectroscopy technique by providing

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ultra-sensitive and inherently chemical fingerprint information. The sensitivity of biological samples directly measured for complex systems is relatively limited, and signals need to be transduced by labels to obtain extremely high sensitivity. Labels can use dye molecules. Therefore, rapid and sensitive SERS detection technology for disease diagnosis is becoming more and more popular.^{17–20} Some studies have shown that immunoassay technology for PSA detection based on SERS can improve the diagnostic accuracy of PSA.^{21,22} The gold-up conversion nanoparticle assembled pyramid structure constructed by Hao et al.²³ can simultaneously detect PSA and thrombin with high sensitivity. The process of assembling the pyramid was complicated and took more than 8 hours. Ouhibi et al.²⁴ utilized a substrate modified with silver nanoparticles on silicon nanowires to achieve highly sensitive and selective detection of PSA with good stability. It can be detected within 30 minutes with a detection limit of 0.1 µg/L. Turan et al.²⁵ combined magnetic molecularly imprinted polymers with SERS, improving the specificity and accuracy of detection with a detection limit as low as 0.9 pg/mL. The whole test process takes more than 90 minutes. Wang et al.²⁶ formed a DNA nano firecracker structure through a concatenated hybridization chain reaction to realize ultrasensitive detection with a detection limit of 0.94 fg/mL. The whole process involves multiple steps and takes more than 6 hours. Zhao et al.²⁷ constructed a SERS immunosensor based on magnetic beads. They used the biotin-streptavidin interaction to amplify the signal for detecting PSA, with a detection limit as low as 0.87 pg/mL. The whole test process takes more than 2 hours. Our detection method is similar to this work, and we have the advantage of being able to detect accurate results in a shorter time. These research achievements provide more accurate and efficient detection means for the early diagnosis of prostate cancer and are expected to promote the development of clinical detection technology. However, the shortcomings of these methods are that the material preparation process is complex, time-consuming, and the operation method is also cumbersome. Our proposed detection approach features straightforward operation. It merely requires the addition of diverse components into the system for reaction, eliminating the necessity of performing multiple steps under varying conditions. The entire detection process can be completed within 10 minutes. This method is characterized by low cost, simplified detection procedures, rapid response, high sensitivity, and strong selectivity. We conducted blind tests on dozens of human blood samples from hospitals, and the experimental results showed stability and reliability.

SERS has been widely used in the analysis of complex biomolecules because of its non-destructive, ultra-sensitive, reliable and rapid detection ability.^{28,29} Immunoassays rely on specific interactions between antibodies and complementary antigens to identify, detect, and quantify a wide range of clinical and environmental analytes with a high degree of specificity through direct or indirect detection of antigens. This is a mature bioanalysis tool. It has become a powerful analytical tool for clinical diagnosis, biochemical analysis and environmental monitoring.^{30,31} Because this method of analysis is based on the

formation of an immune complex between one or two antibodies and antigens, and then the signal generated by the tag attached to the reagent is measured. The combination of SERS and sandwich principle has attracted great attention from researchers.^{32,33} Here, we performed SERS-based PSA biomarker immunoassay. Rapid mixing and incubation of aqueous phases including PSA biomarkers, magnetic beads (MB) and SERS tags. The immune complex was isolated by permanent magnet and the PSA biomarker level was measured by SERS. The advantages of high sensitivity and fast detection speed of SERS were combined with the advantages of high affinity and strong specificity of antigen and antibody. IR808 with a large Raman cross-section is selected as a signal molecule to provide a positive signal. Through the enrichment of magnetic beads, the signal is amplified, and the sensitivity of detection is improved.^{34–37} The ultra-fast and sensitive detection results were achieved within 10 minutes, and the experimental results were highly consistent with the results of electrochemical luminescence in the hospital. Compared with the standard assay in the hospital, the operation of this study was simpler and faster, and the detection Raman instrument was small in size and simple in operation, which could be extended to the early screening of prostate cancer in towns and villages.

Experimental

Materials and reagents

Chloro-auric acid hydrate (HAuCl₄·4H₂O) and trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Boric acid buffer (BB) and Dynabeads MyOne Carboxylic acid (1 µm, 10 mg/mL) were purchased from Thermo Fisher Sciences. Disodium hydrogen phosphate (Na₂HPO₄) and disodium hydrogen phosphate (NaH₂PO₄) were produced by Xilong Technology Co., LTD., China. Bovine Serum albumin (BSA), purchased from Sigma-Aldrich Corporation. Tween-20 and 2-Morpholinoethanesulfonic acid (MES) were acquired from Beijing Inno Chem Science & Technology Co., Ltd. (China). 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC) was purchased from Sangon Biotech (Shanghai) Co., Ltd. N-hydroxysuccinimide (NHS) was from Shanghai yuanye Bio-Technology Co., Ltd. PSA antibodies and antigens were purchased from Abclonal Technology (China). Near infrared fluorescent cyanamide dye (IR808) was purchased from Shaanxi Xinyan Bomei Biotechnology Co., LTD. The water used in the experiment was ultrapure Milli-Q water (18.2 MΩ·cm).

Equipment

The UV–vis absorption spectra were measured with a UV–vis spectrophotometer (Shimadzu, UV 1280). Field emission scanning electron microscopy (SEM) images were obtained using a Zeiss Gemini SEM 500 instrument (Germany) at an accelerating voltage of 5 kV. Transmission electron microscopy (TEM) images were obtained by using JEOL JEM-2100plus microscope under 200 kV acceleration voltage. Dynamic light scattering (DLS) measurements were performed with a Zetasizer NANO-ZS instrument (Malvern, U.K.). The portable



Raman spectrometer (SHINS-P785V) laser of Xiamen SHINS Technology Co., Ltd. was used for Raman spectrum acquisition.

Synthesis of Au Nanoparticles (AuNPs)

According to Frens method³⁸ as described in the literature, 55 nm AuNPs were prepared by slightly adjusting the method. To put it simply, 100 mL of an aqueous solution of HAuCl₄ with a mass fraction of 0.01% is heated to boiling, and a freshly prepared 1% trisodium citrate solution is rapidly added to the continuously stirred solution. The colour of the solution slowly changed from the previous light yellow to black, and quickly changed to brownish red after 2 to 3 minutes. After that, the solution was boiled for another 40 minutes to stop the reaction. The solution was continuously stirred and cooled to room temperature, resulting in a red AuNPs with a diameter of about 55 nm.

Preparation of PSA detection antibodies conjugated SERS tags

The Raman signal detector of the coupled antibody was prepared with reference to the procedure reported³⁰ and with minor modifications. To fix the Raman reporter modification to the AuNPs surface, 4 μ L of 0.75 mM IR808 was dripped into 1 mL of 55 nm AuNPs with 1 OD UV absorption, and the AuNPs and IR808 mixture was gently stirred for 5 minutes. After the reaction, the mixture was centrifuged at 8000 rpm for 10 min to remove the free signal molecules and separate the modified AuNPs. Wash with 1.0 mL 2 mM BB (pH 7.4). Finally, the mixture was centrifuged at 8000 rpm for 10 min. After removing the supernatant, the precipitation was washed twice with 2 mM BB (pH 7.4), continuously centrifuged, and then dispersed. The obtained nanoprobe were stored at 4 °C and could be stably stored for about two weeks. The signalling molecule IR808 contains two carboxylic acid functional groups, which are covalently coupled to the amino group of the antibody by activating the carboxylic acid functional group. Freshly prepared EDC (5 μ L, 25 mM) and NHS (5 μ L, 25 mM) were vigorously mixed with modified nanoprobe at 25 °C for 30 min. The excess EDC and NHS were washed three times from the activated nanoparticles and re-suspended in PBS (10 mM, pH 7.4). AuNPs with activated carboxyl group and PSA detection antibody (4 μ L, 1 mg/mL) were reacted in a centrifuge tube at 4 °C for 8 h. The excess antibodies were removed by 3 rounds centrifugation (8000 rpm, 10 min) and re-suspended in PBS (10 mM, pH 7.4). The modified antibody nanoprobe were stored at 4 °C and could be stably stored for about two months.

Preparation of PSA capture antibodies conjugated magnetic beads

The preparation of the trapping probe is achieved by covalently coupling an amino group on the antibody with a carboxyl group on the surface of the magnetic bead, using EDC and NHS as coupling agents. First, EDC (5 μ L, 0.1 M) and NHS (5 μ L, 0.1 M) solutions were added to the magnetic beads diluted to a certain concentration, respectively, and the magnetic beads were incubated for 30 min to activate carboxyl groups. After incubation, the magnetic beads were separated with permanent magnets, and then washed twice with PBS (10 mM, pH 7.4). The magnetic beads were resuspended with 0.5 mL PBS

(10 mM, pH 7.2), gently shaken with 5 μ L and 1 mg/mL PSA to capture the antibodies and incubated at 4 °C for 8 hours. Finally, the capture magnetic beads are obtained with the aid of permanent magnet. Unbound antibodies were washed twice with PBS (10 mM, pH 7.4). The unreacted COOH group was inactivated with 10 μ L 1% BSA solution for 2 h. The MB of the final antibody coupling was stored in 10 mM PBS (pH 7.4) at 4 °C for the next step.

BCA Protein Quantification Kit

1. Preparation of BCA Working Solution: According to the number of samples, prepare an appropriate amount of BCA working solution by mixing 50 volumes of BCA Reagent A with 1 volume of BCA Reagent B (50:1), and mix well.
2. Drawing of Standard Curve: Take an 96-well plate and dilute the reagent according to a certain concentration gradient.
3. Sample Preparation: Dilute the protein sample to be tested with deionized water to an appropriate concentration. Take 20 μ L of the sample and add 200 μ L of BCA working solution.
4. Oscillation and Mixing: After oscillation and mixing, place at 37 °C for 20 - 30 minutes.
5. Measurement with Enzyme-Linked Immunosorbent Meter: Measure the absorbance value at A562 nm with an enzyme-linked immunosorbent meter, using the absorbance value without BSA as the blank control.
6. Drawing of Standard Curve: Take the protein content (μ g) as the abscissa and the absorbance value as the ordinate to draw the standard curve.
7. Calculation of Protein Content in Samples: According to the measured absorbance value, the protein content in the sample can be calculated on the standard curve.
8. Calculation of Protein Concentration: Divide the measured protein content by the sample volume of 20 μ L and multiply by the corresponding dilution factor to obtain the actual concentration of the sample to be tested.

Assay procedure

First, 40 μ L SERS labels and 20 μ L magnetic beads were re-suspended in incubation buffers containing different concentrations of antigens. Mix the resulting solution together and incubate for a few minutes. Finally, the mixture is separated with a permanent magnet and washed twice with PBST (TW-20 0.4%) buffer. After separation, 10 μ L PBS solution was added into the mixture, and 3 μ L heavy suspension was absorbed for detection, and SERS signal was obtained.

Preparation of clinical serum samples

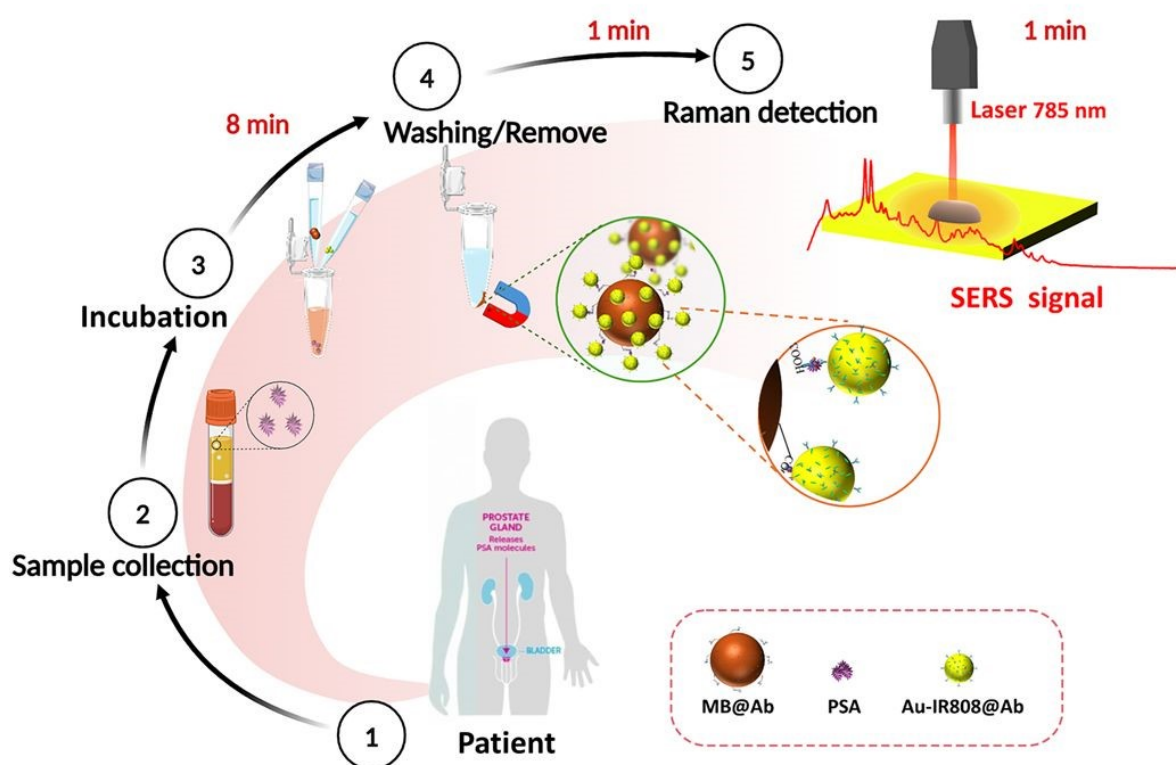
In order to evaluate the practicability of the SERS sensing platform developed, we collaborated with the First Hospital of Xiamen University to obtain serum from hospital patients. Blood samples are processed within 4 hours of collection according to the hospital's protocol, and final serum samples are collected for study. The serum samples were stored in the refrigerator at -80 °C, and all the tested serum samples were only subjected to one freeze-thaw cycle.



Results and discussion

The basic principle of this study was based on double antibody sandwich immunoassay combined with SERS technology, which achieved the advantages of high sensitivity, fast detection speed and strong specificity.¹⁸ In this study, IR808, which has a large Raman cross-section and resonates with 785 nm laser, was selected as a signal molecule to provide positive detection signals.³⁹ The UV-vis absorption spectrum of IR808 is shown in Figure S1. The capture antibody modified magnetic beads (MB) were used as the carrier, and the detection antibody modified gold nanoparticles were used as the sensing probe. MB with a surface-modified carboxyl group is selected as the trapping

substrate, which is easy to collect and concentrate. The surface modified carboxyl group can be activated and coupled to antibody to prepare antibody coupled magnetic beads MB@Ab. Enrichment of magnetic beads can achieve the purpose of signal collection and amplification. Au NPs had the function of signal enhancement and antibody loading to provide enhanced IR808 Raman signal for the system and was prepared into SERS labels Au-IR808@Ab to improve the detection sensitivity. The experimental process is shown in Scheme 1, including incubation, washing, magnetic absorption, detection and other steps. Ultra-fast and sensitive test results are achieved within 10 minutes.



Scheme 1 Schematic diagram of PSA biosensor detection process based on SERS.

Figure 1 shows the sequential process of preparing PSA antibody coupled SERS nanotags and PSA antibody coupled carboxyl magnetic beads for the detection of PSA antigen in serum. In Figure 1A, the signal molecule IR808 is fixed to the AuNPs surface by electrostatic adsorption. The green structure in the upper right corner of Figure 1E is IR808 signalling molecule. Because IR808 itself has carboxyl groups, it can directly conjugate with the amino group on the antibody to form peptide bonds. The PSA antibody was fixed to the AuNPs surface by 1-ethyl-3-(3-(dimethylamino-propyl) carbodiimide (EDC)/ N-hydroxysuccinimide (NHS) promoting the coupling reaction.⁴⁰ In this study, carboxylic acid functionalized magnetic beads were used to prepare the trapping substrate, and the magnetic beads were coupled with antibodies through the

carboxylic groups on their surface. The magnetic bead surface was activated using EDC and NHS, and then the PSA antibody was fixed to the magnetic bead surface, as shown in Figure 1B. These PSA antibodies capture the PSA antigen in the serum through an antibody-antigen reaction. Figure 1D shows a scanning electron microscopy (SEM) image of SERS nanotags, and the small image in its upper right corner is a transmission electron microscope (TEM) image. The diameter is estimated to be 50 ± 5 nm. The particle size increases from 52 nm to 56 nm after the gold nanoparticle binds the signal molecule IR808, and AuNPs increases from 56 nm to 64 nm after coupling with the antibody, which indicates that the modification of the signal molecule and antibody is successful as shown in Figure S2. UV-vis measurements also confirmed the size distribution of AuNPs,



and antibody conjugated SERS nanotags as shown in Figure 1C. The stability of Au nanoparticles itself is due to the double charge of negative ions on the surface. After being modified by signal molecules and antibodies, the particle size of the modified particles changes somewhat due to the certain structural size of the signal molecules and antibodies. As we all know, a certain amount of protein has a good protective effect on stable nanoparticles, and antibodies themselves are proteins. And the antibodies have a certain mutual repulsion between the electrical properties, so they can effectively stabilize the particles.^{30,41–43} Figure 1C also shows that although the modified particles mIR808 and Au-IR808@Ab can still disperse stably in solution, they show an obvious new peak at

700 nm, which may be caused by charge-induced micro clusters of nanoparticles during the modification process. Interestingly, the absorption peak of the particle near 700 nm is close to the 785 nm incident laser used in the experiment, and the absorption of the signal molecule IR808 is very close, which realizes the resonance effect of Raman. The sensitivity of the detection system is significantly improved.³⁰ After the basic characterization of the synthesized particles, we obtained the Raman signals before and after particle modification. As shown in Figure 1E, the signal molecule IR808 was successfully modified and showed its unique Raman signal at 555 cm^{-1} .³⁹

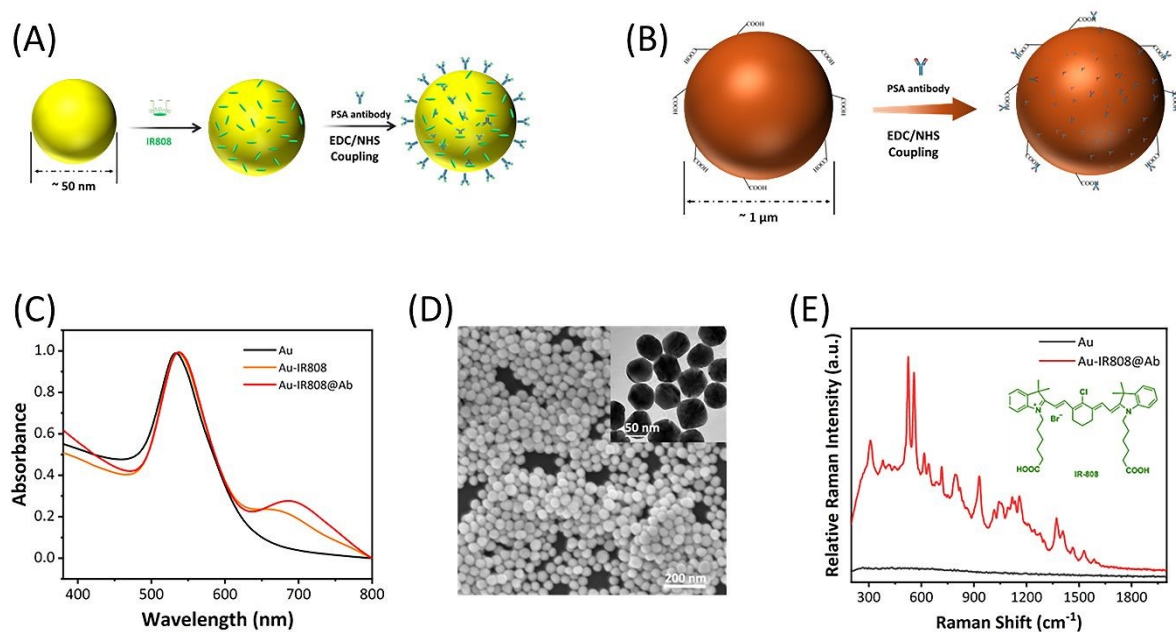


Figure 1 (A) Sequential process for preparing antibody conjugated SERS nanotags. (B) The antibody is coupled to the surface of the magnetic bead. (C) UV-vis spectra of AuNPs (black), Au-IR808 (yellow) and Au-IR808@Ab (red). (D) SEM image of SERS nanotags, and the upper right corner small image is TEM image. (E) Raman spectra of AuNPs (black) and Au-IR808@Ab (red), with IR808 structural formula in the upper right corner.

In order to obtain better performance of SERS biosensor detection system, we explored and improved the detection conditions. The experiment explored the amount of signal molecules added, including the concentration of signal molecules and the volume of molecules added. The volume of gold nanoparticles was fixed to be 1 mL, and other conditions remained unchanged during the particle preparation process, and the added volume of fixed molecules was 4 μL . The concentration of signal molecules was explored, as shown in Figure 2A. When the concentration of signal molecules was 0.75 mM, the SERS signal particles prepared had the best detection signal. Using the optimal concentration of signal molecules, other conditions remain unchanged, change the volume of the added signal molecules. The investigation results are shown in Figure 2B. When the amount of signal molecules added is 4 μL , the signal particles have the best detection performance. It is

well known that pH has a large impact on the immune detection system. We need to consider the pH of the test system. The experiment mainly uses the specific recognition between antigen and antibody, so the experiment first needs to control the pH value of the solution. Figure S3 further confirms that the particle detection process of antibody modification with different content shows the best detection effect in the pH 7.5 environment system. After optimizing the conditions, the subsequent experiments were carried out in this pH system. On the premise of determining the above conditions, we further explore the amount of antibody modification of AuNPs and magnetic beads. The experimental results of AuNPs are shown in Fig 2D. When other conditions are consistent, the positive signal reaches the maximum strength when the amount of antibody reaches 20 $\mu\text{g}/\text{mL}$. At this time, the number of antibodies continued to be added, and the Raman positive



signal did not further improve significantly. This indicates that 20 $\mu\text{g}/\text{mL}$ antibody can already saturate the number of surface modified antibodies of nanoparticles, and further addition of antibodies is ineffective for signal enhancement. The experimental results of antibody modification on the surface of magnetic beads are shown in Fig 2E. The magnetic beads we used were 1 mg/mL, and the optimal amount of antibody modification was 40 $\mu\text{g}/\text{mL}$. Therefore, we fixed the amount of gold antibody modification of the signal at 20 $\mu\text{g}/\text{mL}$ and the amount of magnetic bead modification at 40 $\mu\text{g}/\text{mL}$ in subsequent experiments to meet our experimental requirements. At 4 $^{\circ}\text{C}$, the modified antibody probe can maintain signal stability for a minimum of two months as shown in Figure S4. It is well known that the specificity of SERS biosensors is crucial to the whole system. As shown in Figure 3F, several commonly used antigens were selected for specific detection under the PSA antigen detection system. Bovine

serum albumin (BSA), COVID-19 Spike protein (S Pro), COVID-19 Nucleocapsid Protein (N Pro), cardiac troponin I (cTnI), creatine kinase isoenzyme (CKMB), epithelial adhesion molecule (Ep-CAM) and cytokeratin 19 (CK19) 7 groups of analytes were negative control group, PSA was test group, female serum was blank control group. As shown in Figure 2F, the signal difference between the experimental group and the control group was significant, and the results showed that the antibody had good specificity and no cross-reactivity with the common antigens measured. As shown in Figure S5, we investigated the influence of the reaction time of the experiment in the detection system on the detection signal. It can be easily observed from the figure that the signal of the positive sample will gradually increase with the extension of time in a certain time range, and the signal saturation is basically reached at 60 min, and continuing to increase the reaction time does not significantly help the signal.

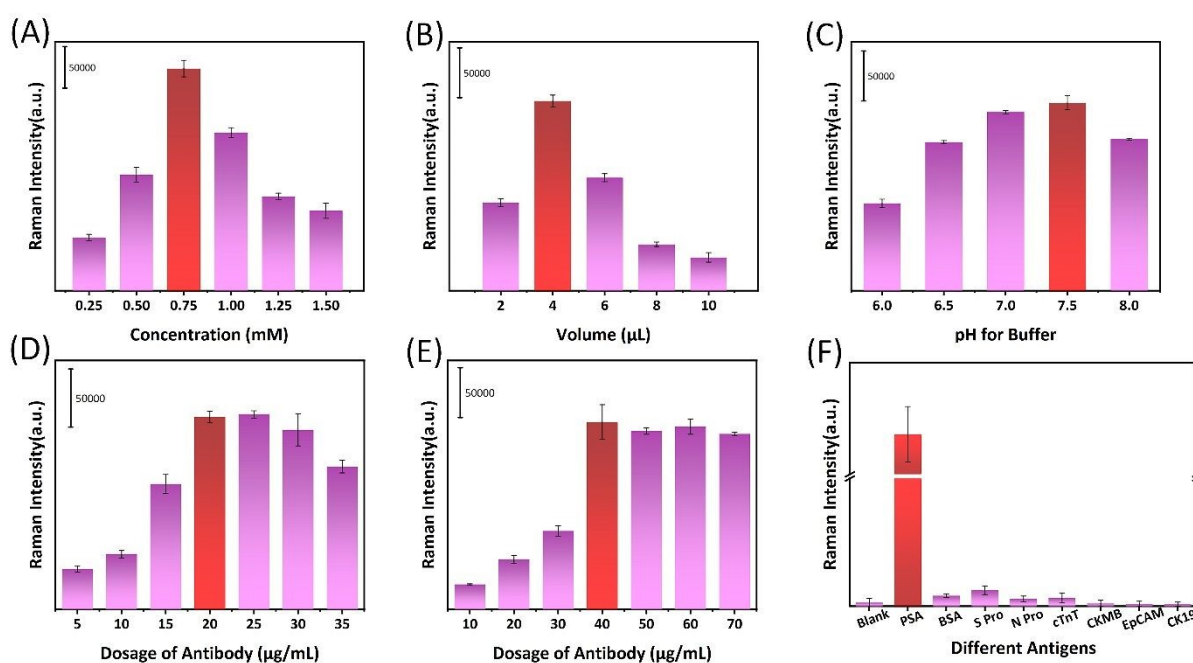


Figure 2 Optimize testing. (A) Influence of signal molecule concentration on detection. (B) Influence of signal molecule dosage on detection. (C) The influence of pH of the detection system on the detection, and the PSA target concentration (6 ng/mL). (D) The influence of the number of gold-modified antibodies on the detection. (E) Influence of the amount of magnetic bead modified antibody on detection. (F) Specificity of the test system. The antigen concentration was 6 ng/mL, and the blank was female serum. The error bar represents the standard deviation of the five measurements.

Therefore, in theory, the best detection results can be obtained when the reaction begins to detect for an hour. However, considering that the experiment needs to achieve rapid detection while ensuring enough sensitivity, we determined the incubation time of 8 minutes as the point for subsequent experiments. For more than six times the time effort, the signal strength increased by only three times. Therefore, according to the requirements of our work plan and the performance demonstrated by the experimental results, we chose an

incubation time of 8 minutes for the following experiments because it balances the need for rapid detection and sensitivity.

In order to more intuitively observe the impact of the presence or absence of antigens on the detection system. PBS blank samples and clinical serum containing PSA antigen were mixed with antibody-conjugated magnetic beads in microtubules, respectively. The antigen-containing serum is captured on the surface of the magnetic bead by antibody-antigen reaction, and Au-IR808@Ab particles are added to the sample respectively, where they form sandwich immune



complexes. As shown in Figure 3A, the blank sample could not bind the signal. The sample containing PSA antigen had IR808 Raman signature peak, as shown in Figure 3B. Magnetic immune complex was separated by magnet to prepare SEM samples for imaging. As shown in Figure 3C, no PSA antigen is able to bind signal AuNPs, while Figure 3D contains antigen binding signal gold to form sandwich immune complexes.

After a series of optimization conditions, the experimental detection system was determined. We verified the sensitivity of the system by detecting different concentrations of PSA. The target PSA antigen was diluted step by step with female serum solution, by which 0-20 ng/mL samples were obtained. Five sets of parallel samples were tested for each concentration, and the

peak area between the 540 cm^{-1} peak and the 570 cm^{-1} peak was calculated. The experimental spectral results are shown in Figure 4A. The peak area intensity at 540 cm^{-1} and 570 cm^{-1} gradually increased with the increase of PSA antigen addition, and the relationship between the two was linear. As shown in Figure 4B, the linear simulation equation is $Y = 15580X - 333.4$ ($X = \text{PSA concentration}$, $R^2 = 0.9897$). ($Y_{\text{LOD}} = \mu_{\text{blank}} + 3\sigma$, where Y_{LOD} denotes the LOD in the signal domain, μ_{blank} is the expected mean blank value, σ is the standard deviation of the blank.) The theoretical detection limit of 0.029 ng/mL is calculated. Current tests used in hospitals typically take up to two hours to produce results.

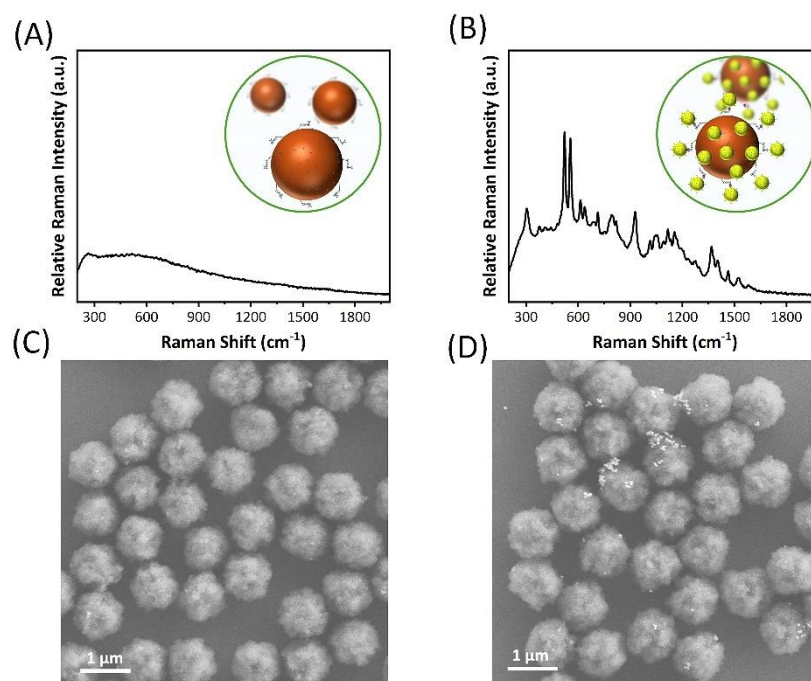


Figure 3 (A) Post-reaction Raman signals in blank samples without PSA. (B) Raman signal after reaction of a positive sample containing PSA. (C) SEM images of magnetic beads of magnetic immune complex formed after reaction of blank samples without PSA. (D) SEM images of magnetic beads forming magnetic immune complex after reaction of positive samples containing PSA.

In contrast, our system can deliver results within 10 minutes, significantly reducing patient wait times. As a real-time detection method, the efficiency and applicability of clinical detection are very important. Therefore, the detection method designed by us has the advantages of simple operation, short detection time (10 min) and high sensitivity. The clinical reference value of PSA was 4 ng/mL and applying our detection system to actual sample detection, we tested 110 samples from volunteers, thus verifying the accuracy and applicability of the method. The study was approved by the Ethics Committee of Xiamen University. Informed consent was obtained from all patients in accordance with guidelines for conducting clinical studies. Among the 110 serum samples, 35 were from female serum, as shown in Figure S6. The content of PSA in female serum is extremely low, and the detection results can exclude the interference of the substance of the serum itself on the experimental results. As shown in Figure 4C, 75 male clinical

samples were respectively detected by our SERS immune method and commercial ECL in the hospital, the latter being the gold standard method in the hospital. By comparing the two assays, the results show good agreement between the two analysis methods, as the data points show a strong linear relationship. Our results found only two cases of bias due to sample concentrations too close to clinical reference values (4 ng/mL, 10 ng/mL). The accuracy of our assay for these serum samples was 97.3%, $R^2 = 0.9823$. In addition, the test results were statistically processed to analyse the accuracy of each sample. Figure 4D shows that out of 75 samples, 62 samples have an absolute error value of less than 10% and 70 samples have an absolute error value of less than 20%. The results were 82.67% and 93.33% respectively, indicating that our detection method was accurate in the detection of PSA in actual samples of human serum. Therefore, our research method provides a



convenient and rapid way for the accurate detection of PSA targets.

Conclusions

Here, our research successfully developed a sensor based on SERS technology with high sensitivity and accuracy for detecting PSA. By using SERS tags that resonate with a 785 nm laser, we were able to quickly complete the detection in 10 min to meet the detection needs of the hospital. The specific recognition of PSA is achieved through the interaction of antigen and antibody. The optimal sensitivity scheme of the sensor is determined through the systematic research and optimization

of various parameters of the detection system. Under the conditions of our study, the detection limit of 0.029 ng/mL was reached, which was higher than the clinical requirements. The immune SERS platform was used for clinical study of detecting PSA targets in human serum, which was of great significance for the popularization of prostate cancer early screening in township hospitals across the country because of its simplicity and speed and low equipment requirements. This work not only provides a powerful and sensitive platform for biomarker detection but is a promising method for early screening of prostate cancer. It also provides a valuable reference for similar research in this field.

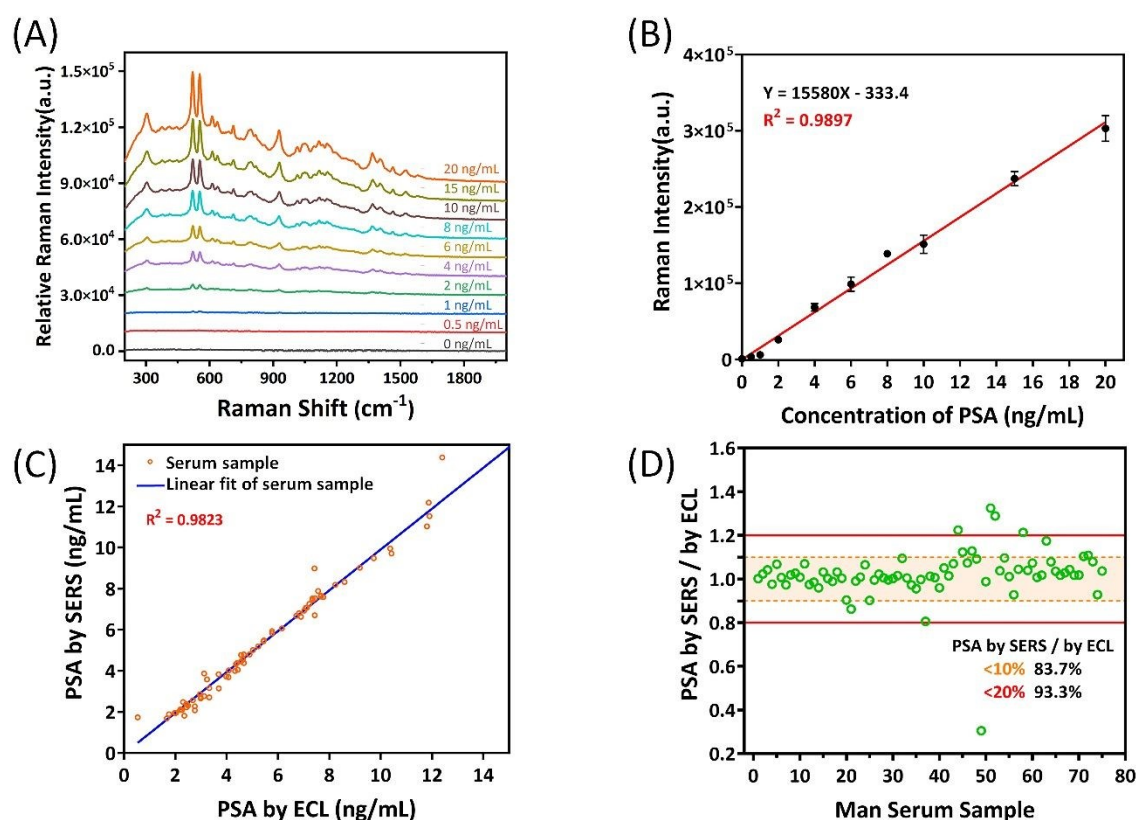


Figure 4 (A) SERS spectrum based on PSA concentration and (B) corresponding calibration curve. The concentration of PSA in human serum ranges from 0 to 20 ng/mL. The change of SERS intensity of 555 cm^{-1} peak was used for quantitative analysis of PSA. (C) Data analysis was conducted on 75 male serum samples with SERS and ECL, and the comparison charts of serum samples with different PSA target concentrations were used to verify the accuracy of SERS clinical detection. (D) Accuracy analysis of clinical test data. The error bar represents the standard deviation of the five measurements.

Author contributions

T. Wang, Y.-J. Zhang and J.-F. Li directed these studies. S.-Y. Fu conducted all the experiments, collated the results, and wrote the first draft. S.S. Xu, H.M. Li, X.-M. Guo, J.-S. Lin, B. Guan, B. Chen discussed the data and wrote the paper. All the authors reviewed the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The datasets generated for this study are available on request to the corresponding author.



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The authors confirm that the data supporting the findings of this study are available within the article [and/or its supplementary materials].

