An ultrasensitive biosensing flexible chip using a novel silver@Prussian blue core-shell nanocube composite

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A B S T R A C T

A real-time, portable, ultrahigh sensitive and selective biosensing chip is desired to satisfy the urgent requirement of the practical application. In this study, a novel three-dimensional (3D) silver nanocubes@Prussian blue (AgNCs@PB) core-shell material has been designed to develop an ultrasensitive biosensing chip by a seed-mediated interfacial assembly approach. A Prussian blue (PB) shell with the 30.12 nm thickness was precisely controlled to in-situ grow on the whole surface of AgNCs through the electrostatic interaction. A flexible and portable biosensing chip was printed and functionalized by using this 3D AgNCs@PB biosensing material to integrate the superior conductivity from AgNCs and electrocatalytic activity from PB for the remarkable performance enhancement. This chip can realize about four times increase of the sensitivity compared with the other PB based screen-printing electrode (SPE), as well as a very wide linear response from 0.01 mM to 1.3 mM and an ultralow limit of detection (LOD) of 0.005 mM for the glucose detection with an excellent anti-interference ability. Especially, it also presented an ultra-accurate detection of the blood sugar in a real serum with a low deviation (3.37%).

1. Introduction

Biosensor with the low-cost, high accuracy and fast response has aroused more and more attentions in the various fields of clinic diagnosis, pollution monitor and food safety evolution [1–6]. Normally, sensitivity and selectivity are considered as two essential parameters of the prepared electrochemical biosensor [7,8]. Therefore, as a core component of the biosensor, electrode material is always required to possess high electrocatalysis and anti-interfering ability [9,10]. However, in the practical application and product transfer, reproducibility and stability of the material are more important to ensure the detection accuracy in the different target systems [11,12]. In recent years, much higher requirements are proposed, such as the stable and large-scale preparation strategy for the high-performance biosensing material [13]. Screen-printing technology has been widely considered as an effective method for the large-scale fabrication of biosensors [14]. Normally, the electrocatalytic materials contained printing ink is the key to the performance of the prepared biosensor [15,16]. Although various nano-materials have been adopted to synthesize the ink, severe aggregation and weak stability of these materials always result in the low electrocatalysis and conductivity, consequently affecting the biosensor performance [17,18]. Hence, a novel nanomaterial which simultaneously possesses high electrocatalysis, conductivity and stability will be expected for the preparation of a high-performance sensing ink. Also, its preparation method is required to be large-scale and facile.

Prussian blue (PB) is an excellent electron mediator with the high electro-catalytic activity to hydrogen peroxide (H₂O₂) [19–21], hence, it is called as an “artificial peroxidase” [9]. Most oxidases can produce hydrogen peroxide as the by-product during their bio-reactions. Therefore, based on this character, various biosensors have been constructed through combining with PB and different enzymes. However, PB belongs to the semiconductor with a band gap of 1.43 eV [22], hindering the electrons transfer [20]. Hence, in order to address this issue, some high conductive materials have been ever adopted to combine with PB, which reduced the resistance of the prepared biosensor in the recent researches, such as graphene and nano-metals [23,24]. Graphene is a layer-structure material which easily aggregates together [25], hence, it is difficult to control the graphene thickness during the combination with PB. Although metallic nanomaterials possess both the high conductivity and catalysis, these materials are extremely active, which easily suffers severe aggregation and oxidation to lose the activity [26]. In this case, the combination of PB and metallic nanomaterials is required to solve above problem to keep the stabilities of both nanostructure and function.

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In this work, we designed a novel core-shell structure of AgNCs@PB nanocomposites by using a facile seed-mediated interfacial assembly approach. A silver nanocube with 200 nm was uniformly dispersed into the base solution by an ultrasound approach. Subsequently, PB was immediately deposited the surface of AgNCs to form a thin shell by electrostatic interaction. This novel core-shell nanostructure can integrate both advantages of PB and Ag together: the shell (PB) possesses high catalytic activity to produce electrons, meanwhile, the core (Ag) possesses high conductivity to promoting the electrons transfer. The AgNCs@PB nanocomposite was served as the biosensing material for the preparation of a miniature biosensor chip. The prepared chip showed ultra-high sensitivities to the detections of H$_2$O$_2$ and glucose, as well as good selectivity, anti-interference and stability under a very low potential $\approx$ 0.05 V. Meanwhile, this chip can be of the wider range of detection (ROD) and lower limit of detection (LOD). The high-performance has been also demonstrated in the test of rabbit serum samples.

2. Experimental section

2.1. Reagents and materials

Potassium ferrocyanide trihydrate (K$_3$[Fe(CN)$_6$]$\cdot$3H$_2$O), iron(III) chloride hexahydrate (FeCl$_3$$\cdot$6H$_2$O), ethylene glycol (EG), polyvinylpyrrolidone (PVP) (Mr $\approx$ 55000), potassium chloride (KCl), sodium chloride (NaCl), glucose and glucose oxidase (GOD) from aspergillus niger (180200 U/g) were purchased from Sigma-Aldrich. Silver nitrate (AgNO$_3$) was purchased from the Shanghai Chemical Reagent Company. Glutaraldehyde 25% (v/v), hydrogen peroxide (H$_2$O$_2$, 30%, w/v, solution) and hydrochloric acid (HCl) were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Ascorbic acid (AA), uric acid (UA) and the similar sugars (such as maltose, sucrose, fructose, lactose, and mannose) were purchased from the Shanghai Chemical Reagent Co. Ltd. (China). Carbon ink (Acheson Electrotag 423SS) was bought from Loctite Corporation (Germany). Silver chloride (AgCl) ink was obtained from Yingman Nano Technology Jiangsu Co. Ltd.

2.2. Apparatus

The biosensor chips were fabricated with a 245 DEK screen-printing machine (Weymouth, UK). The scanning electron microscopic images and energy-dispersive X-ray (EDX) spectrum were tested using a field emission scanning electron microscope (FESEM) (Hitachi, ModelS-4800II and Japan). High-resolution transmission electron microscopy (HRTEM), transmission electron microscopy (TEM) and selected area electron diffraction (SAED) analysis were executed with a JEOL, JEM-2010 UHR. The enzyme solution was immobilized on the working electrode with NORDSON EFD dotting-enzyme machine (JR-V2303MI, Taiwan). The glucose concentration in rabbit serum (Beijing Huao Ke’an Technology Co. Ltd.) was analyzed by using a glucometer (ACCU-CHERK, Roche Diagnostics GmbH and Germany). The spectroscopy of carbon ink and AgNCs@PB biosensing materials were tested with Fourier-transform infrared (FTIR) (Thermo Electron, Nicolet-8700 and USA). The X-ray diffraction (XRD) of PB, AgNCs, carbon ink and biosensing materials were investigated on an X-ray diffractometer (D/MAX 2500V/PC) with a Cu-Ka line (0.15419 nm). All of the electrochemical measurements were executed by using electrochemical workstation (CHI 660E, Shanghai Chenhua Instrument Co. Ltd. China). The table model high speed centrifuge (TGL-18MS, Shanghai Lu Xiangyi Centrifuge Instrument Co. Ltd.) was used to obtain AgNCs and AgNCs@PB. The ultrasonic cleaner (Runshan Ultrasonic Apparatus Co. Ltd. China; volume: 4L; power: 100 W; frequency: 40 KHz) was introduced to obtain more uniform dispersion of AgNCs.

2.3. Synthesis of the silver nanocubes

The polyol method is a typical synthesis of AgNCs by Xia et al. with slight modifications [27,28]. The 6 mL EG was placed in the 24 mL of the vial with stirring and heated to 150 °C until the temperature was stable in oil bath for 60 min. Then 80 μL EG solution of Na$_2$S·9H$_2$O (C = 3 mM) was quickly added into the vial, which S$^{2-}$ were used to facilitate the etched ability of the metallic silver seed and further decrease the total generation rate of agglomerate and irregular particle. After 8–9 min, 1.5 mL EG solution containing polyvinylpyrrolidone (C = 0.18 M) were added into the vial with the speed of 600 μL/min by using a syringe pump. Afterwards, immediately pipette 0.5 mL of the AgNO$_3$(C = 0.28 M) EG solution into the vial. Placing the cap loosely back on the top of reaction vial. Through the following reactions the silver ions are reduced elemental silvers with ethylene glycol (EG).

$$2\text{HOCH}_2\text{CH}_2\text{OH} \rightarrow 2\text{CH}_3\text{CHO} + 2\text{H}_2\text{O} \quad (1)$$

$$2\text{Ag}^+ + 2\text{CH}_3\text{CHO} \rightarrow \text{CH}_3\text{CHO} - \text{OHCCH}_2 + 2\text{Ag} + 2\text{H}^+ \quad (2)$$

When the solution of AgNO$_3$ was injected, the phenomenon went through a series of color changes that included milky white, light yellow, transparent, red, and khaki. The reaction will be complete in 15–20 min. The AgNCs were washed with acetone for three times and twice with ethanol at ten minutes each time by using the centrifugation to collect substrate. Furthermore, the dependence of morphology on the reaction time and the concentration of Na$_2$S·9H$_2$O were also examined in the Fig. S1.

2.4. Synthesis of the AgNCs@PB nanocomposites

A chemical method was used to synthesize PB, which two precursor solutions of solution A: 10 mM K$_4$[Fe(CN)$_6$] + 100 mM KCl + 100 mM HCl, and solution B: 10 mM FeCl$_3$ + 100 mM KCl + 100 mM HCl were preliminarily prepared. PB was synthesized to comply with the following reactions:

$$3[\text{Fe(II)}\text{(CN)}_6]^{4-} + 4\text{Fe}^{3+} \rightarrow \text{Fe}_3\text{(III)}[\text{Fe(II)}\text{(CN)}_6]_3 \quad (3)$$

The solution A and solution B were respectively poured into the syringe of 100 ml, which to fix on the two-channel syringe pump. Afterwards, 0.5 g AgCNs was uniformly dispersed into the 50 mL distilled water by ultrasonic generator, which it as the base solution was placed in the heating magnetic stirrer with stirring and heated to 35 °C. Once the temperature of base solution was stable, the solutions A and B with the speed of 0.5 mL/min was injected into the beaker by the two-channel syringe pump. The extent of the growth of PB on the surface of AgNCs can be controlled by controlling the synthetic time. Finally, the AgNCs@PB nanocomposites were obtained by centrifuging AgNCs@PB solution with the speed of 7000 rpm.

2.5. Preparation of the biosensing ink

The screen-printing was highly required for the viscosity of the materials. Therefore, the biosensing ink was formed by mixing the AgNCs@PB biosensing materials and the carbon ink. The proportion of the AgNCs@PB materials and carbon ink was constantly changed to reach the most appropriate viscosity of biosensing ink. Meanwhile, a small quantity of alcohol was added into mixture ink to help to uniformly blend. Importantly, the AgNCs@PB materials and carbon ink were uniformly blended by constantly stirring the biosensing ink with the magnetic stir bar. Specially, the biosensing ink stopped stirring until the viscosity was suitable for printing.

2.6. Fabrication process of the flexible chips

The flexible chip was created from prepared biosensing ink to screen-printed chip by using the screen-printing machine. A
commercial polyethylene glycol terephthalate (PET) plate was used as the substrate. The biosensing ink was used to fabricate the working electrode (WE), carbon ink was used to print the counter electrode (CE) and connector, silver chloride was used to produce the reference electrode (RE). The CE, RE and WE were sequentially printed by using corresponding printing plate.

2.7. Enzyme immobilization

By using cross-linking method, the enzyme solution contained 0.25% glutaraldehyde (v/v) was immobilized onto the surface of the working electrode, in which 1.8 U/μL GOD were included. Then, enzyme solutions of 5 μL were dotted onto the center of the working electrode of flexible chip by dotting-enzyme machine, preserved under the temperature of 4 °C.

3. Results and discussion

3.1. Nanostructure evolution of the 3D AgNCs@PB nanocomposites

Seed-mediated interfacial assembly approach was extensively applied in the nanocomposites synthesis to address the interfacial binding issues [29]. Here, AgNCs was firstly synthesized as a growth seed for the further crystallization of PB, which inducing the formation of 3D AgNCs/PB nanocubic structure. As shown in Fig. 1A, before the screen-printing process, a biosensing ink is required to fabricate by using the prepared nanocomposite. Then, the glutaraldehyde is used as a crosslinker to immobilize the oxidase on the printed working electrode. The –CHO groups at the terminal of glutaraldehyde would respectively interact with the –COOH groups from biosensing ink and the –NH₂ groups from oxidase to prevent enzyme shedding during testing. As shown in Fig. 1B, relied on the 3D architecture of the AgNCs/PB, PB can produce electrons during the redox under a very low potential. Then, the electrons enable the fast transfer through the AgNCs. Thereby, performance of the flexible chip can be extremely enhanced.

Following above design, the nanocomposite has been prepared by a two-step chemical reaction method. According to the FESEM images in Fig. 2A, a kind of monodisperse AgNCs was first obtained to show a well-defined cubic structure with a 200 ± 25 nm size. After the PB formation, the crystals can still keep the cubic shape, but the size increases to 225 ± 25 nm (Fig. 2B). Moreover, if further improving the deposition time of PB to 4 h, much more and bigger AgNCs@PB crystals were aggregated together to increase the resistance (shown in Fig. S2). Under the sunlight, the prepared AgNCs solution shows khaki, and the AgNCs@PB nanocomposites solution is ultramarine (Fig. 2C). To confirm the core-shell structure, TEM was respectively applied to investigate the single crystals of AgNCs and AgNCs@PB nanocomposites (Fig. 2D and E). For the AgNCs, its cube was solid with the clear edges. However, a PB layer film covered all cube surfaces after the PB growth, which showing a core-shell cubic structure. Besides, as shown in the HRTEM analysis (Fig. 2F) and the SAED pattern (inset of Fig. 2F), singly oriented lattice fringes and electron diffraction spots of square array were presented, which demonstrating the single crystal of the AgNC. Meanwhile, the electron diffraction pattern was recorded by directing the electron beam perpendicular to the (100) planes of a single AgNC. HRTEM image of a single AgNC clearly shows the continuous fringes with the interference distance of 0.181 nm which corresponded to the (200) planes in the face-centered cubic (FCC) AgNC [27]. This also proves the single crystal structure of AgNC. As reported, the single crystal has more advantages than polycrystalline structure in electrochemical applications, such as low resistance, high conductivity and fast electron transfer rate. The essential reason is that the single crystal has a simple crystal structure due to the single crystal consist of arrayed a single oriented crystal fringes.

3.2. Structure optimization of the 3D AgNCs@PB nanocomposites

According to the Zeta potential testing (Fig. 3E), it can illustrate that the surface of the PB material is negative electricity (−12.2 ± 0.8 mV) and AgNCs is positive electricity (−0.8 ± 0.01 mV). The negative
electricity of PB attributed to the abundant $-\text{CN}^-$ functional group in the Fe(II)-CN-Fe(III) section, the positive electricity of AgNCs can be attributed to the $\text{H}^+$ and $\text{Ag}^+$ in solution (see in Eq. 2). Therefore, due to this electrostatic attraction, a PB shell can be tightly combined on the surface of the AgNC. Herein, the shell thickness will determine the catalytic area and electron resistance, which affecting the biosensing performance. Therefore, we carefully investigated the relations between the PB shell thickness and electrocatalytic performance. The thickness can be controlled by the synthesis time of PB. As shown in Fig. 3A–D, different PB shell thickness of ca. 10.13 nm, 30.12 nm, 40.02 nm and 55.72 nm were obtained when the synthesis time increased from 30 to 120 min. In the absence of AgNCs, the pure PB nanocubes with 350 nm in edge length were formed (Fig. S3). Hence, AgNC is served as a template for the formation of PB shell. These nanocomposites with different shell thickness were respectively served as the ink to fabricate different biosensing chips for the electrocatalysis testing. Same amount of glucose oxidase were immobilized on above chips as the recognition component to the bio-reaction. As shown in Fig. 3G, the performance was quite different for the different shell thickness based nanocomposites. Firstly, all kinds of nanocomposites can produce much higher response currents than the pure PB nanocubes during the reaction between oxidase and glucose. This can be attributed

Fig. 2. (A, B) FESEM images of AgNCs and AgNCs@PB core-shell structure; Photograph of (C) was drawing model of AgNC, AgNC@PB and digital photo of prepared AgNCs solution, AgNCs@PB solution; (D, E) TEM image of AgNC and AgNC@PB core-shell structure; (F) HRTEM image of AgNC. The insets of (A) (B) respectively were the magnified image of AgNCs and AgNC@PB. The inset of (F) was SAED pattern recorded by aligning the electron beam to one of the face-centered cubic AgNC. The bar of inset (A) was 50 nm and the bar of inset (B) was 100 nm.

Fig. 3. HRTEM images of AgNC@PB with different PB thickness of (A) 10.13 nm, (B) 30.12 nm, (C) 40.02 nm and (D) 55.72 nm; (E) Zeta potentials of PB, AgNCs and AgNCs@PB; (F) The cyclic voltammograms (CV) of with screen-printed AgNCs@PB of different PB thickness and PB. (G) Calibration curves of response current vs. glucose concentration with screen-printed AgNCs@PB of different PB thickness and PB. (H) The sensitivities with different PB thickness and PB were shown.
to the high conductivity of the AgNC. Current strength is depended on the electron amount and transfer rate. In this core-shell structure, the Ag core possesses a larger volume than the PB shell, increasing the electrons transfer rate for the current enhancement. This conclusion can also be confirmed by the CV results in Fig. 3F. With the increase of PB layer thickness, the potential difference of oxidation and reduction peaks (ΔE) continuously increased. The data were calculated as 281 mV, 104 mV, 192 mV and 231 mV for the shell thickness of 10.13 nm, 30.12 nm, 40.02 nm and 55.72 nm, respectively. These results illustrate that PB possesses weak conductivity which leads to the resistance increase with the coverage increase. It is also interesting that ΔE (ΔE = 304 mV) of the pure PB nanocubes shows even higher value than any AgNCs/PB nanocomposites, confirming the superior electron transfer rate of AgNCs. Moreover, the sensitivities of the pure PB nanocubes was calculated as 71.52 μA mM⁻¹ cm⁻², and the sensitivities of these AgNCs@PB nanocomposites were calculated as 85.52, 115.73, 103.02 and 93.21 μA mM⁻¹ cm⁻² (see in Fig. 3G and H), corresponding to the different shell thickness of 10.13 nm, 30.12 nm, 40.02 nm and 55.72 nm. It is interesting that the medium thickness exhibits the best electrocatalysis. It may be resulted from the tradeoff between the catalytic area and electron resistance. With the increase of thickness, the surface area of PB also increases to provide more active sites. However, PB is a semi-conductor with higher resistance than Ag. Too much surface area of PB also increases to provide more active sites. However, with the increase of thickness, the potential change rate of PB and AgNCs@PB nanocomposite also increased to provide more active sites.

3.3. Characterisation of the AgNCs@PB nanocomposites

The synthesized AgNCs@PB nanocomposites were mixed with a commercial carbon ink to reach the requirement of the printing viscosity. However, stability of the nanocomposites is essential for the performance during the mixing process. Therefore, we tested the XRD patterns of the AgNCs, pure PB, carbon ink and the prepared biosensing ink. As shown in Fig. 4A, pure PB nanocubes showed six peaks at 2θ = 17.69°, 25.00°, 35.56°, 39.89°, 51.12°, 54.36°, and 57.63° which were assigned to the (200), (220), (400), (420), (440), (600), and (620) reflections of PB (Powder Diffraction Standards card, No. 73-0687). For the AgNCs, two peaks at 2θ = 38.12° and 44.28° was presented, which corresponds to (111) and (200) planes of Ag crystal. It should be noticed that the peak strength of (200) diffraction is obviously higher than (111). This indicated that the formation of nanocubic structure was attributed to the preferential orientation with their (100) planes parallel to the supporting substrates [27]. After the mixture with carbon ink, the prepared ink can also keep all typical peaks of PB and AgNCs, confirming the stability of the core-shell nanocomposites in the ink. In order to further study the change of functional groups, the AgNCs@PB nanocomposites, carbon ink and the biosensing ink were characterized by FTIR. According to the comparisons in Fig. 4B, both of the AgNCs@PB nanocomposites and biosensing ink exhibited a strong absorption peak at 2900 cm⁻¹ which belonged to the stretching absorption band of the −CN− functional group in the Fe(II)-CN-Fe(III) section of PB [30]. This indicated that the structure of PB can retain stability in the carbon ink. The applied carbon ink presented a peak at around 1700 cm⁻¹, corresponding to the stretching absorption band of the −COO− (enlarged image of Fig. 4B). This peak is also clear after the nanocomposites addition, illustrating the rare reaction happened during the mixing. Besides, the abundant −COOH functional group can facilitate the covalent immobilization of the enzyme to enhance the stability of the biosensing chips. XPS was used for evaluating the composition and element valence state of the AgNCs@PB and PB. According to the Fig. 4C, comparison of the two curves reveals that the AgNCs@PB only adds the Ag peak. As shown in the Fig. 5A, two curves of AgNCs and AgNCs@PB are nearly same, both showing the binding energies of Ag3ds₂ and Ag3da₂ at about 367.16 and 373.48 eV, respectively. Furthermore, in Fig. 5B–D, the Fe2p, C1s (284.4 eV) and N1s (397.29 eV) core-level spectra of PB and AgNCs@PB also show rare deviations, and the binding energies of Fe2p₁₂ and Fe2p₃₂ locates at about 708.1 and 721.2 eV [31], respectively. Above results can demonstrated that the valence states of each element have not changed after the synthesis of AgNCs@PB, and the core-shell structure is connected by the electrostatic interaction rather than the chemical bond.

By using a screen-printing technology, a miniature and flexible biosensing chips were fabricated to integrate the three-electrode system. Working, counter and reference electrodes were prepared by the biosensing ink, commercial carbon ink and silver ink, respectively. As shown in Fig. 6A, the surface of working electrode (WE) has not shown any cracks because of the excellent ductility and adhesive quality of the prepared biosensing ink. Moreover, the distributions of iron and silver elements were characterized by the EDX mapping. As shown in Fig. 6C and D, AgNCs@PB nanocomposites can be uniformly dispersed in the carbon ink, which is beneficial for the resistance decrease and usage stability.

3.4. Electrochemical performance of the biosensing chips

In order to confirm the superior conductivity of the AgNCs@PB based chip, PB nanocubes were also applied to print a chip under the same conditions. Two chips were studied by the CV method, and the results were compared in Fig. 7A. Under the changing potential, PB and its reduction state (Prussian White, PW) will be mutually transformed as the following equation:

$$\text{Fe}_3(\text{III})[\text{Fe}(\text{II})(\text{CN})_6]_3^+ + 4e^- + 4\text{K}^+ \leftrightarrow K_2[\text{Fe}_2(\text{II})(\text{CN})_6]_3$$ (4)

As shown in the results of Fig. 7A, both chips presented a couple of redox peaks, indicating the electron transfer behaviors of PB reduction and PW oxidation. However, the potential differences (ΔEₚ) of redox peaks of two chips were quite different. Their values were calculated as 118 mV and 304 mV for AgNCs@PB nanocomposites and PB cubes based chips, respectively. Besides, the peak shape of AgNCs@PB nanocomposites was much sharper. These evidences can demonstrate that the introduction of AgNCs can obviously improve the conductivity of PB to allow the fast transfer of electrons.

Due to the excellent electrocatalysis to H₂O₂, PB is considered as an “artificial peroxidase”. Hence, we adopted H₂O₂ as the target to test the electrocatalytic activity of the chip. CV curves of the chip were scanned before and after the addition of 1 mM H₂O₂ (Fig. 7B). It was shown that the oxidation peak decreased and reduction peak enhanced when H₂O₂ existed in the system. This indicated the electrochemical reduction of H₂O₂ to OH⁻ by the PB shell of nanocomposites. Besides, Fig. 7C shows the kinetic control experiment of the chips, which was continuously operated under the different scanning rates. According to the linear fitting in Fig. 7D, peak currents (Iₚ) and (Iₚ') linearly increased with the scan rate to achieve the excellent correlation coefficients (R² = 0.9965 and 0.9963), indicating that electrochemical reaction of the flexible chips is a surface controlled redox process [32]. Furthermore, with the increase of the scan rate (10–100 mV/s), the potential difference (ΔEₚ) was increased from 93 to 228 mV with its current ratio, illustrating that the electrochemical reaction of the flexible chips is the quasi-reversible redox process [33]. As shown in Fig. 3E, the weak electrostatic attraction is used as the combination force between the core and shell, and its stability is required to test. As shown in Fig. 8, oxidation peak weakly decay 14.2 μA (ca. 1.6%) and reduction peak weakly change 15 μA (ca. 1.46%) after the 100 scanning cycles of the AgNCs@PB chip. This result shows that the AgNCs@PB materials can provide an excellent stable as the promising candidate for the construction of a sensitive and stable biosensor.

Specific surface area is one of the most significant factors to affect the catalytic activity of catalysts. From the N₂ adsorption/desorption curves recorded by BET (Fig. 9A), we can find that the specific surface...
Fig. 4. (A) XRD patterns of biosensing ink, PB, carbon ink and AgNCs. (B) FTIR spectra of AgNCs@PB nanocomposites, carbon ink and biosensing ink. (C) XPS spectrum of AgNCs@PB and PB.
area is significantly increased after the PB modification surrounding the surface of AgNCs. The BET surface area of AgNCs@PB and PB were respectively 187.43 and 68.22 m² g⁻¹. Thus, the high specific surface areas of AgNCs@PB may provide more active centers to improve the catalytic capability.

The biosensing performance of the chip was investigated by the chronoamperometry test. When the equivalent concentration of H₂O₂ was continuously injected at each same time interval, the current steps were rapidly generated for both kinds of chips (Fig. 9B). However, the step strength and stability of the AgNCs@PB based chip were superior than those of the PB based chip. According to the calibration curves (the inset of Fig. 9B), sensitivities of the AgNCs@PB and PB chips were calculated as 1822.96 and 450.55 μA mM⁻¹ cm⁻², respectively. It is more than four times increasing after the formation of the PB shell. Moreover, the nanocomposite based chip also exhibited a wider linear response from 0.01 mM to 2.0 mM with an ultralow limit of detection (LOD = 2 μM), as well as an excellent linear relation with R-Square = 99.37% for H₂O₂ detection. Above remarkable performance...
can be attributed to the synergetic effects of the high electrocatalysis and conductivity from the core-shell nanostructure of AgNCs@PB. One creates signal, and the other transfers the signal.

Most oxidase reactions can produce H$_2$O$_2$, hence, PB based biosensors can be applied in detections of various physiological analytes by the immobilization of different oxidases. Here, we applied glucose oxidase as the recognition component to test the performance under a very low potential $-0.05$ V. As shown in Fig. 9C, the continuous additions of 0.1 mM glucose can also arouse the current response to form steps, and its response time is less than 3 s (see in inset of Fig. 9C). With the same phenomenon in the H$_2$O$_2$ detection, AgNCs@PB also exhibited much higher current response than PB nanocubes. Sensitivities of AgNCs@PB and PB based chips were calculated as 115.73 and 71.52 μA mM$^{-1}$ cm$^{-2}$ for the glucose detection. Besides, As shown in Fig. 9D, AgNCs@PB based chips exhibited a wider linear response from 0.01 mM to 1.3 mM with an ultralow LOD = 5 μM, and an excellent linear relation with R-Square = 99.84%. However, PB nanocubes based chips can only possess a linear response from 0.05 mM to 0.9 mM with an LOD = 10 μM. Above results can demonstrate that the introduction of AgNC as a core can greatly accelerate the electron transfer rate to improve the biosensing performance. Meanwhile, the comparison of the electrochemical performance of the AgNCs@PB electrode with the reported PB-modified electrodes is listed in Table S1. The sensitivity of AgNCs@PB modified electrode is considerably higher compared to other PB material based electrodes due to the synergic effects of PB and AgNCs.

3.5. Anti-interference ability, reproducibility and stability of the biosensing chips

The accuracy of biosensor is an essential parameter for the practical application. Normally, the real target system contains various complex components which can easily produce the interference signal. For instance, the most common electrochemical interferential species are ascorbic acid (AA) and uric acid (UA). Besides, for glucose detection, other reducing sugars, such as maltose, sucrose, fructose, lactose, and mannose, may also affect the accuracy of the chip. In order to investigate the anti-interference ability of the self-made biosensing chips, UA and AA were successively added after the addition of glucose with the same concentration. As shown in Fig. 10A, there is rare current increase after the addition of AA and UA except for the addition of...
glucose. This is attributed to the lower operation potential of the AgNCs@PB nanocomposite (−0.05 V) than the oxidation potentials of AA and UA (0.2 V, 0 V) [34,35], avoiding other electrocatalytic reactions during the detection process. Moreover, we also investigated the effects of other similar sugars during the glucose detection (Fig. 10B). After we continuously added five kinds of sugars, there is no obvious current response until again addition of glucose. This phenomenon is due to the specificity of the immobilized glucose oxidase to the glucose oxidation. Above results can confirm the excellent anti-interference ability of the prepared chips during the glucose detection.

Furthermore, the reproducibility of the chips was estimated by the testing of twenty chips prepared in the different batches. The relative standard deviation (RSD) of the sensitivity was 5.6%, indicating a satisfactory reproducibility. The use stability is essential for the practical application, which is mainly relied on the electrode material stability during the electrochemical operations. This character can be also revealed from the CV and chronoamperometry results in Figs. 8 and 9c. After the continuous 100 times CV scanning, the as-prepared nanocomposite can still provide rare change of the redox peaks and the similar curve shape. Furthermore, during the chronoamperometry test, each glucose addition with the same concentration can arouse a stable and obvious current step, confirming the excellent use stability. Besides,

Fig. 9. (A) Nitrogen adsorption and desorption isotherms of AgNCs@PB nanocomposites and PB. Chronoamperometry curves of the chips based on AgNCs@PB nanocomposites and pure PB for the detection of (B) H2O2 and (C) glucose in 0.05 M PBS solution at – 0.05 V. Calibration curves of response current vs. the inset of (B) H2O2 and (D) glucose concentration according to the chronoamperometry results. The inset of (C) showed the current response time after the addition of 0.1 mM glucose.

Fig. 10. Amperometric responses to the successive addition of interferential compounds such as (A) 0.1 mM AA, 0.1 mM UA and (B) 0.1 mM maltose, sucrose, fructose, lactose, mannose, as well as 0.1 mM glucose into a stirring PBS solution at – 0.05 V.
Table 1
Concentration of blood glucose in rabbit serum obtained by flexible chips and the glucometer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucometer (mM)</th>
<th>Ratio</th>
<th>Flexible chip (mM)</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.6</td>
<td>5.83</td>
<td>5.83</td>
<td>4.11%</td>
</tr>
<tr>
<td>2</td>
<td>5.8</td>
<td>5.65</td>
<td>5.65</td>
<td>2.59%</td>
</tr>
<tr>
<td>3</td>
<td>5.8</td>
<td>5.63</td>
<td>5.63</td>
<td>4.66%</td>
</tr>
<tr>
<td>4</td>
<td>5.7</td>
<td>5.81</td>
<td>5.81</td>
<td>2.11%</td>
</tr>
</tbody>
</table>

Ratio = $\Delta i_{\text{serum}} / \Delta i_{\text{glucose}}$

one chip was stored in the refrigerator at 4 °C for 30 days, and then its performance was again examined. The stored chip can retain about 77.26% of the initial sensitivity, which to show considerably stability of chip. Above stable performance is attributed to the stability of the designed core-shell structure. PB, which is a very stable material, covered the whole surface of Ag to protect the core AgNC away from redox.

3.6. Blood glucose detection of the biosensing chip in the real serum

In order to investigate chip performance in the practical application, the flexible chip was applied to measure blood glucose levels in the real rabbit serum [36]. As shown in Fig. 11, 1 μl glucose (1 M) was first added into 10 ml PBS as the standard sample, then 1 ml rabbit serum was added into system to calculate the current increase ratio (Ratio = $\Delta i_{\text{serum}} / \Delta i_{\text{glucose}}$). According to the equation (Eq. 5), the concentration of the tested blood glucose was 5.83 mM, and the glucometer detection level was 5.6 mM, showing the 4.11% deviation.

Where $C_{\text{serum}}$ is uncharted concentration of rabbit serum and $C_{\text{glucose}}$ is known concentration of glucose. $\Delta i_{\text{serum}}$ is the steady current steps difference of rabbit serum and $\Delta i_{\text{glucose}}$ is the steady current steps difference of glucose. $V_{\text{glucose}}$ is volume of added glucose and $V_{\text{serum}}$ is volume of added rabbit serum. Above experiment was repeated for four times, and the data were listed in Table 1. We can find that the average deviation of detection in the rabbit serum was 3.37%, demonstrating the excellent accuracy of the biosensing chips.

4. Conclusions

In summary, we have developed an ultrasensitive flexible chip using a novel 3D AgNCs@PB core-shell composite by a facile and large-scale synthesis strategy. This architecture can integrate both advantages of PB and Ag to possess a synergetic effect of high electrocatalysis and conductivity. This nanocomposite was further applied to screen-print a miniature and flexible biosensing chips. The as-prepared chips have exhibited a superior sensitivity, reproducibility and stability for the detections of H$_2$O$_2$ and glucose under the very low potential. Besides, the chip also showed an excellent anti-interference ability to the UA, AA and five kinds of sugars. Especially, in the real rabbit serum, this biosensing chip can accurately detect the level of blood glucose with low deviation compared with the glucometer. This synthesis route can be promising to extending more core-shell nanocomposites with regular and uniform nanostructure, and the biosensing chips can be further used in the detections of more physiological analyses in the practical applications.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2018.08.070.

References


