ChemComm

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ROYAL SOCIETY OF CHEMISTRY

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ROYAL SOCIETY OF CHEMISTRY View Article Online
DOI: 10.1039/C9CC04049H

Journal Name

COMMUNICATION

A Highly Sensitive Photoelectrochemical $VEGF_{165}$ Biosensor with Dual Signal Amplification Strategy by using $AgVO_3$ as Photoactive Material

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x
www.rsc.org/

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We developed a novel "signal-on" photoelectrochemical (PEC) aptasensor with near-zero background signal by using $AgVO_3$ as single photoactive material for the sensitive detection of vascular endothelial growth factor ($VEGF_{165}$).

Photoelectrochemical (PEC) bioanalysis is a recently developed analytical method with unique properties of the separation of excitation source and the detection signal, which can reduce the background noise to improve the sensitivity for detecting of DNA sequences, heavy metal ions, and biomarkers.[1-9] In PEC bioanalysis, photoactive material is a critical component to generate the initial photocurrent signal.[10-13] To increase the initial photocurrent signal, multiple photoactive materials were usually combined to obtain co-sensitized systems.[27,28] The sensitivity can be improved by co-sensitized structure. However, the sensitivity enhancement is still limited because of the high background signal.[20-22] Therefore, it is really important to reduce the background signal and improve the detection sensitivity for PEC biosensor.

To achieve low background signal for PEC biosensor, excellent photoactive material without sensitizer is necessary to generate detectable signals. Recently, vanadate has drawn widespread attention because of the excellent visible-light-driven photoelectrochemical and photocatalytic performance.[14-16] Therefore, it has great potential to be used as the photoactive material for PEC sensor. However, there are some intrinsic limitations and disadvantages of the reported vanadate materials, such as poor dispersity and large particle size, making them unqualified to be applied in PEC biosensor.[17-19] Therefore, we propose a novel surfactant assisted method to prepare $AgVO_3$ nanoparticles with decreased particle size and good dispersibility.

To further improve the detection sensitivity of target $VEGF_{165}$, an important biomarker for breast cancer and lung cancer, Exonuclease III-assisted target recycling and hybridization chain reaction (HCR) dual amplification strategies were intelligently combined. $VEGF_{165}$ is often over expressed in cancer cells during the tumor growth and inflfluences the lymph-angiogenesis and the metastasis of cancers for the abnormally fast growth and division.[29] Firstly, the target $VEGF_{165}$ would be converted to multiplex secondary target DNA by Exonuclease III-aided target recycling, which could be further used to trigger the HCR reaction and to provid an excellent platform for the immobilization of photoactive $AgVO_3$ material for generating strong detectable signals with near-zero background signal. The sensitivity of PEC biosensor would be greatly improved by the reduced background signal and the dual signal amplification.

Herein, a novel "signal on" PEC biosensor based on $AgVO_3$ with near-zero background signal and dual nucleic acid signal amplification was developed. As shown in Scheme 1, Exonuclease III is an enzyme which has a high activity for duplex strands in the direction from 3′ to 5′ terminus and limited activity on single strand. Exonuclease III-aided target recycling was triggered by the target $VEGF_{165}$ to generate multiplex secondary target DNA (S1). Subsequently, S1 was used to hybridize with HP3 immobilized on the electrode surface to trigger the hybridization chain reaction in the presence of S2 and S3. The structure formed by HCR provided an excellent platform for the immobilization of photoactive $AgVO_3$ nanoparticles to generate strong detectable signals.

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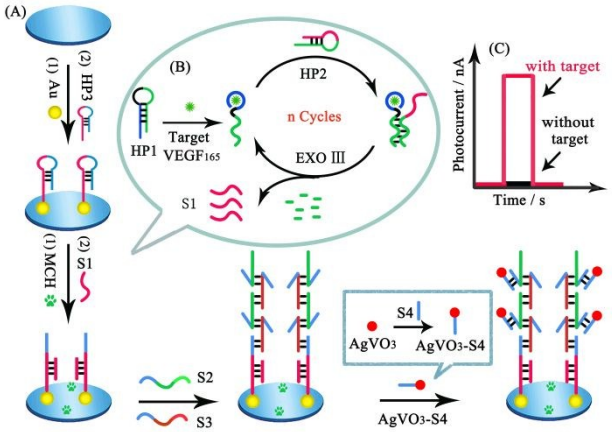
Electronic Supplementary Information (ESI) available: Experimental details and electrochemical characterizations. See DOI: 10.1039/x0xx00000x

Since the signal material $AgVO_3$ could not be immobilized on the surface of electrode in absence of target $VEGF_{165}$, the PEC biosensor showed near-zero background signal, which could remarkably improve the detection sensitivity. Therefore, the novel "signal on" PEC biosensor exhibits a wide linear range from 10 fM to 10 nM with a detection limit of 3 fM . This strategy provides a promising platform for sensitive detection of various biomarkers for early diagnoses of diseases.



Scheme 1 (A) Fabrication of the PEC $VEGF_{165}$ biosensor including Exonuclease III-assisted target recycling and HCR. (B) Exonuclease III-aided target $VEGF_{165}$ recycling. (C) The diagram of photocurrent variation.

In order to prepare $AgVO_3$ with decreased particle size and good dispersibility for the DNA modification, Poly acrylic acid (PAA) was used as surfactant for the nucleation of $AgVO_3$ and induce the carboxyl group simultaneously. The morphology of the as-prepared $AgVO_3$ was investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown in Fig. S1, $AgVO_3$ nanoparticles with poor dispersity and large particle size can be observed in the absence of PAA. However, $AgVO_3$ nanoparticles with size range from 2 nm to 10 nm can be obtained when PAA was added, as shown in Fig. 1A. The prepared sample shows a very good dispersibility. A lattice distance about 0.242 nm can be observed from the HRTEM images (Fig. 1B) of $AgVO_3$ corresponding to the (-602) plane of $AgVO_3$.[17]

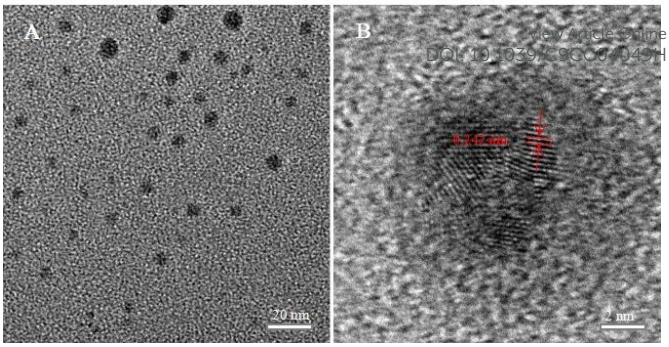


Fig. 1 TEM (A) and HRTEM image (B) of $AgVO_3$ prepared by using PAA as surfactant.

The "signal-on" PEC biosensor was based on the immobilization of $AgVO_3$. Therefore, the photocurrent intensity should be directly correlated with the concentration of $AgVO_3$ and indirectly correlated with the concentration of $VEGF_{165}$. The relationship between the photocurrent response and the concentration of $VEGF_{165}$ is demonstrated. As shown in Fig. 2A, the PEC signal increased along with the increasing of $VEGF_{165}$ concentration. The quantitative relationship confirmed that the photocurrent signal exhibited logarithmically proportional to $VEGF_{165}$ concentrations over the range from 10 fM to 10 nM with a detection limit of 3 fM (S/N = 3). The linear equation was $I = 42.75\lg c + 336.29$ with a correlation coefficient of 0.9989. (The photocurrent was collected in 10s.) In addition, the as-prepared PEC biosensor was compared with other reported methodologies, as depicted in Table 1. The proposed PEC biosensor presented lower detection limit and wider linear range for $VEGF_{165}$.

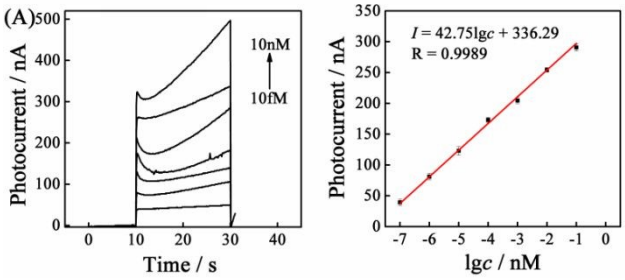


Fig. 2 (A) Photocurrent response and (B) calibration curve of the PEC biosensor to different concentrations of $VEGF_{165}$.

It is great important to validate the stability and selectivity of biosensors. The proposed PEC biosensor was investigated under periodically light for 10 cycles in order to evaluate the stability. As shown in Fig. 3A, there is no obvious change of the photocurrent over 10 cycles when $VEGF_{165}$ concentration is 1 pM, indicating the excellent stability. The selectivity of the biosensor for $VEGF_{165}$ was further investigated by using 10-fold excess of different interferences such as $VEGF_{121}$, PDGF-BB (platelet derived growth factor BB), prostate specific

antigen (PSA), β2-microglobulin (β2-MG) and human immunoglobulin (lgG). As shown in Fig. 3B, obvious photocurrent increase was observed for 10 nM $VEGF_{165}$ (ΔI=320 nA). The corresponding photocurrent of 100 nM $VEGF_{121}$, PDGF-BB, PSA, β-Mg and lgG were negligible, indicating that the biosensor exhibits high specificity for $VEGF_{165}$.

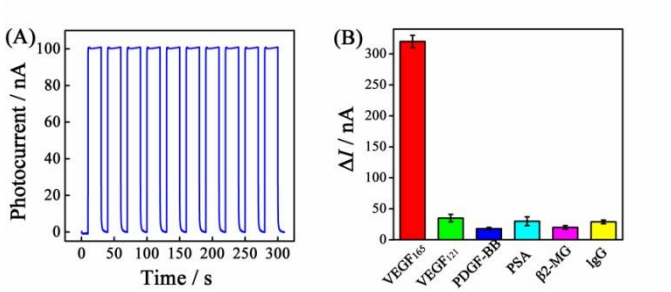


Fig. 3 (A) Stability of the PEC biosensor. (B) Specificity of the PEC biosensor for $VEGF_{165}$ by comparing it to the 10-fold excess of other interfering substances.

In summary, we developed a novel "signal on" $VEGF_{165}$ PEC biosensor with near-zero background signal based on $AgVO_3$ nanoparticles as photoactive material. By using PAA as surfactant, the dispersiblity of the photoactive $AgVO_3$ was remarkably improved and carboxyl group was introduced for the further DNA modification. In the presence of the $VEGF_{165}$, Exo III-aided target recycling and HCR were triggered successively to immobilize $AgVO_3$ nanoparticles as photoactive material, which generated strong detectable signals with near-zero background signal. The fabricated PEC biosensor provides a wide range of linearship and show high sensitivity, specificity and stability for the detection of traget $VEGF_{165}$. In addition, this novel "signal on" PEC biosensor $VEGF_{165}$ can be extended to detect other biomarkers for early diagnoses of diseases.

Table 1. Comparison of the analytical performance of other $VEGF_{165}$ biosensors.

Analytical method	Linear range (pM)	Detection limit (pM)	Ref.
FL	100–1.6×10^4	80	23
ECL	1–2×10^4	0.2	24
ELISA	–	0.03	25
SWV	1.3-2.6×10^3	–	26
PEC	0.1–1×10^4	0.03	20
PEC	0.01–1×10^4	0.003	This work

Abbreviations: fluorescence (FL); electrochemiluminescent (ECL); enzyme-linked immunosorbent assay (ELISA); Square wave voltammetry (SWV).

This work was financially supported by the National Natural Science Foundation of China (21501081, 21575116, 21775124) and the Fundamental Research Funds for the Central Universities (XDJK2017C021, XDJK2018AA003).

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