Nanoscale Advances

PAPER

Check for updates

Cite this: Nanoscale Adv., 2023, 5, 830

Direct fabrication of NbS₂ nanoflakes on carbon fibers by atomic layer deposition for ultrasensitive cardiac troponin I detection[†]

Yazhou Huang, ^(b) *^a Yunfei Zhang,^a Junyan Lv,^a Yinfeng Shao,^a Dongfang Yang^b and Yuan Cong^c

The sensitive detection of cardiac troponin I (cTnI) is of great significance for the early diagnosis of acute myocardial infarction (AMI). Herein, in order to fabricate an electrochemical biosensor for ultrasensitive cTnI detection, atomic layer deposition (ALD) was employed to directly deposit NbS₂ nanoflakes (NFs) on carbon fiber paper (CFP). Due to the self-limiting reaction of ALD, NbS₂NFs were deposited uniformly and accurately on the surface of carbon fibers by controlling the number of ALD cycles, which ensured ultrasensitive detection. Precise regulation of the nanoscale morphology and electrochemical performance of NbS₂ nanoflakes *via* ALD cycles was observed in depth. Owing to the high surface area and conductivity, an anodic/cathodic current of \sim 3.01 mA of NbS₂NFs/CFP can be obtained. Subsequently, an electrochemical biosensor based on the excellent performance of NbS₂NFs/CFP was fabricated. The ultrasensitive detection of cTnI in a linear range of 1 fM to 0.1 nM with a detection limit of 0.32 fM was achieved.

Received 21st November 2022 Accepted 8th January 2023

DOI: 10.1039/d2na00827k

rsc.li/nanoscale-advances

1. Introduction

Acute myocardial infarction (AMI) with high mortality and morbidity is an extremely time-sensitive disease.1-3 Cardiac troponin I (cTnI) has been proved to be a typical biomarker of AMI owing to its superior sensitivity and specificity for myocardial damage.⁴ Therefore, the sensitive detection of cTnI is of great significance in the early diagnosis of AMI. Among many cTnI detection methods such as enzyme-linked immunosorbent assay (ELISA),5,6 field-effect transistor-based biosenfluorescence,8 electrochemiluminescence sors,7 (ECL), radioimmunoassay (RIA)10 and surface plasmon resonance (SPR) biosensors,11 electrochemical (EC) detection is one of the most promising choices owing to the high sensitivity and low background noise.¹²⁻¹⁷ As it should be, the high sensitivity of electrochemical detection highly depends on the surface material on the working electrode and its manufacturing method.

Two-dimensional (2D) transition metal dichalcogenide (TMDC) materials¹⁸ have promising prospects in detecting

biological molecules including miRNA,19-22 protein23 and others,24-27 because of their excellent stability, biological affinity, and large surface area. However, there are obvious problems for traditional 2D TMDCs such as MoS₂,²⁸ WS₂ (ref. 29) and ReS₂.³⁰ Firstly, the detection response current of the electrode is insufficient owing to their poor conductivity from the semiconductor structures. In order to improve the response current, they are often mixed with other high conductivity materials, such as graphene,³¹ MXenes,²¹ etc.,³² which leads to a decrease in the detection accuracy and stability. In addition, due to the 2D structure, conventional modification methods that physically adsorb materials on the electrode surface by van der Waals force, such as drop coating,33,34 chemical vapor deposition (CVD)^{35,36} and hydrothermal,^{37,38} easily lead to the agglomeration of materials on the electrode surface rather than uniform distribution. More seriously, the agglomerations induce additional impedance and make the material easy to fall off from the electrode surface during the detection process, which destroy the detection accuracy and stability of the electrode. Therefore, it is necessary to find a new material and manufacturing method to promote 2D TMDCs in detecting biomolecules.

Hence, NbS_2 , a new type of 2D TMDC, is paid attention to. It not only has the advantages of traditional 2D TMDCs, including stability, biological affinity and a large surface area, but also has the high conductivity of metal owing to its zero band gap structure, which makes it an ideal material to build an electrochemical detection platform.^{39,40} In addition, the development of atomic layer deposition (ALD) technology makes it

View Article Online

View Journal | View Issue

[&]quot;Industrial Center, Nanjing Institute of Technology, Nanjing 211167, People's Republic of China. E-mail: huangyazhou@njit.edu.cn

^bSchool of Energy and Power Engineering, Nanjing Institute of Technology, Nanjing 211167, People's Republic of China

^cSchool of Materials Science and Engineering, Nanjing Institute of Technology, Nanjing 211167, People's Republic of China

[†] Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d2na00827k

Paper

possible to manufacture 2D material sensing electrodes.^{41,42} Thanks to self-limiting chemical reactions, ALD can accurately control the deposition process and avoid the agglomeration of materials on the surface of the electrode.⁴³⁻⁴⁶ Therefore, it is timely and meaningful that employing ALD to manufacture a NbS₂ sensing electrode for ultrasensitive cTnI detection.

In this work, ALD was employed to directly and precisely deposit NbS₂ nanoflakes (NFs) on carbon fiber paper (CFP) to fabricate an electrochemical biosensor for ultrasensitive cTnI detection. Precise regulation of the nanoscale morphology and electrochemical performance of NbS₂NFs *via* ALD cycles was observed. Then, the structure, mechanism and sensitivity of the biosensor were observed by various means.

2. Experimental

2.1. Materials

Carbon fiber paper (CFP, TGP-H-60) was purchased from Toray. Potassium chloride (KCl, 99.5%) and potassium hexacyanoferrate-II trihydrate (K₄[Fe(CN)₆]·3H₂O, 99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Niobium chloride (NbCl₅, 99.8%), hydrogen tetrachloroaurate trihydrate (HAuCl₄·3H₂O, 99.9%) and potassium hexacyanoferrate-III (K₃[Fe(CN)₆], 99.5%) were purchased from Aladdin (Shanghai, China). Phosphate buffer solution (PBS, pH 7.4) and 6-mercaptohexanol (MCH) were purchased from Sigma Aldrich (Shanghai, China). Nitrogen (N₂, 99.999%) and hydrogen sulfide (H₂S, 99.5%) were purchased from Nanjing Special Gas Co. Ltd Cardiac troponin I (cTnI), horseradish peroxidase (HRP), myoglobin (MB), nucleolin (NCL), and thiolated cTnI aptamer (AcTnI): 5'-SH-(C)6-CGTGCAGTACGC-CAACCTTTCTCATGCGCGCTGCCCCTCTTA-3' were purchased from Sangon Biotech Co., Ltd (Shanghai, China).

2.2. Fabrication of the electrochemical biosensor based on ALD-constructed NbS_2NFs/CFP

As shown in Fig. 1, NbS₂ NFs were firstly deposited on the surface of carbon fiber paper (CFP, 0.25 cm^2) by ALD. NbCl₅ and H₂S were selected as Nb and S sources for ALD respectively. An ALD cycle consists of four steps: feeding NbCl₅ (0.5 s), purging NbCl₅ (10 s), feeding H₂S (0.5 s), and purging H₂S (10 s). The corresponding two self-limiting reactions can be given as follows.

(A)
$$Nb - SH^* + NbCl_5 \rightarrow Nb - S - NbCl_4^* + HCl$$

(B) NbCl* + H₂S
$$\rightarrow$$
 Nb–SH* + HCl + S

where * denotes the surface species. Due to the self-limiting chemical reactions, the depositing process of NbS₂NFs can be accurately controlled by the number of ALD cycles. During the ALD process, the reaction chamber and NbCl₅ were kept at 430 and 130 °C respectively. In addition, the pressure of the reaction chamber was controlled at 10 hPa, and 100 sccm of N₂ was used as the carrying and purging gas.

In order to assemble the cTnI aptamer (AcTnI) probe, Au nanoparticles (AuNPs) were electrodeposited on NbS₂NFs/CFP in 20 mL electrolyte solution composed of 0.02 g HAuCl₄·3H₂·O and 0.15 g KCl at a constant potential of -0.2 V for 200 s. Then, 15 μ L AcTnI probe (1 μ M) solution was dripped and assembled on the surface of NbS₂NFs/CFP with AuNPs (named AcTnI/Au/NbS₂NFs/CFP). The unbound AuNPs were filled by 6-mercaptohexanol (MCH) to avoid nonspecific adsorption, through adding 15 μ L MCH solution (1 μ M) on AcTnI/Au/NbS₂NFs/CFP (named MCH/AcTnI/Au/NbS₂NFs/CFP). Thus, the



Fig. 1 Schematic showing the fabrication of the electrochemical biosensor and its detection of cTnl.

sensing platform was built. Different concentrations of cTnI can be captured by the AcTnI probe owing to the specific binding (named cTnI/MCH/AcTnI/Au/NbS₂NFs/CFP), and then detected through the decrease of electrochemical current owing to the steric hindrance effect.

2.3. Techniques

The morphology, chemical elements and crystal structures of as-constructed NbS₂NFs/CFP were observed by various means, including atomic force microscopy (AFM, Dimension Icon, Bruker), scanning electron microscopy (SEM, S-4800, Hitachi), high resolution transmission electron microscopy (HRTEM, Talos F200X G2, FEI), energy dispersive spectroscopy (EDS, S-4800, Hitachi), X-ray photoelectron spectroscopy (XPS, EscaLab-250Xi, Thermo Fisher), Raman spectroscopy (Reflex, Renishaw) and X-ray diffraction (XRD, D8 Advance, Bruker). Before TEM characterization, the samples were ground into powder and transferred on a copper grid. HRTEM was performed using an accelerating voltage of 200 kV. Raman spectroscopy was carried out by using excitation light of a 532 nm laser with 0.8 mW power and 1 µm spot. XRD was carried out with Cu K α radiation ($\lambda = 1.54$ Å) at 40 mA and 45 kV. The XPS source is Al K α (hv = 1486.6 eV) with a power of 22.8 W.

All the electrochemical tests were carried out by using a three-electrode electrochemical cell (CHI660E, CH Instruments), which employed a saturated calomel electrode (SCE) and a Pt wire as reference and counter electrodes, in an electrolyte solution composed of 5 mM $[Fe(CN)_6]^{3-/4-}$, 0.1 M KCl and 0.1 M PBS. The electrochemical properties of NbS₂NFs/CFP were observed by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and chronocoulometry (CC), respectively. CV was performed in a range of -0.2 and 0.6 V at a scan rate of 50 mV s⁻¹. EIS was performed from 0.1 to 100 kHz at the open circuit voltage. Chronocoulometry was performed in an electrolyte solution composed of 0.1 mM $[Fe(CN)_6]^{3-}$ and 1 M KCl with an initial potential of 0 V and a final potential of 0.4 V. Finally, the detection performance of the sensor for cTnI was evaluated by using the peak current of differential pulse voltammetry (DPV) in a range of -0.2 and 0.6 V.

Results and discussion

3.1. Deposition of NbS₂ nanoflakes by ALD

In order to evaluate ALD, NbS_2 was firstly deposited on flat SiO₂. The deposition process of NbS_2 can be accurately controlled by the ALD cycle, because of the self-limiting chemical reactions. NbS_2 films obtained by 10, 30 and 50 ALD cycles on SiO₂ substrates were scanned by AFM. As shown in Fig. 2(a)–(c) and S1,† with the increase of ALD cycles from 10 to 50, NbS_2 showed the typical process of nucleation, growth, and film formation. Then, the changes of the thickness, particle diameter and coverage of NbS_2 with the number of ALD cycles were observed



Fig. 2 AFM images of NbS₂ films obtained by 10 (a), 30 (b), and 50 (c) ALD cycles. (d) Thickness of the NbS₂ film against the number of ALD cycles.

Paper

in detail. When the number of ALD cycles increases from 10 to 30, the thickness, particle diameter and coverage increase from ~1.5 nm, ~3.3 nm and 18% to ~14.5 nm, ~23 nm and 48% respectively. After adding another 20 cycles, the thickness, particle diameter and coverage increase to ~23.7 nm, 30 nm and 83%. Growth-per-cycle (GPC) was usually employed to evaluate ALD. As shown in Fig. 2(d), the GPC of 0.58 nm is close to the single layer thickness (~0.6 nm) of natural NbS₂,⁴⁴ indicating the excellent performance of ALD.

Then NbS₂ was deposited on carbon fiber paper (CFP) to construct the sensing electrode. The SEM images of bare CFP and NbS₂NFs/CFP obtained by 10, 30, 50, 70 and 100 ALD cycles are shown in Fig. 3(a)-(f), which exhibit the precise growth process of NbS₂ nanoflakes under the control of the ALD cycle. According to Fig. 3(b), NbS₂ firstly nucleates uniformly on the CFP surface by 10 ALD cycles. It was further grown into NbS₂ nanoparticles after another 20 cycles, and its density also increased significantly at the same time (as shown in Fig. 3(c)). Then, when the number of ALD cycles reached 50 (Fig. 3(d)), NbS₂ nanoflakes of \sim 300 nm appeared on the basis of NbS₂ nanoparticles. This is an important moment meaning that NbS₂ nanoflakes have been successfully built on the CFP surface by ALD. However, it is obvious that the CFP substrate is not completely covered by the NbS2 nanoflakes. As shown in Fig. 3(e), the NbS₂ nanoflakes increased to \sim 500 nm after 70

ALD cycles, and they almost completely and uniformly covered the CFP substrate. Undesirably, the agglomeration of NbS₂ nanoflakes appeared on the surface of CFP when the ALD cycles increased to 100 (as shown in Fig. 3(f)), which is not recommendable. Therefore, NbS₂ nanoflakes on CFP obtained by 70 ALD cycles were preliminarily employed as the sensing electrode.

The NbS₂ nanoflake structure may significantly increase the surface area of CFP and bind more AcTnI probes, thus improving the sensitivity in the electrochemical detection process. In addition, compared with the traditional 2D TMDCs such MoS₂, WS₂, ReS₂, *etc.*, NbS₂NFs will hardly induce additional resistance and reduce the response current of electrochemical detection, owing to its high conductivity.⁴⁷ Therefore, the excellent properties of NbS₂NFs/CFP including stability, biological affinity, a large surface area and high conductivity make it an ideal electrochemical sensing electrode. SEM results show that NbS₂ nanoflakes can be accurately constructed by ALD by controlling the number of cycles, thus avoiding the agglomeration of materials on the electrode surface and providing a guarantee for the accuracy and stability of the subsequent electrochemical biosensor.

In addition, the chemical elements of as-deposited NbS_2 nanoflakes by 70 ALD cycles were observed by EDS. As shown in Fig. 3(g), the S/Nb ratio is 1.56 indicating that the NbS_2



Fig. 3 SEM images of bare CFP (a) and NbS₂NFs/CFP obtained by 10 (b), 30 (c), 50 (d), 70 (e), and 100 (f) ALD cycles. ESD (g), Raman (h) and XRD (i) spectra of 70-NbS₂NFs/CFP.

nanoflakes are sulfur deficient. This can be attributed to the low temperature (450 °C) and the high efficiency (21 s per cycle) of the ALD process.⁴² Raman (Fig. 3(h)) and XRD (Fig. 3(i)) of NbS₂NFs/CFP and bare CFP were also carried out at room temperature. As shown in Fig. 3(h), the NbS₂ peak at 321 cm⁻¹ belongs to E_2 mode, and the peak at 381 is from its E_1 mode. According to Fig. 3(i), the (002) peak at 14.6° in the XRD spectrum confirms the 2H phase structure of NbS₂.⁴⁷

The structures of the as-deposited NbS₂ nanoflakes by 70 ALD cycles were further observed by TEM. Fig. 4(a) shows the NbS₂ nanoflakes with a thickness of \sim 300 nm on the CFP. The elemental mapping under HAADF confirms the compositions of Nb and S of NbS₂ (Fig. 4(b)). According the HRTEM images in Fig. 4(c) and (d), a layer structure with a spacing of 0.6 nm can be clearly observed. In addition, the chemical elements were

also observed by XPS. According to Fig. 4(e) and (f), the peaks of Nb $3d_{3/2}$, Nb $3d_{5/2}$, S $2p_{1/2}$ and S $2p_{3/2}$ are clearly shown at 210.68, 207.78, 165.08 and 163.88 eV respectively, which correspond to Nb⁴⁺ and S²⁻ of NbS₂.⁴⁷ Hence, the XPS results indicate that NbS₂ nanoflakes have been successfully constructed on the CFP.

3.2. Electrochemical performance of the NbS₂NFs/CFP electrode obtained by ALD

In order to find the most suitable one to fabricate the cTnI sensor, the electrochemical performance of bare CFP and asdeposited NbS₂NFs/CFP by different ALD cycles was evaluated by a cyclic voltammetry (CV) test. According to the CV curves shown in Fig. 5(a), for bare CFP, it can be observed that anodic/



Fig. 4 (a) TEM image of NbS₂NFs/CFP. (b) High-angle annular dark-field (HAADF) images of the area in (a) and its elemental mapping images of C, Nb, and S, respectively. (c and d) HRTEM images of the local area in (a). XPS spectra of Nb 4f (e) and S 2p (f).

Paper



Fig. 5 (a) CV, (b) EIS, (c) Q-t, and (d) $Q-t^{1/2}$ curves of bare CFP and NbS₂NFs/CFP obtained by different ALD cycles. 0, 10, 30, 50, 70 and 100 are bare CFP, 10, 30, 50, 70 and 100 ALD cycles respectively.

cathodic current peaks with an intensity of ~1.03 mA appear at 0.32 and 0.16 V respectively. However, the currents were only \sim 0.2 mA while a conductive glass plane with the same size (0.25 cm²) was selected as the electrode substrate.⁴¹ The reason why CFP has such a high current intensity is its high specific surface area from a large number of voids scattered between the carbon fibers, which can improve the contact area between the electrode and the electrolyte. Therefore, the CFP electrode substrate ensures the high sensitivity of the subsequent sensors. However, by conventional methods, it is difficult to modify materials accurately and uniformly on the substrate surface with a high specific surface area like CFP. ALD can effectively solve this problem owing to the self-limiting reaction (shown in SEM images in Fig. 3). When NbS₂ nanoflakes were deposited on the surface of CFP by different ALD cycles, there was a significant increase in the currents. As the number of ALD cycles increases from 10 to 70, the intensity gradually enhances from \sim 1.51 to \sim 3.01 mA, because the NbS₂ nanoflakes with high conductivity further improve the contact between the electrode and the electrolyte. However, when the ALD cycle increases to 100, the current decreases by \sim 0.17 mA. This can be attributed to the agglomeration of NbS2 nanoflakes on the CFP surface (shown in Fig. 3(f)), which reduces the contact between the electrode and the electrolyte, thus hindering the transfer of charge in the redox process.

The electrode dynamics of as-deposited NbS2NFs/CFP were further observed by electrochemical impedance spectroscopy (EIS). EIS and the equivalent circuit are shown in Fig. 5(b) and S2.† The $R_{\rm CT}$, meaning the resistance of charge transport at the interface between the electrode and the electrolyte, is indicated by the semicircle in the high-frequency region in EIS. For bare CFP, $R_{\rm CT}$ is 26.29 Ω. When 10 ALD cycles of NbS2 nanoflakes were deposited on the surface of CFP, $R_{\rm CT}$ dropped significantly to 20.06 Ω . Then, as the number of ALD cycles increases, the R_{CT} keeps decreasing and reaches the minimum (0.82 Ω) at 70 cycles, but there is an increase at 100 cycles (1.66 Ω). Obviously, the trends of the redox current and the $R_{\rm CT}$ are opposite with the number of ALD cycles. In other words, the increase of the redox current is due to the reduction of the R_{CT} by depositing NbS₂ nanoflakes on CFP. The results of EIS indicate that the alteration of the current is mainly caused by the change of the interface resistance, and ALD can precisely modify the electrode by controlling the number of cycles.

In addition, the effective surface area (ESA) between the electrode and the electrolyte was evaluated by using Anson's equation:⁴⁸ $Q = 2nFAcD^{1/2}t^{1/2} + Q_{dl} + Q_{ads}$. The Q-t and Q- $t^{1/2}$ curves of bare CFP and NbS₂NFs/CFP from chronocoulometry tests are shown in Fig. 5(c) and (d). Then, the ESA was obtained by the linear fitting of the Q- $t^{1/2}$ curve. The ESA of bare CFP is 1.28 cm², which is much larger than its geometry size (0.25 cm²), owing to the large number of voids between the carbon

8

fibers in the CFP. The ESA of NbS₂NFs/CFP obtained by 10, 30, 50, 70 and 100 ALD cycles is 3.08, 5.37, 7.51, 9.26 and 8.62 cm² respectively. Obviously, NbS₂ nanoflakes on the surface of CFP further increase the ESA, and the NbS₂ nanoflakes obtained by 70 cycles get the largest one. Hence, the improvement of the interface resistance $R_{\rm CT}$ of NbS₂NFs/CFP is mainly due to the increase of the ESA by depositing NbS₂ nanoflakes.

Moreover, as one of the traditional 2D TMDCs, the electrochemical performance of MoS_2 nanoflakes obtained by 70 ALD cycles had also been observed for a contrast (Fig. S3†). Due to the poor conductivity, the current (~1.06 mA) of MoS_2NFs/CFP is much smaller than that of NbS_2NFs/CFP (~3.01 mA). Therefore, the reason why NbS_2NFs/CFP has such an excellent electrochemical performance is the large surface area and the high conductivity coming from the CFP substrate and the 2D NbS_2 nanoflake structure, which ensure high sensitivity for the subsequent cTnI sensor. ALD-deposited NbS_2NFs/CFP by 70 cycles was chose as the electrode for the subsequent fabrication of the cTnI sensor.

3.3. Sensitivity and selectivity for cTnI sensing

As shown in Fig. 1, based on the ALD-deposited NbS₂NFs/CFP electrode, an electrochemical cTnI sensor was fabricated from modified AuNPs (Au/NbS₂NFs/CFP), an AcTnI probe (AcTnI/Au/TaS₂NFs/CFP) and MCH (MCH/AcTnI/Au/NbS₂NFs/CFP),

respectively. Then, the sensor was used to detect cTnI (10⁻¹⁰ M, cTnI/MCH/AcTnI/Au/NbS₂NFs/CFP). The related DPV and EIS curves are shown in Fig. 6(a) and (b). The peak current of DPV and the interface resistance R_{CT} for NbS₂NFs/CFP, Au/ NbS2NFs/CFP, AcTnI/Au/NbS2NFs/CFP, MCH/AcTnI/Au/NbS2-NFs/CFP and cTnI/MCH/AcTnI/Au/NbS2NFs/CFP are 1.79, 1.86, 1.62, 1.45, and 1.06 mA, and 0.82, 0.62, 23.75, 32.35, and 70.97 Ω , respectively. The results of DPV and EIS were coincident with each other, indicating that the electrochemical sensor for cTnI detection has been successfully fabricated. When cTnI is fed to it, cTnI can be captured by the AcTnI probe on the surface of the sensor owing to specific binding, and then detected through the decrease of DPV current from the steric hindrance effect. Moreover, as shown in Fig. 6(c) and (d), when 10^{-10} M cTnI was added into the sensor, the time and temperature of hybridization between cTnI and the AcTnI probe were optimized based on the peak current of DPV. It can be observed that the optimal hybridization time and temperature are 120 min and 37 °C, which are set as the working parameters of the sensor.

The detection performance of the as-fabricated biosensor was further evaluated. cTnI with different concentrations from 10^{-10} to 10^{-15} M was detected by the sensor. According to Fig. 7(a), as the concentration of cTnI increases, the DPV current gradually decreases, due to the increase of the charge transport resistance from the steric hindrance caused by more cTnI. The peak current *I* against the logarithm concentration *c* of cTnI is



Fig. 6 (a) DPV and (b) EIS curves of different modifiers based on NbS₂NFs/CFP. The equivalent circuit is shown in Fig. S2.† Influence of the hybridization time (c) and temperature (d) on peak current (n = 3).



Fig. 7 (a) DPV response of the biosensor to various concentrations of cTnl (from 0 to 10^{-10} M). (b) The response current of (a) as a function of logarithm of concentration (n = 3). (c) Comparison of cTnl response current with other proteins (HRP, MB and NCL), indicating the excellent selectivity of the fabricated biosensor.

Table 1	Comparison	of biosensing	results for	cTnl
---------	------------	---------------	-------------	------

Materials	Detection methods	Linear range (ng L^{-1})	$\rm LOD \ (ng \ L^{-1})$	Ref.
N, Zn-G QDs	ECL	0.01-1000	0.005	13
BN QDs	DPV	10-5000	0.5	14
g-C ₃ N ₄ QDs	Fluorescence	1-10 000	0.227	49
DPVBi	ECL	0.1-20 000	0.043	50
$\rm NbS_2NFs/CFP$	DPV	0.022–2200 (1 fM–0.1 nM)	0.007 (0.32 fM)	This work

shown in Fig. 7(b). According to the linear relationship between *I* and *c*, *I* = 0.34–0.07 lg *c* can be obtained with a correlation coefficient $R^2 = 0.99$ and a limit of detection (LOD) of 0.32 fM (S/ N = 3). The selectivity of the sensor was evaluated by using three other types of protein including horseradish peroxidase (HRP), myoglobin (MB) and nucleolin (NCL) at 10^{-10} M. According to Fig. 7(c), the DPV response ΔI of cTnI (0.39 mA) is much higher than that of HRP (0.098 mA), Mb (0.08 mA) and NCL (0.065 mA), indicating that the sensor has a high selectivity for cTnI. Moreover, compared with previous reports for cTnI detection (Table 1), the sensor based on ALD-constructed NbS₂NFs/CFP is potentially useful for the early diagnosis of AMI. Meanwhile, ALD is a simple and controllable method that can be employed in large-scale manufacturing of the electrochemical sensor.

3.4. Stability and serum sample analysis

Stability is important to the actual work of the sensor. In order to evaluate the stability, the as-fabricated sensor was kept at 4 $^{\circ}$ C for 0–8 days. As shown in Fig. S4,† after 8 days, the current response lost only 12.26%, indicating good stability.

Finally, in order to evaluate the possibility of application in real samples, the performance of the as-fabricated sensor was observed in serum. As shown in Table S1,† recoveries for cTnI with different concentrations (100 pM, 1 pM and 10 fM) are 101.61, 93.67 and 99.23 respectively, and the relative standard deviation (RSD) is less than 5%, indicating the effective detection of cTnI in real samples.

4. Conclusion

In summary, an electrochemical biosensor based on ALD-deposited NbS_2NFs/CFP for AMI-related cTnI detection has

been successfully fabricated. Due to the self-limiting reactions, the nanoscale morphology and electrochemical performance of NbS₂ nanoflakes can be accurately and directly regulated by controlling the ALD cycles. Therefore, the agglomerations can be effectively avoided. An anodic/cathodic current of \sim 3.01 mA of NbS₂NFs/CFP deposited by 70 cycles can be obtained owing to its high surface area and conductivity. Then, the ultrasensitive detection of cTnI in a linear range of 1 fM to 0.1 nM with a detection limit of 0.32 fM was achieved at 37 °C with a hybridization time of 120 min. Hence, it is potentially useful for the early diagnosis of AMI and can be employed in large-scale manufacturing.

Author contributions

Y. H. conceptualization, writing-original draft, methodology, and writing-review & editing. Y. Z. formal analysis and methodology. J. L. and Y. S. methodology and data curation. D. Y. and Y. C. resources and conceptualization.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (51905259), the Natural Science Foundation of Jiangsu Province (BK20191017), the Qing Lan Project of Jiangsu Province, and the College Students' Practice and Innovation Training Program of Jiangsu Province (202211276020Z).

References

- 1 U. Friess and M. Stark, Cardiac Markers: A Clear Cause for Point-Of-Care Testing, *Anal. Bioanal. Chem.*, 2009, **393**(5), 1453–1462.
- 2 X. Liu, Y. Wang, P. Chen, A. McCadden, A. Palaniappan, J. Zhang and B. Liedberg, Peptide Functionalized Gold Nanoparticles With Optimized Particle Size and Concentration for Colorimetric Assay Development: Detection of Cardiac Troponin I, *ACS Sens.*, 2016, 1(12), 1416–1422.
- 3 X. Han, S. Li, Z. Peng, A. M. Othman and R. Leblanc, Recent Development of Cardiac Troponin I Detection, *ACS Sens.*, 2016, **1**(2), 106–114.
- 4 M.-I. Mohammed and M. P. Y. Desmulliez, Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers: a review, *Lab Chip*, 2011, **11**(4), 569–595.
- 5 E. Dempsey and D. Rathod, Disposable Printed Lateral Flow Electrochemical Immunosensors for Human Cardiac Troponin T, *IEEE Sens. J.*, 2018, **18**(5), 1828–1834.
- 6 I.-H. Cho, E.-H. Paek, Y.-K. Kim, J.-H. Kim and S.-H. Paek, Chemiluminometric enzyme-linked immunosorbent assays (ELISA)-on-a-chip biosensor based on cross-flow chromatography, *Anal. Chim. Acta*, 2009, **632**(2), 247–255.
- 7 K. Kim, C. Park, D. Kwon, D. Kim, M. Meyyappan, S. Jeon and J.-S. Lee, Silicon nanowire biosensors for detection of cardiac troponin I (cTnI) with high sensitivity, *Biosens. Bioelectron.*, 2016, 77, 695–701.
- 8 P. Kar, A. Pandey, J. J. Greer and K. Shankar, Ultrahigh sensitivity assays for human cardiac troponin I using TiO₂ nanotube arrays, *Lab Chip*, 2012, **12**(4), 821–828.
- 9 C. A. Marquette, F. Bouteille, B. P. Corgier, A. Degiuli and L. J. Blum, Disposable screen-printed chemiluminescent biochips for the simultaneous determination of four pointof-care relevant proteins, *Anal. Bioanal. Chem.*, 2009, **393**(4), 1191–1198.
- 10 F. S. Apple, A. Falahati, P. R. Paulsen, E. A. Miller and S. W. Sharkey, Improved detection of minor ischemic myocardial injury with measurement of serum cardiac troponin I, *Clin. Chem.*, 1997, 43(11), 2047–2051.
- 11 Y. Yanase, T. Hiragun, T. Yanase, T. Kawaguchi, K. Ishii and M. Hide, Application of SPR Imaging Sensor for Detection of Individual Living Cell Reactions and Clinical Diagnosis of Type I Allergy, *Allergol. Int.*, 2013, **62**(2), 163–169.
- 12 N. Singh, P. Rai, M. A. Ali, R. Kumar, A. Sharma, B. D. Malhotra and R. John, A hollow-nanosphere-based microfluidic biosensor for biomonitoring of cardiac troponin I, *J. Mater. Chem. B*, 2019, 7(24), 3826–3839.
- 13 M. Liu, R. Jiang, M. Zheng, M. Li, Q. Yu, H. Zhu, H. Guo and H. Sun, A sensitive ratiometric biosensor for determination cardiac troponin I of myocardial infarction markers based on N, Zn-GQDs, *Talanta*, 2022, 249, 123577.
- 14 M. L. Yola and N. Atar, Development of cardiac troponin-I biosensor based on boron nitride quantum dots including

molecularly imprinted polymer, *Biosens. Bioelectron.*, 2019, **126**, 418–424.

- 15 D. Sun, Z. Luo, J. Lu, S. Zhang, T. Che, Z. Chen and L. Zhang, Electrochemical dual-aptamer-based biosensor for nonenzymatic detection of cardiac troponin I by nanohybrid electrocatalysts labeling combined with DNA nanotetrahedron structure, *Biosens. Bioelectron.*, 2019, **134**, 49–56.
- 16 I. Grabowska, N. Sharma, A. Vasilescu, M. Iancu, G. Badea, R. Boukherroub, S. Ogale and S. Szunerits, Electrochemical Aptamer-Based Biosensors for the Detection of Cardiac Biomarkers, ACS Omega, 2018, 3(9), 12010–12018.
- 17 D. Sun, X. Lin, J. Lu, P. Wei, Z. Luo, X. Lu, Z. Chen and L. Zhang, DNA nanotetrahedron-assisted electrochemical aptasensor for cardiac troponin I detection based on the co-catalysis of hybrid nanozyme, natural enzyme and artificial DNAzyme, *Biosens. Bioelectron.*, 2019, **142**, 111578.
- 18 Q. H. Wang, K. Kalantar-Zadeh, A. Kis, J. N. Coleman and M. S. Strano, Electronics and optoelectronics of twodimensional transition metal dichalcogenides, *Nat. Nanotechnol.*, 2012, 7(11), 699–712.
- 19 S. Su, W. Cao, W. Liu, Z. Lu, D. Zhu, J. Chao, L. Weng, L. Wang, C. Fan and L. Wang, Dual-mode electrochemical analysis of microRNA-21 using gold nanoparticle-decorated MoS₂ nanosheet, *Biosens. Bioelectron.*, 2017, 94, 552–559.
- 20 S. Su, Y. Wu, D. Zhu, J. Chao, X. Liu, Y. Wan, Y. Su, X. Zuo, C. Fan and L. Wang, On-Electrode Synthesis of Shape-Controlled Hierarchical Flower-Like Gold Nanostructures for Efficient Interfacial DNA Assembly and Sensitive Electrochemical Sensing of MicroRNA, *Small*, 2016, 12(28), 3794–3801.
- 21 L. Liu, Y. Wei, S. Jiao, S. Zhu and X. Liu, A novel label-free strategy for the ultrasensitive miRNA-182 detection based on MoS₂/Ti₃C₂ nanohybrids, *Biosens. Bioelectron.*, 2019, 137, 45–51.
- L. Liu, M. Kong, Y. Xing, Z. Wu and Y. Chen, Atomic Layer Deposition-Made MoS₂-ReS₂ Nanotubes with Cylindrical Wall Heterojunctions for Ultrasensitive MiRNA-155 Detection, ACS Appl. Mater. Interfaces, 2022, 14(8), 10081–10091.
- 23 S. Su, X. Han, Z. Lu, W. Liu, D. Zhu, J. Chao, C. Fan, L. Wang, S. Song, L. Weng, *et al.*, Facile Synthesis of a MoS₂–Prussian Blue Nanocube Nanohybrid-Based Electrochemical Sensing Platform for Hydrogen Peroxide and Carcinoembryonic Antigen Detection, *ACS Appl. Mater. Interfaces*, 2017, 9(14), 12773–12781.
- 24 N. Rohaizad, C. C. Mayorga-Martinez, Z. Sofer and M. Pumera, 1T-Phase Transition Metal Dichalcogenides (MoS₂, MoSe₂, WS₂, and WSe₂) with Fast Heterogeneous Electron Transfer: Application on Second-Generation Enzyme-Based Biosensor, *ACS Appl. Mater. Interfaces*, 2017, **9**(46), 40697–40706.
- 25 T. Yang, M. Chen, F. Nan, L. Chen, X. Luo and K. Jiao, Enhanced electropolymerization of poly(xanthurenic acid)– MoS₂ film for specific electrocatalytic detection of guanine and adenine, *J. Mater. Chem. B*, 2015, 3(24), 4884–4891.

- 26 N. Li, F. Zhang, W. Sun, L. Zhang and X. Su, Redox reactionmodulated fluorescence biosensor for ascorbic acid oxidase assay by using MoS₂ quantum dots as fluorescence probe, *Talanta*, 2021, 222, 121522.
- 27 X. Lin, Y. Ni and S. Kokot, Electrochemical cholesterol sensor based on cholesterol oxidase and MoS₂-AuNPs modified glassy carbon electrode, *Sens. Actuators, B*, 2016, 233, 100–106.
- 28 B. Radisavljevic, A. Radenovic, J. Brivio, V. Giacometti and A. Kis, Single-layer MoS₂ transistors, *Nat. Nanotechnol.*, 2011, 6(3), 147–150.
- 29 R. J. Toh, C. C. Mayorga-Martinez, Z. Sofer and M. Pumera, 1T-Phase WS₂ Protein-Based Biosensor, *Adv. Funct. Mater.*, 2017, 27(5), 1604923.
- 30 L. Liu, Y. Yao, K. Ma, C. Shangguan, S. Jiao, S. Zhu and X. Xu, Ultrasensitive photoelectrochemical detection of cancerrelated miRNA-141 by carrier recombination inhibition in hierarchical Ti₃C₂@ReS₂, *Sens. Actuators, B*, 2021, 331, 129470.
- 31 J. Zhou, Y. Zhao, J. Bao, D. Huo, H. Fa, X. Shen and C. Hou, One-step electrodeposition of Au-Pt bimetallic nanoparticles on MoS₂ nanoflowers for hydrogen peroxide enzyme-free electrochemical sensor, *Electrochim. Acta*, 2017, 250, 152– 158.
- 32 J. Yoon, T. Lee, B. Bapurao G, J. Jo, B.-K. Oh and J.-W. Choi, Electrochemical H₂O₂ biosensor composed of myoglobin on MoS₂ nanoparticle-graphene oxide hybrid structure, *Biosens. Bioelectron.*, 2017, **93**, 14–20.
- 33 J. Mei, Y.-T. Li, H. Zhang, M.-M. Xiao, Y. Ning, Z.-Y. Zhang and G.-J. Zhang, Molybdenum disulfide field-effect transistor biosensor for ultrasensitive detection of DNA by employing morpholino as probe, *Biosens. Bioelectron.*, 2018, **110**, 71–77.
- 34 B. Wang, X. Zhong, Y. Chai and R. Yuan, An ECL biosensor for sensitive detection of concanavalin A based on the ECL quenching of Ru complex by MoS₂ nanoflower, *Sens. Actuators, B*, 2017, 245, 247–255.
- 35 J. Liu, X. Chen, Q. Wang, M. Xiao, D. Zhong, W. Sun, G. Zhang and Z. Zhang, Ultrasensitive Monolayer MoS₂ Field-Effect Transistor Based DNA Sensors for Screening of Down Syndrome, *Nano Lett.*, 2019, **19**(3), 1437–1444.
- 36 T. Pham, Y. Chen, J. Lopez, M. Yang, T.-T. Tran and A. Mulchandani, Effect of Al₂O₃ Passive Layer on Stability and Doping of MoS₂ Field-Effect Transistor (FET) Biosensors, *Biosensors*, 2021, **11**(12), 514.
- 37 S. Sri, D. Chauhan, G. B. V. S. Lakshmi, A. Thakar and P. R. Solanki, MoS₂ nanoflower based electrochemical biosensor for TNF alpha detection in cancer patients, *Electrochim. Acta*, 2022, 405, 139736.
- 38 Y. J. Zhai, J. H. Li, X. Y. Chu, M. Z. Xu, F. J. Jin, X. Li, X. Fang, Z. P. Wei and X. H. Wang, MoS₂ microflowers based electrochemical sensing platform for non-enzymatic glucose detection, *J. Alloys Compd.*, 2016, 672, 600–608.

- 39 J. Shi, X. Wang, S. Zhang, L. Xiao, Y. Huan, Y. Gong, Z. Zhang, Y. Li, X. Zhou, M. Hong, *et al.*, Two-dimensional metallic tantalum disulfide as a hydrogen evolution catalyst, *Nat. Commun.*, 2017, 8(1), 958.
- 40 Y. Zhang, B. Zheng, C. Zhu, X. Zhang, C. Tan, H. Li, B. Chen, J. Yang, J. Chen, Y. Huang, *et al.*, Single-Layer Transition Metal Dichalcogenide Nanosheet-Based Nanosensors for Rapid, Sensitive, and Multiplexed Detection of DNA, *Adv. Mater.*, 2015, 27(5), 935–939.
- 41 L. Liu, S. Zhu, Y. Wei, X. Liu, S. Jiao and J. Yang, Ultrasensitive detection of miRNA-155 based on controlled fabrication of AuNPs@MoS₂ nanostructures by atomic layer deposition, *Biosens. Bioelectron.*, 2019, **144**, 111660.
- 42 Y. Huang, L. Liu and X. Liu, Modulated electrochemical oxygen evolution catalyzed by MoS_2 nanoflakes from atomic layer deposition, *Nanotechnology*, 2019, **30**(9), 095402.
- 43 L. K. Tan, B. Liu, J. H. Teng, S. Guo, H. Y. Low and K. P. Loh, Atomic layer deposition of a MoS_2 film, *Nanoscale*, 2014, **6**(18), 10584–10588.
- 44 X. Peng, G. Wan, L. Wu, M. Zeng, S. Lin and G. Wang, Peroxidase-like activity of Au@TiO₂ yolk-shell nanostructure and its application for colorimetric detection of H₂O₂ and glucose, *Sens. Actuators, B*, 2018, (257), 166–177.
- 45 L. Yu, G. Wan, Y. Qin and G. Wang, Atomic layer deposition assisted fabrication of high-purity carbon nanocoil for electrochemical energy storage, *Electrochim. Acta*, 2018, (268), 283–294.
- 46 L. Wu, G. Wan, S. Shi, Z. He, X. Xu, Y. Tang, C. Hao and G. Wang, Atomic layer deposition-assisted growth of CuAl LDH on carbon fiber as a peroxidase mimic for colorimetric determination of H₂O₂ and glucose, *New J. Chem.*, 2019, **43**, 5826–5832.
- 47 Z. Li, W. Yang, Y. Losovyj, J. Chen, E. Xu, H. Liu, M. Werbianskyj, H. A. Fertig, X. Ye and S. Zhang, Largesize niobium disulfide nanoflakes down to bilayers grown by sulfurization, *Nano Res.*, 2018, **11**(11), 5978–5988.
- 48 F. C. Anson, Application of Potentiostatic Current Integration to the Study of the Adsorption of Cobalt(III)-Ethylenedinitrilo(tetraacetate) on Mercury Electrodes, *Anal. Chem.*, 1964, **36**(4), 932–934.
- 49 L. Miao, L. Jiao, Q. Tang, H. Li, L. Zhang and Q. Wei, A nanozyme-linked immunosorbent assay for dual-modal colorimetric and ratiometric fluorescent detection of cardiac troponin I, *Sens. Actuators, B*, 2019, **288**, 60–64.
- 50 M. Yan, S. Feng, L. Yu, Y. Xue, J. Huang and X. Yang, Labelfree immunosensor for cardiac troponin I detection based on aggregation-induced electrochemiluminescence of a distyrylarylene derivative, *Biosens. Bioelectron.*, 2021, **192**, 113532.