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Free-standing MXene/chitosan/Cu_2O electrode: an enzyme-free and efficient biosensor for simultaneous determination of glucose and cholesterol

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1 Introduction

The incidence of "three highs", referring to hyperglycemia, hypertension, and hyperlipidemia, has been increasing rapidly all over the world (Dey and Raj, 2013; Gao et al., 2019). These illnesses may be attributed to the perturbations of blood metabolites which are small molecules within biofluids (Patil et al., 2018). Among them, the presence of cholesterol and glucose in blood at abnormal levels highly increases the risk of cardiac and brain vascular diseases, and diabetes (Li et al., 2019). Furthermore, it has been confirmed that high glucose concentration is one of the main causes of atherosclerosis, by causing the accumulation of cholesterol in macrophages (Henry et al., 2002). Therefore, for early diagnosis of these diseases, the development of a low-cost, simple, and efficient strategy for simultaneous, accurate, and rapid detection of multiple metabolites is greatly needed for on-site disease monitoring in-home healthcare.

Electrochemical biosensors are widely involved in the detection of glucose and cholesterol due to their simplicity, low cost, and high sensitivity (Shumyantseva et al., 2004; Li et al., 2005; Fan et al., 2017). In particular, enzymatic biosensors with the prominent features of specificity and fast response have been widely used. These biosensors are modified with at least one type of enzyme, glucose oxidase (GOx) or cholesterol oxidase (ChOx) (Jaime et al., 2017; Gao et al., 2019; Phetsang et al., 2019; Wu et al., 2019). Also, because of the complicated immobilization procedure and high environmental sensitivity of enzyme-based biosensors, non-enzymatic biosensors are in high demand for their compromise between sensing performance and the practical aspects of low cost, reproducibility, operational simplicity, and robustness (Yang et al., 2012; Raj et al., 2014). Recent decades have witnessed the development of enzyme-free biosensors for determination of a single analyte. However, little work has been aimed at developing enzyme-free biosensors that can realize simultaneous detection of multiple analytes with satisfactory performance (Bairagi and Verma, 2018; Ahmad et al., 2020). Therefore, more research devoted to developing non-enzymatic electrodes with high surface area and physical rigidity, coupled with chemical stability, is very worthwhile.

MXene, a new member of the 2D material family, composed of transition metal carbides, carbonitrides, and nitrides, was discovered in 2011 (Naguib et al., 2011). MXenes have inherently tunable surface functional groups (Khazaei et al., 2013), ultra-high water solubility (Sinha et al., 2018), and intercalation ability (Mashtalir et al., 2013), and have displayed great potential for electrochemical catalysis. MXenes are formed by selective etching of the A element layers from the ternary carbides and nitrides $M_{n+1}AX_n$, where M stands for an early transition metal, X stands for C and/or N, and A stands for a group IIIA or IVA element (Naguib et al., 2011; Dong et al., 2018). As a

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typical and the most studied case, $Ti_3C_2T_x$ exhibits great biocompatibility and metallic conductivity (up to 6500 S/cm), where T stands for a surface functional group, typically F^-, O^{2-}, or OH^- (Liang et al., 2015; Lu et al., 2018), which can easily induce an oxidation reaction in water and an oxygen environment at positive potential (Wang et al., 2016; Anasori et al., 2017). Moreover, MXene (Ti_3C_2) can be easily assembled with conducting polymers for enhanced electrical conductivity (Ling et al., 2014), of which chitosan (CTS), a non-toxic, biologically compatible, and chemically versatile polymer composite can further greatly enhance the mechanical properties of electrodes (Oh et al., 2009).

Recently, a group of transition metal oxides and sulfides has also attracted much attention as biosensors because of their ease of preparation, good reproducibility and reversibility, high stabilities, and fast response (Li ZJ et al., 2016; Li X et al., 2019). The gold working electrode, modified by NiO nanosheets, established abundant active sites for glucose sensing with no enzymes (Bairagi and Verma, 2018; Ahmad et al., 2020). The NiO/graphene (Rengaraj et al., 2015) and Cu_2S/Cu (Ji et al., 2014) exhibited excellent detection of cholesterol with a combined effect. Cuprous oxide (Cu_2O), a p-type semiconducting material with a cubic lattice (Grozdanov, 1994; Pagare and Torane, 2016), has demonstrated superior catalytic abilities towards cholesterol detection at a negative potential range (Khaliq et al., 2020), which can be separated from a glucose conventional sensing potential in the positive.

Herein, a free-standing and non-enzymatic MXene/CTS/Cu_2O electrode for simultaneous detection of glucose and cholesterol was fabricated by taking full advantage of MXene, CTS, and Cu_2O nanomaterials. The as-prepared electrode was formed through electrostatic interaction of MXene and CTS with opposite charges, followed by the electrodeposition of Cu_2O, and was utilized directly as the working electrode in a three-electrode electrochemical system. MXene demonstrated excellent bio-compatibility and electrical conductivity, acting as a steady substrate for electrochemical sensing with the combination of CTS. Cu_2O nanoparticles provided more catalytic active edges to enhance the sensitivity while contributing to the separation of the reaction potential. Effective interfacial junctions were formed through the synergistic effect of three components, facilitating charge transfer during reactions and providing easy access to detected species. The as-synthesized electrode was characterized by scanning electron microscopy (SEM), transmission electron microscope (TEM), X-ray diffraction data (XRD), and X-ray photoelectron spectroscopy spectra (XPS), and the sensing performance was examined by cyclic voltammetry (CV). This work offered a reliable approach for multi-metabolite sensing, and demonstrated remarkable sensitivity, selectivity, and repeatability for the simultaneous determination of glucose and cholesterol, with a great potential for clinical application.

2 Results and discussion

2.1 Structural characterization of MXene/CTS/Cu_2O

The schematic illustration for the prepared MXene/CTS/Cu_2O film is shown in Fig. 1 (details are provided in Figs. S1–S5 of electronic supplementary materials). A self-assembled ternary enzyme-free glucose and cholesterol electrocatalyst was successfully fabricated through a simple vacuum filtering and electrodepositing method, which was cheap and robust. The morphology of the as-synthesized film was characterized by TEM and SEM (Figs. S1a–S1e), revealing single and a few MXene layers of flat sheets, and verifying the successful mixture of CTS and the Cu_2O modification by electrodeposition (details are provided in Fig. S6). Furthermore, the crystal structure of the as-prepared samples and the chemical states and valence of composites were investigated by XRD and XPS, respectively (details are provided in Figs. S1f–S1h, S7, and S8).

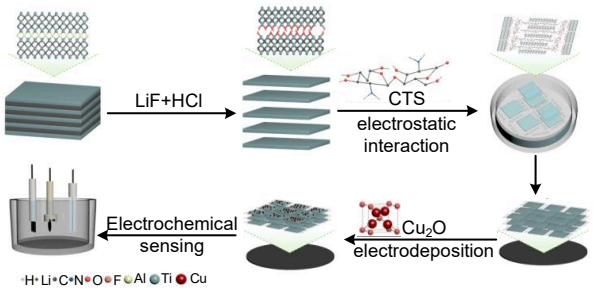

Fig. 1 Schematic illustration for the fabrication process of the self-assembled MXene/CTS/Cu_2O

2.2 Electrocatalytic characterization

The CV technique was used to investigate the electrocatalytic performance of the modified electrode towards the oxidation of glucose and cholesterol. Sodium hydroxide (NaOH) solution of 1 mol/L was chosen as the electrolyte (Fig. S4). To investigate the potential range, a series of tests was performed with the high potential from 0.35 to 0.80 V. As shown in Fig. S5, the range of −0.80 to 0.40 V was proved to be an appropriate range with a relatively high current response. To assess the electrocatalytic characteristics towards glucose and cholesterol sensing, CV curves of binary and ternary electrodes are compared in Fig. 2. The MXene/CTS electrode did well in glucose sensing, whereas it had no current response to cholesterol. As shown in Fig. 2b, with the aid of Cu_2O, the ternary electrode can establish a clear current change with the addition of cholesterol and glucose at peak I and peak VI, respectively, which were selected as the detection peaks (details are provided in Figs. S6, S7, and S9).

For the simultaneous detection of glucose and cholesterol in biofluids, non-interference between substances is essential for the multi-analyte electrochemical electrode. As shown in Fig. 3, the mutual interference between glucose and cholesterol was investigated in a mixture solution under the optimized experimental conditions, where the concentration of one analyte was changed while that of the other was kept constant. The results show that the currents (I) at peak VI increase linearly with increasing concentrations (C) at a constant cholesterol concentration of 0.1 mmol/L, resulting in a corresponding linear regression equation:

$$I\,(\text{mA})=0.0573\times C\,(\text{mmol/L})+0.370\ (R^2=0.99207). \quad (1)$$

This shows a good linear fitting curve with a sensitivity of 60.295 μA·L/(mmol·cm^2) in the linear range of 52.4 to 2000 μmol/L with the limit of detection (LOD) at 52.4 μmol/L (signal-to-noise ratio (SNR)=3). Similarly, the currents at peak I of cholesterol sensing are determined at a constant glucose concentration of 1 mmol/L. The linear regression equation is as follows:

$$I\,(\text{mA})=0.205\times C\,(\text{mmol/L})-0.444\ (R^2=0.99838). \quad (2)$$

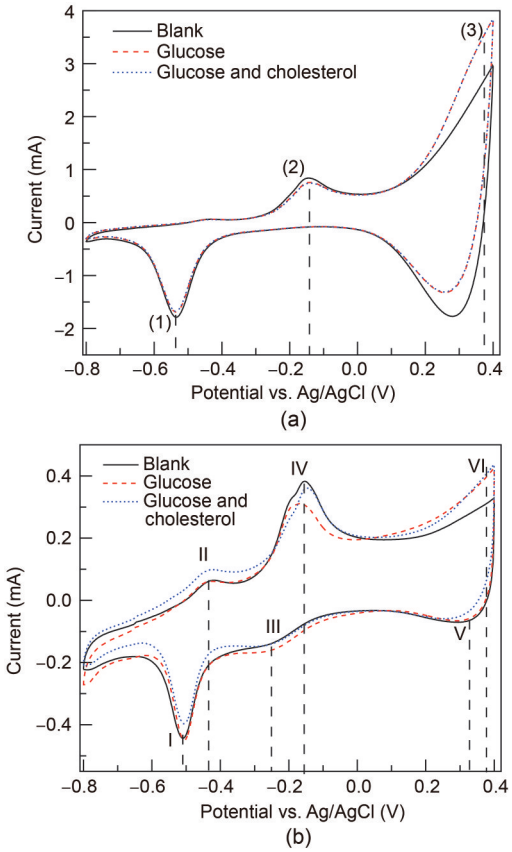

Fig. 2 CV curves of MXene/CTS (a) and MXene/CTS/Cu_2O (b) electrodes for 1 mmol/L glucose and 0.2 mmol/L cholesterol detecting with labeled peaks (peaks (1) and (2): pseudocapacitance of MXene; peak (3): Ti^{2+} to Ti^{4+} at 0.35 V; peak I: Cu^+ to Cu; peak II: Cu to Cu^+; peak III: Cu^{2+} to Cu^+; peak IV: Cu^+ to Cu^{2+}; peak V: Ti^{4+} to Ti^{2+}; peak VI: Ti^{2+} to Ti^{4+} at 0.35 V) (Wang et al., 2015; Boota et al., 2017)

The LOD is 49.8 μmol/L (SNR=3) and the sensitivity reaches 215.71 μA·L/(mmol·cm^2) in the linear range of 49.8 to 200 μmol/L. It is shown that the MXene/CTS/Cu_2O electrode can discriminate the potentials of glucose and cholesterol for electrochemical detection in the mixture with no synergistic effect, thus ensuring the validity of the sensor.

As shown in Fig. 4, the ability for simultaneous determination by the MXene/CTS/Cu_2O electrode was further investigated by CV measurements with the concentrations of the two analytes varying at the same time. Both the peak currents increase gradually with increasing concentrations of glucose and cholesterol, which confirms that the proposed electrode can be used as an electrocatalyst and dual-function biomimetic nanoenzyme, showing good sensitivity and selectivity for the detection of glucose and cholesterol.

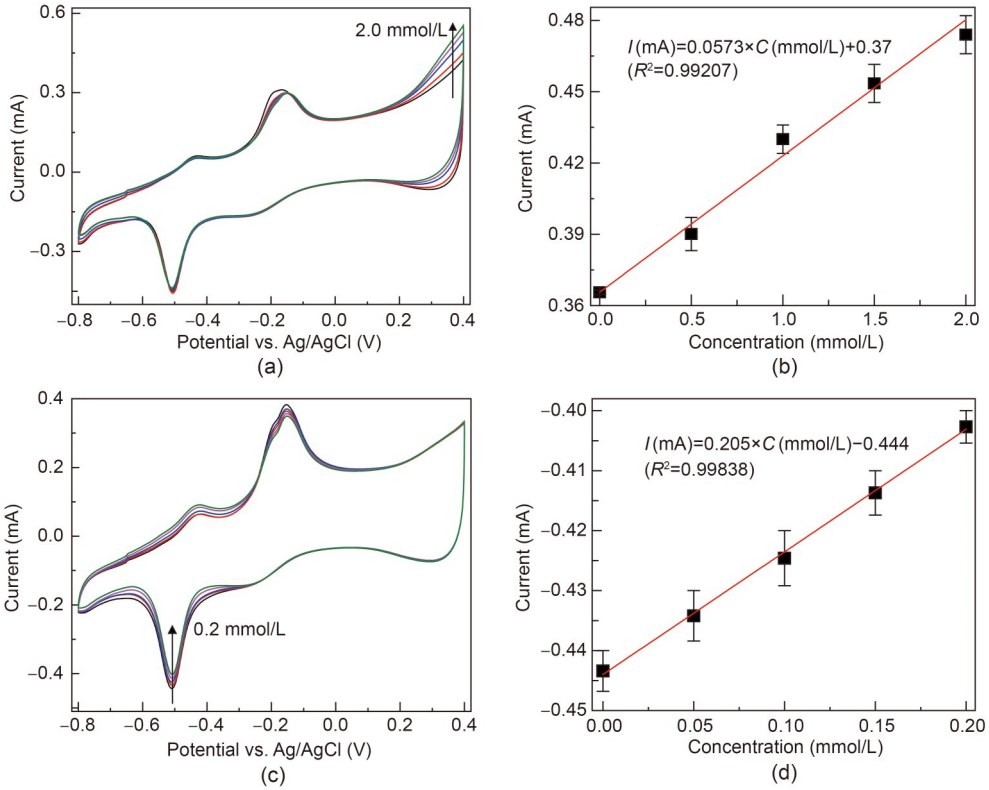

Fig. 3 CV curves of MXene/CTS/Cu_2O with 0.1 mmol/L cholesterol towards glucose sensing (glucose: from 0 to 2 mmol/L at 0.5 mmol/L interval) (a) and with 1 mmol/L glucose towards cholesterol sensing (cholesterol: from 0.0 to 0.2 mmol/L at 0.05 mmol/L interval) (c); plotted relationship curves of MXene/CTS/Cu_2O between electrocatalytic current and glucose concentration (b), and between electrocatalytic current and cholesterol concentration (d)

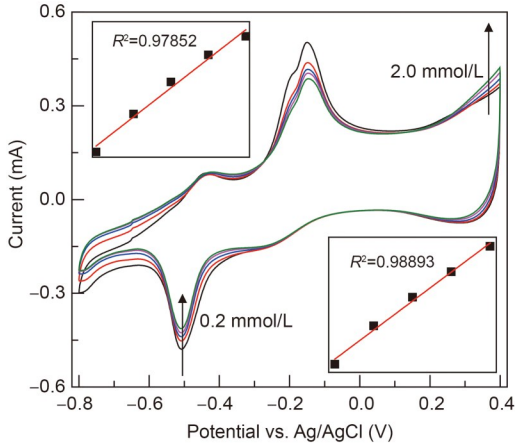

Fig. 4 CV responses of MXene/CTS/Cu_2O for simultaneous detection of glucose and cholesterol (adding 0.5 mmol/L glucose and 0.05 mmol/L cholesterol each time). The inset plots are corresponding peak current fitted lines (top: glucose; bottom: cholesterol)

2.3 Electrocatalytic mechanism

The obtained results demonstrate that the ternary MXene/CTS/Cu_2O electrode exhibits superior electrocatalytic performance towards glucose and cholesterol sensing and so has the potential for on-site and real-time multi-analyte monitoring. The irreversible oxidation of MXene upon exposure to an anodic potential over 0.2 V is overcome by the addition of CTS (Lorencova et al., 2017), which is attached to MXene layers through electrostatic and hydrogen-bonding interactions between its active amino groups and the OH, O, and F groups on the surface of the MXene nanosheets (Song and Wang, 2020). The increased specific surface area ensures the reversible redox action of elemental Ti for glucose detection. Based on the results, we propose the following equation for the oxidation of glucose at peak VI:

$$2TiO_2+C_6H_{12}O_6\ (\text{glucose})+2H_2O\rightarrow 2Ti^{3+}+6OH^-+C_6H_{10}O_6\ (\text{glucolactone}). \quad (3)$$

For cholesterol sensing, Cu_2O nanoparticles act as the electrocatalyst and play vital roles in efficient electron transport. The predominance of transition

metal species in electrocatalysts arises because of their unpaired d-electrons and unfilled d-orbitals which are available for forming bonds with absorbates, which can be considered as ligands to the central transition metal ion acting as the catalyst center (Pletcher, 1984). Moreover, the low redox potential for cholesterol sensing at peak I can greatly reduce the interference of other common species in biofluids, increasing the practicality of the biosensor for clinical application. There is published work supporting the view that hydroxyl radicals (·OH) can initiate the cholesterol autooxidation to produce oxysterols (Lee et al., 1997; Khaliq et al., 2020). Through the analysis of experimental phenomena (details are provided in Figs. S8 and S10), the redox action can be deduced as follows:

$$Cu^{+}+OH^{-}+e^{-}\rightarrow Cu+\cdot OH, \quad (4)$$

$$\cdot OH+Cholesterol\rightarrow Oxysterol. \quad (5)$$

In short, well-dispersed Cu_2O nanoparticles on an MXene/CTS substrate can provide abundant metal active edges and promote heterogeneous charge transfer and reactivity. With increasing concentrations of cholesterol, more Cu_2O particles are consumed, leading to a decreased signal. MXene/CTS film with high specific surface area supplies more channels for diffusion of ions and establishes easy access for glucose and cholesterol, accelerating the electron transfer rate and enhancing the conductivity of the composite. The positively charged CTS acts as an effective binder to enhance the interfacial interaction between the negatively charged MXene sheets, greatly improving the mechanical strength of the electrode (Fig. S9). The synergistic effect among these three components contributes to the particular electrocatalytic performance of the MXene/CTS/Cu_2O electrode. Table S1 shows the comparison of key performance indicators between our electrode and other biosensors. Evidently, the coupling and coordinated nanoarchitecture exhibits competitive electrocatalytic performance, and meets the need for non-enzymatic multianalyte detection. The CV curves measured at different scan rates from 10 to 60 mV/s are shown in Fig. S10, illustrating the diffusion-controlled process for glucose and cholesterol detection.

2.4 Interference studies and practical applications

Interference tests were conducted to evaluate the selectivity of the MXene/CTS/Cu_2O towards glucose and cholesterol. The experiments were conducted with additions of 0.5 mmol/L sucrose (SC), uric acid (UA), acetaminophen (APAP), lactose (LT), NaCl, ascorbic acid (AA) or L-cysteine (L-c). The amperometric response curves were recorded at peak VI and peak I (vs. Ag/AgCl) with 0.5 mmol/L glucose and 0.05 mmol/L cholesterol as the final addition, respectively. The peak current value of 1 mol/L NaOH electrolyte was selected as the blank current. As shown in Fig. 5, considering the excessive concentration of interfering species added, the current responses of glucose and cholesterol are still the most significant,

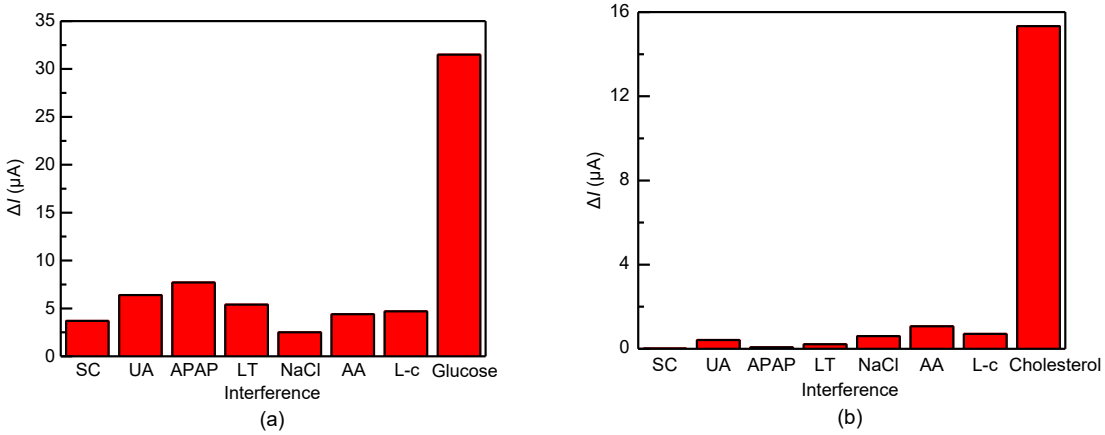

Fig. 5 Current responses of the MXene/CTS/Cu_2O electrode to the addition of interfering agents (0.5 mmol/L): sucrose, uric acid, acetaminophen, lactose, NaCl, ascorbic acid, and L-cysteine at the potential for: (a) glucose sensing (0.5 mmol/L); (b) cholesterol sensing (0.05 mmol/L)

demonstrating selectivity toward glucose and cholesterol detection.

The practical application of MXene/CTS/Cu_2O biosensor was investigated by testing the glucose and cholesterol concentrations in human serum samples which were provided by Zhongda Hospital in Nanjing, China. The normal concentrations of glucose and cholesterol in the human body are 5.0 and 5.2 mmol/L, respectively. The total cholesterol contains a mixture of free and esterified cholesterol in serum. Less than 10% of cholesterol exists in the free state (Shih et al., 2009; Raj et al., 2014) and it can be oxidated directly by the proposed non-enzymatic sensor without the need for cholesterol esterase and cholesterol oxidase (Huang et al., 2011; Dey and Raj, 2013; Patil et al., 2018). Therefore, the serum sample can be tested within the linear range at 20-fold dilution, ensuring minimal sample requirements. The spike and recovery method was employed and the analytical results are listed in Table 1. The results obtained suggest that the developed biosensor based on MXene/CTS/Cu_2O electrode exhibits suitability for simultaneous detection of glucose and cholesterol in actual samples and offers high potential for practical applications in clinical diagnosis.

Table 1 Simultaneous detection of glucose and cholesterol samples in 5% human serum with the MXene/CTS/Cu_2O electrochemical biosensor

Sample	Metabolite	Concentration (mmol/L)		Recovery (%)
		Added	Measured	
1	Glucose	0.5	0.505	100.92
	Cholesterol	0.1	0.098	98.04
2	Glucose	0.5	0.494	98.71
	Cholesterol	0.1	0.103	102.94

3 Conclusions

In summary, a free-standing MXene/CTS/Cu_2O electrode was formed through electrostatic interaction of MXene and CTS with opposite charges, followed by the electrodeposition of Cu_2O. Taking advantage of the synergistic function of MXene/CTS layers and Cu_2O nanoparticles, this ternary electrode exhibits excellent sensing capabilities for glucose and cholesterol with preferable linear ranges that can cover the full concentration range in clinical diagnosis. For glucose sensing, the sensitivity was 60.295 μA·L/(mmol·cm^2) with LOD being 52.4 μmol/L (SNR=3), while a sensitivity up to 215.71 μA·L/(mmol·cm^2) and LOD low to 49.8 μmol/L (SNR=3) were achieved for cholesterol detection. Additionally, this biosensor possesses superior anti-interference ability and reproductivity, and thus exhibits great potential for genuine sample analysis. Accordingly, the as-prepared enzyme-free MXene/CTS/Cu_2O electrode acts as a biomimetic electrocatalyst with excellent performance for analysis of multiple metabolites, and overcomes the disadvantages of an enzyme-based biosensor. This work has proposed a versatile strategy for designing and fabricating self-assembled nanocomposite materials with tuned structural and functional properties. It is a first attempt which could be easily integrated into portable electrochemical devices, facilitating effective routine monitoring of blood metabolites and paving the way for commercialization and point-of-care testing.

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Author contributions

Tao HU designed the research. Man ZHANG processed the corresponding data. Hui DONG and Tong LI participated in the methodology and discussion. Xiao-bei ZANG provided the original technique of experiments and reviewing. Man ZHANG wrote the first draft of the manuscript. Zhong-hua NI and Xiao LI helped to organize the manuscript. Man ZHANG and Xiao LI revised and edited the final version.

Conflict of interest

Tao HU, Man ZHANG, Hui DONG, Tong LI, Xiao-bei ZANG, Xiao LI, and Zhong-hua NI declare that they have no conflict of interest.

Ethical approval

All procedures were in accordance with the ethical standards of the Responsible Committee on Human Experimentation (Institute of Process Engineering, Chinese Academy of Sciences, China) and with the Helsinki Declaration of 1975, as revised in 2008(5). Informed consent was obtained from all patients for being included in the study.

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Electronic supplementary materials

Table S1, Fig. S1, Fig. S2, Fig. S3, Fig. S4, Fig. S5, Fig. S6, Fig. S7, Fig. S8, Fig. S9, Fig. S10