Rapid and selective electrochemical detection of Pb^{2+} ions using aptamer-conjugated alloy nanoparticles

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Abstract

Heavy metals are an important class of technogenic toxicants that pollute rivers and underground water. Here we proposed the use of electrochemical methods using silver (Ag)–gold (Au) alloy nanoparticle (NP)–aptamer-modified glassy carbon electrode (GCE) for the detection of lead ions (Pb^{2+}). Alloy Ag–Au NPs were electrodeposited on the electrode using the potentiostatic double-pulse method. The NPs were characterized by high-resolution transmission electron microscopy, which revealed that the average NP size was 44 nm. The binding of Pb^{2+} induced conformational changes of the aptamer and corresponding modulation of electrochemical signals. As a result, we were able to detect Pb^{2+} with a straight slope of 0.01–10 $\mu\text{g/L}$ with limits of detection (LOD) of $0.03 \times 10^{-2} \mu\text{g/L}$ for CV, DPV and $0.04 \times 10^{-2} \mu\text{g/L}$ for EIS. The minimum detectable Pb^{2+} concentration was calculated to be 0.8 μM .

Graphic abstract

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1 Introduction

The analysis of heavy metals at very low levels in drinking water has become a globally high-priority area of research [1, 2]. Metal quantification at the microgram and nanogram level in water was a challenging assignment before the development of such systems as flameless atomic absorption spectrophotometry and differential pulse anodic stripping. These methods can measure the total metal contents in a sample. However, assessing the bioavailable concentration of heavy metals in a more convenient way remains a great challenge. The scientific community has turned its attention towards nanobiosensor research to overcome this challenge.

In general, metal nanoparticles (NPs) exhibit very good conductivity and catalytic properties. These two features make them appropriate to improve the electron exchange between redox-active groups in biomolecules and electrode surfaces. Metal NPs can also act as catalysts, promoting electrochemical reactions. Recently, there have been some reports on the evaluation of the application of nanoparticles to electrochemical biosensors [3–6].

Recently, alloy, core–shell nanoparticles and biometallic nanostructured material played important role for design of electrochemical biosensor because of their excellent optical, magnetic, and catalytic properties. They offer better sensing properties as compare to single nanometallic material [7–9]. The conjugation of metal particles Pb^{2+} with bioreceptors, for example, proteins, antibodies, or nucleic acids, can increase the selectivity and lower the limit of detection (LOD) of nanobiosensors [10, 11].

The reasons for selecting silver (Ag) and gold (Au) to develop bimetallic NPs are (i) a metal of a higher valency can dissolve in one with lower valency (i. e. valency of Ag and Au are 1 and 3, respectively) [12] and (ii) the tunability of bimetallic NPs means they show potential applications in biosensors, drug delivery, and cell labeling [13, 14]. Recently, several developments were aimed at the detection of lead ions (Pb^{2+}) in water [15–19]. For example, Shen et al. [20]. Constructed an electrochemical biosensor using an Au NP–oligonucleotide-modified glassy carbon electrode (GCE) for detection of Pb^{2+} in water. The electrode achieved a low LOD of 1 nM. However, electron flow prompted direct oxidation of nucleotides, which resulted in incorrect estimation of Pb^{2+} levels. Currently, biosensors have constraints regarding strength, reaction time, and precision, so their further development is required.

Different DNAzyme-based biosensors have been produced for detection of Pb^{2+} , including ones designed

detection of Pb^{2+} and including those designed for colorimetric electrochemical and fluorescence detection [21–25]. These sensors do not require complicated pretreatment, costly delivery of samples to central research facilities with a long turnaround time, or rather complex laboratory techniques. However, the LODs of conventional sensors are not satisfactory. Thus, the development of a high sensitivity nanobiosensor for Pb^{2+} in water with good stability and low LOD is still desired.

The novelty of present work is that Ag-Au modified electrode possesses excellent biocompatibility with a variety of biomolecules, such as aptamers, antibodies, enzymes, DNA, cells, and proteins [26].

Aptamers are short, single-stranded oligonucleotides (DNA or RNA) that bind to targets with high affinity and specificity by folding into tertiary structures as compare to traditional nucleic acids. A combinational selection process called systematic evolution of ligands by exponential enrichment (SELEX). The SELEX is process used to select aptamer from large oligonucleotide library. There are several advantages in using aptamers instead of enzymes/antibodies, including having the ability to be modified chemically, chemical stability, and flexibility for being used in biosensors [27].

An aptamer contains various groups that can interact with metal particles, such as exocyclic keto, ribose hydroxyl, ring nitrogen, and negatively charged phosphate-based groups [28]. For example, the N_7 atoms of purines can directly bind to bases and indirectly bind to phosphate groups. These binding sites of aptamers are responsible for their ability to sense metal ions. A literature survey revealed that electrochemistry is a useful technology for studying bimolecular interactions based on ligand binding interaction-induced changes in electrode properties.

The many metals are known to be of specific physiological function to life systems [29]. These metals show preference for certain types of ligands. The most common types of ligands are the amino acids, especially cysteine and the porphyrins. The main function of the porphyrins is to bind metal atoms which act as centres for significant electrochemical or biochemical events. Electrochemical reduction or oxidation occurs at the surface of ligand modified electrode for detection heavy metals is reported by many researcher [30].

The nanobiosensor has great potential for on-site detection of multiple heavy metals. The nanotechnology provides the solution of challenges faced by traditional techniques. The nanobiosensor could able to

improve the sensitivity, limit of detection, selectivity, and reproducibility. Nanoparticle-modified electrodes have higher surface area to volume ratio, improved electron transfer rate, increased mass-transport rate, less solution resistance, and higher signal-to-noise ratio [31–33]. The nanoparticle-aptamer modified glassy carbon electrode significantly reduces the memory effect, foreign ions, detection limit and sensitivity of nanobiosensor [34, 35]. For example, the Au NP-modified glassy carbon electrode significantly lowered the LOD toward Hg^{2+} and As^{3+} and its sensitivity is much higher than other sensor [36, 37]. Recently, bimetallic nanoparticles are applied for modification of surface of GCE and showed excellent results for detection of heavy metals using different bioreceptors due to higher electroactive surface area.

The objective of the present work is to detect Pb^{2+} in drinking water by cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) using a GCE modified with Au–Ag alloy NPs and an aptamer.

2 Materials and methods

2.1 Materials and reagents

Silver nitrate (AgNO_3) and chloroauric acid were purchased from Sigma-Aldrich (India). An aqueous solution of Pb^{2+} (1 g/L) was purchased and used as a stock solution. (Thiol-C6 GGGTGGGTGGGTGG) was obtained from Sigma-Aldrich. G-quadruplex electrochemical biosensors have received particular attention, since the electrochemical response is particularly sensitive to the DNA structural changes from single-stranded, double-stranded, or hairpin into a G-quadruplex configuration. Furthermore, the development of an increased number of G-quadruplex aptamers that combine the G-quadruplex stiffness and self-assembling versatility with the aptamer high specificity of binding to a variety of molecular targets allowed the construction of biosensors with increased selectivity and sensitivity [38]. The stock solution of aptamer sequence Thiol-C6-GGGTGGGTGGGTGG (40 mg/L) in phosphate buffered saline (PBS, pH = 7.4) was kept at -20°C . All experiments were performed at room temperature using Milli-Q water with a conductivity of less than $1\ \mu\text{g/L mho}$ and pH of 5.6. Detection of Pb^{2+} from the real water samples was carried out with the help of CV, DPV and EIS methods. The optimization experiments are shown in Fig. 1. (a) Optimization pH value (b) Optimization of aptamer different concentration for sensing of Pb^{2+} (c) time on the stability of (Ag–Au) NP-aptamer-modified GCE.

2.2 Apparatus

A Palmsan3 potentiostat system with microcode version 1.8 was used to conduct CV, DPV, and EIS measurements. The size and shape of the synthesized NPs were characterized by high-resolution transmission electron microscopy (HRTEM) using a Techani G230S electron microscope (TWIN Company FEI).

2.3 Synthesis and characterization of the bimetallic alloy NPs

Solutions of AgNO_3 and chloroauric acid at 1:3 volume ratio were mixed in a flask and purged with nitrogen gas continuous 10 min. To prevent the photochemical reaction of AgNO_3 , the flask was completely covered with black cloth during the reaction. The solution was stirred for 10 min using a magnetic stirrer. Deposition of bimetallic alloy NPs on the GCE was conducted in 0.1 M NaNO_3 solution by differential pulse voltammetry (DPV) [39]. In this experiment the potential range from 0 to +800 mV was selected using sweep rate of $100\ \text{mVs}^{-1}$ for 20 cycles.

2.4 Surface modification and characterization of the modified GCE

The surface modification of the GCE disk working electrodes was polished with $0.05\ \mu\text{m}$ silver powder. The experiment was carried out in aqueous media in the potential range from 0 to +800 mV using a sweep rate of $100\ \text{mVs}^{-1}$ for 20 cycles. The thiolated aptamer ($3\ \mu\text{g/L}$) was pipetted onto the GCE modified with Au–Ag NPs and then incubated overnight at 25°C in 100% humidity. After flushing with 10 mM PBS (pH 7.4), the electrode was washed with Milli Q water to rinse away nonspecific adsorption sites. The modified electrode was used for direct detection of Pb^{2+} in real-life water samples.

2.5 Electrochemical detection of Pb^{2+}

The interaction of Pb^{2+} with the modified electrode was measured in terms of oxidation and reduction peak currents, the peak current in CV, and DPV and peak value in EIS measurements. All responses were measured until saturation of the sensor signal. The electrochemical reactions of Pb^{2+} in Milli Q water and environmental water tests were recorded in 0.1 M citrate solution (pH 6). For each experiment, a fresh electrode was prepared.

2.6 Recovery tests and assay of real water samples

Real water samples were collected from the Agra ground water, Mathura ground water, Chambal river water, Gwalior

Fig. 1 **a** Optimization of pH value **b** Optimization of aptamer different concentration for sensing of Pb²⁺ **c** time on the stability of (Ag–Au) NP–aptamer-modified GCE

water, Delhi Ground water and Meerut ground water (north region of India). Each sample was collected in plastic tubes and kept frozen at –20 °C prior to analysis. Before the electrochemical experiments, all the water samples were heated at 25 °C and centrifuged 15 min at 8500 rpm to remove denaturated high molecular organic compounds. The supernatant was filtered through 0.22 μm filter Millex GP by Millipore. The prepared water samples were tested by Atomic absorption spectroscopy (AAS) method to estimate Pb²⁺ concentration. The present value Pb²⁺ in all water samples are presented in Table 1.

3 Results and discussion

3.1 TEM studies of native and modified NPs

HRTEM was used to investigate the structure, morphology, and size distribution of the Ag–Au bimetallic NPs and their binding with the aptamer. Figure 2a shows an HRTEM image of the spherical NPs, which are homogeneously dispersed with an average size of 44 nm. The NPs were physically conjugated with the aptamer. Figure 2b shows the diameter of NPs increases when bound with aptamer and Pb²⁺.

Table 1 Determination of Pb²⁺ in real water samples by CV, DPV, and EIS

Sample	Present (µg/L)	Added (µg/L)	CV cycle found (µg/L)	Recovery (%)	DPV Found (µg/L)	Recovery (%)	EIS Found (µg/L)	Recovery (%)
Milli-Q water	0	0.1	0.096	96	0.093	93	0.107	107
		0.5	0.49	98	0.48	96	0.49	98
Agra water	167	0.1	166.9	93	166.9	99	166.9	94
		0.5	166.5	94	166.4	102	166.5	97
Mathura water	321	0.1	320.8	102	320.9	93	320.9	99
		0.5	320.5	97	320.4	106	320.4	105
Chambal water	623	0.1	622.9	92	622.9	98	622.9	93
		0.5	622.5	95	622.5	92	622.5	99
Gwalior water	123	0.1	122.9	98	122.9	95	122.9	92
		0.5	122.5	94	122.5	98	122.5	99
Delhi water	350	0.1	349.9	93	349.9	96	349.8	102
		0.5	349.5	96	349.5	95	349.5	96
Meerut water	231	0.1	230.9	92	230.9	92	230.9	92
		0.5	230.5	97	230.4	103	230.5	94

Fig. 2 **a** HRTEM image of bimetallic alloy (Ag-Au) NPs without aptamer. **b** TEM image of NPs with aptamer and bound Pb²⁺

3.2 Molecular mechanism of the modified electrode action

A schematic diagram of the interaction of Pb²⁺ with the Ag-Au NP–aptamer-modified GCE is shown in Fig. 3. The electronic configurations of Ag and Au are 4d¹⁰5s¹ and 6s¹, respectively, so they both have their valence electron in s¹. The oxidation potentials of Ag and Au are –0.799 and –1.50 V vs SHE, respectively. Ag and Au can easily form alloys because of their similar atomic radii. In addition, Ag and Au both easily lose an electron, resulting in favorable electrochemical properties and endowing sensors with high sensitivity and selectivity. The electrostatic attractive force between the bimetallic NPs and GCE aided the uniform deposition of NPs. More details of the Pb²⁺–aptamer interaction can be found elsewhere [40–45].

The overall reaction and its standard cell potential at 25 °C and 1atmospheric pressure for the reaction involving the galvanic cell in which Pb metal is oxidized to 1 M Pb ion and a half-cell consisting of an silver electrode in 1 M. This could be taken relative to standard hydrogen electrode (SHE). The reduction of Pb²⁺_(aq) to Pb_(s) is a straight transfer of two electrons shown in Eq. (3.1):

Differential pulse voltammetry markedly increased the signal-to-noise ratio for lead deposition and better allowed detection of low levels of lead in the solution and its corresponding binding with modified electrode

Fig. 3 Schematic illustration of the alloy (Ag–Au) NP–aptamer-modified GCE for Pb^{2+} detection

because it successfully minimized the double layer capacitive current [46].

The Ag–Au bimetallic NPs have an electronic polarity as a result of the $5s^1$ electron of Ag and $6s^1$ electron of Au. This polarity attracts the charged aptamer and promotes a loading platform for the attachment of the aptamer on the surface of the GCE. This process formed a large number of binding sites for metal ions for electrochemical detection. These binding sites help to increase the sensitivity and stability of the electrode and lower its LOD for metal ions. In the present case, Pb^{2+} bind negatively charged oxygen atoms of the phosphate backbone and with selected groups of nucleobases (e.g., N_7 and O_6 of guanine) to form $\text{Pb}(\text{OH})^+$ [47].

In the aptamer, the electrostatic repulsion between the phosphate groups is responsible for its conformation. This repulsion is alleviated by binding of Pb^{2+} , which leads to a change of aptamer structure. The binding of Pb^{2+} with phosphate groups in the G-quadruplex aptamer showed different electrochemical responses due to the different labeling positions and G-quadruplex structures. G-quadruplex secondary structures (G_4) are formed in nucleic acids by sequences that are rich in guanine. They are helical structures containing guanine tetrads that can form from one, two or four strands.

3.3 Detection of Pb^{2+} by CV

The modified electrode was used to investigate redox processes, reaction intermediates, quantitative analyte determination, and the stability of reaction products. Three electrode system (i.e. modified GCE as a working

electrode, pt as a counter electrode and Ag/AgCl as a reference electrode) have been used for detection of Pb^{2+} in water. The varying potential was applied to the modified GCE in both forward and reverse directions at 0.05 s.

In this process, the change in the measured current upon exposure of the electrode in different concentrations of Pb^{2+} has been monitored. The CV response of the electrode to different concentrations of Pb^{2+} in Milli Q water and real water samples is shown in Fig. 4a. The peak current increases gradually as the concentration of Pb^{2+} increases. The calibration plot in Fig. 4b revealed the good linear relationship between its electrochemical response (i.e., current) and Pb^{2+} concentrations ranging from 0.10 to 10 $\mu\text{g/L}$, with a coefficient of determination of 0.97. The LOD for Pb^{2+} was found to be $0.03 \times 10^{-2} \mu\text{g/L}$ by CV. Good recoveries of Pb^{2+} from different water samples of between 100 and 105% were obtained (Table 1).

3.4 Detection of Pb^{2+} by DPV

In the DPV method, the potential is scanned with a series of pulses. The electrochemical current is estimated at two points for each pulse; one is before the pulse and the other is toward the end of a pulse. These two points are used to measure the decay of the charging current. The difference of potential between these two points was determined and plotted against the base potential for different concentrations of Pb^{2+} (Fig. 5a). The DPV calibration plot in Fig. 5b showed a linear relationship between charging current for different Pb^{2+} concentrations ranging from 0.10 to 10 $\mu\text{g/L}$ with a coefficient of determination of 0.97. The scan rate

Fig. 4 Cyclic voltammetry measurements showing Pb^{2+} detection by the modified electrode: **a** Cyclic voltammetry measurements showing Pb^{2+} detection by the modified electrode and **b** calibration

plot of cyclic voltammetry measurements showing Pb^{2+} detection by the modified electrode

Fig. 5 DPV measurements showing Pb^{2+} detection by the modified electrode: **a** DPV measurements showing Pb^{2+} detection by the modified electrode and **b** calibration plot of DPV measurements showing Pb^{2+} detection by the modified electrode

was 50 mVs. All experiments were carried out at 25 °C. This result shows that the charging current is functions of Pb^{2+} concentration and pulse potential. The LOD for Pb^{2+} for the modified electrode in DPV measurements calculated from the calibration plot in Fig. 5b was $0.03 \times 10^{-2} \mu g/L$. The recoveries for this method ranged from 92 to 106% (Table 1).

3.5 Detection of Pb^{2+} by EIS

EIS measurements provided the change in impedance of the modified electrode at different concentrations of Pb^{2+} . The semicircle part of the Cole–Cole plot in Fig. 6a shows the electron transfer resistance in the higher frequency region, whereas the linear region corresponds to that of

the diffusion-limited process. The diameter of the semi-circle part increased with Pb^{2+} concentration. This result indicates that the electron transfer resistance increased because Pb^{2+} hindered the access to the redox probe at the modified electrode surface, reflecting the change in the interfacial charge-transfer resistance (R_{ct}) of the aptamer-conjugated bimetallic Ag–Au NP-modified GCE. R_{ct} is an important parameter to achieve a low LOD for Pb^{2+} . The calibration plot obtained from the EIS data is presented in Fig. 6b. We have measure the height of high peak in Fig. 6a at the scan rate was 50 mV s. The calibration plot is a straight line that implies that the impedance increases with the concentration of Pb^{2+} in the water samples. A proportional relationship was achieved between R_{ct} and Pb^{2+} concentration in the range of 0.01–1 $\mu\text{g/L}$ with

a coefficient of determination of 0.97. The LOD was calculated to be 0.04 $\mu\text{g/L}$ based on 3 s per slope, where S is the standard deviation of a blank sample measured ten times. The recoveries (Table 1) for real-life water samples ranged from 92 to 107%, indicating the suitability of the modified GCE for detection of Pb^{2+} .

3.6 Analytical parameters

Analytical parameters such as coefficient of determination, LOD, and LOQ were evaluated from calibration plot [48]. Linear calibration plots were obtained over the concentration range from 0.01 to 10 $\mu\text{g/L}$ using ten standard solutions and three replicates per standard. The linearity of standard curves obtained for CV, DPV, and EIS

Fig. 6 **a** Electrochemical impedance spectroscopy (EIS) measurements showing Pb^{2+} detection by the modified electrode and **b** calibration plot of EIS measurements showing Pb^{2+} detection by the modified electrode

Table 2 Analytical parameters using the peak area in pb^{2+}

Calibration plot and analytical parameters using the peak area in Pb^{2+}			
Using the peak area	Cyclic voltametry	DPV	EIS
coefficient of determination (r^2)	0.97	0.99	0.99
LOD ($\mu\text{g/L}$)	0.03×10^{-2}	0.03×10^{-2}	0.002×10^{-2}
LQD ($\mu\text{g/L}$)	0.09×10^{-2}	0.1×10^{-2}	0.006×10^{-2}

Table 3 Limits of detection (LOD) of different sensor systems for Pb^{2+}

S.N	Species	Detection method	LOD ($\mu\text{g/L}$)	Linear range ($\mu\text{g/L}$)	Refs
1	Pb(II)	Electrochemical	1.05	0–100	[45]
2	Pb(II)	Electro-chemiluminescence	0.011	0.25–1.0	[46]
3	Pb(II)	DLS detection	0.035	0.1–1.0	[47]
4	Pb(II)	Photoelectrochemical	0.1	0.5–20	[48]
5	Pb(II)	Photoelectrochemical	0.05	0.1–50	[49]
6	Pb(II)	Present work	0.03×10^{-2}	0.1–10	

measurements was confirmed by their high r^2 values, as shown in Table 2. This conversion is a purely statistical that allows for the direct comparison of the results with standard values. The units of a standard deviation are identical to the base units of the data from which the standard deviation was calculated. Table 3 lists the LODs of different sensor systems for Pb^{2+} and demonstrates competitive advantages of the proposed nanobiosensor as compared with the existing ones.

3.7 Selectivity and interference studies

The effects of interfering elements on Pb^{2+} detection by the modified GCE were evaluated. The response of the modified electrode was recorded in Pb^{2+} solutions containing other ions. In this experiment, Cd^{2+} , Cr^{3+} , Na^+ , K^+ , Ni^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , and Cu^{2+} were chosen as interfering ions to investigate the selectivity of the modified electrode. The results demonstrated that the intensities of signals from these interfering elements were very small compared with those for Pb^{2+} . However, Cd^{2+} interfered slightly with Pb^{2+} detection.

3.8 Reproducibility and storage stability

The reproducibility of the response of the modified GCE was evaluated by comparing the anodic behavior in CV, DPV and EIS. The current in DPV was 0.069 μA and the imaginary part of impedance in EIS was 8.78 for 0.01 $\mu g/L$ Pb^{2+} solution under the optimized conditions. The relative standard deviation (RSD) calculated from the calibration plot drawn from the electrochemical techniques was around 0.98% for the modified electrode, revealing that the electrode possessed good reproducibility. The storage stability of the modified electrode was investigated by repeated DPV and EIS measurements of a solution containing Pb^{2+} . The electrode was stored at 4 $^{\circ}C$ and used for 20 days consecutively. DPV and EIS measurements were conducted under the same experimental conditions each day. The results displayed an RSD of 0.5%. Therefore, the modified GCE showed good storage stability and reproducibility.

4 DNA conformational changes improve the interaction between lead (Pb^{2+}) and aptamer

Pb^{2+} induced G-quadruplex (G4) complex have high sensitivity and selectivity (because of its smallest diameter of Pb^{2+} 130 pm) as compared with other heavy metal ions [49]. Pb^{2+} induced aptamer conformational changes from random coil to G-quadruplex (G4) with Ag–Au alloy

nanoparticles. This means that structural changes of aptamer, when Pb^{2+} attached with it. Or coil structure attain the quadruplex structure with alloy nanoparticles after binding of Pb^{2+} ions. Pb^{2+} induced aptamer conformational changes from random coil to G-quadruplex (G4) with Ag–Au alloy nanoparticles.

On the surface of modified electrode aptamer was self assembled by hybridizing with the G- rich and forming aptamer double helix structure. The Pb^{2+} -induced G-rich DNA aptamer duplex is formed by addition of Pb^{2+} , which is unwinds and forms a stabilizing G-quadruplex. Overall, Ag–Au alloy and aptamer modified GC electrode offer good charge transport capacity and large surface area which make sensor highly unique and highly sensitive and selective.

5 Conclusions

An Ag–Au alloy NPs aptamer-modified GCE with good storage stability and reproducibility was developed and used for detection of Pb^{2+} in real water samples without effects from interfering metal ions. The modified electrode contained numerous binding sites for Pb^{2+} because of the bimetallic NPs conjugated with an aptamer on the GCE surface. The interaction between Pb^{2+} and aptamer confirm the formation of G quadruplex structures by enhancement of electrochemical signal with concentration of Pb^{2+} . Therefore, Pb^{2+} diffused around the aptamer because of the strong electrostatic interactions between these materials and bound to the phosphate backbone, which stabilized the aptamer. The use of an aptamer and bimetallic NPs markedly enhanced the sensitivity of the GCE to around ten times that of a bimetallic NP-modified GCE without aptamer. The modified GCE has potential to contribute to new sensing applications in the field of nanobiosensor research. The developed nanobiosensor is rapid among all the existing techniques, due to its ease of use, high sensitivity and responsiveness.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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