Research Article

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Zeolite nanomaterial-modified dielectrode oxide surface for diagnosing Alzheimer's disease by dual molecular probed impedance sensor

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Abstract

Objectives: Alzheimer's disease (AD) is an irreversible and progressive neurogenerative disorder, which affects the learning part of brain. It mainly affects the aged population and becoming a global health issue, expecting to increase more in near future. Late diagnosis of AD worsens the situation and difficult to treat the patient. Various biosensing techniques with suitable biomarkers have been developed by researchers to diagnose the earlier stages of AD.
Methods: This research was focused to develop a highly sensitive zeolite-dual probe-modified impedance biosensor for identifying AD biomarker, Aβ Oligomer (AβO). The sensing surface was initially modified with zeolite through the chemical linker and then a dual probe of anti-AβO aptamer and anti-AβO antibody were attached to the surface of the zeolite.
Results: On these dual probe-modified surfaces, AβO was quantified to diagnose AD. Further, AβO spiked artificial CSF was identified by dual probes without any interference, indicating the selective identification of AβO. In addition, control experiments with non-immune, complementary, and control proteins failed to show the increment of responses, confirming the specific detection of AβO.
Conclusions: This zeolite-dual probe-modified biosensor helps to lower the limit of detection to 0.1 pM and diagnose AD at the earlier stages.

Keywords: Alzheimer's disease; Aβ Oligomer; biosensor: nanomaterial; impedance spectroscopy

Introduction

Alzheimer's disease (AD) is a neuro-disorder, a common most cause of memory loss and other cognitive-related abilities [1, 2]. AD is responsible for 60–80 % of dementia, begins in the learning part of the brain and advanced AD caused severe symptoms, which include memory loss, behavior and mood changes, confusion, and difficulty walking, speaking, and swallowing [3]. AD is not happening suddenly, changes in the brain occur a decade or more before the symptom appears. During the earlier stages of AD, toxic changes such as abnormal protein build-ups form the tau tangles and amyloid plaques in the brain. In addition, healthy neurons stop their function, lose connection with neurons, and sometimes die. Various complex brain changes have occurred during the process of AD. If more neurons die, other parts of the brain also die and started to shrink. Identifying AD at its earlier stages helps to prevent the death of neurons and extend the patient's lifespan with a healthy lifestyle. Various biosensors are developing with different

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biomarkers to identify AD at its earlier stages [4–9]. Improving biosensors is mandatory to enhance analytical performances. In this research, authors introduced a highly sensitive zeolite nanomaterial-based impedance spectroscopy to identify the AD biomarker Aβ Oligomer (AβO).

Application of nanomaterials in biosensor enhance the biomolecule interaction on the sensing surfaces [10–12]. Nanomaterial-based biosensors utilize unique physical and biological properties to facilitate analyte recognition, resulting in a measurable change of the signal, which can be detected by using the transducers. Nanomaterials proved the improvement of electrochemical, mechanical, magnetic, and optical properties of biosensors, which helps to develop a high-affinity biosensor [13, 14]. A wide range of nanomaterials are effectively used for surface functionalization, and sensor chip preparation to develop a highly sensitive biosensor [15–17]. Here, zeolite nanomaterial is used to attach the probe molecule to the impedance electrode for detecting the AD biomarker. Zeolites are inorganic solids having a larger surface area and structure with uniform cavities, channels, or cages of monodisperse dimensions [18]. The application of zeolite in biosensors are developed in recent days for surface functionalization and biomolecular immobilization [19]. In particular, enzyme immobilization is challenging on the sensor chip, zeolite is a promising material for the immobilization of coenzymes due to its mechanical stability, larger surface area, high thermal ability, controllable hydrophobicity, and capacity of ion exchange. Apart from that, using a zeolite various biosensors were developed to identify various targets [20–23]. In this work, zeolite is for surface functionalization to attach the aptamer and antibody for identifying AβO.

Selection of probe and immobilization is playing a major role in improving sensor. In this research, the combination of aptamer and antibody was developed as the detection molecule to detect the AD biomarker. Aptamer is an artificial nucleic acid, coined from a randomized pool of DNA molecules by using the method called 'SELEX', which mainly involved the steps of binding, separation, and amplification [24–26]. The aptamers selected from the process shows a higher affinity to the target, and are used for various medical applications, such as biosensors, imaging, drug delivery, and drug screening [27, 28]. Since most of the aptamers binding region with its target varies from the antibody, the aptamer–antibody sandwich assay was developed by researchers for identifying the particular target. Both aptamers and antibodies bind with their target to the various region, it is commonly used to identify the target by a sandwich assay. Apart from that, aptamers are specially bound with the smaller region of the target, which can be commonly used to capture the target especially, and antibodies are used as the detection probe. But this type of sandwich assay need a lot of steps for target identification. To minimize the procedure, a single probe with antibody–aptamer on the zeolite was developed in this study. This probe is expected to attract a high number of AβO and lowered the detection limit of AβO.

Materials and methods

Reagents and biomolecules

Anti-Aβ antibody was ordered from Abcam, England. (3-Aminopropyl)-trimethoxysilane, was ordered from Sigma Aldrich, USA. Aβ (1–42) was bought from DGPeptides Co., Ltd, China. The following COOH-ended aptamer sequence was commercially synthesized and received from a local supplier. (5′-COOH-GCCTGTGTTGGGGCGGGTGCG). Artificial cerebrospinal fluid (CSF) was prepared by a mixture of 1 mM phosphate, 150 mM NaCl, 3.0 mM KCl, and 1.4 mM $CaCl_2{\cdot}2H_2O$. Zeolite was synthesized as described previously [29].

Sensing electrode fabrication

The impedance electrode was prepared as described previously [30]. At first, the length, gap size, and thickness of the sensing electrode were optimized with AutoCAD software. After that, the following methods were used to fabricate the impedance electrode, (i) Base substrate of the silica (Si) wafer was cleaned with distilled water; (ii) Si was transformed into SiO_2 through thermal oxidation at 500 degrees Celsius; (iii) aluminium (Al) was coated on SiO_2 using an aluminium coil and thermal evaporator; (iv) positive photoresist was coated on Al using the spin coating method; (v) the desired pattern was transferred on the electrode with UV light exposure; and (vi) the electrode was cleaned with acetone and water after being dipped in an Al etching solution.

Aβ Oligomer preparation

The Aβ monomer was prepared by dissolving 1 mg/mL of Aβ in 1 mg/mL of HFIP (1,1,1,3,3,3-hexafluoroisopropanol) and stored the mixture at room temperature for an entire night while shaking. Here, the pre-existing structural inhomogeneity of Aβ was eliminated using HFIP. The HFIP solvent was evaporated the following day using N_2 gas, and the mixture was then combined with 1 mM NaOH in PBS to produce the Aβ monomer. In order to generate the AβO, the Aβ monomer solution was left at 37 °C overnight. The insoluble aggregates were separated by centrifugation at a speed of 10,000×g for 10 min. For later usage, the finished solution was maintained at 20 °C.

Preparation amine modified zeolite

Antibody was attached to the surface of the zeolite through the amine modification. At first, zeolite was initially modified into amine by APTES linker. For this, 1 mg/mL of zeolite was dispersed in 1 % KOH for 10 min and then washed thoroughly with distilled water and separated by centrifugation. The KOH-treated zeolite was mixed with 2 % of APTES based on preliminary optimization and kept overnight at 25 °C. The

amine-modified zeolite nanoparticle was washed with 30 % ethanol and recovered by centrifugation.

Sensing surface functionalization with dual probe

The sensing surface was functionalized with aptamer and antibody through the amine-modified zeolite. At first, the impedance sensor surface was immersed in 1 % of diluted KOH for 10 min and then 1 mg/mL of amine modified zeolite was added and rested it for 3 h. Further, 200 nM of anti-AβO antibody was dropped on the surface for the interaction antibody with the amine on the zeolite. After that, 1 µM of COOH ended anti-AβO aptamer was introduced on the sensing surface and rested it for 30 min to fulfil the amine gap on the zeolite surface. In between each step, the sensing surface was washed with PBS to remove the unbound molecules. The changes of real impedance part Z′ vs. imaginary impedance part Z″ were recorded after each immobilization process. This dual probe of aptamer and antibody on zeolite was used to quantify the level of AβO on impedance spectroscopy.

Detection of AβO by the dual probe

AβO was detected by dual probe-modified zeolite on impedance spectroscopy. Before the detection, the sensing electrode was covered with the blocking agent PEG-COOH to reduce the signal-to-noise ratio. For this purpose, 1 mg/mL of diluted PEG polymer was dropped on the zeolite-probe immobilized surface for 30 min to cover the excess sensing surface. After that AβO concentrations from 0.1 to 1,000 pM were diluted in PBS and dropped on the surface independently and rested it for 30 min. The changes of real impedance part Z′ vs. imaginary impedance part Z″ were recorded before and after adding each AβO concentration. The difference of changes in Z′ was calculated and plotted in excel to calculate the limit of detection of AβO.

Selective and specific detection of AβO in spiked CSF and other proteins

AβO concentrations from 0.1 pM to 1 nM were spiked in artificial CSF and dropped on zeolite-probe modified surfaces and waited for 30 min. After that, the surface was washed with buffer to remove the unbound AβO and then the reading was taken with impedance spectroscopy. Further to confirm the selectivity, AβO concentrations from 0.1 pM to 1 nM were mixed in other proteins namely presenilin, interleukin-6, and monocyte chemoattractant protein-1, dropped on zeolite-probe modified surfaces and waited for 30 min. After that, the surface was washed with buffer to remove the unbound AβO and then the reading was taken with impedance spectroscopy.

Results and discussion

Figure 1 shows schematic of the surface functionalization of AβO impedance biosensor on a zeolite nanomaterial-modified sensor surface. As shown in the Figure, the impedance surface was treated with KOH and APTES-modified zeolite was added. Usage of KOH enhances the surface hydroxyl groups for silanization, which facilitates a better reaction between sensing surface and APTES. Further, anti-AβO antibody was introduced on the surface and then COOH ended anti-AβO aptamer was added. Both antibody and aptamer can attach on the surface through the interaction of amine with COOH in aptamer and antibody. In most of the cases, aptamer or antibody was used as the capture or detection probe, and for a sandwich assay aptamer was used as the capture molecule and antibody as the detection molecule [31]. Both aptamer and antibody have their unique features, researchers combined these for various biosensing applications [32–35]. Apart from that, aptamer and antibody have various binding sites with their target, various biosensors were developed with aptamer and antibody as the detection and capture probe. As stated in Figure 1, aptamer binds among antibodies with the available free spaces. Due to a huge difference between the sizes of antibody (150,000 Da) and aptamer (7,000 Da), smaller sized aptamer easily occupies the free spaces. Different aptamers with varying sizes may not give significant variation with the attachment on zeolite. Herein, a single probe was developed with aptamer and antibody to quantify the level of AβO. At first, the antibody was attached to the surface of the zeolite and then the excess zeolite surface was covered with aptamer. Since the size of the antibody form the gap between them on the zeolite surface, aptamer filled that gap and form the combined probe with aptamer and antibody. This dual probe attracts a higher

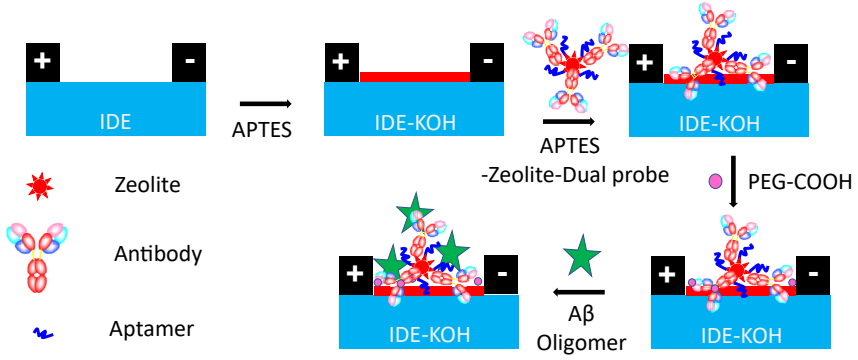

Figure 1: Schematic of AβO impedance biosensor. The impedance surface was treated with KOH and the APTES-modified zeolite was added. Further, anti-AβO antibody was introduced and then COOH ended anti-AβO aptamer was added. The excess surface was blocked with PEG-COOH and then AβO was added to the surface to detect.

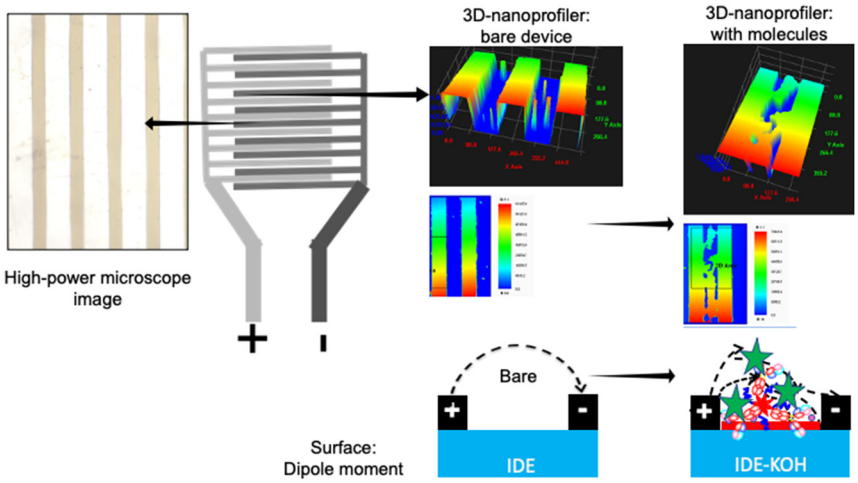

Figure 2: Surface on interdigitated electrode. High-power microscope and 3D-nanoprofiler images are shown. Discriminated the surface with molecular attachment. In addition, surface mechanism is shown.

number of AβO and lowered the limit of detection of AβO. Before being modify the surface with zeolite, the intactness of electrodes was verified under high-power microscope (Figure 2). The attachment of molecules on the surface is confirmed by 3D-nanoprofiling images. As shown in the Figure, when there is a surface modification or molecular attachment/interaction, dipole mechanism will be occurred due to the ionic changes which leads the alteration in current flow towards the electrodes.

Zeolite-probe immobilization on impedance sensor

The process of zeolite-probe immobilization was monitored by impedance spectroscopy. Figure 3A shows the process of Nyquist plot for probe immobilization. The Nyquist plot represents negative imaginary vs. real parts of the complex impedance electrode. The impedance results were analyzed based on the changes of real part Z′ upon the biomolecules binding on the electrode. The KOH-treated impedance shows the Z′ value of 1.2 E10 Ω, after the zeolite modification, Z′ value was changed to 1.09 E10 Ω. Further, upon adding an antibody to the surface, Z′ value was changed to 9.06 E09 Ω. This change of Z′ confirms the binding of the antibody on the surface of the zeolite. Finally, aptamer was introduced on the surface, and Z′ value was further decreased to 7.38 E09 Ω. The clear difference in Z′ value was recorded after each immobilization of biomolecules (Figure 3B). This result confirms the functionalization of zeolite–antibody–aptamer on the impedance electrode, which is used to detect the AβO.

Detection of AβO by the zeolite-dual probe

AβO was quantified on a zeolite-probe modified impedance electrode. On the zeolite-probe modified electrode, various concentrations of AβO was dropped, and analyzed the Z′ value. At first, the lowest concentration of 0.1 pM was added, and Z′ value was recorded as 6.24 E07 Ω, which confirms the binding of AβO with its aptamer and antibody (Figure 4A). Further, increasing the concentrations to 1, 10, 100, 1,000, and 2,000 pM, the Z′ value was decreased to 5.23, 4.81, 4.12, 3.65, and 2.83 E07, respectively. It was noted that with increasing AβO concentration, the Z′ value decreased gradually (Figure 4B). The difference in Z′ value was calculated and

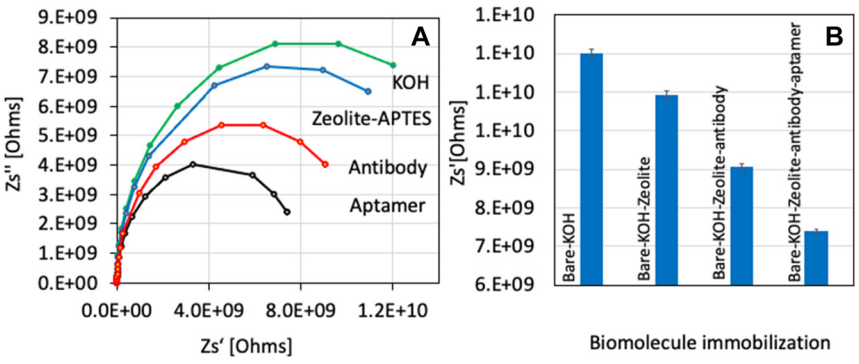

Figure 3: (A) Nyquist plot for probe immobilization. The Nyquist plot represents negative imaginary vs. real parts of the complex impedance electrode. Clear changes of Z′ was noted after each immobilization. (B) Value of Z′ for probe immobilization. The clear difference in Z′ value was recorded after each immobilization of biomolecules confirming the functionalization of zeolite–antibody–aptamer.

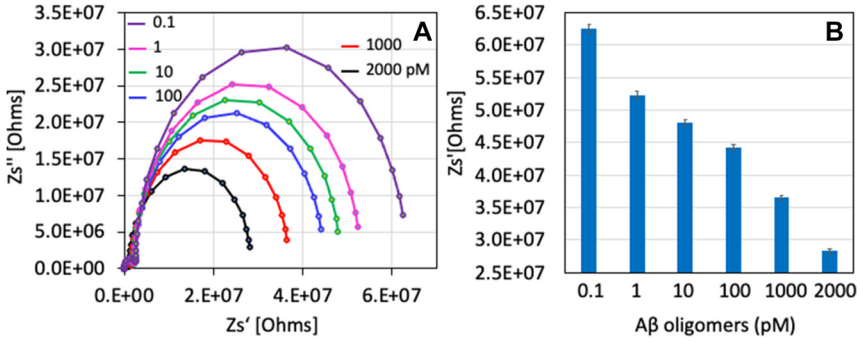

Figure 4: (A) AβO quantification on zeolite-probe modified impedance electrode. Clear changes of Z′ was noted after adding each AβO concentration. (B) Z′ value for each concentration of AβO. With increasing AβO, the Z′ value decreased gradually.

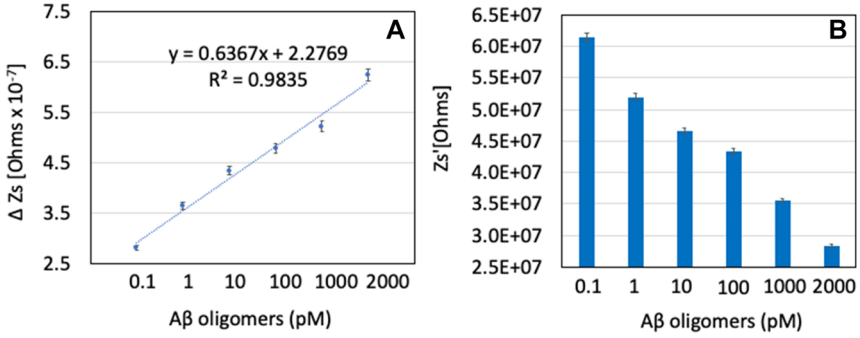

Figure 5: (A) Limit of detection of AβO. The difference in Z′ value was calculated and plotted in a excel to calculate the detection limit. The detection limit from the graph was calculated as 0.1 pM with an R^2 value of 0.9835. (B) Detection of AβO in spiked CSF. AβO concentrations from 0.1 pM to 2 nM were spiked in artificial CSF and dropped on zeolite-probe modified surfaces and the changes of Z′ value was analysed. The value of Z′ was decreased gradually when increasing AβO, confirming the detection of AβO spiked in CSF without any interferences.

plotted in an excel to calculate the detection limit. The detection limit from the graph was calculated as 0.1 pM with the R^2 value of 0.9835 (Figure 5A).

Identification of AβO in spiked CSF and high-performance analysis

Detecting the analyte molecule in the biological sample such as serum, blood, urine, and sweat is mandatory to identify diseases in the real-life sample. High-sensitive and selective biosensors can identify the smaller amount of targets in the crude sample. To confirm the detection of AβO in the biological sample, AβO concentrations from 0.1 pM to 2 nM were spiked in artificial CSF and dropped on zeolite-probe modified surfaces, and the changes of Z′ value were analysed. As shown in Figure 5B, the value of Z′ decreased gradually when increasing AβO concentrations. From this result, it was confirmed that AβO can detect selectively in CSF without any interferences. Similarly, AβO mixed control proteins did not interfere to the interaction of AβO with its aptamer and antibody indicating the specific detection of AβO (Figure 6A). The repeatability and reproducibility performances of sensing devices fabricated from the same batch of

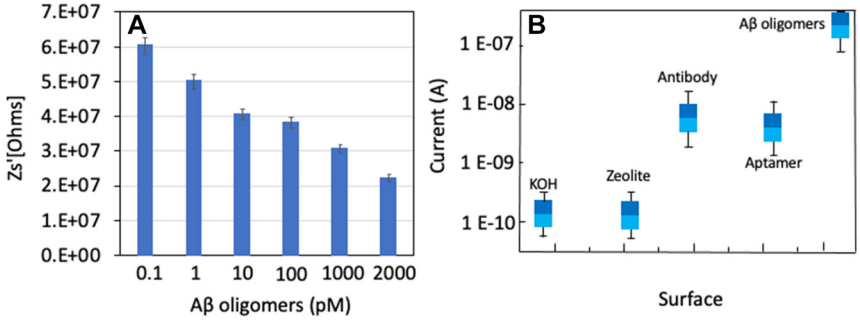

Figure 6: (A) Selective detection of AβO. AβO concentrations from 0.1 pM to 1 nM were mixed with relative proteins namely presenilin, interleukin-6, and monocyte chemoattractant protein-1, which are dropped on zeolite-probe modified surfaces. And the changes of Z′ values were registered. The value of Z′ was decreased gradually when increasing AβO, confirming the detection of AβO in mixed sample without any interferences. (B) Reproducibility test. 10 different devices fabricated from the same batch were tested with surface modifications/interactions desired and averaged the obtained values.

preparation were carried out as shown in Figure 6B, indicates the reliability of this sensing system for high-performance analysis. Evaluation on the regeneration with the same sensing surface was carried out by denaturing aptamer by warm water and found that the fabricated surface can reuse for 5 times. Further, the surface at lower temperature with wet-condition was noticed to be stable for 2 months and shown 90 % activity. After that, the surface molecular activity was declined drastically and reached 30 % within a month. Considering our previous studies with either antibody or aptamer as a single probe, the current study with dual probes yielded an enhanced impedance signal. However, it is hard to discriminate specifically whether the signal arises from antibody or aptamer. Overall, this sensing set-up with dual probes provide a high-performance signalling with enhanced sensitivity.

Conclusions

Alzheimer's disease (AD) is a brain disorder, that destroys thinking skills, memory, and eventually the inability to carry a simple task. In most cases, AD is the major cause of dementia. Since AD is irreversible, diagnosing AD in later stages worsens the situation and difficult for recovery. So that, identifying AD and its symptoms at the earlier stages helps to provide the necessary treatment and improve the patient's lifestyle. Further, it is crucial to highlight a general overview of Alzheimer's disease, and that current research may lead to more insights and advances in understanding and treating this complicated disorder. This research experiment was focusing developing a highly sensitive impedance biosensor for detecting the AD biomarker Aβ Oligomer (AβO). Zeolite nanomaterial was used to immobilize the probe of aptamer and antibody on the sensing electrode. Antibody was first attached to the zeolite through the amine linker and then the free space on the zeolite was covered by aptamer. This aptamer–antibody–modified zeolite was used to quantify AβO and the limit of detection was lowered to 0.1 pM. Further, AβO was identified in the artificial CSF spiked AβO, indicating the specific detection of AβO in the biological samples without any disturbances. This AβO biosensor helps to diagnose AD and its progress at its earlier stages and this platform is suitable for a wide range of clinical and non-clinical targets. Overall, the platform's future is bright, with possible applications in healthcare, environmental monitoring, food safety, and beyond. Biosensors have the potential to revolutionise different fields as technology improves and interdisciplinary collaborations continue, contributing to enhanced diagnostics, personalised medicine, and general well-being.

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Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
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Data availability: Not applicable.

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