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Formaldehyde Detection by a Combination of Formaldehyde Dehydrogenase and Chitosan on a Sensor Based on an Organic Field-Effect Transistor

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Abstract: Formaldehyde is utilized for the preservation of materials due to its strong bactericidal effects. As formaldehyde is also a harmful substance that causes health hazards, the quantitative monitoring of formaldehyde in natural and living environments is desirable. For the rapid and easy detection of formaldehyde, in this study we applied an organic field-effect transistor (OFET)-based sensor that can function as a potentiometric device for electrochemical measurements. A polyion-complex gel of formaldehyde dehydrogenase (FDH) and chitosan (CT) was constructed on a gold electrode. When the FDH/CT gel-coated electrode was connected to an OFET device it could detect formaldehyde in an aqueous solution, in which the amino groups of chitosan would protonate during the enzymatic reaction. The limit of detection was calculated to be 3.1 μM (93 ppb), demonstrating the applicability of the film-type OFET sensor to environmental monitoring.

Keywords: chitosan; formaldehyde dehydrogenase (FDH); formaldehyde detection; organic field-effect transistor (OFET); polyion-complex gel

1. Introduction

Formaldehyde, which is inexpensive and has strong bactericidal effects, is utilized for the preservation of materials such as building materials, resins, and textile products [1]. However, it is also a harmful substance that causes health hazards, such as mucosal irritation and skin allergic diseases, with exposure at or higher than a certain concentration. International research institutions, including the International Agency for Research on Cancer (IARC), the Environmental Protection Agency, and the European Chemicals Agency, have identified the environmentally toxic and carcinogenic substances that are related to formaldehyde [1–4]. In fact, the World Health Organization (WHO), and other organizations in many countries, define the allowable regulatory values of formaldehyde concentration in the environment to avoid its influence on organisms and people [5,6]. Based on the risk assessment, it is desirable to quantitatively monitor formaldehyde in the field.

In conventional measurements, direct detection of formaldehyde by liquid chromatography [7], and indirect detection of a formaldehyde derivative with acetylacetone by spectrophotometry [8,9] or with 2,4-dinitrophenylhydrazine by gas chromatography [10] have been used, as these methods are quantitative and reliable. However, these methods are not suitable for monitoring formaldehyde levels in the field because of the time-consuming nature of the analysis and the requirement of a bench-top device with high analytical capabilities. Although it has been reported that formaldehyde could be detected in an aqueous solution at the limit of detection of 1.2 μM by the amperometric method using formaldehyde dehydrogenase (FDH) and quinone as a mediator, it would be difficult use this method in the field because the current limit of detection is at the nanoampere level [11]. Thus, a sensing device that uses a disposable and inexpensive sensor chip for detecting formaldehyde rapidly and easily is required. One such candidate is an organic field-effect transistor (OFET)-based sensor, which has the potential to be a film-type device [12].

We developed an OFET-based sensor that can function as a potentiometric device for electrochemical measurements [13–15]. Previously, we succeeded in detecting electrically charged molecules, such as organic acid molecules [16], and enzymatic oxidations of saccharides [17] and lactic acid [18]. We achieved this by using the substance-specific oxidase immobilized on a Prussian blue (PB) electrode, on which hydrogen peroxide as a by-product from an oxidase reaction can oxidize Fe^{2+} ions in PB into Fe^{3+} ions. Here, we focused on the development of a formaldehyde-specific sensor based on an OFET device, which not only has the advantages of being suitable for fabrication on a flexible plastic film and low-cost production using inkjet-printing technology [12], but also has the potential to be incorporation into portable devices used in the field. In this study, we demonstrate the detection of formaldehyde using a combination of a polyion-complex gel of FDH and chitosan (CT) on a gold electrode connected with an OFET device. Chitosan, which is an aminated polysaccharide, was applied for two roles: (1) as a matrix to hold the enzyme which was captured by an electrostatic interaction with the cationic amino groups of chitosan and (2) as a material to be charged by a proton produced as a by-product from the enzyme reaction at the non-protonated amino groups in this matrix (Figure 1).

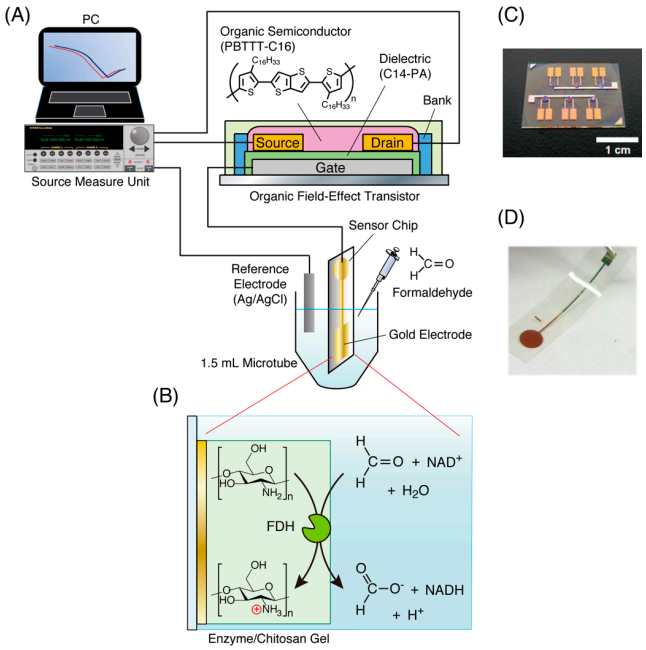

Figure 1. (**A**) Setup of an organic field-effect transistor (OFET)-sensor for a formaldehyde detection system using a polyethylene naphthalate (PEN) film sensor chip equipped with a gold electrode, on which the formaldehyde dehydrogenase (FDH)/chitosan gel was coated. (**B**) Schematic of the reaction process in the enzyme/chitosan gel on the sensor chip. (**C**) Photograph of six OFET devices on a plate. (**D**) Photograph of a sensor chip.

2. Materials and Methods

2.1. Materials

Chemicals used for this study were commercially available and used as supplied. Formaldehyde dehydrogenase (FDH) from *Pseudomonas* sp. (EC 1.2.1.46) (4.92 units/mg, FRD-201) was purchased from TOYOBO Co., Ltd. (Osaka, Japan). β-Nicotinamide adenine dinucleotide (NAD) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Tetradecylphosphonic acid (C14-PA) and Dulbecco's phosphate-buffered saline (0.1 M, pH 7.4) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Chitosan (molecular weight > 100,000) was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Formaldehyde solution (36.0–38.0%) was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). A polyethylene naphthalate (PEN) film was purchased from Teijin Film Solutions Ltd. (Tokyo, Japan). Poly-2,5-bis(3-hexadecylthiophene-2-yl)thieno[3,2-b]thiophene) (PBTTT-C16) was purchased from Merck Japan Ltd. (Tokyo, Japan). Cytop^{TM} (CTL-809M), an amorphous fluoropolymer, was purchased from Asahi Glass Co., Ltd. (Tokyo, Japan). Gold and aluminum were purchased from Tanaka Kikinzoku Kogyo K.K. (Tokyo, Japan) and Furuuchi Chemical Co., Ltd. (Tokyo, Japan), respectively. Type1 ultrapure water (MilliQ^{TM}) was used in all the experiments.

2.2. Fabrication of an OFET Device

An OFET device was fabricated as described in previous papers [12,16]. An aluminum thin film of 30 nm thickness was deposited on a glass plate as a gate electrode by thermal evaporation with monitoring of its thickness with a quartz crystal microbalance. The oxidation of the aluminum electrode was carried out by an oxygen-plasma treatment (300 W) and then the coating of the gate electrode with a SAM (self-assembled monolayer) formed from a 2-propanol solution including tetradecylphosphonic acid (C14-PA) was performed to form a 5 nm thickness of dielectric layer on the gate electrode. Subsequently, two gold electrodes, acting as source and drain electrodes, were attached by gold-thermal evaporation on the gate insulating layer, which was patterned using a shadow mask. The width and length of the channel composed of the source and drain electrodes were 1010 and 18 μm, respectively. A bank layer surrounding the channel was formed by printing of a 1 wt% solution of Teflon AF1600 (DuPont-Mitsui Fluorochemicals Co., Ltd., Tokyo, Japan) in Fluorinert (FC-43, 3M Japan Ltd., Tokyo, Japan) using a dispenser device (Image Master 350 PC, MUSASHI Engineering, Inc., Tokyo, Japan). Under a nitrogen atmosphere, a 1,2-dichlorobenzene solution of PBTTT-C16 (0.03 wt%) was drop cast onto the square area surrounded by the bank layer, in which the source and drain electrodes exist, followed by heat treatment at 150 °C for 30 min to form a semiconductor layer. Finally, a protective film of an amorphous fluorinated polymer (Cytop, CTL-809M, AGC Inc., Tokyo, Japan) was formed by spin-coating to improve the device stability.

2.3. Preparation of a Sensor Chip with an Enzyme Electrode

An electrode for an enzyme electrode on a sensor chip was prepared as an electrode of a 50 nm thick gold film, which was deposited onto a PEN film with a thickness of 125 μm by thermal evaporation. A 0.1 wt% chitosan solution was prepared by adding chitosan powder to a 0.05 M HCl aqueous solution stirring the solution for 30 min using a Planetary Centrifugal Mixer (Thinky Co., Tokyo, Japan). The chitosan solution was adjusted to pH 5.4 using 1 M NaOH aqueous solution and then filtered through a 0.45 μm PTEF syringe filter. A mixture of 10 μL of 0.1 wt% chitosan solution and 1.5 μL of FDH solution (1 unit/μL in Phosphate buffered saline) was formed to obtain a polyion-complex solution. The polyion-complex solution was deposited on the electrode by a drop casting method, followed by incubation for 3 h at 30 °C to dry the complex. Then, the enzyme-coated electrode was soaked in a phosphate buffer solution (10 mM, pH 7.4) for 1 h to remove loosely bound enzymes.

2.4. Observation of the Formaldehyde Detection on OFET

The procedure for the detection of formaldehyde is described below. The enzyme-coated electrode on a sensor chip and the reference electrode (Ag/AgCl, RE-1 B, BAS Inc., Tokyo, Japan) were immersed in a 1.5 mL microtube filled with 500 μL of 10 mM phosphate buffer. The electrode on the sensor chip was connected to a gate electrode of the OFET device. The source and drain electrodes of the OFET device and the reference electrode were connected to a source measure unit (Keithley 2636B SYSTEM SourceMeter, Tektronix Inc., Beaverton, OR, USA). In measurements using the OFET device, the transfer characteristic curves were obtained when the gate voltage (V_{GS}) was swept from 1 V to –2 V (0.1 V interval) at a –2 V of voltage between the source and drain electrodes (V_{DS}). Prior to the formaldehyde detection, the stability of the OFET device was confirmed by repeating the measurements of the transfer characteristic curve for 1 h, resulting in a standard deviation of ±7 mV fluctuation against V_{GS} in the curves. After the addition of a formaldehyde solution to the microtube, in which the sensor chip was immersed, and incubation for 10 min, the shifts in the transfer characteristic curve responding to each added concentration were observed.

3. Results and Discussion

3.1. Observation of the Transfer Characteristic Curve of the OFET for Formaldehyde Detection

We observed the shifts in the transfer characteristic curve after incubation for 10 min from the addition of a formaldehyde solution to the measurement microtube and the immersion of the FDH/CT gel-coated electrode connected to the OFET device. Afterwards, we confirmed that the curve position was constant. When the formaldehyde concentration to be added was varied in the range of 0 to 2.6 mM, a shift up to approximately 200 mV in the negative voltage direction of the transfer characteristic curves was observed to be dependent on the concentration, as shown in Figure 2A. These shifts indicate that a positively charged layer had formed on the gold electrode of the sensor chip, which was put between the gate electrode and the reference electrode. It can be interpreted that it was necessary to apply a lower gate voltage to obtain the same I_{DS} value on account of the positively charged layer. On the other hand, in the absence of NAD^+, which is necessary for formaldehyde oxidation by FDH, the curves slightly shifted by only about 9 mV with the addition of formaldehyde in the same concentration range (Figure 2B). The 9 mV value was approximately equal to the standard deviation (±7 mV) of the shift at the stable state of the OFET device (Figure 3). These results show that NAD^+ was required for the curve shift. In addition, no shift was observed in the case of electrodes coated with only chitosan, excluding FDH. Thus, the observed large shift of ~200 mV in the negative direction of the transfer characteristic curve was considered to be due to the enzymatic reaction. FDH oxidizes formaldehyde to formic acid with the reduction from NAD^+ to NADH, in which two protons are produced as by-products (Figure 1). The localized cationic protons near the electrode and/or protonated amino groups of the chitosan in the polyion-complex gel on the electrode could cause a curve shift in the negative voltage direction due to the formation of a positively charged layer.

The sensor chip could be used repeatedly in the one-day experiment. However, after the second day the response decreased, probably due to desorption of the enzyme from the electrode surface. The reusability of the sensor chip could be improved by crosslinking the polyion-complex [19]. In the measurements we used an incubation time of 10 min to reach the equilibrium state of the enzyme reaction as the reaction took several minutes to reach saturation in the previous report [11]. The sensor response could be improved by optimizing the measurement conditions.

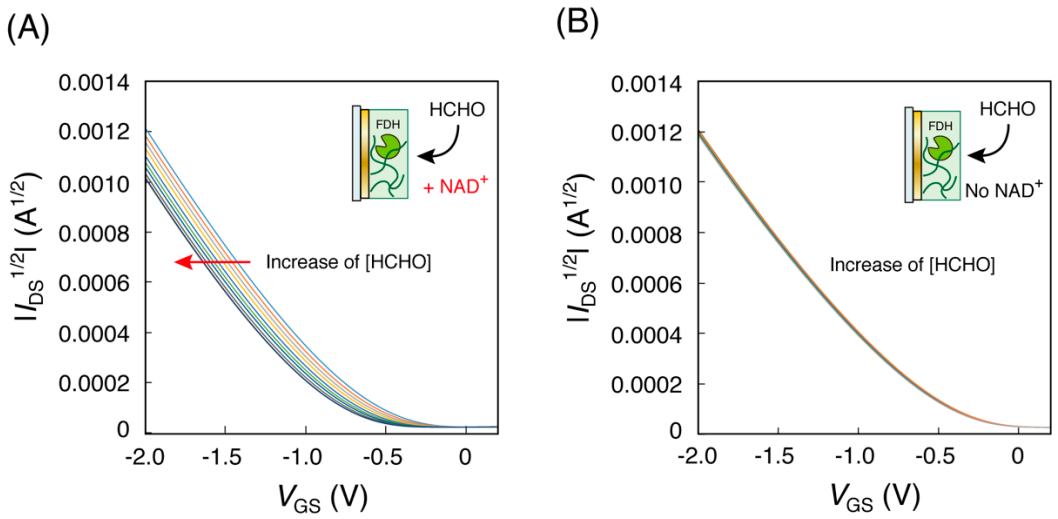

Figure 2. Transfer characteristic curves of the OFET-based sensor with a gold electrode coating FDH/chitosan (CT) gel, responding to the addition of formaldehyde solution at different concentrations (0–2.6 mM) (**A**) in the presence of and (**B**) in the absence of NAD^+. Experimental condition: 1 or 0 mM of [NAD^+] in 10 mM phosphate buffer (pH 7.4) at 25 °C.

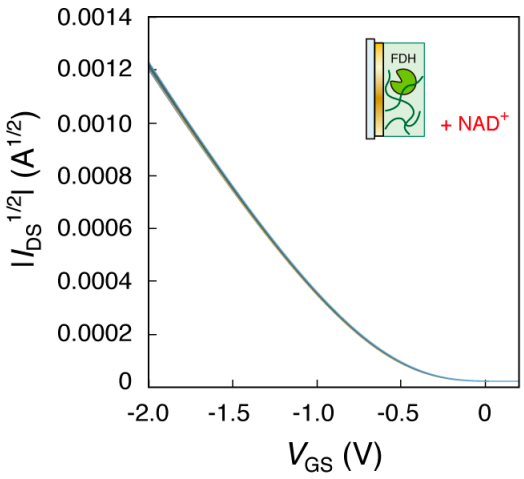

Figure 3. Transfer characteristic curves of the OFET-based sensor with a gold electrode coating FDH/CT gel, scanned 13 times for 1 h in 10 mM phosphate buffer (pH 7.4) including 1 mM of [NAD^+] at 25 °C.

3.2. Dependence of Curve Shift on Formaldehyde Concentration

Figure 4A shows the plots of the V_{GS} value shifts (ΔV_{GS}) as a function of the formaldehyde concentration ([HCHO]). ΔV_{GS} in the absence of NAD^+ did not change, even when the formaldehyde concentration was increased to 2.6 mM (Figure 4A, curve *b*). In contrast, ΔV_{GS} in the presence of NAD^+ increased depending on the formaldehyde concentration and was saturated at approximately 0.5 mM (curve *a*). In the FDH/CT gel on the electrode surface, the amino groups of chitosan could dominate and determine the pH due to the larger number of amino groups compared to the molar concentration of the buffer component. Therefore, the proton production by the enzyme in the gel could directly affect the cationization of chitosan. The concentration of protons in the FDH/CT gel would depend on the balance between the rate of generation by the enzyme reaction, determined based on the formaldehyde concentration in the substrate and the rate of elimination from the diffusion into the bulk solution, resulting in a constant value (when equilibrium was reached). According to the proton concentration in the FDH/CT gel, the cationic charge density would become a certain value. As a result, a change in the ΔV_{GS} value, depending on the formaldehyde concentration, could be obtained.

On the other hand, the addition of an acid to the measurement cell would be equilibrated by the buffer components before reaching the electrode.

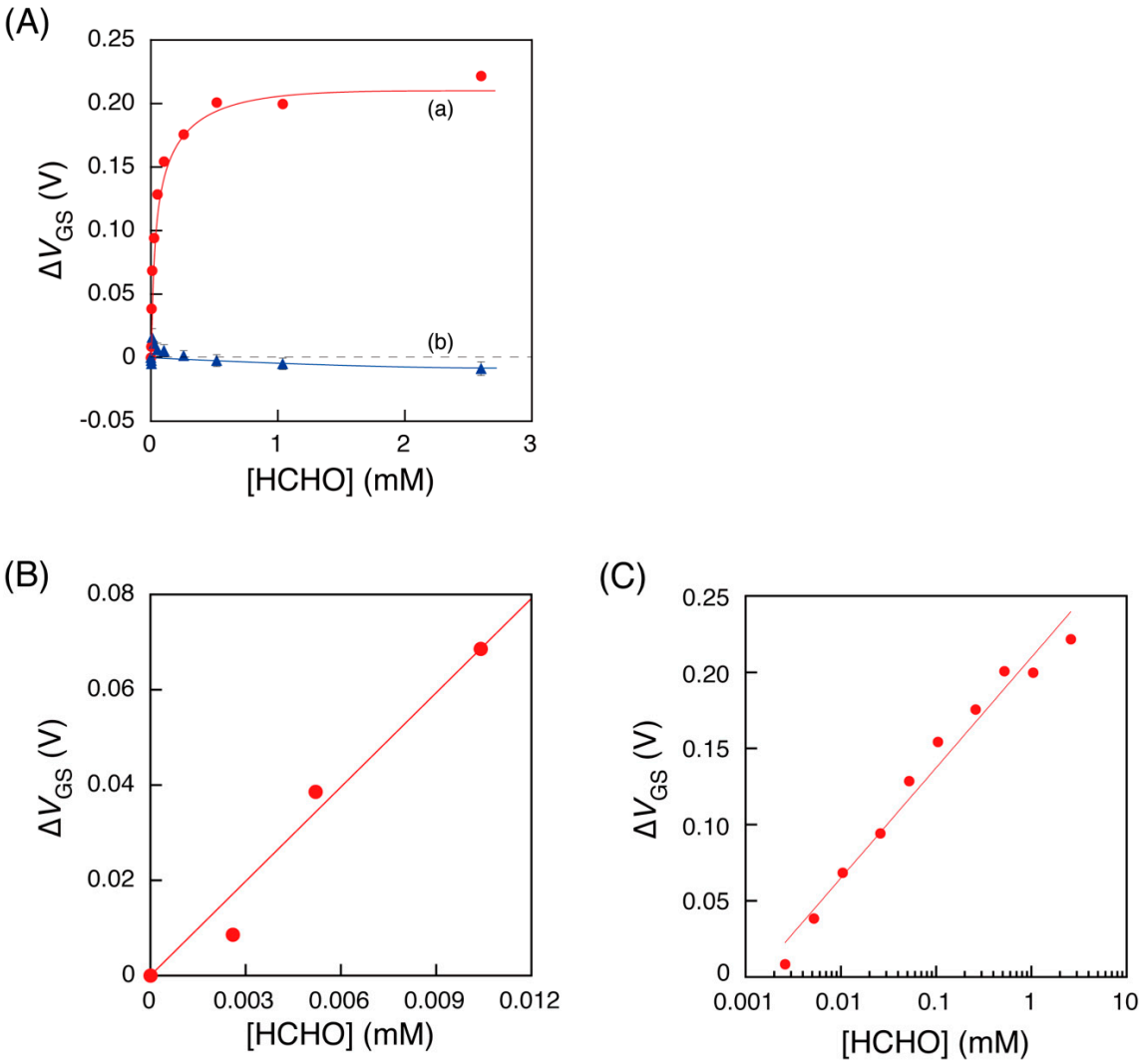

Figure 4. (**A**) Dependence of the gate voltage shifts (ΔV_{GS}) of transfer characteristic curves on the concentration of formaldehyde ([HCHO]); (a: circles) in the presence of NAD^+ and (b: triangles) in the absence of NAD^+. (**B**) Linear approximation in the range of 0–10 μM formaldehyde. (**C**) Linear approximation to logarithm of the concentration of formaldehyde in the range of 0–2.6 mM. The maximum and average values of the SD for each plot were approximately ±7 mV and ±3 mV, respectively.

3.3. Limit of Detection in Formaldehyde Sensing Using the OFET Device

To calculate the limit of detection (LOD) according to Equation (1) [20], the 0–10 μM linear range at low concentrations was plotted as shown in Figure 4B:

$$LOD = 3 \times SD/Slope \quad (1)$$

where *SD* is the standard deviation at 0 μM of [HCHO] and *Slope* is the slope value of the straight line. The LOD was calculated to be 3.1 μM with *SD* = 7.2 mV and *Slope* = 6.9 mV/μM. This is sensitive enough to measure the formaldehyde concentration in the environment at the allowable regulatory value level, such as 33 μM in the aqueous solution of a river based on the Environment Basic Act in Japan. In addition, there is a linear response to the logarithm of [HCHO] in the range of 0–2.6 mM (Figure 4C). In a previous report on the potentiometric detection of formaldehyde, using an ion-sensitive field-effect

transistor (ISFET), the linear response of the sensor to up to 200 μM formaldehyde with a 10 μM LOD were reported [21]. Compared to the ISFET device, a sensor using an FDH/CT gel-coated PEN film as a chip connecting the OFET device possesses not only comparable sensitivity and a linear response, but also disposability and easy preparation.

4. Conclusions

We developed an OFET-based sensor for the detection of formaldehyde in an aqueous solution at a few μM (LOD = 3.1 μM) with a linear response to the logarithm of the concentration (0–2.6 mM). The system consisted of an OFET device, working as a potentiometer at a low voltage of 2 V, and a PEN film coated with FDH/CT gel as a sensor chip, which could be prepared with a simple operation to drop cast the polyion-complex solution. The mechanism of the electrochemical detection of formaldehyde using the FDH/CT gel was determined to be the increase in the positive charge density in the gel on the sensor electrode from the protonation of the amino groups of the chitosan, due to the protons generated as by-products of the enzyme reaction. Based on this mechanism, a potentiometric sensor including an OFET device can also be applied for the sensing of other dehydrogenases by combining the gelation with chitosan. We believe that OFET-based sensors, which can be lightweight and inexpensive film-type devices, produced from an OFET circuit to an enzyme electrode by printing technology, can be applied to measurements using combinations of not only oxidases and Prussian blue but also dehydrogenase and chitosan.

Author Contributions: K.T. performed the measurements and analysis of the OFET experiments. H.F. supervised the entire project. A.N. and H.M. fabricated the OFET device and advised on the device handling. M.N. and S.T. participated in comprehensive discussion and provided helpful suggestions. K.T. wrote the original draft and all the authors reviewed and edited the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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