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# Label−free and facilely electrochemical biosensing using carbon nanotubes for malondialdehyde detection

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## Experimental section

**Chemicals and materials.** Human complement factor H (CFH) elisa kit and malondialdehyde (MDA) elisa kit were purchased from Shanghai Fengxiang biotechnological Co., Ltd (Shanghai, China). Human 4−hydroxynonenal elisa kit was purchased from Shanghai Jijin chemical Co., Ltd (Shanghai, China). Multiwalled carbon nanotubes (MWNTs, φ = 10−20 nm) were purchased from Nanotech Port Co., Ltd. (Shenzhen, China). Chitosan (CS), glutaraldehyde (GLD) solution (50%), bovine serum albumin (BSA) and 1, 1, 3, 3−tetraethoxypropane (TEP) were purchased from Sigma−Aldrich Chemicals Co. (St. Louis, MO, USA) and used without further purification. 2−thiobarbituric acid (TBA) was purchased from sinopharm chemical reagent Co., Ltd (Shanghai, China). Trichloroacetic acid (analytical grade) was purchased from Shanghai Linfeng chemical reagent Co. Ltd (Shanghai, China). N−butyl alcohol was purchased from Shanghai Shenbo chemical Co. Ltd (Shanghai, China). Human serum samples were kindly provided by Jiangsu Institute of Cancer Prevention and Cure and they were obtained by centrifugation of blood for about 5 min with the rotation rate of 3000–4000 rpm. All experiments with human serum samples were carried out in compliance with laws concerning the patients' right to know

and the guidelines for blood collection for experiments given by the Pharmacology Experimentation Committee, Jiangsu Institute of Cancer Prevention and Cure. Informed consent was obtained from all patients who provided serum samples for these experiments. All other chemicals were of analytical grade. Aqueous solution was prepared with double−distilled water (DDW) and the pH value of phosphate buffered saline (PBS) was 7.4 unless indicated otherwise.

**Instruments.** Electrochemical measurements including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 660D electrochemical workstation (CH Instruments Inc., USA). The electrochemical impedance measurements (EIS) were carried out with an Autolab PGSTAT12 (Eco Chemie, BV, Netherlands) and controlled by GPES 4.9 and FRA 4.9 software. A three−electrode electrochemical system was composed of a platinum wire as the auxiliary electrode, a saturated calomel electrode (SCE) as the reference electrode and a bare or modified glassy carbon electrode (GCE) (d=3 mm) as the working electrode. Ultraviolet-visible (UV-vis) absorption spectra were recorded using a Cary 60 UV-vis spectrometer (Agilent, Australia). The thickness and morphologies of the formed complex film were characterized by a JSM-7600F field emission scanning electron microscopy (SEM) (JEOL, Japan) at an accelerating voltage of 10 kV.

**Preparation of MWNTs−CS suspension.** The suspension of MWNTs−CS was prepared according to the literature[S1] with minor modification. First, a certain amount of MWNTs were purified through refluxing in concentrated $HNO_3$−$H_2SO_4$ (V/V, 1:1) mixture for 12 h at 55 °C and dried after washing with DDW until pH 7.0. Then 1 mg purified MWNTs were put into 2 mL DDW and ultrasonicated to obtain a uniform and black MWNTs suspension. 0.5 mg CS were put

into 2 mL 1% acetic acid solution and ultrasonicated for 3 hours to obtain a uniform CS solution. Finally, 2 mL MWNTs suspension was mixed with 2 mL CS solution and ultrasonicated to obtain a uniform MWNTs−CS suspension.

**Preparation of the modified electrodes.** The GCE was firstly polished with 1.0, 0.3, and 0.05 μm α−$Al_2O_3$ powder successively. After sonication in water, the electrode was rinsed with DDW and allowed to dry at room temperature. 8 μL MWNTs−CS suspension was dropped on the pretreated GCE and dried in a desiccator to obtain GCE/MWNTs−CS. Then 8 μL GLD solution (1%) was dropped on GCE/MWNTs−CS for 2 hours to obtain GCE/MWNTs−CS/GLD. Following a rinse with 0.01 M pH 7.4 PBS and DDW, 8 μL CFH (100μg $mL^{-1}$) was dropped on GCE/MWNTs−CS/GLD and kept at 4 ˚C overnight to obtain GCE/MWNTs−CS/GLD/CFH. Then 8 μL BSA (0.25%) was dropped on GCE/MWNTs−CS/GLD/CFH and incubated an hour to obtain GCE/MWNTs−CS/GLD/CFH/BSA. After that, MDA was dropped on GCE/ MWNTs−CS/GLD/CFH/BSA and incubated 38 minutes at 37 ˚C to obtain GCE/ MWNTs−CS/GLD/CFH/BSA/MDA. Finally, 8 μL CFH (200μg $mL^{-1}$) was dropped on GCE /MWNTs−CS/GLD/CFH/BSA/MDA and incubated 38 minutes at 37 ˚C to obtain GCE/ MWNTs−CS/GLD/CFH/BSA/MDA/CFH.

**Measurements.** The following parameters were employed for CV: scan rate was 100 mV/s, potential scanning range was 0~0.5 V. The DPV experimental parameters: potential scanning range was 0~0.4 V, amplitude was 50 mV. The EIS experiments were carried out under open−circuit conditions, the AC voltage amplitude was 5 mV and the voltage frequencies ranged from 0.1 Hz to $10^5$ Hz. All of the supporting electrolyte was 5 mmol $L^{-1}$ $K_3[Fe(CN)_6]$ and 5

mmol $L^{-1}$ $K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 mol $L^{-1}$ KCl. UV-vis absorbance was recorded at wavelength from 700 nm to 400 nm.

## The morphologies and thickness of MWNTs−CS/GLD/CFH/BSA/MDA/CFH complex film

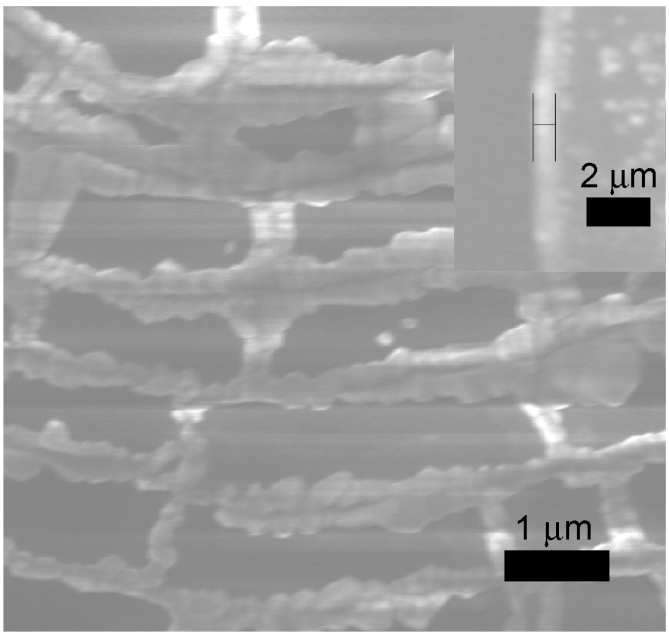


***Fig. S1*** The SEM images of MWNTs−CS/GLD/CFH/BSA/MDA/CFH complex film. Inset: magnified SEM image for the edge of MWNTs−CS/GLD/CFH/BSA/MDA/CFH complex film.

## The effects of incubation temperature and time on the biosensor responses

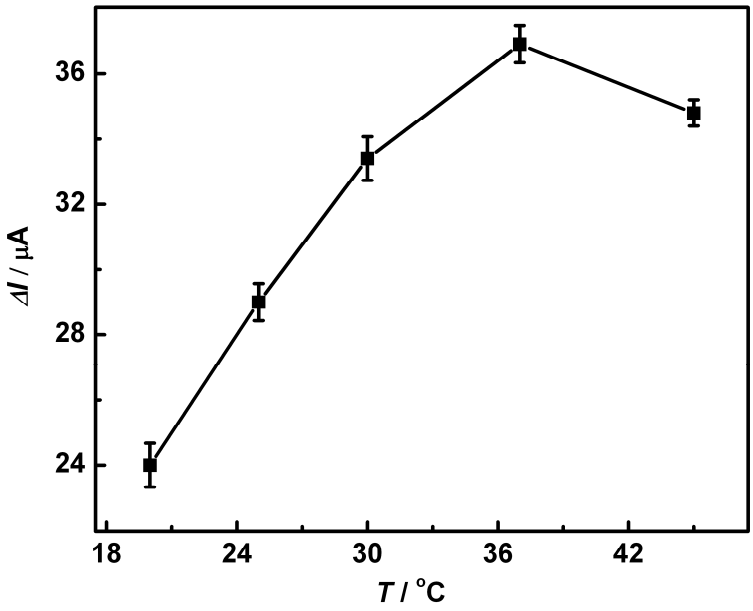


***Fig. S2*** The effect of incubation temperature on the biosensor response towards 9 μmol $L^{-1}$ MDA in 5 mmol $L^{-1}$ $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 mol $L^{-1}$ KCl.

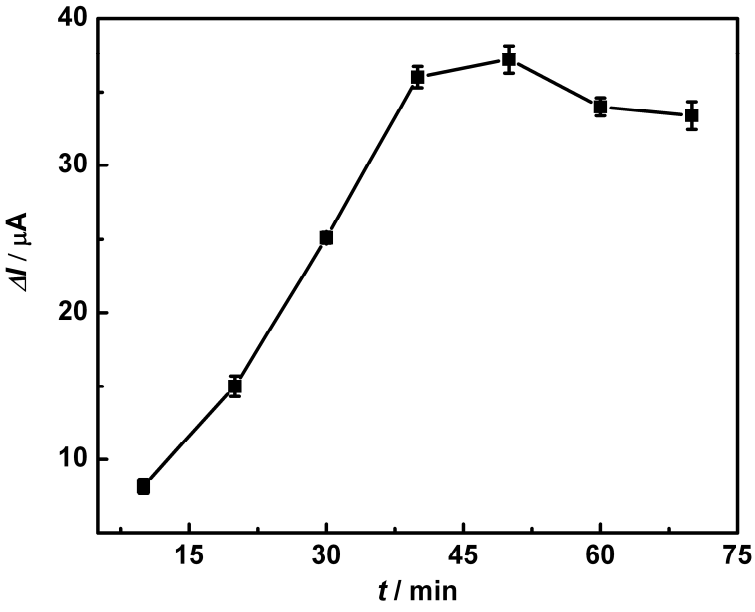


***Fig. S3*** The effect of incubation time on the biosensor response towards 9 μmol $L^{-1}$ MDA in 5 mmol $L^{-1}$ $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 mol $L^{-1}$ KCl.

## The responses of the biosensor towards different MDA concentrations in serum

Firstly, the healthy human serum was centrifuged at 14000 rpm for 5 min. Then, the supernatant of the human serum was spiked with 0.1, 0.3, 0.9, 5, 9, 30, 90, 300 and 500 μmol $L^{-1}$ MDA respectively. Finally, the responses of the biosensor towards different MDA concentrations in serum were investigated by DPV method. The concentration of serum which was not spiked with MDA was determined as the basal level. The calibration curve by plotting the current shift ($\Delta I$) ($\Delta I = I_0 - I$, when the MDA concentration was zero, the current response was recorded as $I_0$, when the MDA concentration changed, the current response was recorded as $I$) with MDA concentration was presented in the insert graph of Fig. S4. It was found that $\Delta I$ had a linear dependence on the logarithmic concentration of MDA in the range of 0.1−90 μmol $L^{-1}$. The regression equation was expressed as $\Delta I$ (μA) = 23.78 + 18.92 log$C$ (μmol $L^{-1}$) and the regression coefficient ($\gamma$) was 0.9985. The detection limit was estimated to be 0.042 μmol $L^{-1}$ with 3σ (where σ was the relative standard deviation of 11 parallel measurements in the blank solution).

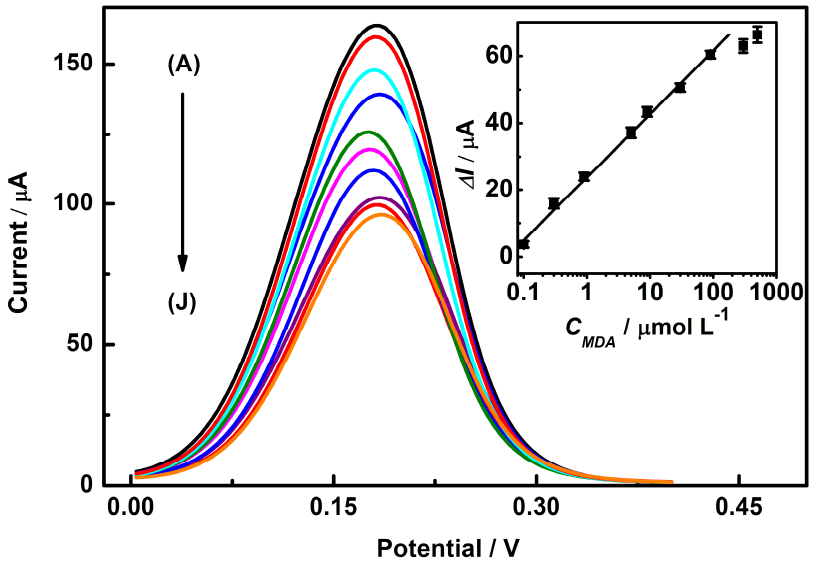


***Fig. S4*** DPVs for the relationship between the current and MDA concentration in serum. The concentrations of MDA were 0 (A), 0.1 (B), 0.3 (C), 0.9 (D), 5 (E), 9 (F), 30 (G), 90 (H), 300 (I) and 500 μmol $L^{-1}$ (J) in 5 mmol $L^{-1}$ $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 mol $L^{-1}$ KCl. Inset was the corresponding calibration curve.

## The selectivity of the proposed biosensor

Firstly, 9 μmol $L^{-1}$ MDA was mixed with 2.13 mmol $L^{-1}$ $Ca^{2+}$, 0.84 mmol $L^{-1}$ $Mg^{2+}$, 1.15 mmol $L^{-1}$ $PO_4^{3-}$, 8 μmol $L^{-1}$ 4−hydroxynonenal[S2] and 1.96 mmol $L^{-1}$ glutaraldehyde respectively. Then, the five kinds of mixed solution replaced 9 μmol $L^{-1}$ MDA to prepare the modified electrodes. Finally, the modified electrodes were put into 5 mmol $L^{-1}$ $K_3[Fe(CN)_6]$ and 5 mmol $L^{-1}$ $K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 mol $L^{-1}$ KCl and DPV experiments were done. The results were shown in Fig. S5. The current was increased 1.18% after MDA was mixed with $Ca^{2+}$, and it was decreased 1.42%, 15.80%, 11.91% and 3.33% after MDA was mixed with $PO_4^{3-}$, $Mg^{2+}$, 4−hydroxynonenal and glutaraldehyde, respectively, indicating acceptable selectivity of

the proposed biosensor.

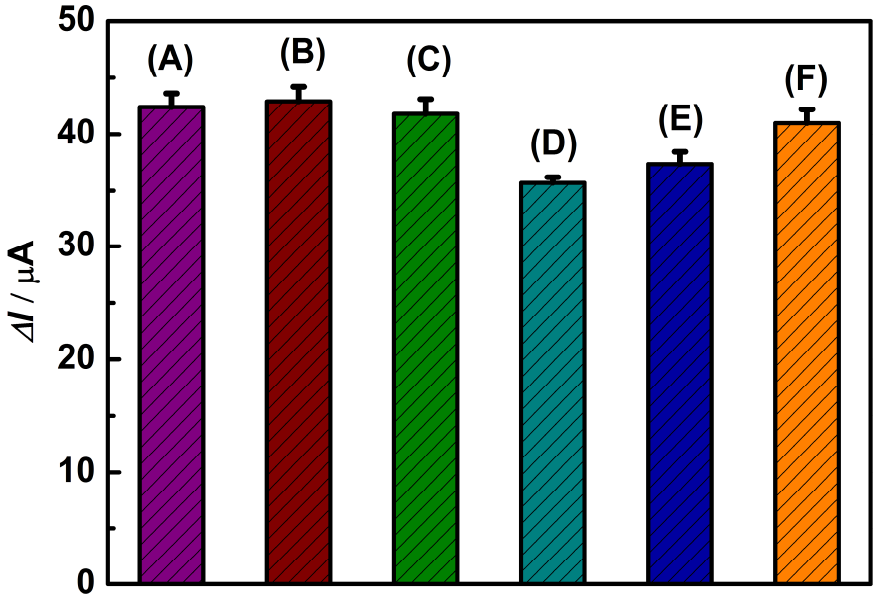


***Fig. S5*** Selectivity analysis of the biosensor tested in 9 μmol $L^{-1}$ MDA without (A) and in the presence of 2.13 mmol $L^{-1}$ $Ca^{2+}$ (B); 1.15 mmol $L^{-1}$ $PO_4^{3-}$ (C) ; 0.84 mmol $L^{-1}$ $Mg^{2+}$ (D); 8 μmol $L^{-1}$ 4−hydroxynonenal (E) and 1.96 mmol $L^{-1}$ glutaraldehyde (F), respectively.

## Ultraviolet−visible spectrophotometry for detecting MDA content

The spectrophotometric method based on the reaction between MDA and TBA[S3]. Briefly, 0.5 mL different concentrations of MDA standard solution were mixed with 3 mL of 0.2% TBA. The mixtures were heated in a hot water bath at 95 °C for 1 hour. After cooling on ice, the resulting chromogens were extracted with 4 mL n−butyl alcohol. The organic phase was separated by centrifugation at 3000 rpm for 10 min and the absorbance was recorded at wavelength from 700 nm to 400 nm. The peak occurred at 534 nm. MDA standard solution was made freshly by the hydrolysis of TEP. The responses towards different MDA concentrations were shown in Fig. S6. The calibration curve by plotting each absorbance with different MDA concentration was

presented in the insert graph of Fig. S6. Absorbance had a linear dependence on the concentration of MDA in the range of 0.5−150 μmol $L^{-1}$. The regression equation was expressed as $A$ = 0.03193+ 0.01792 $C$ (μmol $L^{-1}$) and the regression coefficient ($\gamma$) is 0.9747.

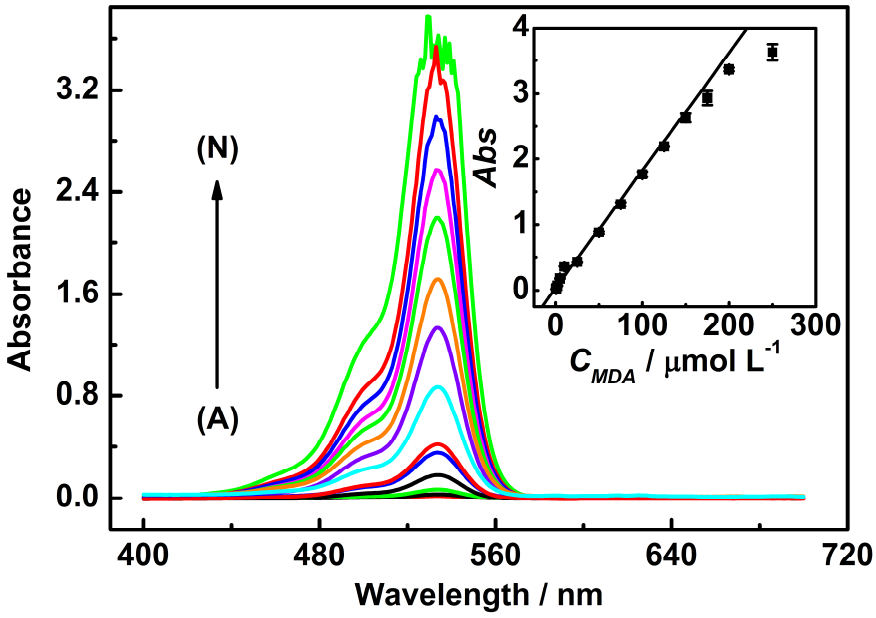


***Fig. S6*** UV−vis absorption spectra of different MDA concentrations. The concentrations of MDA were 0.5 (A), 1 (B), 2 (C), 5 (D), 10 (E), 25 (F), 50 (G), 75 (H), 100 (I), 125 (J), 150 (K), 175 (L), 200 (M) and 250 μmol $L^{-1}$ (N). The insert graph: the calibration curve of different MDA concentrations.

## The comparison of different methods for measurement of MDA

***Table S1*** The detection limit of different methods for measurement of MDA.

| Method | Detection limit (μmol $L^{-1}$) | Ref. |
|---|---|---|
| Reversed−phase high−performance liquid chromatography and ultraviolet −visible spectrophotometry detection | 0.056 | S4 |
| Fluorimetric–liquid chromatographic detection based on assays of MDA–TBA adduct | 0.128 | S5 |
| Fluorimetric–liquid chromatographic detection based on the labeling of MDA with 2-aminoacridone | 0.0018 | S6 |
| High−performance liquid chromatography −visible detection | 0.050 | S7 |
| Proposed method | 0.042 | Our work |

## Analysis in human serum samples

The feasibility of the bioassay system for clinical applications was investigated by analyzing several real samples, in comparison with the UV−vis method. Before detection, the serum samples were pretreated. Briefly, 0.5 mL each of the three serum samples was mixed with 2.5 mL trichloroacetic acid (3%) and the precipitate was separated by centrifugation at 3000 rpm for 10 min. Then the precipitate was dispersed in 2.5 mL acetic acid (1%) and the supernatant was collected. Finally, the MDA contents of the three treated serum samples were detected by UV−vis method. The MDA contents of the supernatant were called free MDA, the MDA contents of the precipitate were called bound MDA. The total MDA contents were the sum of free MDA and bound MDA. When the MDA contents were detected by DPV method, a series of

preparation should be done as follows: in order to detect total MDA, 0.5 mL each of the three serum samples was hydrolyzed for 1h at 60 ˚C in the presence of 2.5 mL NaOH[S8] (1mol $L^{-1}$); 0.5 mL each of the three serum samples was mixed with 2.5 mL DDW to detect free MDA after the solution was centrifuged at 14000 rpm for 5min.

## Reference

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