Supporting Information (SI)

Electrostatic Assemblies of Molecularly Imprinted Polymers on the surface of Electrospun Nanofibers Membranes for Point-of-care Detection of Thiodiglycol, a Sulfur Mustard Poisoning Metabolic Marker

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1. Experimental Section

1.1. Reagents and apparatus

TDG was purchased from Alpha Chemical Co., Ltd. (Zhengzhou, China).Dopamine (DA) was purchased from Huaxia Company (Shandong, China). Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4 \cdot 3H_2O$, >99%) was purchased from Sinopharm Group Chemical regent Co., Ltd. (Shanghai, China).α-methacrylic acid (MAA), glutamic acid (Glu), phenylalanaine (Phe), leucine (Leu), aspartic acid (Asp) and cysteine (Cys), sodium boro-hydride ($NaBH_4$), ethylene imine polymer (PEI, average M_W=70 000), cetyltrimethylammonium bromide (CTAB) and glutaric dialdehyde (GA, 50%) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Ethyleneglycol dimethacrylate (EGDMA) and 2,2'-Azobis(2-methylpropionitrile) (AIBN) were purchased from Yuanye Bio-Technology Co., Ltd (Shanghai, China). Uric acid (UA), alanine (Ala) and poly(vinyl alcohol) powder (PVA, 99% hydrolyzed, average M_W=89 000−98 000) were purchased from Sigma-Aldrich (Shanghai, China). Urea (UR), sodium penthydrodithiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) and citric acid were purchased in Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Leucine (Leu) was purchased from Guangfu Chemical Reagent Co., Ltd. (Tianjin, China). Hydroquinone was purchased from Acros Organics (Belgium). Silver nitrate ($AgNO_3$) was purchased in Shenbo Chemical Co., Ltd. (Shanghai, China). L-ascorbic (l-AA) purchased in Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Trichloromethane was purchased in Chuandong Chemical Group Co., Ltd. (Chongqing, China). Nitrocellulose membrane was purchased in Millipore. Ultrapure water (18.2mΩ, Barnstead EasyPure Rodi) was used to prepare all samples.

1.2. Apparatus

FT-IR spectroscopy (4000-500 cm^{-1}) was measured by Fourier Infrared Spectrometer (Shimadz Company, Japan). S-4800 Field Emission Scan Electron Microscope (Hitachi, Japan) was used to characterize MIPs/PEI/PVA NFS. Hitachi U-

3010 spectrophotometer (Hitachi, Japan) was used to measure absorption spectrum. MX-E-type vortex mixer (Haimen Company, China) was used for mixing solution. A dynamic light scattering particle nanosizer (ZEN3600, Malvern, U.K.) was used for measuring zeta potentials.

1.3. Preparation of MIP

The imprinted polymers were prepared by the thermal polymerization processes. Briefly, 100 μL of the template molecule TDG and 168 μL of the functional monomer MAA (1: 2) was dissolved in 30 mL of methanol solution and was mixed by ultrasonication for 10 min. Then 2 mL of crosslinking agent EGDMA and 200 mg initiator AIBN were added to the above solution. The prepolymerization solution was sealed with ultrasonic waves after 30 min. Then the mixture was stirred in the oil bath for 24 h. The obtained imprinted polymers were milled, extracted with acetic/methanol (1:9, v/v) and then dried in an oven at 60 °C. The NIP preparation step is the same as that of the MIP without the addition of TDG [1].

1.4. Preparation of AuNPs

First, 5.0 mL solution of gold seeds were prepared by reducing 250 μM $HAuCl_4$ with freshly prepared 3.0 mM ice-cold $NaBH_4$ in the presence of 75 mM CTAB. After being mixed vigorously for about 30 s, the mixture rapidly changed into light-brown suspension. This mixture could be used as seeds for further synthesis of AuNPs after 2 h aging. Second, 10.0 mL growth solution was prepared by the reduction of 0.2 mM $HAuCl_4$ with freshly prepared 6.0 mM L-AA in the presence of 1.6 mM CTAB, which was gently mixed by inversion, with the color changing from orange to colorless immediately. Then, followed by adding 2 h-aged 120 nM gold seed solution prepared in the first step to the growth solution and inversing it once more, and then the color of mixture became red gradually. At last, the mixture was left undisturbed for 24 h [2].

1.5. Electrospinning

Typically, PVA powder was dissolved in deionized water (DIW) for 3 h at 90 °C with the concentration of 12 wt %. PEI (50 wt %) was mixed with the former solution

(the PEI/PVA mass ratio of 1:3) under vigorous stirring at room temperature for about 8 h. Then, the homogeneous and viscous electrospun solution was filled into a 10 mL plastic syringe with a 0.8 mm diameter blunt-ended needle. All nanofibers were electrospun prepared using commercial electrospinning equipment (DNF−001,Beijing Kaiweixin Technology Co., Ltd., China) at the high voltage of 25.0 kV. The needle was located at a distance of 20 cm from the grounded collector wrapped with an aluminum (Al) foil. The rate of the syringe pump was set to 1.8 mm/h. Finally, the PEI/PVA nanofibers were cross-linked using GA vapor in a vacuum desiccator for 1 h at 60 °C. Then, the nanofibrous membranes were washed with distilled water three times to remove redundant GA. The cross-linked PEI/PVA nanofibrous mats were dried under vacuum and stored in a desiccator before use.

1.6. TDG analysis in urine samples

Three samples of human urine were obtained from volunteers in accordance with the institutional committee of Southwest University. All three samples were only employed in this work. All of the experimental steps were performed in accordance with the relevant laws and the institutional regulations of ethical standards of the institutional committee of Southwest University (approval number: yxy2021123).

The TDG standard solution was added to the human urine sample from a healthy volunteer, which was passed through a membrane filter (regenerated cellulose, a pore diameter of 0.45 μm, Phenomennex, USA). The filtrate was then diluted 10 times with 100 mM PBS (pH 7.4), and TDG in the urine was detected by the above method.

2. Figures

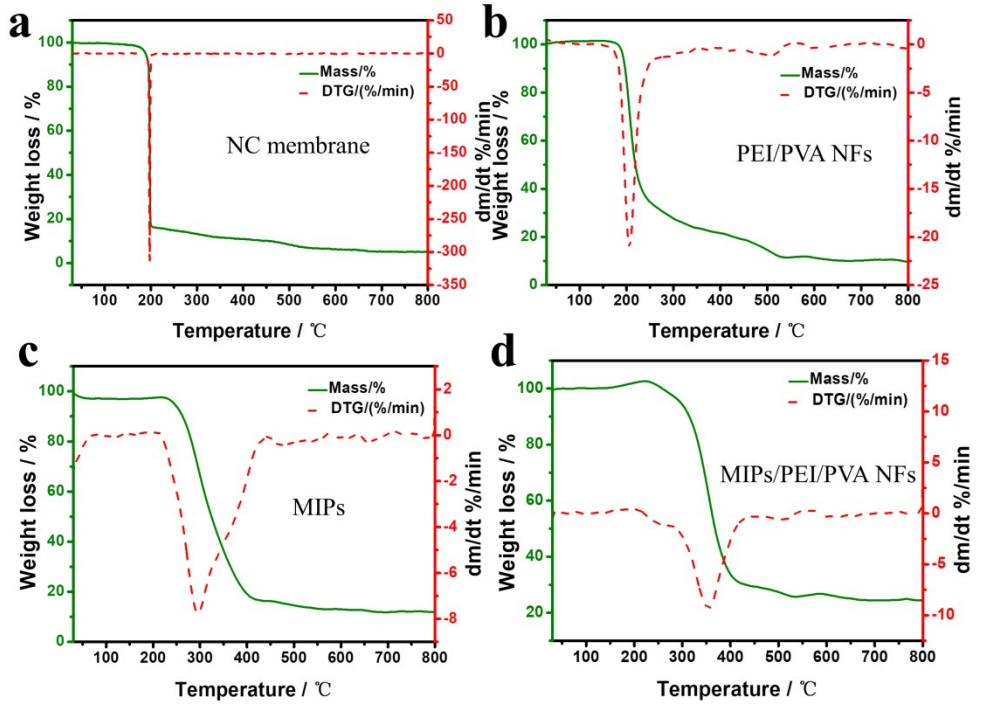

Fig. S1. TGA curves of (a) nitrocellulose membrane, (b) PEI/PVA NFs, (c) MIPs and (d) MIPs/PEI/PVA NFs at a heating rate of 20 °C/min from room temperature to 800 °C under air atmosphere.

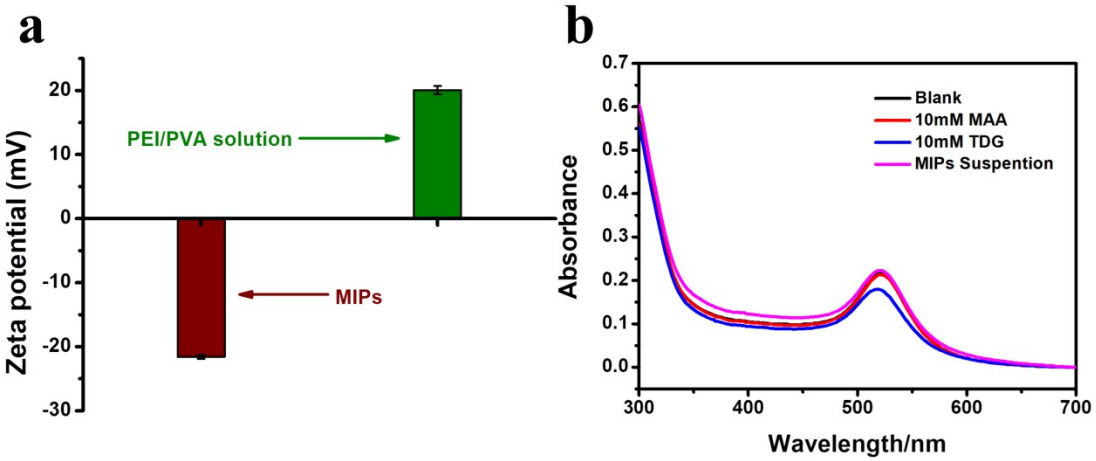

Fig. S2. (a) ζ-potential measurements of PEI/PVA electrospun and MIPs solution. (b) The effect of 10mM TDG solution, functional monomer and MIPs on AuNPs

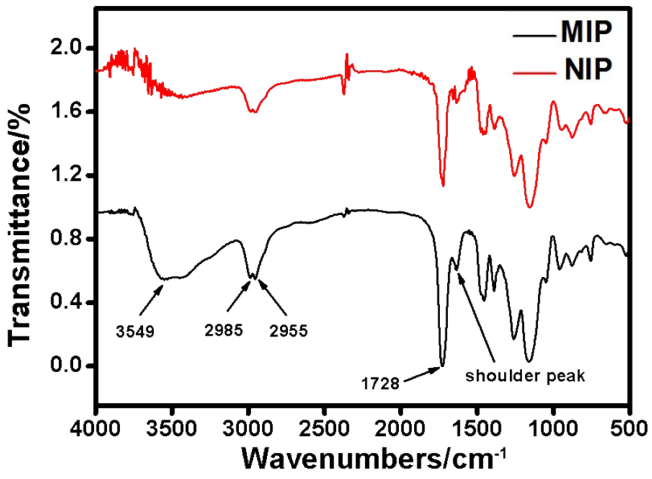

Fig. S3. FT-IR spectrum of MIPs and NIPs.

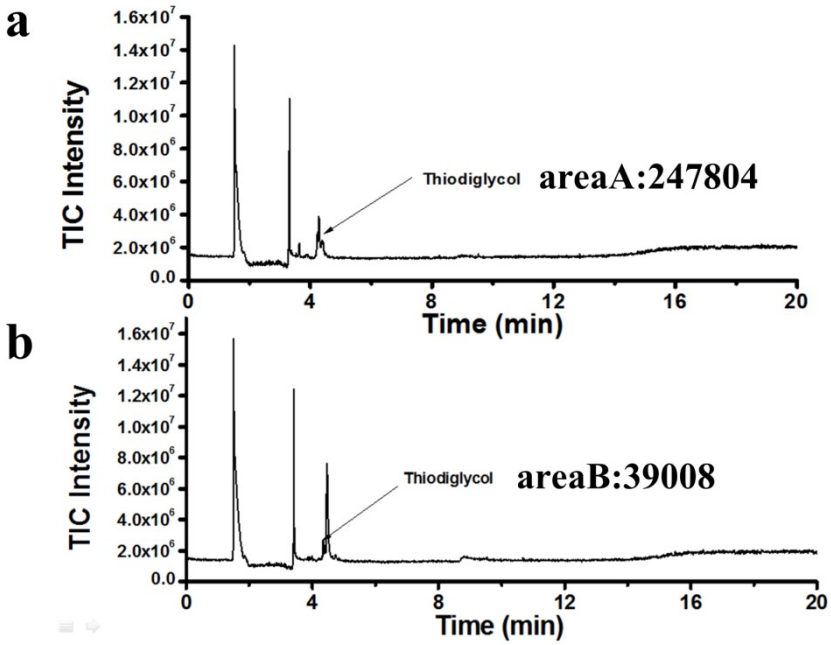

Fig. S4. GC-MS image (a) before 100 μg/mL TDG was specifically adsorbed by MIPs, (b) after 100 μg/mL TDG was specifically adsorbed by MIPs.

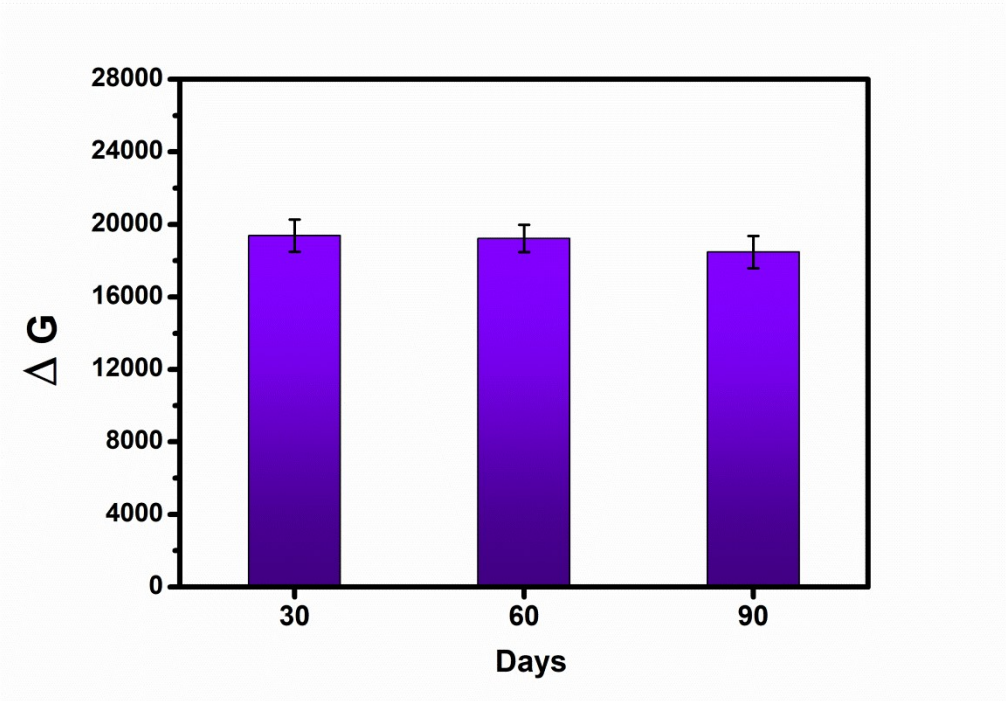

Fig. S5. Stability of the molecularly imprinted polymers electrospun nanofibers composed membrane.

3. Table Section

Table S1. Comparison of different TDG detection methods

Method	Detection limit	Samples	References
GC-MS	1.13 mg/g	Soil	[3]
LC-MS	0.2 g/mL	Urine	[4]
GC-MS-MS	0.5 ng/mL	Urine	[5]
GC-MS-MS	0.01 ng/mL	Urine	[6]
GC-MS-MS	0.1 ng/mL	Urine	[7]
LC-MS	250 ng/mL	Water	[8]
HPLC-MS/MS	0.03 ng/mL	Urine	[9]
LC-MS	0.1 ng/mL	Urine	[10]
LC-MS	0.25 ng/mL	Urine	[11]
MIP/PVA/PEI	38 pg/mL	Urine	This work

4. Reference

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