Supporting Information

Self-service aptamer-free molecularly imprinted paper-based sensor for high-sensitivity visual detection of influenza H5N1

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Chemicals and Materials

H5N1 (molecular weight: 2.5×10^8 g/mol), H1N1, Freeze-dried vaccine, was supplied by Harbin Veco Biotechnology Co., Ltd. HBV, Freeze-dried vaccine, was supplied by Shenzhen Kangtai Biology Products Co., Ltd. JEV, Freeze-dried vaccine, was supplied by Wuhan Institute of Biological Products Co., Ltd. HAV, Freeze-dried vaccine, was supplied by Zhejiang Pukang Biotechnology Co., Ltd. $FeCl_3\cdot6H_2O$, PEG-2000, NaAc and tetraethyl orthosilicate (TEOS) were purchased from Sarn Chemical Technology (Shanghai) Co., Ltd. $HOCH_2CH_2OH$, 3-aminopropyltriethoxysilane (APTES) and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Shanghai Bide Pharmaceutical Technology Co., Ltd. EtOH was purchased from Hunan Huihong Reagent Co., Ltd. Whatman 1 qualitative filter paper was purchased from Cytiva. $NH_3.H_2O$ was purchased from Xilong Chemical Co., Ltd. sodium dodecyl sulfate (SDS) and acetic acid were purchased from Tianjin Chemiou Chemical Reagent Co., Ltd. H_2O_2 (30.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd. PBS was purchased from Hubei Dongcao Chemical Technology Co., Ltd.

Instruments

The fluorescence spectra and intensity measurements were recorded with Hitachi fluorescence spectrophotometer F-4600 (Hitachi Technology Co., Ltd., China). The pH value of all solutions is adjusted by a digital pH meter (pHs-25, INESA Instrument Co., Ltd., Shanghai, China). The morphology of the prepared nanoparticles was characterized by a scanning electron microscope (SEM, JSM-6610LV, Japan). The centrifugal process adopts high-speed centrifuge (H1650, Hunan Xiangyi Laboratory Instrument Co., Ltd. China).

Optimization of the dose ratio of TEOS, APTES and $NH_3\cdot H_2O$

The thickness of the imprinting layer is directly related to the performance of the sensor,

so the dosage of monomers and initiator during imprinting is optimized at first (**Fig. S1A**). It was found that the IF value obtained in the second dosage group is the best, so the optimal dosage was 100 μL TEOS, 80 μL APTES and 100 μL $NH_3 \cdot H_2O$ respectively.

Optimization of the template dosage

The optimum dosage of template virus in the imprinting process was then explored (**Fig. S1B**). WP@MIP was prepared with 0, 5, 10, 15, 20, 25 and 30 μL H5N1 (2.9 μg/mL) [1, 2] as template. It was found that the ΔF value increased before the template dosage was 20 μL, and then tends to be stable, so 20 μL was selected as the best dose.

Optimization of buffer type

Several buffer with a pH value of 7.4 were screened for the detection of H5N1, including H_2O, phosphate buffer saline (PBS), phosphate buffer (PB), acetic acid-sodium acetate (NaAc-HAc) and citric acid-sodium citrate (SSC). As can be seen from the results, PBS was the best (**Fig. S1C**).

Optimization of imprinting time

The imprinting time is an important factor affecting silicon imprinting, so it was optimized from 1 to 12 hour (**Fig. S1D**). It was found that the ΔF and IF value increased before 8 h, and then reduced, so 8 hour was selected as the best imprinting time.

Optimization of elution times

The elution times will affect the elution rate of the template virus and even the integrity of the imprinted cavity. Therefore, the detection effect of WP@MIP was studied after elution for 0-10 times (**Fig. S1E**). It was found that the ΔF value increased before elution for 5 times, and then tends to be stable, until after elution for 7 times, the ΔF value decreased, which might be caused by the damage of imprinting cavities. After 5 times of elution, the virus was almost completely washed away, so 5 times was finally

chosen as the optimal elution times.

Optimization of incubation time

The incubation time from 10 to 90 minutes in the detection process was studied (**Fig. S1F**). It was found that the ΔF value increased before 50 minutes, and then tends to be stable, indicating that the adsorption begins to reach equilibrium, so 50 minutes is finally chosen as the best incubation time for the detection process.

Notes and references

1 S. Y. Yeung, A. Mucha, R. Deshmukh, M. Boutrus, T. Arnebrant, and B. Sellergren, ACS Cent. Sci. 2017, 3, 1198−1207.

2 P. Kramberger, M. Ciringer, A. Štrancar, M. Peterka, Virol. J. 2012, 9, 265.

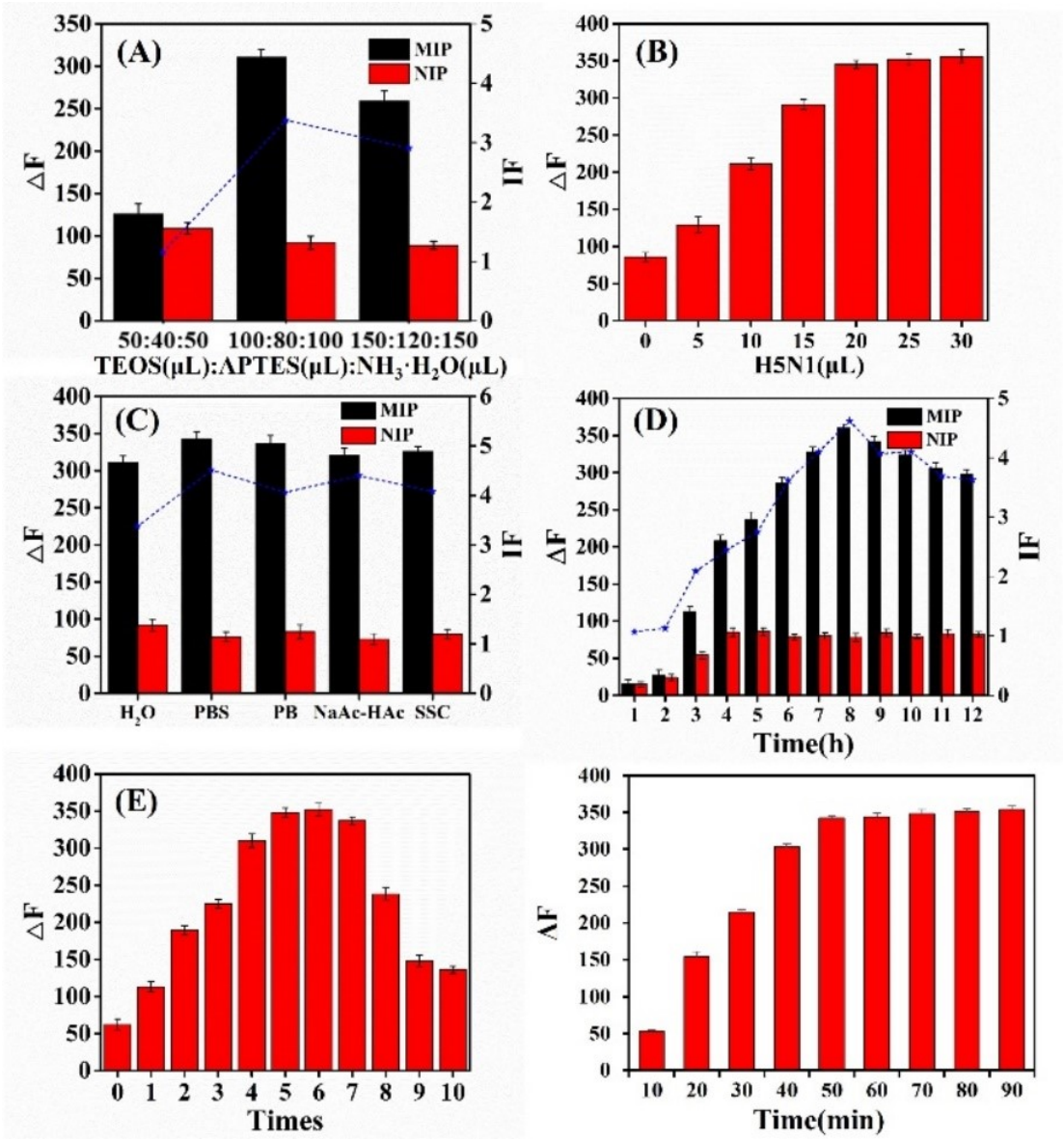

Fig. S1. Effect of the dosage of monomers and initiator used in imprinting (A), the dosage of template virus (B), buffer solutions (C), imprinting time (D), elution times (E) and incubation time (F) (error bar is the standard deviation of three parallel tests).

Table S1 Cost accounting for the WP@MIPs sensor.

Materials	Source	Price (Yuan RMB)	Dosage each sensor/cost (Yuan RMB)
$FeCl_3 \cdot 6H_2O$	Sarn Chemical Technology (Shanghai) Co., Ltd.	28/500 g	8.26×10^{-4} g /4.62×10^{-5}
$HOCH_2CH_2OH$	Shanghai Bide Pharmaceutical Technology Co., Ltd.	32.25/1000 mL	2.45×10^{-2} g /7.90×10^{-4}
PEG-2000	Sarn Chemical Technology (Shanghai) Co., Ltd.	32/500 g	6.12×10^{-4} g /3.92×10^{-5}
NaAc	Sarn Chemical Technology (Shanghai) Co., Ltd.	36/1000 g	2.20×10^{-3} g /7.92×10^{-5}
EtOH	Hunan Huihong Reagent Co., Ltd.	45/2500 mL	6.12×10^{-3} mL/ 1.10×10^{-4}
Whatman 1 qualitative filter paper	Cytiva	0.6/38 cm^2	1.0 cm^2 /1.58×10^{-2}
H5N1	Harbin Veco Biotechnology Co., Ltd.	240/2000 μL	0.53 μL /6.36×10^{-2}
APTES	Shanghai Bide Pharmaceutical Technology Co., Ltd.	115/100 g	2.10×10^{-3} g /2.42×10^{-3}
TEOS	Sarn Chemical Technology (Shanghai) Co., Ltd.	36/500 mL	2.63×10^{-3} mL /1.90×10^{-4}
$NH_3 \cdot H_2O$	Xilong Chemical Co., Ltd.	9/500 mL	2.63×10^{-3} mL /4.73×10^{-5}
SDS	Tianjin Chemiou Chemical Reagent Co., Ltd.	69/1000 mL	1.32×10^{-2} mL /9.11×10^{-4}

AcH	Tianjin Chemiou Chemical Reagent Co., Ltd.	50/500 mL	1.58×10^{-4} mL /1.58×10^{-5}
TMB	Shanghai Bide Pharmaceutical Technology Co., Ltd.	329.25/25 g	5.00×10^{-3} g /6.58×10^{-2}
H_2O_2(30.0%)	Sinopharm Chemical Reagent Co., Ltd.	24/500 mL	1 mL/4.8×10^{-2}
PBS	Hubei Dongcao Chemical Technology Co., Ltd.	18/500 mL	1 mL/3.6×10^{-2}
			Total: 4 cents (0.23 yuan) per sensor