Electronic Supplementary Information (ESI)
This journal is (c) The Royal Society of Chemistry 2019

Three-dimensional macroporous gold superior to conventional gold disk electrode in the construction of electrochemical immunobiosensor for *Staphylococcus aureus* detection

Hui Wang, Xiao Zhao, Hui Yang, Lin Cao, Wenfang Deng, Yueming Tan*, and Qingji Xie

Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education of China), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, China

*Corresponding author.

E-mail address:tanyueming0813@hunnu.edu.cn

Instrumentation and reagents

Transmission electron microscopy (TEM) images were obtained with a Philips TECNAI F-30 transmission electron microscope. Scanning electron microscopy (SEM) images were obtained with a JSM-6360 field emission scanning electron microscope. Fourier transform infrared (FTIR) spectra were collected on Nicolet Nexus 670 Fourier transform infrared spectrometer. Ultraviolet-visible (UV-Vis) absorption spectra were recorded on UV-2450 spectrometer. Nitrogen adsorption and desorption isotherms and pore size distribution spectra were collected on Tristar 3000 automatic specific surface area and pore analyzer. All electrochemical experiments were performed on a CHI760E electrochemical workstation in a three-electrode system. The three-electrode system consists of a modified Au electrode (diameter 3 mm) as the working electrode, a KCl-saturated calomel electrode (SCE) as the reference electrode, and a Pt wire as the counter electrode.

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Chitosan (CS), chloroauric acid, tetraethyl silicate, and silver nitrate were purchased from Aladdin Chemistry. Nitric acid, sulfuric acid, potassium nitrate, ammonia, ethanol, ether, sodium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, hydrochloric acid, sodium hydroxide, potassium chloride, acetic acid and hydrogen peroxide are purchased from China National Pharmaceutical Group Co., Ltd. (Shanghai, China). Baird-Parker agar plates and sodium chloride broth (7.5 wt.%) were purchased from Qingdao Haibo Biological Company. 3-aminopropyltriethoxysilane (APTES, 98 wt.%) was purchased from Macleans. Calf intestinal ALP and L-ascorbic acid-2-phosphate trisodium salt (AAP) were purchased from Beijing

Libing Biotechnology Co., Ltd. Phosphate buffer solution (PBS, 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.4) used in the experiments was autoclaved at 125 °C for 30 min and the storage time was less than three weeks. Bacterial suspensions of different concentration gradients were configured using sterilized PBS. *S. aureus*, *Escherichia coli* O157:H7 (*E. coli* O157:H7), *Escherichia coli* K12 (E. coli K12), and *L. monocytogenes* were purchased from the United States Culture Collection (ATCC, USA). Anti-*S. aureus* polyclonal antibody (molecular weight: 40-60 kDa) was purchased from Beijing Boaosen Biological Co., Ltd. All other reagents were analytical grade and without further purification. Milli-Q ultrapure water (Millipore, ≥18 MΩ cm) was used throughout.

Bacterial growth

S. aureus, *E. coli* O157:H7 and *E. coli* K12 grew in sterile Luria-Bertani (LB) media (2.0 g bactotryptone, 1.0 g bacto-yeast extract powder, 2.0 g NaCl dissolving in 200 mL distilled water, and adjusted to pH 7.4 with 3.0 M NaOH) in an incubator-shaker at 37 °C to reach the growing stationary phase. After overnight incubation, 1 mL of bacterial cells was centrifuged (6000 rpm, 10 min) to remove the supernatant and washed 3 times with PBS (0.1 M, pH 7.4), and resuspended in 1 mL of PBS. Howbeit, *L. monocytogenes* grew in Nutrient-Broth (NB) media (2.0 g bacto-peptone, 0.6 g bacto-beef extract, 1.0 g NaCl dissolving in 200 mL distilled water, and adjusted to pH 7.4 with 3.0 M NaOH). By plating bacteria on LB or NB plates, the amount of bacteria per milliliter can be acquired by counting related colony forming units (CFU) after incubation overnight at 37 °C.

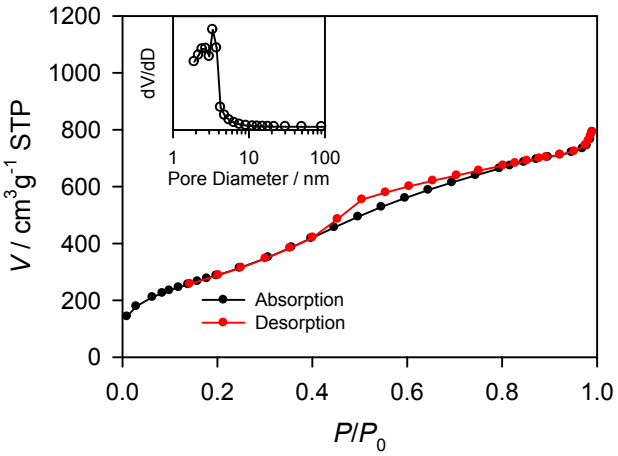

Fig. S1 Nitrogen adsorption and desorption isotherms of MSNs. Inset shows pore size distribution curve.

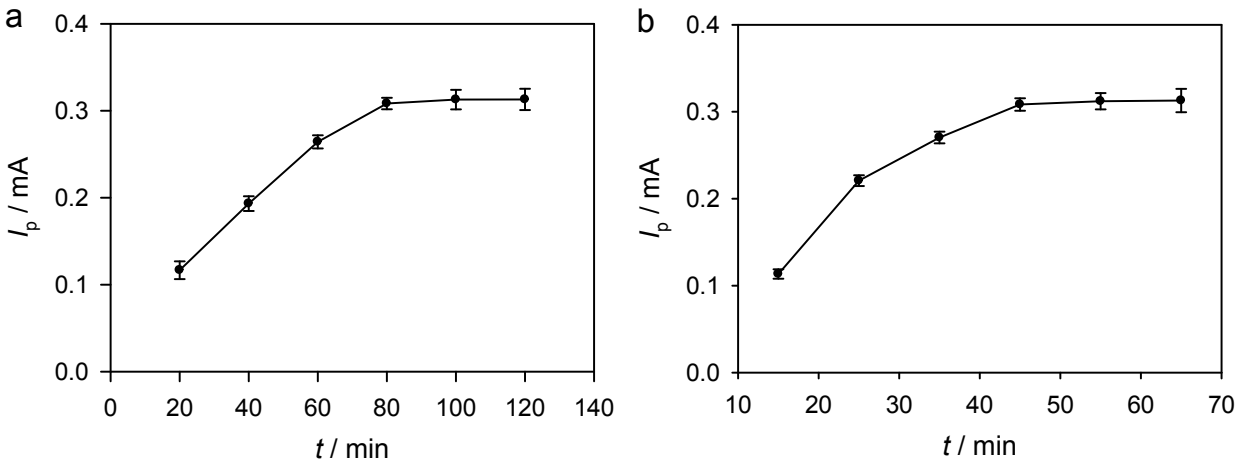

Fig. S2 The effects of *S. aureus* capture time (a) and ALP-MSNs-Ab_2 labeling time (b) on the resulting current responses for the detection of *S. aureus* (10^3 CFU mL^{-1}).

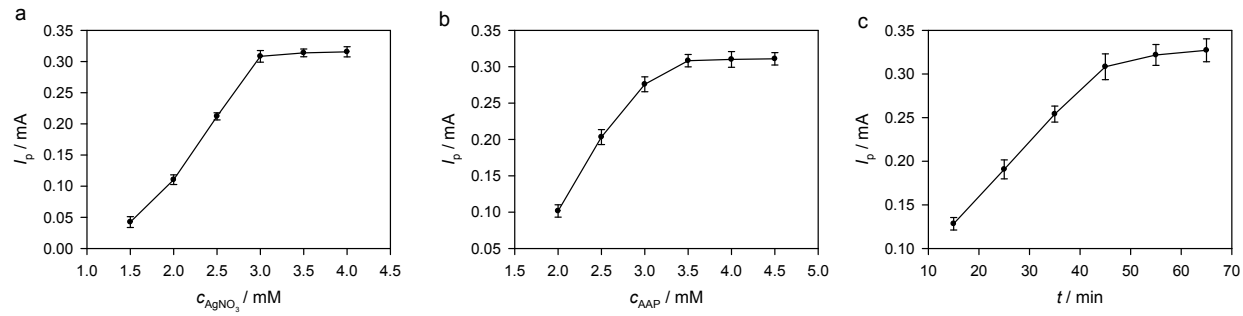

Fig. S3 The effects of $AgNO_3$ concentration (a), AAP concentration (b), and enzymatic reaction time (c) on the resulting current responses for the detection of *S. aureus* (10^3 CFU mL^{-1}).

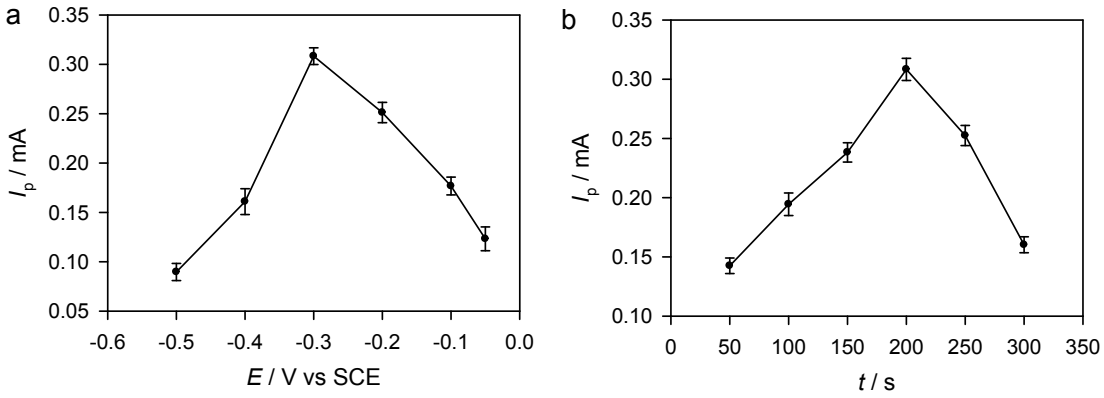

Fig. S4 The effects of deposition potential (a) and deposition time (b) on the resulting current responses for the detection of *S. aureus* (10^3 CFU mL^{-1}).

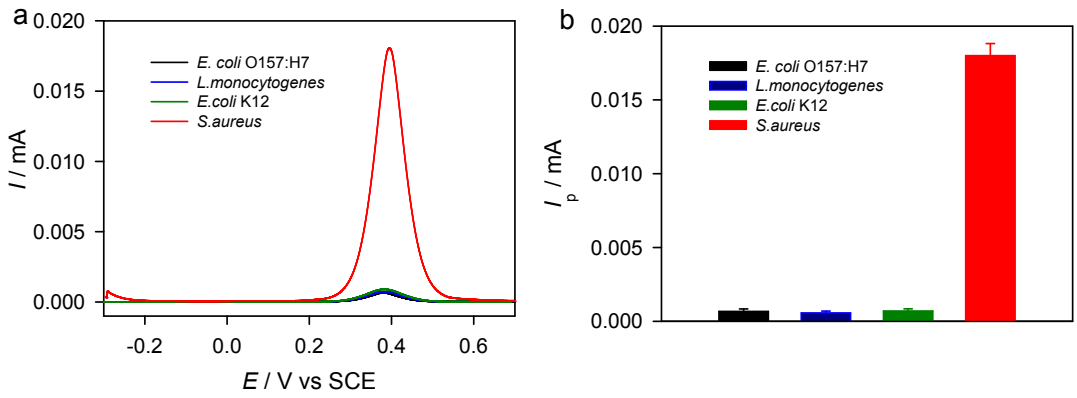

Fig. S5 DPVs (a) and peak currents (b) for detecting different pathogens the Au disk electrode-based biosensor. The concentrations of all pathogens are 10^3 CFU mL^{-1}.

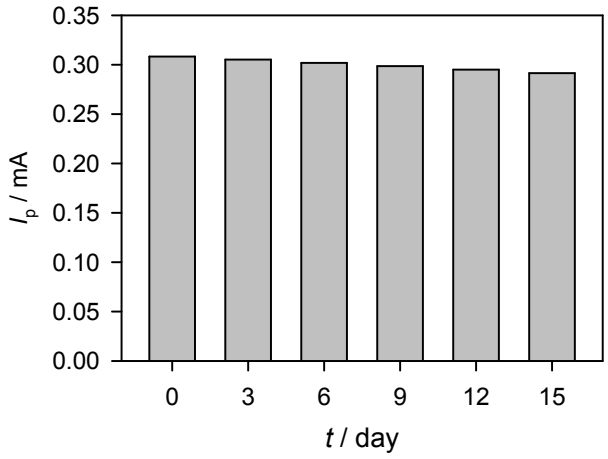

Fig. S6 Current responses for the detection of 10^3 CFU mL^{-1} *S. aureus* by 6 immunoelectrodes (prepared under the same conditions) at different time.

Table S1. Performance comparison of the detection of *S. aureus* in the literature.

Detection Method	Linear range (CFU mL^{-1})	Detection limit (CFU mL^{-1})	Ref
Electrochemical impedance spectroscopy	$10\text{-}10^7$	10	1
Electrochemical piezoelectric quartz crystal	$42\text{-}4.1\times10^5$	41	2
Electrochemical impedance spectroscopy	$10\text{-}10^6$	10	3
Electrochemical impedance spectroscopy	$10^2\text{-}10^7$	100	4
Amperometric detection	$10\text{-}10^8$	10	5
Amperometric detection	$4.4\times10^5\text{-}1.8\times10^7$	1.7×10^5	6
Potentiometric detection	$10^3\text{-}10^8$	8×10^2	7
Conductometric detection	$4.1\times10^3\text{-}4.1\times10^8$	4.1×10^3	8
Differential pulse voltammetric detection	$10\text{-}10^7$	1	9
Roll-to-roll point of care electrochemical device	-	200	10
Electrochemiluminescence	$1.0\times10^3\text{-}1.0\times10^9$	3.1×10^2	11
Colorimetry	$50\text{-}10^4$	20	12
Colorimetry	10-2000	10	13
Surface-enhanced Ranman scatting	50-800	17.8	14
Fluorescence	$80\text{-}8\times10^6$	39	15
Polymerase chain reaction	$5.0\times10^1\text{-}5.0\times10^8$	50	16
Differential pulse voltammetric detection	$5\text{-}10^9$	2	This work

Table S2. Results for detection of *S. aureus* in milk samples using 3DMG electrode-based biosensor and Au disk electrode-based biosensor.

Samples	3DMG electrode-based biosensor				`Au disk electrode-based biosensor			
	Original (CFU mL^{-1})	Added (CFU mL^{-1})	Found (CFU mL^{-1})	Recovery (%)	Original (CFU mL^{-1})	Added (CFU mL^{-1})	Found (CFU mL^{-1})	Recovery (%)
Milk #1	Not found	10	9±1	90	Not found	10	-	-
		100	95±5	95		100	93±5	93
		1000	970±20	97		1000	1020±30	102
Milk #2	Not found	10	9±2	90	Not found	10	-	-
		100	105±10	105		100	108±10	108
		1000	1020±20	102		1000	1010±50	101

Table S3. Results for detection of *S. aureus* in milk samples with the 3DMG electrode-based biosensor and a reported method that combines immunomagnetic separation with fluorescent immunoassay.[17]

	3DMG electrode-based biosensor				Immunomagnetic separation - fluorescent immunoassay			
Samples	Original (CFU mL^{-1})	Added (CFU mL^{-1})	Found (CFU mL^{-1})	Recovery (%)	Original (CFU mL^{-1})	Added (CFU mL^{-1})	Found (CFU mL^{-1})	Recovery (%)
Milk#1	Not found	10	9±1	90	Not found	10	9±2	90
		100	95±5	95		100	98±10	98
		1000	970±20	97		1000	1040±20	104
Milk#2	Not found	10	9±2	90	Not found	10	9±3	90
		100	105±10	105		100	108±10	108
		1000	1020±20	102		1000	950±50	95

References

1. K. Bekir, H. Barhoumi, M. Braiek, A. Chrouda, N. Zine, N. Abid, A. Maaref, A. Bakhrouf, H. Ben Ouada, N. Jaffrezic-Renault and H. Ben Mansour, *Environ.Sci. Pollut. Res.*, 2015, **22**, 15796-15803.

2. Y. Lian, F. He, H. Wang and F. Tong, *Biosens. Bioelectron.*, 2015, **65**, 314-319.

3. F. Jia, N. Duan, S. Wu, X. Ma, Y. Xia, Z. Wang and X. Wei, *Microchim. Acta*, 2014, **181**, 967-974.

4. J. H. Yoon, Y. D. Han, P. C. Lee and H. C. Yoon, *Biochip J.*, 2013, **7**, 344-352.

5. T. Majumdar, R. Chakraborty and U. Raychaudhuri, *Food Biosci.*, 2013, **4**, 38-45.

6. V. Escamilla-Gomez, S. Campuzano, M. Pedrero and J. M. Pingarron, *Anal. Bioanal. Chem.*, 2008, **391**, 837-845.

7. G. A. Zelada-Guillén, J. L. Sebastián-Avila, P. Blondeau, J. Riu and F. X. Rius, *Biosens. Bioelectron.*, 2012, **31**, 226-232.

8. X. Zhang, X. Wang, Q. Yang, X. Jiang, Y. Li, J. Zhao and K. Qu, *Microchim. Acta*, 2020, **187**.

9. A. Abbaspour, F. Norouz-Sarvestani, A. Noori and N. Soltani, *Biosens. Bioelectron.*, 2015, **68**, 149-155.

10. M. Safavieh, M. U. Ahmed and M. Zourob, *Sensors*, 2013, 1-4.

11. H. Yue, Y. Zhou, P. Wang, X. Wang, Z. Wang, L. Wang and Z. Fu, *Talanta*, 2016, **153**, 401-406.

12. S. Wang, W. Deng, L. Yang, Y. Tan, Q. Xie and S. Yao, *Acs Appl. Mater. Interfaces*, 2017, **9**, 24440-24445.

13. H. Shen, J. Wang, H. Liu, Z. Li, F. Jiang, F.-B. Wang and Q. Yuan, *ACS Appl. Mater. Interfaces*, 2016, **8**, 19371-19378.

14. A. Zhang, G. Tao and J. Wang, *J. Nanopart. Res.*, 2018, **20**.

15. R. Cai, F. Yin, Z. Zhang, Y. Tian and N. Zhou, *Anal. Chim. Acta*, 2019, **1075**, 128-136.

16. Y. Zeng, Y. Wan and D. Zhang, *Microchim. Acta*, 2016, **183**, 741-748.

17. W. Deng, C. Cheng, H. Yang, H. Wang, Y. Tan, Q. Xie, M. Ma and S. Yao, *Talanta*, 2019, **202**, 244-250.