Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Materials

Combination assay of lung cancer associated serum markers by surface-enhanced Raman spectroscopy

Chunyuan Song, Yanjun Yang, Boyue Yang, Linghua Min and Lianhui Wang

Table S1. Comparison of the proposed work with some reported results (markers in buffer)

Detection method	Detection range	LOD	Ref
fluorescence	AFP: 1.0 - 100 ng mL^{-1}	AFP: 0.72 ng mL^{-1}	[1]
	CEA:1.0 - 120 ng mL^{-1}	CEA: 0.89 ng mL^{-1}	
	0.5 - 500 ng mL^{-1}	CEA: 0.36 ng mL^{-1}	[2]
		α-AFP: 0.28 ng mL^{-1}	
	3 - 100 ng mL^{-1}	CEA: 1 ng mL^{-1}	[3]
		NSE: 1 ng mL^{-1}	
field effect transistor	1 ng mL^{-1} - 1 ug mL^{-1}	CYFRA 21-1: 1 ng mL^{-1}	[4]
		NSE: 10 ng mL^{-1}	
electrochemiluminescence	0.01 - 50 ng mL^{-1}	CEA: 0.006 ng mL^{-1}	[5]
		PSA: 0.003 ng mL^{-1}	
		α-AFP:0.005 ng mL^{-1}	
	0.01 - 0.1 pg mL^{-1}	CEA: 0.4 fg mL^{-1}	[6]
		AFP: 0.4 fg mL^{-1}	

electrochemistry	0.02 pg mL^{-1} - 5.0 ng mL^{-1}	CEA: 0.093 pg mL^{-1} AFP: 0.061 pg mL^{-1}	[7]
	0.01 - 100 ng mL^{-1}	CEA: 0.003 ng mL^{-1} AFP: 0.002 ng mL^{-1}	[8]
	0.01 - 100 ng mL^{-1}	CEA: 2.7 pg mL^{-1} PSA: 4.8 pg mL^{-1} AFP: 3.1 pg mL^{-1}	[9]
	0.01 - 100 ng mL^{-1}	AFP: 10 pg mL^{-1} CEA: 8.6 pg mL^{-1} PSA: 7.5 pg mL^{-1}	[10]
SERS	2 - 80 ng mL^{-1}	CEA: 0.62 ng mL^{-1} CK-19: 1.01 ng mL^{-1}	[11]
	0 - 100 ng mL^{-1}	CEA: 1.67 ng mL^{-1} AFP: 1.56 ng mL^{-1}	[12]
	1 fg mL^{-1} -1 ng mL^{-1}	CEA: 0.03 fg mL^{-1} NSE: 0.66 fg mL^{-1}	**This work**

Table S2. Comparison of the proposed work with some reported results (markers in serum)

Detection method	**Detection range**	**LOD**	**Ref**
fluorescence	CEA: 2 - 50 ng mL^{-1} CA125: 0 - 400 U mL^{-1}	CEA: 0.02 ng mL^{-1} N/A	[13]
electrochemiluminescence	AFP: 0.5 - 100 ng mL^{-1} CA125:1.0 - 100 U mL^{-1} CA199:0.5 - 100 U mL^{-1}	AFP: 0.15 ng mL^{-1} CA125:0.6 U mL^{-1} CA199:0.17 U mL^{-1}	[14]

	CEA: 1.0 - 100 ng mL^{-1}	CEA: 0.5 ng mL^{-1}	
LSPR	1 fM - 1 nM	AFP: 91 fM CEA: 94 fM PSA: 10 fM	[15]
SERS	CA15-3: 0.1 - 500 U mL^{-1}	CA15-3: 0.99 U mL^{-1}	[16]
	CA27-29: 0.1 - 500 U mL^{-1}	CA27-29: 0.13 U mL^{-1}	
	CEA: 0.1 - 500 ng mL^{-1}	CEA: 0.05 ng mL^{-1}	
	10 pg mL^{-1} - 100 ng mL^{-1}	CEA: 2.82 pg mL^{-1} NSE: 2.04 pg mL^{-1}	**This work**

Table S3. Experiment design for studying the specificity of combined detection of CEA and NSE in BBS buffer

Designations	CEA SERS tags	NSE SERS tags	immune-GMNPs	CEA	NES
blank	√	√	√		
mismatch 1	√		√		√
mismatch 2		√	√	√	
CEA	√	√	√	√	
NSE	√	√	√		√
CEA/NSE	√	√	√	√	√

Table S4. Experiment design for studying the specificity of combined detection of CEA and NSE in human serum

Designations	CEA SERS tags	NSE SERS tags	immune-GMNPs	CEA	NES	AFP
blank	√	√	√			
AFP	√	√	√			√
CEA	√	√	√	√		
NSE	√	√	√		√	
CEA/NSE	√	√	√	√	√	

Table S5. Characterization of LSPR peaks of the Au NFs after each surface modification for preparing CEA SERS tags

Materials	λ (nm)	❒λ (nm)
Au NFs	803	
Au NFs-4MBA	813	10
Au NFs-4MBA（EDC/NHS）	814.5	1.5
Au NFs-4MBA-anti CEA	842	27.5

Table S6. Characterization of LSPR peaks of the Au NFs after each surface modification for preparing NSE SERS tags

Materials	λ (nm)	❒λ (nm)
Au NFs	803	
Au NFs-DTNB	819	16
Au NFs-DTNB (EDC/NHS)	821	2
Au NFs-DTNB-anti NSE	845	24

Table S7. Zeta potential of the Au NFs after each surface modification for preparing CEA SERS tags

Materials	Zeta potential (mV)
Au NFs	46.32 ± 1.02
Au NFs-4MBA	-21.65 ± 1.64
Au NFs-4MBA (EDC/NHS)	-34.12 ± 0.71
Au NFs-4MBA-anti CEA	-45.49 ± 1.32

Table S8. Zeta potential of the Au NFs after each surface modification for preparing NSE SERS tags

Materials	Zeta potential (mV)
Au NFs	46.32 ± 1.02
Au NFs-DTNB	-28.64 ± 2.24
Au NFs-DTNB (EDC/NHS)	-36.81 ± 1.34
Au NFs-DTNB-anti NSE	-50.73 ± 1.38

Table S9. Characterization of LSPR peaks of the GMNPs before and after immobilization of mixed antibodies

Materials	λ (nm)	❒λ (nm)
GMNP	608	
GMNP-anti CEA/anti NSE	616	8

Table S10. Zeta potential of the GMNPs before and after immobilization of mixed antibodies

Materials	Zeta potential (mV)
GMNP	-30.70 ± 1.89
GMNP-anti CEA/anti NSE	-40.47 ± 1.21

References

1. Li, J.; Wang, H.; Dong, S.; Zhu, P.; Diao, G.; Yang, Z., Quantum-dot-tagged photonic crystal beads for multiplex detection of tumor markers. *Chemical Communications* **2014,** *50*, 14589-14592.
2. Tian, J.; Zhou, L.; Zhao, Y.; Wang, Y.; Peng, Y.; Zhao, S., Multiplexed detection of tumor markers with multicolor quantum dots based on fluorescence polarization immunoassay. *Talanta* **2012,** *92*, 72-77.
3. Li, H.; Cao, Z.; Zhang, Y.; Lau, C.; Lu, J., Simultaneous detection of two lung cancer biomarkers using dual-color fluorescence quantum dots. *Analyst* **2011,** *136*, 1399-1405.
4. Cheng, S.; Hideshima, S.; Kuroiwa, S.; Nakanishi, T.; Osaka, T., Label-free detection of tumor markers using field effect transistor (FET)-based biosensors for lung cancer diagnosis. *Sensors and Actuators B: Chemical* **2015,** *212*, 329-334.
5. Zhang, Y.; Liu, W.; Ge, S.; Yan, M.; Wang, S.; Yu, J.; Li, N.; Song, X., Multiplexed sandwich immunoassays using flow-injection electrochemiluminescence with designed substrate spatial-resolved technique for detection of tumor markers. *Biosensors and Bioelectronics* **2013,** *41*, 684-690.

6. Guo, Z.; Hao, T.; Du, S.; Chen, B.; Wang, Z.; Li, X.; Wang, S., Multiplex electrochemiluminescence immunoassay of two tumor markers using multicolor quantum dots as labels and graphene asconductingbridge. *Biosensors and Bioelectronics* **2013,** *44*, 101-107.
7. Lai, G.; Wu, J.; Ju, H.; Yan, F., Streptavidin-Functionalized Silver-Nanoparticle-Enriched Carbon Nanotube Tag for Ultrasensitive Multiplexed Detection of Tumor Markers. *Advanced Functional Materials* **2011,** *21*, 2938-2943.
8. Liu, N.; Liu, Z.; Han, H.; Ma, Z., Graphene oxide reduced directly by redox probes for multiplexed detection of tumor markers. *Journal of Materials Chemistry B* **2014,** *2*, 3292-3298.
9. Xu, T.; Liu, N.; Yuan, J.; Ma, Z., Triple tumor markers assay based on carbon-gold nanocomposite. *Biosensors & Bioelectronics* **2015,** *70*, 161-166.
10. Wang, Z.; Liu, N.; Feng, F.; Ma, Z., Synthesis of cadmium, lead and copper alginate nanobeads as immunosensing probes for the detection of AFP, CEA and PSA. *Biosensors and Bioelectronics* **2015,** *70*, 98-105.
11. Lu, W.; Wang, Y.; Cao, X.; Li, L.; Dong, J.; Qian, W., Multiplexing determination of lung cancer biomarkers using electrochemical and surface-enhanced Raman spectroscopic techniques. *New Journal of Chemistry* **2015,** *39*, 5420-5430.
12. Chon, H.; Lee, S.; Yoon, S.Y.; Chang, S.-I.; Lim, D. W.; Choo, J., Simultaneous immunoassay for the detection of two lung cancer markers using functionalized SERS nanoprobes. *Chemical communications* **2011,** *47*, 12515-12517.
13. Jokerst, J. V.; Raamanathan, A.; Christodoulides, N.; Floriano, P. N.; Pollard, A. A.; Simmons, G. W.; Wong, J.; Gage, C.; Furmaga, W. B.; Redding, S. W., Nano-bio-chips for high performance multiplexed protein detection: determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels. *Biosensors and Bioelectronics* **2009,** *24*, 3622-3629.
14. Ge, L.; Yan, J.; Song, X.; Yan, M.; Ge, S.; Yu, J., Three-dimensional paper-based electrochemiluminescence immunodevice for multiplexed measurement of biomarkers and point-of-care testing. *Biomaterials* **2012,** *33*, 1024-1031.
15. Lee, J. U.; Nguyen, A. H.; Sim, S. J., A nanoplasmonic biosensor for label-free multiplex detection of cancer biomarkers. *Biosensors and Bioelectronics* **2015,** *74*, 341-346.
16. Li, M.; Kang, J. W.; Sukumar, S.; Dasari, R. R.; Barman, I., Multiplexed detection of serological cancer markers with plasmon-enhanced Raman spectro-immunoassay. *Chemical Science* **2015,** *6*, 3906-3914.