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A capillary-driven LoC-SERS device integrated with catalytic hairpin assembly amplification technology for NSCLC-related biomarkers detection

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1.1 Fabrication of LoC system and hydrophilic treatment

The proposed LoC system consisted of two parts: PDMS cover with micro-nano channels and glass slide. A silicon wafer was chosen as the substrate for the preparation of the mold. Hydrophilization treatment can improve the clear water properties of the silicon wafers and improve the spin coating quality of the SU-8 adhesive, resulting in a higher quality male mold. The cleaned silicon wafers were left to dehydrate in a 200 °C drying oven to ensure the adhesion followed by spin-coating a 100-μm thick layer of SU-8 photoresist. Then, the silicon wafer was covered with the mask plate and placed on the lithography bench for exposure. After exposure, removed the mask stencil, put the silicon wafer on the heating plate followed by putting into the developing solution. Then, put the silicon wafer on the heating plate again, and thus the silicon stencil with micro-nano channels structure were prepared. Subsequently, a certain amount of PDMS and the curing agent in the ratio of 10:1 were measured and mixed. Poured the mixture into a transparent plastic cup and stirred quickly. After rapid stirring for about 5 min, the mixture was placed in the vacuum tube of the vacuum annealing furnace for bubble removal. Then, the PDMS mixture gradually returned to the transparent state, and the bubbles inside the colloid disappeared. Finally, poured the PDMS into the silicon template and put it into a blast dryer followed by heating at 70 °C for two hours. Then, the PDMS cover with micro-nano channels structure could be obtained after cooling, cutting and slotting. The prepared PDMS cover and glass slide were soaked into ethanol, isopropyl alcohol and placed in ultrasonic cleaner for 10 min. After drying on the heating plate, PDMS

cover and glass slide were placed in the plasma cleaning machine for plasma treatment (30 min). As PDMS is poorly wettable and highly hydrophobic, and the liquid in the channel is difficult to flow spontaneously. Thus, hydrophilic modification of the inner surface of the channel can reduce the contact angle resulting in the spontaneous flow of liquid along the channel due to the capillary phenomenon. Briefly, the chips were first rinsed with isopropyl alcohol and deionized water for 5 times respectively flollowed by drying and 90 s plasma oxidation. The oxidized chip was immediately submerged in PEG and the surface-treated chip was then heated at 60 °C for 30 min. After heating, the chips were rinsed 5 times using isopropyl alcohol and deionized water, and then the chips were dried on a hot plate at 150 °C. Thus, a LoC system with great hydrophilicity were fabricated.

1.2 Preparation of antibody-DNA conjugates

The antibody-DNA conjugates ($Ab_{1\text{-}1}$@$DNA_{1\text{-}1}$ and $Ab_{2\text{-}1}$@$DNA_{2\text{-}1}$) were prepared as follow. $Ab_{1\text{-}1}$ (4 mg/mL) and $Ab_{2\text{-}1}$ (4 mg/mL) were incubated in PBS with a 20-fold molar excess of SMCC at room temperature for 2 h. Concurrently, 6 mL of 100 μM thiolated oligonucleotide and 4 mL of 100 μM DTT were mixed with PBS at 37 °C for 1 h followed by three times of purification (10000 rpm, 10 min). Thus, the $Ab_{1\text{-}1}$@$DNA_{1\text{-}1}$ and $Ab_{2\text{-}1}$@$DNA_{2\text{-}1}$ were achieved after the incubation antibody overnight at 4 °C and three times of purification. Similarly, the antibody-DNA conjugates ($Ab_{1\text{-}2}$@$DNA_{1\text{-}2}$ and $Ab_{2\text{-}2}$@$DNA_{2\text{-}2}$) were synthesized.

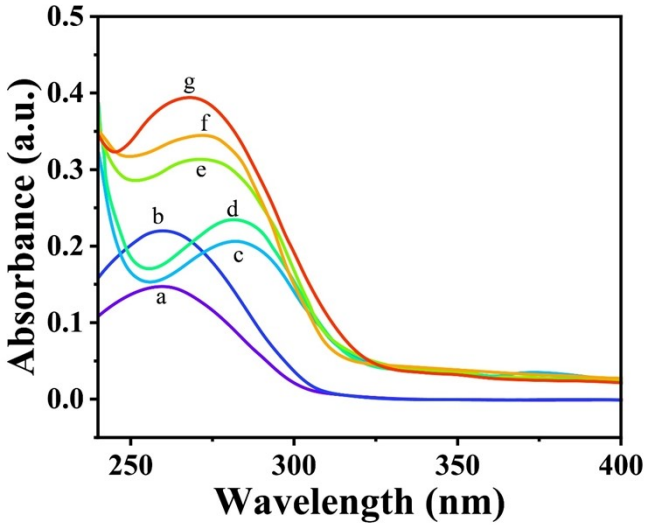

Fig. S1 UV-visible absorption spectrum of (a) DNA1-1 (1 μM), (b) $DNA_{2\text{-}1}$ (1 μM), (c) $Ab_{1\text{-}1}$ (0.2 mg/mL), (d) $Ab_{2\text{-}1}$ (0.2 mg/mL), (e) $Ab_{1\text{-}1}$@$DNA_{1\text{-}1}$, (f) $Ab_{2\text{-}1}$@$DNA_{2\text{-}1}$ and (g) $Ab_{1\text{-}1}$@$DNA_{1\text{-}1}$@$Ab_{1\text{-}1}$@CEA@$Ab_{2\text{-}1}$@$DNA_{2\text{-}1}$.

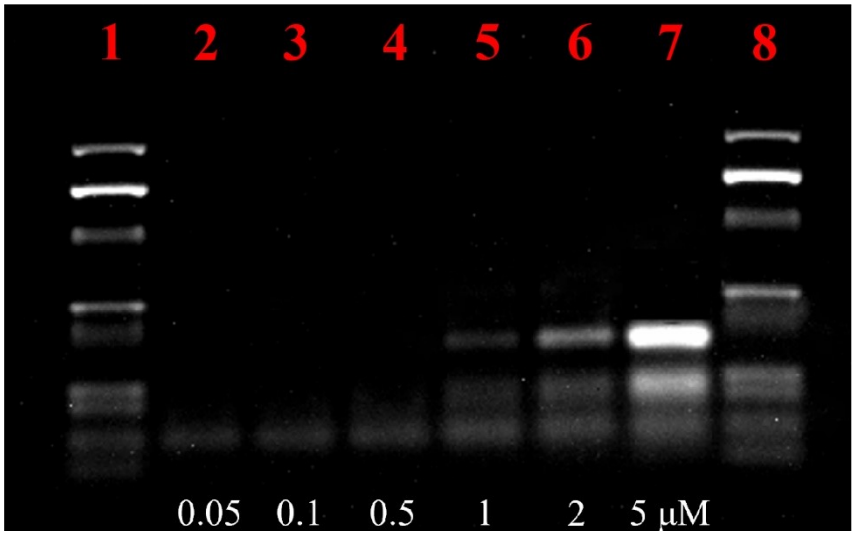

Fig. S2 AGE characterizations of CHA signal amplification strategy. Lane 1: marker; lane 2: $DNA_{1\text{-}1}$+refDNA+$DNA_{2\text{-}1}$+hp1-1+hp2-1 (0.05 μM); lane 3: $DNA_{1\text{-}1}$+refDNA +$DNA_{2\text{-}1}$+hp1-1+hp2-1 (0.1 μM); lane 4: $DNA_{1\text{-}1}$+refDNA+$DNA_{2\text{-}1}$+hp1-1+hp2-1 (0.5 μM); lane 5: $DNA_{1\text{-}1}$+refDNA+$DNA_{2\text{-}1}$+hp1-1+hp2-1 (1 μM); lane 6: $DNA_{1\text{-}1}$+refDNA+$DNA_{2\text{-}1}$+hp1-1+hp2-1 (2 μM); lane 7: $DNA_{1\text{-}1}$+refDNA+$DNA_{2\text{-}1}$ +hp1-1+hp2-1 (5 μM); lane 8: marker.

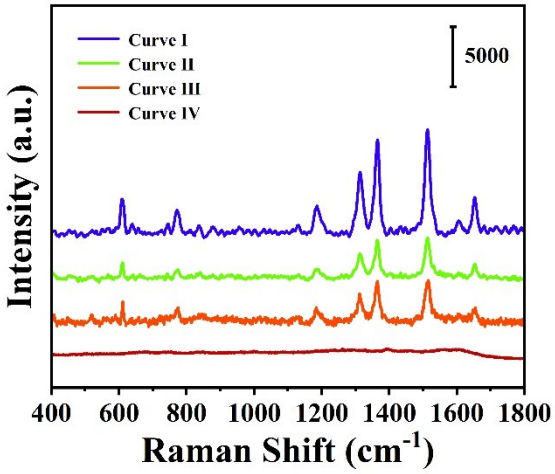

Fig. S3 SERS spectra of Au-AgNBs@R6G@hp1-1 (Curve II), MBs@hp2-1 (Curve IV), $DNA_{1\text{-}1}$+$DNA_{2\text{-}1}$+Au-AgNBs@R6G@hp1-1+MBs@hp2-1 (Curve III) and $DNA_{1\text{-}1}$+refDNA+ $DNA_{2\text{-}1}$+Au-AgNBs@R6G@hp1-1+MBs@hp2-1 (Curve I).

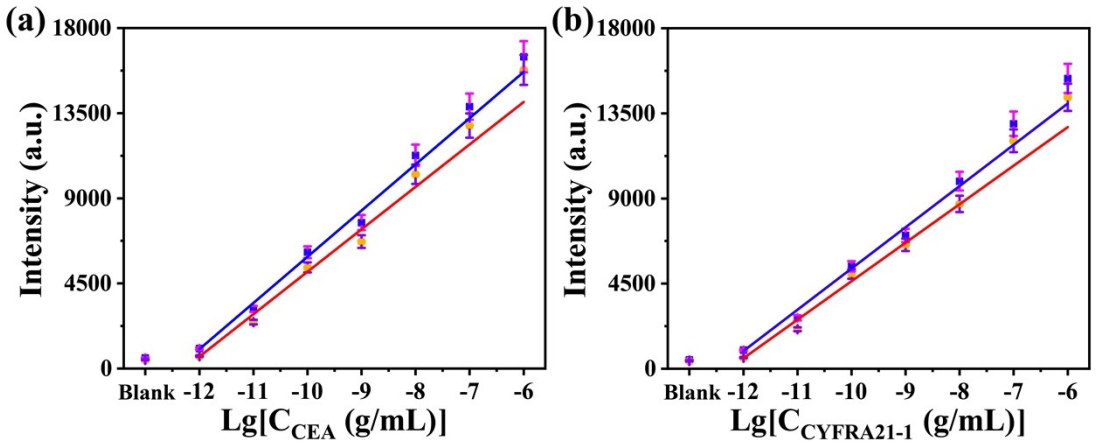

Fig. S4 (a) Calibration curve of peak intensities at 1080 cm^{-1} in PBS buffer and serum versus logarithm of CYFRA21-1 concentration. (b) Calibration curve of peak intensities at 1508 cm^{-1} in PBS buffer and serum versus logarithm of CYFRA21-1 concentration.

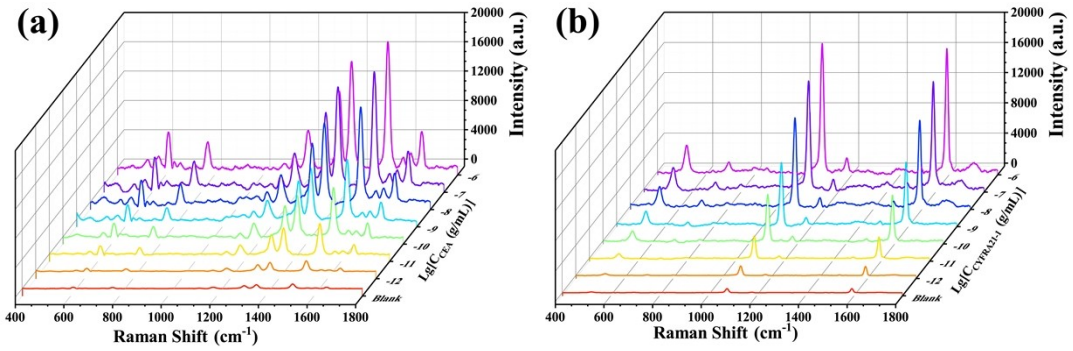

Fig. S5 SERS spectra of (a) CEA and (b) CYFRA21-1 at different concentrations (Blank, 1 pg/mL, 10 pg/mL, 100 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL and 1 μg/mL).

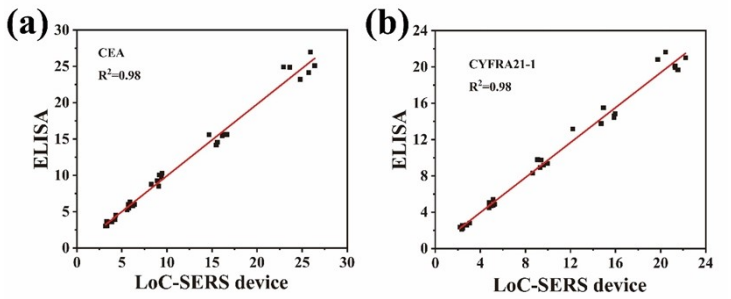

Fig. S6 Linear fitting in the measured relative expression levels of (a) CEA and (b) CYFRA21-1 in all mouse samples at different time between SERS and ELISA.

Table S1 Oligonucleotides applied in the experiment

Name	Sequences (5'-3')
DNA_{1-1}	HS-TACGTCCAGAACTTTACCAATTTTTTTTTTTTTTTGTGAGGCACGTTAGACCACTT
DNA_{1-2}	HS-TTAATCCCATATTTCTGACCATTTTTTTTTTTTTTTAGCTTCCACGTTAGACCACTT
DNA_{2-1}	ATCTCCACAACTGAACCTCACTTTTTTTTTTTTTTTTGGCTCTAGCGTATGCTATTG-SH
DNA_{2-2}	ATCTCCACAACTGAAAACAGGTTTTTTTTTTTTTTTAGATGGTCAGACATATCGGAT-SH
hp1-1	ACAACTGAACACGTTAGACCACTTCCATCCTCGCAAATCTCCACAACTAAGTGGTCTAA CGTGTTCAGTTGTGGAGAT-SH
hp1-2	ACAACTGAACACGTTAGACCACTTCCATCCTCGCAAATCTCCACAACTAAGTGGTCTAA CGTGTTCAGTTGTGGAGAT-SH
hp2-1	H_2N- TGCTCTCCACTTCCATCCTCGCAAATCTCCACAACTGAACACGTTAGACCACTTAGTTGT GGAGATTTGCGAGGATGGAAGTGGTCTAAC
hp2-2	H_2N- ACCTCCACAACTGAACACGTTAGACCACTTAGTTGTGGAGATTTGCGAGGATGGAAGTG GTCTAACAGAAGAAGGTGTTTAAGT
refDNA	TTGGTAAAGTTCTGGACGTATCTCCCATTGTATTATCTTGTATTATCTCTTATCTCCAGTC AATAGCATACGCTAGAGCC

Table S2 Comparison of SERS and ELSIA method in real samples (1 d)

Group	CEA			CYFRA21-1		
	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)
1	3.32	3.65	-9.94	2.19	2.37	-8.22
2	4.22	3.91	7.35	2.39	2.54	-6.28
3	3.18	3.02	5.03	2.35	2.15	8.51
4	4.31	4.53	-5.10	2.78	2.59	6.83
5	3.36	3.07	8.63	2.42	2.27	6.20
6	3.89	3.60	7.46	3.06	2.82	7.84

Table S3 Comparison of SERS and ELSIA method in real samples (7 d)

Group	CEA			CYFRA21-1		
	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)
1	5.76	5.52	4.17	4.98	4.72	5.22
2	5.69	5.99	-5.27	4.78	5.05	-5.65
3	6.21	5.81	6.44	5.12	5.42	-5.86
4	6.41	6.01	6.24	5.26	4.87	7.41
5	5.88	6.34	-7.82	4.78	4.49	6.07

6	5.57	5.26	5.57	5.14	4.79	6.81

Table S4 Comparison of SERS and ELSIA method in real samples (14 d)

Group	CEA			CYFRA21-1		
	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)
1	9.09	8.52	6.27	9.96	9.36	6.02
2	9.15	10.05	-9.84	9.38	9.74	-3.84
3	8.93	9.25	-3.58	9.31	8.92	4.19
4	9.45	10.28	-8.78	8.64	8.32	3.70
5	8.27	8.78	-6.17	9.08	9.79	-7.82
6	9.39	9.72	-3.51	9.61	9.21	4.16

Table S5 Comparison of SERS and ELSIA method in real samples (21 d)

Group	CEA			CYFRA21-1		
	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)
1	15.46	14.18	8.28	14.92	15.52	-4.02
2	16.66	15.63	6.18	12.2	13.17	-7.95
3	14.67	15.61	-6.41	15.85	14.62	7.76
4	16.13	15.44	4.28	14.72	13.77	6.45
5	16.24	15.63	3.76	15.86	14.42	9.08
6	15.59	14.56	6.61	15.95	14.84	6.96

Table S6 Comparison of SERS and ELSIA method in real samples (28 d)

Group	CEA			CYFRA21-1		
	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)	SERS (ng/mL)	ELISA (ng/mL)	Relative error (%)
1	22.93	24.93	-8.72	21.27	20.12	5.41
2	25.72	24.15	6.10	21.53	19.68	8.59
3	26.39	25.09	4.93	22.21	21.01	5.40
4	23.61	24.87	-5.34	20.43	21.64	-5.92
5	24.79	23.21	6.37	21.29	19.91	6.48
6	25.91	26.98	-4.13	19.74	20.82	-5.47