Supporting Information

Multiplexed electrochemical and SERS dual-mode detection of stroke biomarkers: rapid screening with high sensitivity

Jingyi Sun[c], Yi Zhao[b], Yajun Hou[a], Hanxia Li[a], Mingfeng Yang[a], Ying Wang[a]*, Baoliang Sun[ab]*

[a]*Key Lab of Cerebral Microcirculation in Universities of Shandong, Shandong First Medical University & Shandong Academy of Medical Sciences, Taian, Shandong, 271000, China*

[b]*The second Affiliated Hospital, Shandong First Medical University & Shandong Academy of Medical Sciences, Taian, Shandong, 271000, China*

[c]*Wonju Severance Christian Hospital, Yonsei University Wonju College of Medicine, Wonju, Gangwon 220-701, Republic of Korea*

**E-mail: blsun99@163.com*

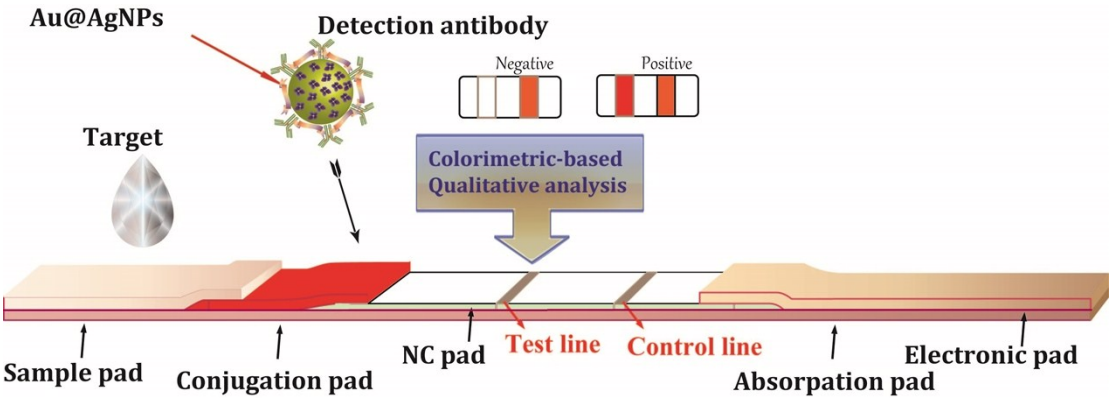

Figure S1 Schematic illustration of the operation principle of protocol LFA.

Figure S2A shows that the current response of NBA@Au@AgNPs@FTO-electrode decreased with the increase of pH, because of the requirement of proton in the process of electrochemical oxidation and reduction of NBA. When pH increases from pH 4.0 to 8.0, the reduction peak current will get lower, due to that there were not enough protons to promote NBA to react at the surface of electrode. Figure S2C shows that the current response of the 4-MBA@Au@AgNPs decorated FTO electrode 4-MBA@Au@AgNPs@FTO-electrode increases from pH 4.0, reaches the maximum value at pH 6.5, and then decreases to pH 8.0. Therefore, pH 6.5 is chosen as the optimal pH of the detection solution to obtain a high sensitivity. Incubation time is another important parameter in the construction of the immunosensor. The effect of incubation time is investigated in the time range of 0-50 min with 10 ng/mL of S100-β. Figure S2B shows that the current response rapidly decreases within the first 30 min and then tends to level off due to the saturated formation of antigen-antibody complex. For the incubation time of NSE, the similar results are observed in Figure S2D. Therefore, the optimal incubation time is set at 30 min.

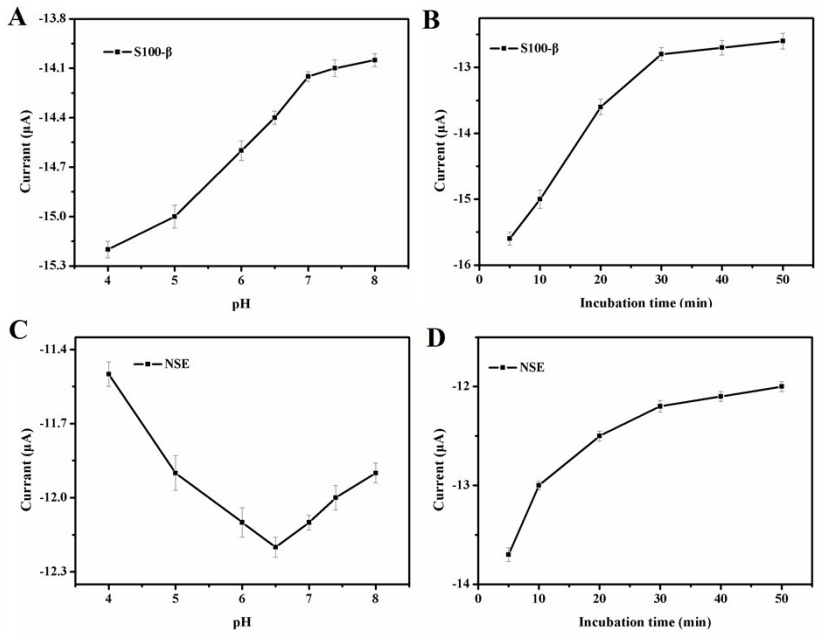

Figure S2 Effects of (A) pH of detection solution and (B) incubation time on SWV responses to 10 ng/mL S100-β at the modified electrode. Effects of (C) pH of detection solution and (D) incubation time on SWV responses to 10 ng/mL NSE at the modified electrode.

The original three stroke biomarkers purchased was diluted to 0.01, 0.1, 0.5, 1, or 5 $ng/mL_{S100-\beta}$ and 0.02, 0.1, 0.5, 1, or 5 ng/mL_{NSE} using PBS (pH 7.4) buffer to form biomarkers stock solutions respectively. The results are presented in Figure S3. As can been seen, the stroke biomarkers obtained from the three individual trials correspond well with each other, indicating good reliability and reproducibility of the SERS protocol.

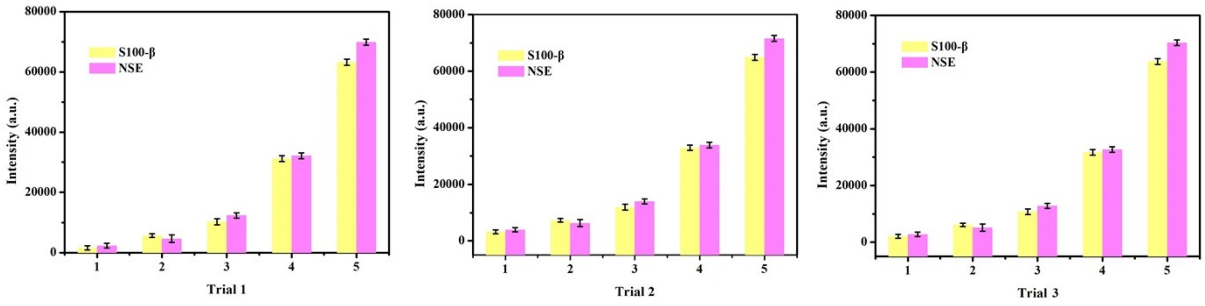

Figure S3 Reproducibility and reliability of the SERS protocol. Two individual stroke biomarkers detection assays were performed using different batches of SERS FLGH. The error bars represent the standard deviation of 5 measurements.

To assess the clinical applicability and diagnostic capability of our SERS LFA, 100 serum samples collected from hospitalized patients suffering from stroke were measured using our SERS LFA and clinical enzyme linked immunosorbent assay (ELISA) method, respectively. For validation of the as-proposed assay technique, PassingBoblok regression and Spearman's rank correlation coefficient were adopted to analyze the linear dependence between the two methods (Figure. S4). The regression equation and Spearman's coefficient of rank correlation of S100-β, NSE and MMP-9 detection between clinical ELISA and SERS LFA are shown in inset. Both the slopes of the three regression equations and the Spearman's coefficients are close to 1, which are included in the 95 % confidence intervals of the corresponding parameters. This indicates that the two methods for S100-β, NSE and MMP-9 detection possess good linear correlation. However, the ELISA needs sample pretreatment (about 1 h), expensive instruments and trained personnel for the assays. In contrast, SERS LFA is free of sample pretreatment in clinical application, low-cost, easy to use, and the reaction result is ready to be read in 15 min per marker which is faster than ELISA, with its sensitivity one order higher than ELISA. This is favorable for the precision stroke diagnosis and prognosis at home or in hospital, which will greatly reduce the stroke mortality. Although the Raman readout time of each test line

is 10 min here. Therefore, the design of a customized Raman reader for SERS LFA is not a problem with the fast-growing demand in POCT and will pave the way for its wide applications.

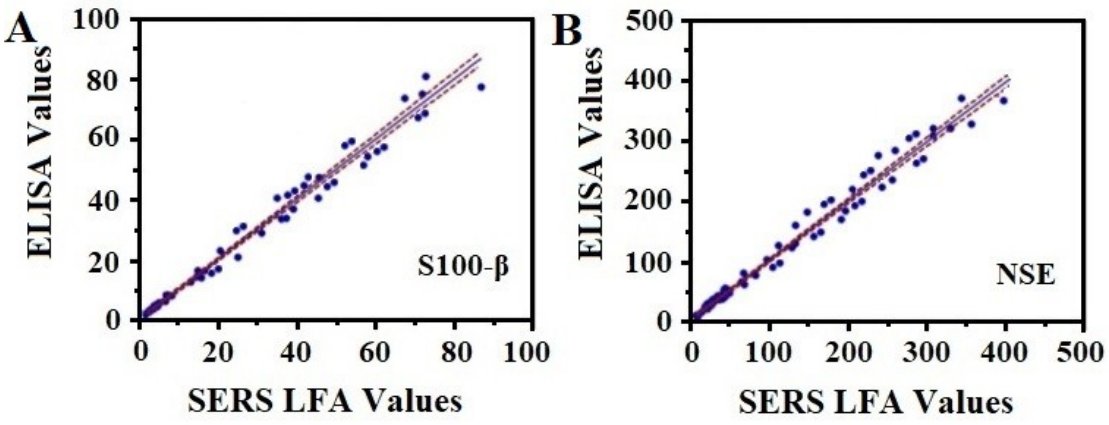

Figure S4 Regression lines between the results obtained from clinical ELISA methods and SERS LFA for (A) S100-β and (B) NSE detection in real serum samples. In the figure, the solid blue lines represent the linear regression line, and the red dash lines show the range of ELISA.

The feasibility of the prepared electrochemical immunosensor for possibly clinical application is investigated by analyzing human serum samples from Affiliated Hospital of Taishan Medical University. The immunoassay for human serum samples is investigated by analyzing three real samples in comparison with ELISA technique. Each human serum sample is analyzed for ten times. The results and the relative errors between the two methods ranged from -2.15 % to 8.51 % for S100-β and from -4.37 % to 8.78 % for NSE. It is demonstrated that there is no signification difference between the results given by the two methods. As a result, the prepared

immunosensor could be reasonably applied in the clinical determination of S100-β and NSE.

Table S1 Assay results of clinical samples using the proposed and the ELISA methods.

Analyte	S100-β			NSE		
Number	Prepared Sensor (ng/mL)	ELISA (ng/mL)	Realative Error (%)	Prepared Sensor (ng/mL)	ELISA (ng/mL)	Realative Error (%)
1	1.79	1.85	-2.87	1.47	1.43	2.11
2	4.64	4.63	1.24	4.92	4.76	3.59
3	9.81	9.35	-1.35	9.23	9.73	-4.67

Table S2 An overview on recently reported nanomaterial

Materials	Method	LODs	References
Au@AgNPs tags	electrochemical and SERS dual-mode detection of stroke biomarkers	0.35 pg·mL^{-1} 0.53 pg·mL^{-1}	
nanocomposite modified ITO electrode and CdS-labeled antibody	sandwich-type photoelectrochemical immunoassay	Range: 0.25-10 ng·mL^{-1}	[1]
carbon nanotube based carbon working electrode	modified electrochemical immunoassay technique	Range: 10 fg·mL^{-1} -10 ng·mL^{-1}	[2]
Titanium (20 nm) and gold (150 nm) formed zigzag pattern electrodes	miniaturized label-free electrochemical biosensor using zigzag electrodes	Range: 10 ng·mL^{-1} - 10ug·mL^{-1} LOD: 10 ng·mL^{-1}	[3]

References

1. Tabrizi MA, Ferré-Borrull J, Kapruwan P, Marsal LF. (2019) A photoelectrochemical sandwich immunoassay for protein S100β, a biomarker for Alzheimer's disease, using an ITO electrode modified with a reduced graphene oxide-gold conjugate and CdS-labeled secondary antibody. Microchimica Acta 186: 117.

2. Mathew AS, Shi X, Yau ST. (2018) Detection of a Traumatic Brain Injury Biomarker at the 10 fg/mL Level. Molecular diagnosis & therapy 22: 729-735.

3. Kuo YC, Lee CK, Lin CT. (2018) Improving sensitivity of a miniaturized label-free electrochemical

biosensor using zigzag electrodes. Biosensors and Bioelectronics 103: 130-137.