Highly-integrated SERS-Based Immunoassay NanoPADs for Early Diagnosis of Alzheimer's Disease

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***Abstract*— Nanocellulose paper (nanopaper) has been widely applied as a promising substrate for biomedical due to its low cost, biocompatibility and high optical transparency. In this report, we first reported surface-enhanced Raman scattering (SERS) immunoassay on the nanopaper-based analytical microfluidic devices (NanoPADs). We detected glial fibrillary acidic protein (GFAP) in human plasma without pretreatment using SERS on NanoPADs for highly sensitive early diagnosis of Alzheimer's disease. For SERS detection, DTNB-labeled uniform gold nanoparticles (AuNPs) were utilized as tags. Additionally, *in-situ* silver nanoparticles (AgNPs) were used as SERS substrates. We detected different concentrations of GFAP and determined the limit of detection as 150 fg/mL, which was 100 times better than commercial analytical techniques.**

I. Introduction

Alzheimer's disease (AD) is a serious degenerative disease of the brain-nervous system that poses a significant social challenge with increasing prevalence each year [1]. Among analytical techniques, surface-enhanced Raman scattering (SERS) has emerged as an excellent choice for simultaneous detection due to its high sensitivity. However, existing SERS-based AD detection substrates such as silicon and glass required complicated fabrication and massive manual operation, which restricted the sensitivity of detection. Thanks to the high optical transparency and easy-to-operation of nanopaper as a SERS substrate, the highly-integrated NanoPADs we developed previously provided a facile and highly sensitive technique to solve these problems [2].

II. Methods

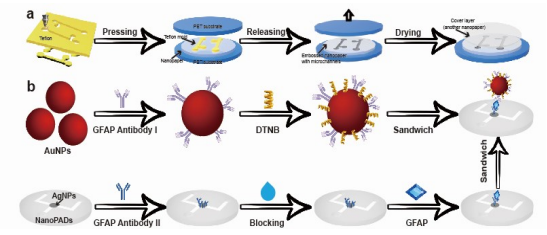

Figure 1. Schematic of SERS-based sandwich AD detection.

Fig. 1a shows the NanoPADs using a facile micro-embossing process with convenient plastic molds. Fig. 1b illustrates a SERS-based sandwich immunoassay for early AD diagnosis. First, to fabricate DTNB-labeled SERS tags, Glial fibrillary acidic protein (GFAP) antibody Ⅰ was added to gold nanoparticles (AuNPs) suspension through mercaptan and ionic interactions. The resulting mixture was centrifugated to remove free antibodies. Subsequently, DTNB was added to the above GFAP-AuNPs solution, and uncombined DTNB was washed by centrifugation after fully stirring. Second, to fabricate the SERS immobilization substrate, GFAP antibody Ⅱ was pipetted into the inlet zone of the NanoPADs with *in-situ* silver nanoparticles (AgNPs) for coating. Before use, the substrate was thoroughly blocked with a blocking solution. Third, different concentrations of GFAP in artificial human serum (1 μg/mL to 1 pg/mL) were added and transferred into the inlet zone of the NanoPADs for incubation. Finally, SERS tags were added in the NanoPADs for sandwich immunoassay.

III. Results

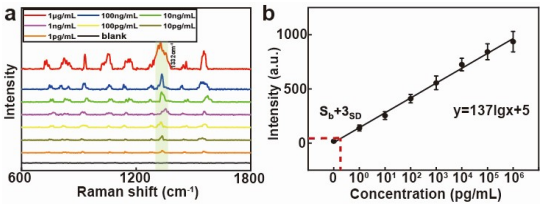

Figure 2. SERS-based NanoPADs for GFAP detection.

The SERS spectra for detecting different concentrations of GFAP in artificial human serum are shown in Fig. 2a. Using 1332 cm^{-1} peak intensity as the reading, the calibration curve is shown in Fig. 2b with 0.99 R^2. Thanks to the excellent optical transparency of the substrate and the highly-integrated technique we developed, the femto-detection LOD (150 fg/mL) of GFAP was achieved, which is 100 times better than the commercial detection.

IV. Discussion & Conclusion

We demonstrated the highly-integrated NanoPADs for early AD diagnosis with high sensitivity, providing a basis for detecting multiple SERS markers in the future.

References

[1] M. Citron, "Alzheimer's disease: treatments in discovery and development," *Nat. Neurosci.*, vol. 5, no. 11, Art. no. 11, Nov. 2002.

[2] W. Yuan *et al.*, "Facile Microembossing Process for Microchannel Fabrication for Nanocellulose-Paper-Based Microfluidics," *ACS Appl. Mater. Interfaces*, vol. 15, no. 5, pp. 6420–6430, Feb. 2023.

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