

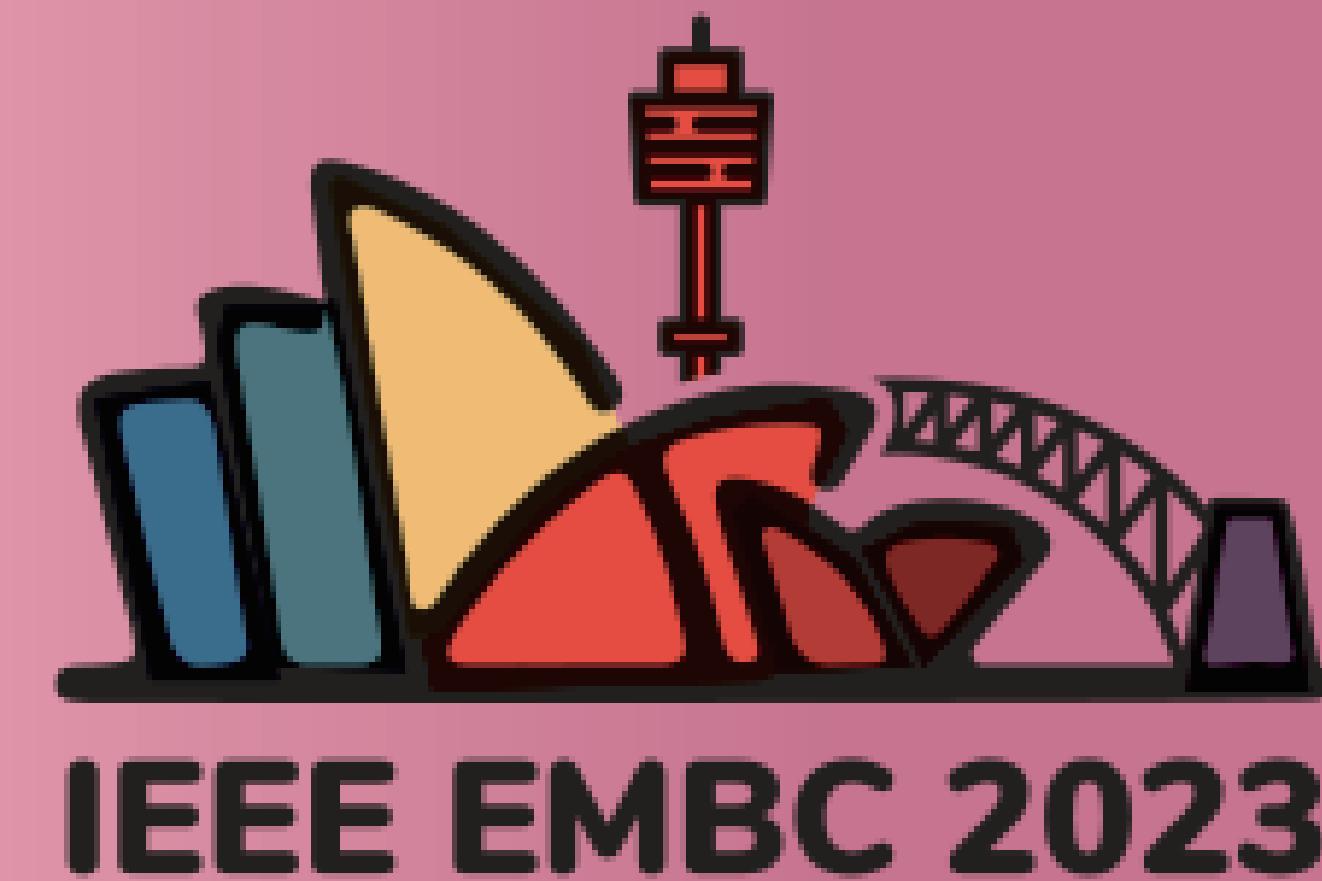
Highly-integrated SERS-Based Immunoassay NanoPADs for Early Diagnosis of Alzheimer's Disease

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ABSTRACT

In this study, 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.

INTRODUCTION

- Nanocellulose paper (nanopaper) has been widely applied as a promising substrate for biomedical due to its low cost, biocompatibility and high optical transparency.
- 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].
- Surface-enhanced Raman scattering (SERS) has emerged as an excellent choice for simultaneous detection due to its high sensitivity.
- Existing SERS-based AD detection substrates such as silicon and glass required complicated fabrication and massive manual operation, which restricted the sensitivity of detection.
- The highly-integrated nanopaper-based analytical microfluidic devices (NanoPADs) we developed previously provided a facile and highly sensitive technique to solve existing problems [2].

RESULTS

- SERS spectra:
 - Fig. 2(a) shows the SERS spectra for detecting different concentrations of GFAP in artificial human serum.
- Calibration curve:
 - Fig. 2(b) shows the calibration curve with $0.99 R^2$ using 1332 cm^{-1} peak intensity as the reading.
- The femto-detection limit of detection (150 fg/mL) of GFAP was achieved, which is 100 times better than the commercial detection.

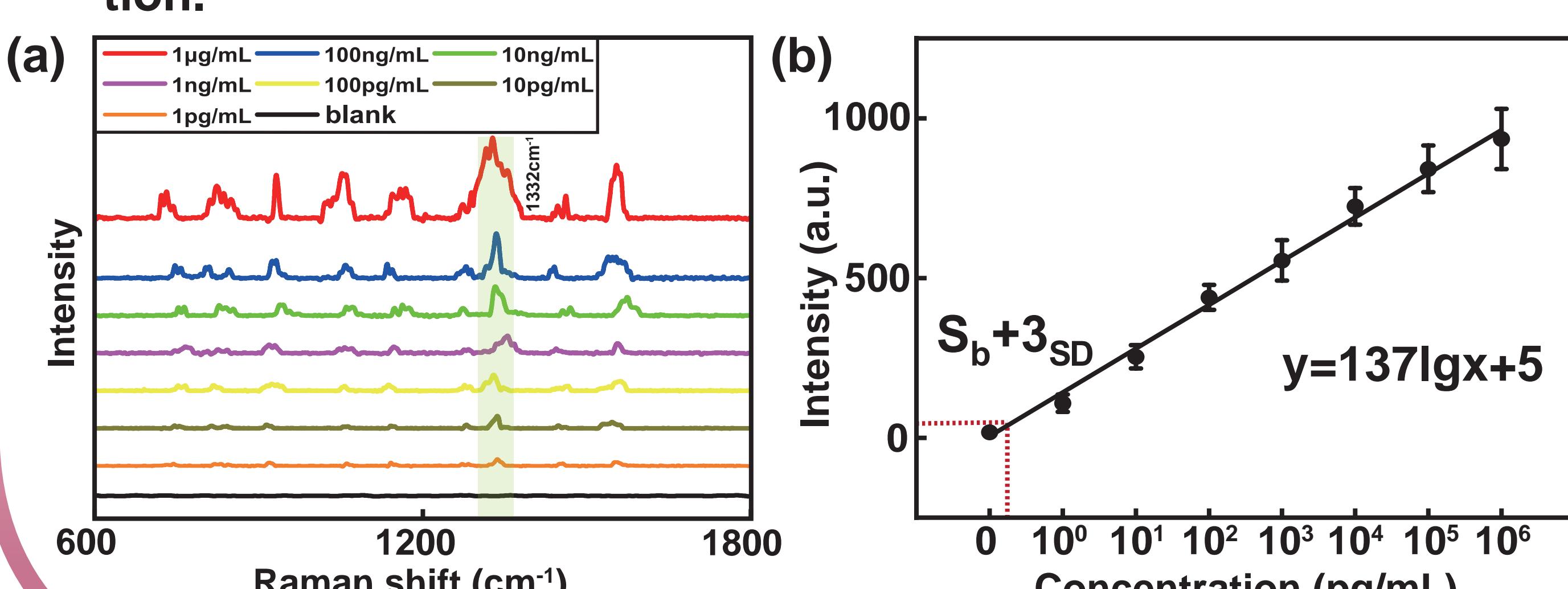


Figure 2. SERS-based NanoPADs for GFAP detection.

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[1] M. Citron, "Alzheimer's disease: treatments in discovery and development," *Nat. Neurosci.*, vol. 5, no. 11, pp. 1055-1057, 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, 2023.

METHODS

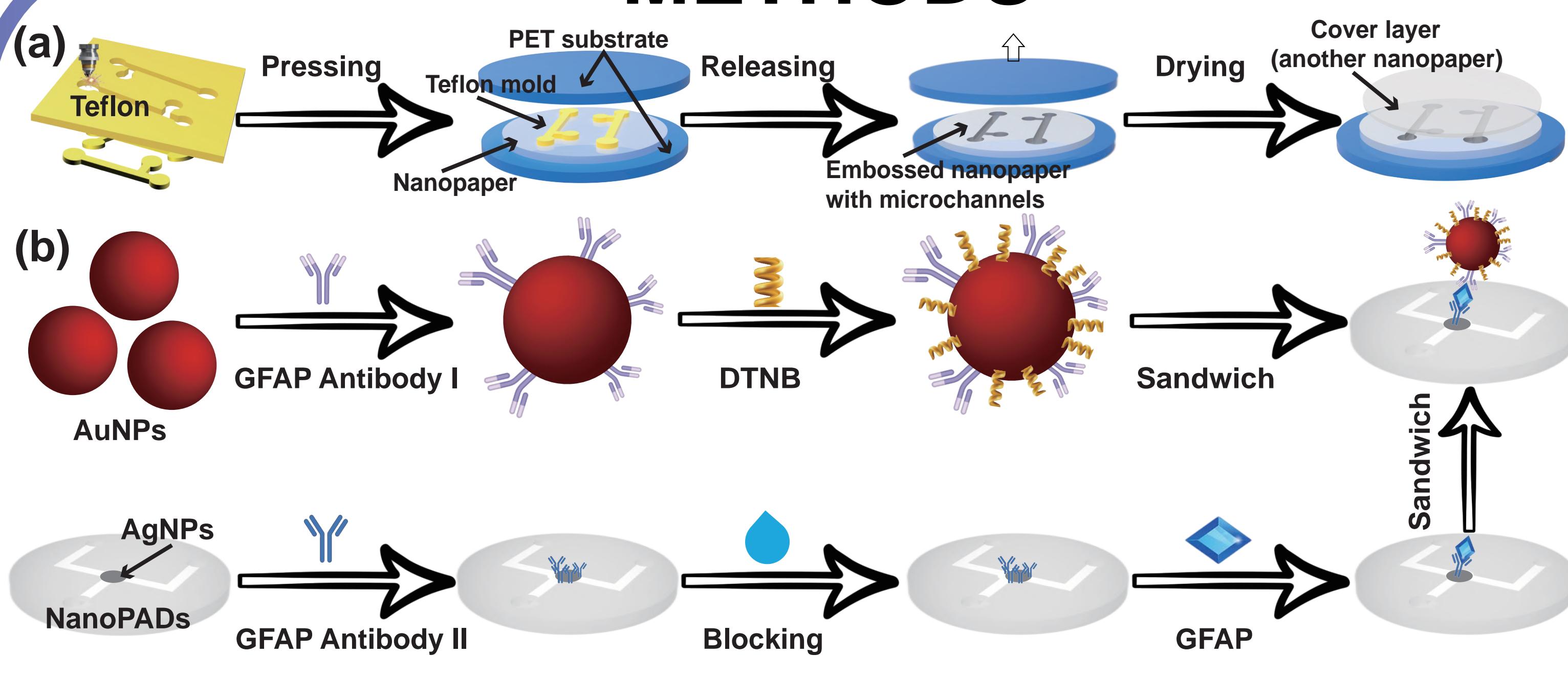


Figure 1. Schematic of SERS-based sandwich AD detection.

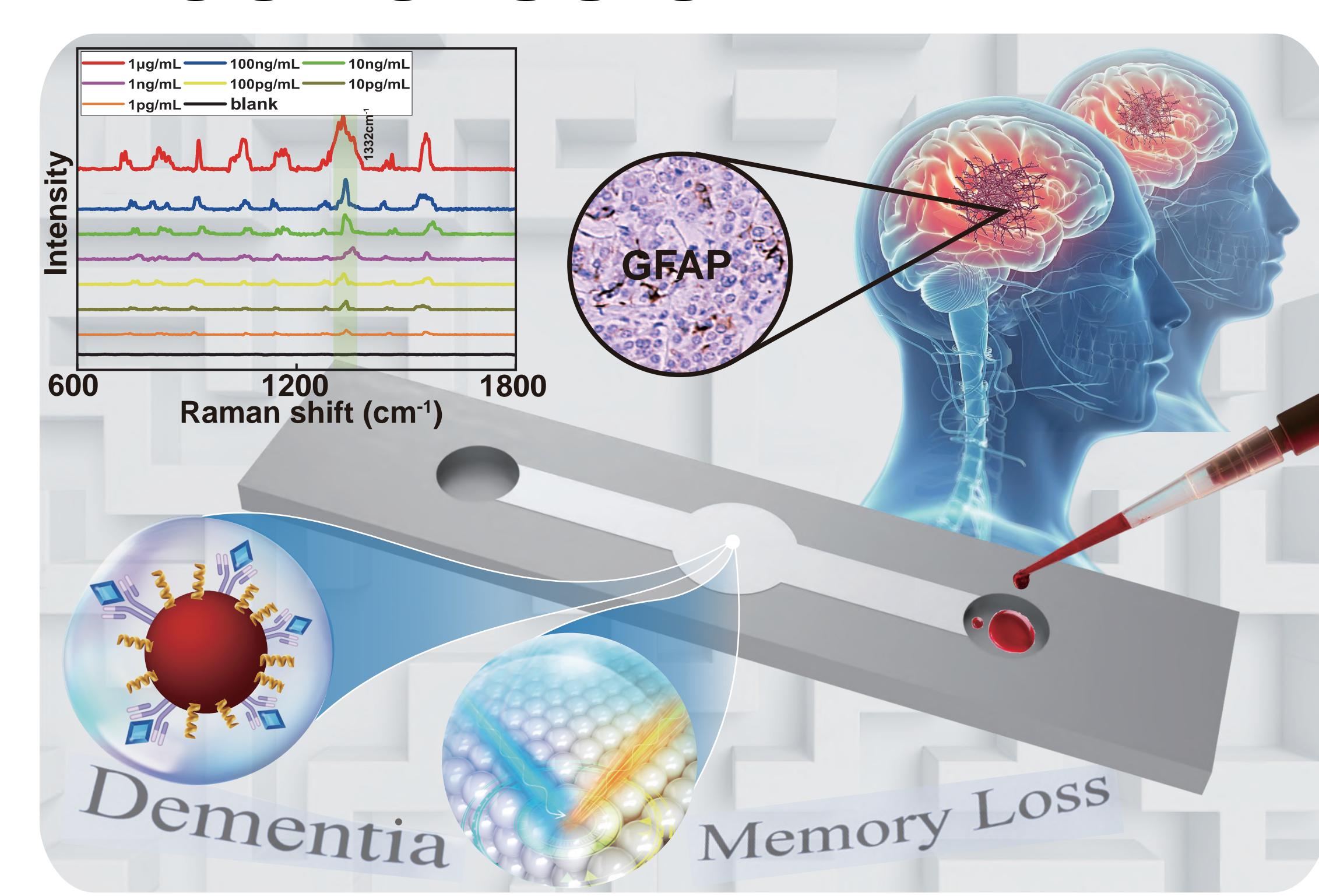
Fabrication of NanoPADs:

- Fig. 1(a) shows the NanoPADs fabricated using a facile micro-embossing process with convenient plastic molds.

SERS-based sandwich AD detection:

- Fig. 1(b) illustrates a SERS-based sandwich immunoassay for early AD diagnosis.
 - To fabricate DTNB-labeled SERS tags, GFAP antibody I was added to AuNPs suspension through mercaptan and ionic interactions. The resulting mixture was centrifuged to remove free antibodies. DTNB was then added to the above GFAP-AuNPs solution, and uncombined DTNB was washed by centrifugation after fully stirring.
 - To fabricate the SERS immobilization substrate, GFAP antibody II was pipetted into the inlet zone of the NanoPADs with *in-situ* AgNPs for coating. The substrate was then thoroughly blocked with a blocking solution. Subsequently, 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.
 - SERS tags were added in the NanoPADs for sandwich immunoassay.

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.