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Serum-Stabilized Pickering Emulsions as Reproducible Liquid SERS Analyzer for Direct Metabolic Fingerprinting

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Experimental

Materials

Thechemicals, including Chloroauric acid ($HAuCl_4$), anhydrous ethanol, and sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, trade name: Cate) were purchased from Sinopharm Chemical Reagent Co., Ltd. Rhodamine 6G ($C_{28}H_{31}ClN_2O_3$), n-hexadecane ($C_{16}H_{34}$), and aspartic acid were supplied by Shanghai Aladdin Biochemical Technology Co., Ltd. All chemicals used were of analytical grade and were not further purified. Solutions were prepared using ultrapure water from the Shanghai Yishuo Micropore Water Purification System. The constant-temperature heatingmagnetic stirrer was provided by Gongyi Yuhua Instrument Co., Ltd., and the portable Ramanspectrometer (AJ16002004) was provided by BWTEK, USA.

Patient information

This study included 60 patients with pathologically confirmed esophageal squamous cell carcinoma (ESCC). All participants had undergone comprehensive treatment primarily consisting of radiotherapy and chemotherapy. Blood samples were collected at a specific time point to assess disease progression. Tumor response was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Among the patients, 28 were classified as having stable disease (SD), where the sum of the maximum diameters of the target tumor lesions either decreased by less than 30% or did not increase enough to meet the criteria for progressive disease (PD). The remaining 32 patients were diagnosed with PD, defined by an increase of at least 20% in the sum of the maximum diameters of the target lesions, or the development of new lesions. All participants were thoroughly informed about the study and provided written informed consent prior to its commencement. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Reference number: PJ2025-08-31) and adhered to the principles outlined in the Declaration of Helsinki.

Fabrication of the gold AuNPs

Weight 200 mL of water into a beaker and position it on a magnetic stirrer. Set the temperature to 150°C, and while stirring, add 200 μL of 250 mm $HAuCl_4$. Once the water begins to boil, introduce 1.4 mL of 1% cate solution. Adjust the temperature to 110°C and maintain boiling for 40 minutes. After this, immediately place the beaker in an ice water bath to cool it to room temperature to obtain 50-nanometer AuNPs. After the reaction is completed, centrifuge again to remove the residual cate. Finally, resuspend the obtained AuNPs in deionized water and store them in a 4 ° C refrigerator until use.

Preparation of human serum

After an overnight fast of 10 hours, each participant was drawn for 3 mL of peripheral blood. The blood was then subjected to centrifugation at 3000 rpm for 10 minutes at 4°C to separate blood cells, fibrinogen, and platelets. The supernatant obtained after centrifugation was collected as the serum sample and stored at -80°C until it was needed for the experiment.

Fabrication of Pickering emulsion

First, centrifuge the synthesized AuNPs at 6,000 rpm for 15 minutes, discard the supernatant, and then dilute the pellet with an appropriate volume of purified water to obtain AuNPs with a concentration of approximately 1.34×10^{-10}. The serum and AuNPs served as the aqueous phase, while ethanol and n-hexadecane were used as the oil phase in a 2:1 ratio. The mixture was then shaken for 30 seconds on a stirring machine, resulting in a stable O/W emulsion.

SERS measurement

The excitation wavelength used is 785 nm, with a silicon wafer serving as the carrier. A 3 μL drop of emulsion is applied to the center of the silicon wafer. To ensure the accuracy and reproducibility of the spectra, measurements are taken while the emulsion remains in a liquid state. A minimum of 10 spectra are collected for each sample.

Data processing and analysis

This study obtained 1,000 SERS spectra from emulsions prepared from serum specimens of 60 esophageal cancer patients and 28 healthy volunteers. Spectral data in the wavenumber range of 600 to 1800 cm^{-1} were selected for analysis. Background subtraction and spectral smoothing preprocessing operations were completed using BWSpec4 software, thereby constructing a comprehensive database suitable for serum SERS analysis. The preprocessed data were incorporated into the machine learning workflow, with initial research focusing on intergroup differentiation using principal component analysis (PCA). As a straightforward and efficient dimensionality reduction technique, PCA maps the original high-dimensional dataset onto a set of principal components (PCs). These principal components are designed to maximally characterize the variance features of the original data, thereby enabling the simplification of complex spectral datasets and facilitating the identification of differences among the various groups. Under the circumstance that PCA did not yield the desired differentiation results, the study further incorporates the FNN—a model recognized as a classical learning method for multilayer neural networks with extensive application potential in the biomedical field, particularly displaying remarkable advantages in medical image analysis, gene data analysis, and drug development. In the process of constructing the FNN model, the research adopted a dataset partitioning strategy of 75% for the training set, 15% for the validation set, and 15% for the prediction set. Furthermore, an additional 50 spectral data points were selected to validate the model's generalization capability, resulting in the acquisition of ideal analytical outcomes.

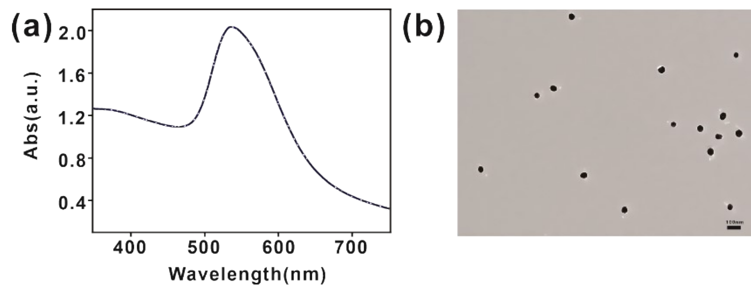

Fig S1. The UV-Vis absorption spectra of AuNPs (a), along with the TEM micrographs of gold nanoparticles(b).

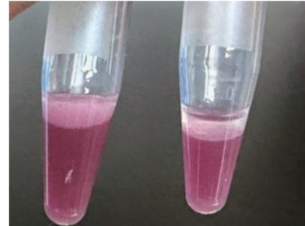

Fig S2. Emulsions prepared with stirring times of 30 s (left) and 50 s (right). Stirring for 30 s was identified as the optimal condition for emulsion formation in the present system.

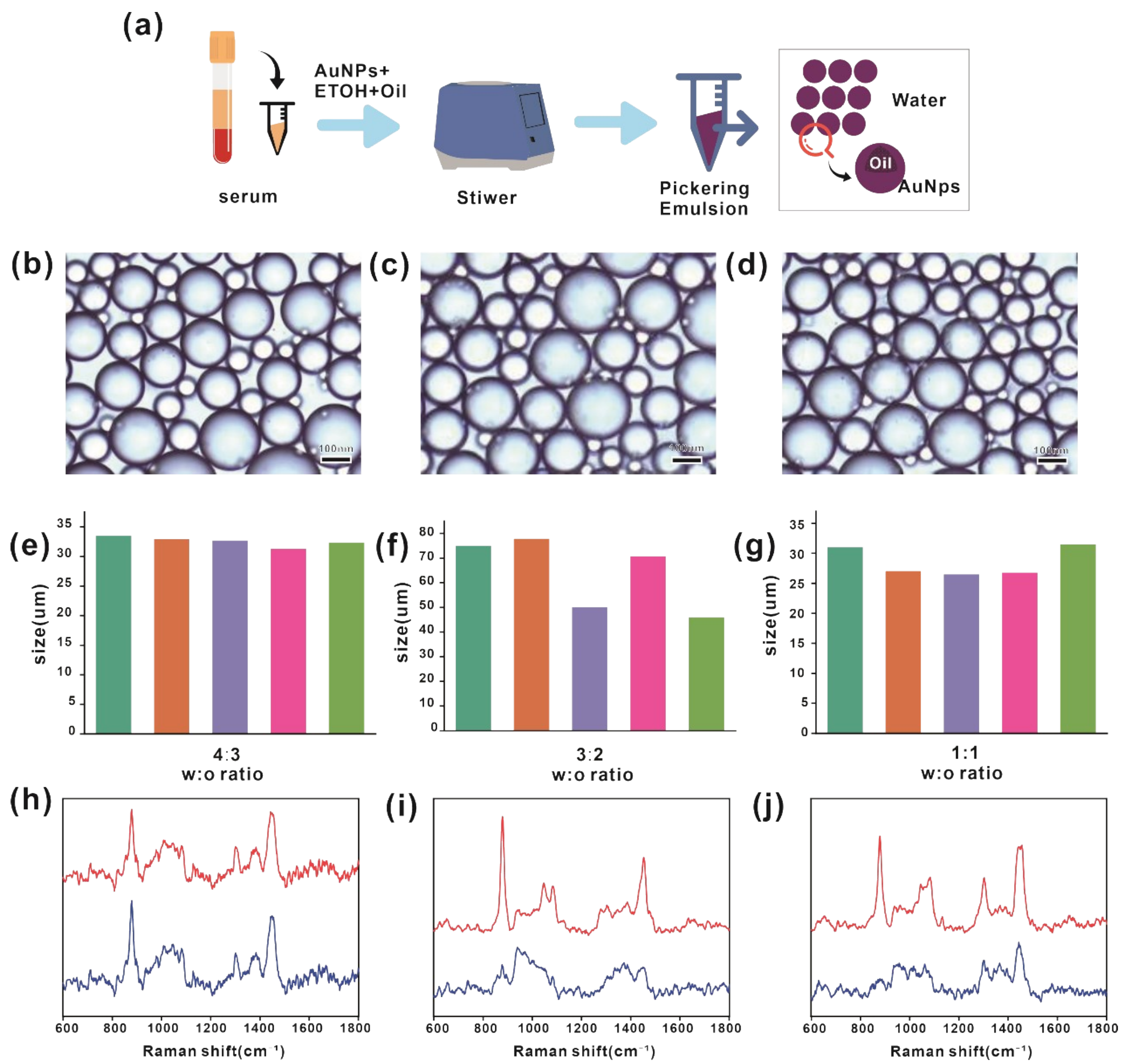

Fig S3. Particle size and spectral stability at w:o ratios of 4:3, 2:1, and 1:1. (a) Emulsion preparation scheme; n-

hexadecane as oil phase. Optical microscopy of emulsions with w:o ratios 4:3 (b), 3:2 (c), 1:1 (d). Based on the optical microscopic images, the particle size distributions of emulsions with w:o ratios of 4:3 (e), 3:2 (f), and 1:1 (g) were statistically analyzed; the emulsion with a 3:2 ratio exhibited larger sizes and a broader distribution. SERS spectra recorded within 5 min for emulsions with w:o ratios of 4:3 (h), 3:2 (l), 2:1 (i), and 1:1 (j), revealing that the 4:3 and 2:1 emulsions exhibit superior stability. freshly prepared serum-Pickering emulsion SERS spectrum (red) and SERS spectrum measured after resting at room temperature for 10 minutes (blue).

The results revealed that, except for the 3:2 ratio emulsion, which exhibited a larger particle size and a broader distribution, the other systems showed good uniformity with average particle sizes ranging from 26 to 33 um(Figure S3e-g). To further validate the spectral stability of the SERS signals in emulsions with varying w:o ratios, this study collected spectral data from both freshly prepared emulsions and those allowed to stand at room temperature for 10 minutes. Ten spectral readings were collected for each group, and after data averaging, a comparative analysis was conducted. The research findings indicate that no significant differences exist in the SERS spectral characteristics of the two emulsions at w:o ratios of 4:3 and 2:1. Conversely, the emulsions with w:o ratios of 1:1 and 3:2 exhibit pronounced fluctuations in their SERS spectral peaks, suggesting a relatively poor stability of the interfacial structure in these emulsions(Figure S3h-j).

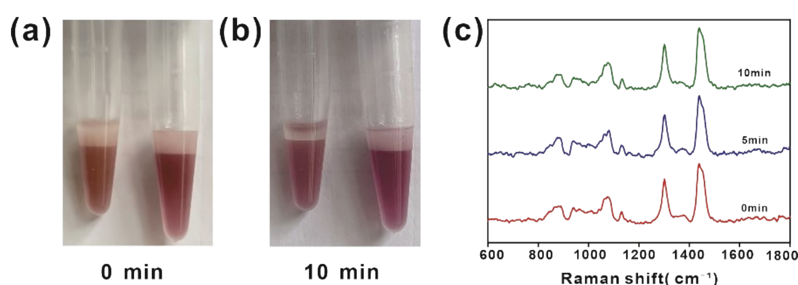

Fig S4. (a-b) w:o ratios of 4:3 (left) and 2:1 (right); the Pickering emulsions of 2:1 and 4:3 placed at room temperature for 10 minutes (a) and the 2:1 Pickering emulsion placed at room temperature for 10 minutes (b). (c) The SERS spectrum of the 2:1 emulsion remains stable after standing at room temperature for 10 minutes.

The emulsion with a w:o ratio of 4:3 exhibited obvious phase separation after standing at room temperature for 10 minutes, with the oil phase and water phase beginning to stratify (Figure S4a, b). This phenomenon would severely affect the operational convenience and reliability of results in practical detection. In contrast, the emulsion with a ratio of 2:1 remained well dispersed under the same conditions, and no detectable changes were observed in its SERS spectra (Figure S4c), demonstrating that this ratio provides superior stability.

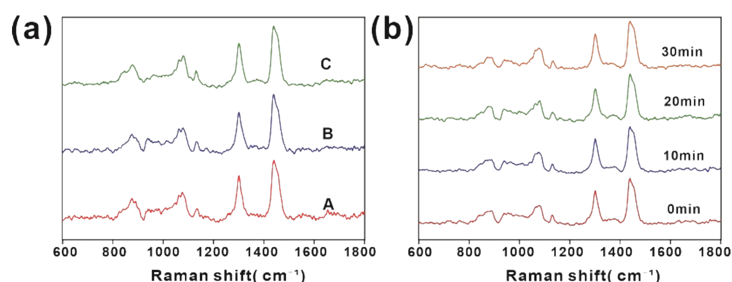

Fig S5. (a) Pickering emulsions with a wate:oil ratio of 2:1 were prepared using sera from three healthy volunteers(A,B,C), and the corresponding spectral results demonstrated good inter-batch reproducibility. (b) The spectra of Pickering emulsions prepared from the same serum sample with a water-to-oil ratio of 2:1 remained stable within 30 min, indicating good long-term stability of the system.

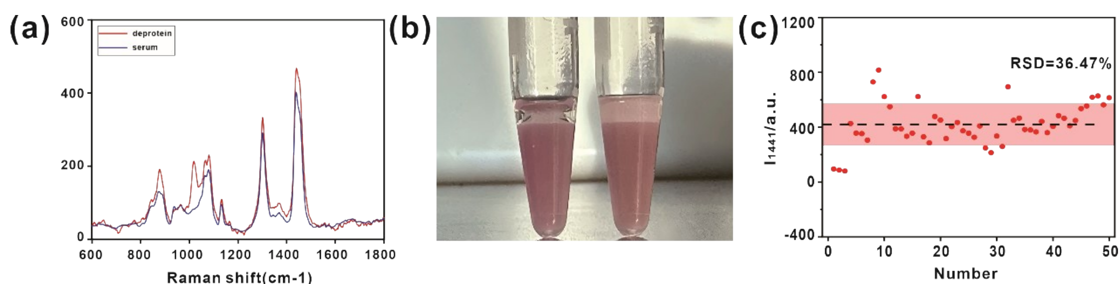

Fig S6. (a) Comparison of SERS between serum-Pickering emulsions and deproteinized serum as the aqueous phase. (b) Comparison of emulsions formed after methanol deproteinization (left) with those that were not deproteinized (right). (c) In the Pickering emulsion with w:o = 2:1, using deproteinized serum as the aqueous phase, the intensity of the characteristic Raman peak located at 1441 cm^{-1} has a calculated RSD of 36.47% (based on spectral data from 50 random sampling points)

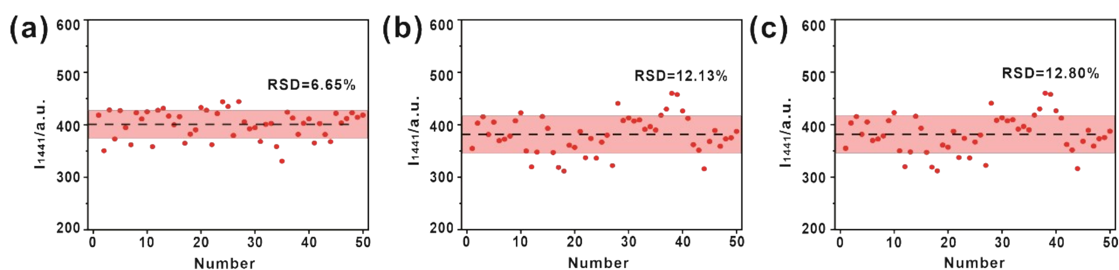

Fig S7. At the same w:o ratio (2:1), SERS spectra were collected from 50 randomly selected points, and the Raman intensity of the characteristic peak at 1441 cm^{-1} was analyzed to calculate the RSD of the peak intensity, assessing the signal reproducibility of the system; (a) The RSD of the serum-Pickering emulsion is 6.65%, confirming the excellent uniformity and intensity stability of the SERS substrate;(b) The RSD with aspartic acid as the aqueous phase is 12.13%. (c) The RSD with R6G as the aqueous phase is 12.80%

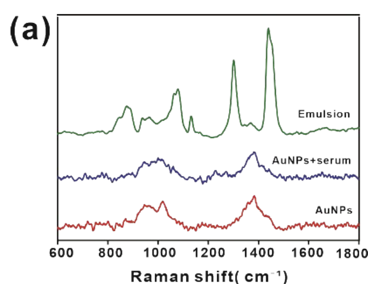

Fig S8. Neither individual AuNPs nor AuNPs-serum mixtures produce recognizable molecular fingerprint signals, while distinct peaks can be clearly detected in the emulsion system.