Supporting Information for

SERS-based immunochromatographic assay with Au^{DTNB}@Ag nanoparticles for the detection of ancient silk residues

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Isolation of silk fibroin from silk cocoon

Silkworms were first removed from the cocoons, which were then cut into small pieces and accurately weighed. The pieces were subjected to degumming by treatment with a 0.5% Na_2CO_3 solution at a bath ratio of 1:50 and a temperature of 98 ± 2 °C for 0.5 hours under constant stirring. This degumming process was repeated twice to ensure the complete removal of sericin. The insoluble silk fibroin was thoroughly rinsed multiple times with deionized water and subsequently dried overnight in an oven at 60 °C for later use. The dried silk fibroin was dissolved in a protein extraction solution at a bath ratio of 1:50 and 98 ± 2 °C for 2 hours with stirring. The solution containing silk fibroin (SF) was cooled to room temperature, filtered, and then dialyzed against deionized water using cellulose dialysis tubing with a molecular weight cut-off (MWCO) of 10,000 to remove calcium and chloride ions. The deionized water was replaced every 4 hours, and after 72 hours, a purified SF solution was obtained. This SF solution was freeze-dried in a vacuum freeze-dryer for 72 hours to obtain solid SF, which was finally ground into a powder and stored in a sealed container for future use.

Preparation of anti-silk fibroin monoclonal antibodies

SF isolated from Bombyx mori silk was used as the antigen for mouse immunization. The SF antigen was dissolved in sterile saline and emulsified with an equal volume of Freund's Complete Adjuvant (FCA) to form the antigen emulsion. Female BALB/c mice (7-10 weeks old) were immunized subcutaneously and intraperitoneally with the prepared emulsion via multiple injections, with a total volume of 100 μL per mouse and an antigen concentration of 1 mg/mL for the primary immunization. Beginning on day 21 after the first immunization, booster injections with a 0.5 mg/mL antigen emulsion (0.1 mL per mouse) were administered every two weeks. Seven days after the fourth immunization, blood was collected from the retro-orbital plexus and centrifuged at 10,000 rpm for 5-10 minutes. The supernatant serum was collected for indirect enzyme-linked immunosorbent assay (ELISA) to determine serum antibody titers. A mouse showing a titer of 1:10,000 was selected, and blood was collected via cardiac puncture to prepare antiserum for use as a positive control.

The spleen was aseptically removed from the selected mouse and placed in a Petri dish containing pre-warmed IMDM culture medium. A small opening was made at one end of the spleen using fine forceps, and splenocytes were gently extruded. The cells were then dispersed by pipetting and collected into a 50 mL centrifuge tube. The harvested splenocytes were mixed with SP2/0 myeloma cells at a ratio of 10:1, and cell fusion was induced by adding 50% polyethylene glycol (PEG). The fused cells were resuspended in HAT selection medium for selective culture. After 7 days, the culture supernatants were screened by indirect ELISA to identify positive hybridomas. If the initially selected positive clones were not monoclonal, subcloning was repeated until monoclonal positive cell lines were obtained. These monoclonal hybridoma cell lines were then expanded in culture; supernatants were periodically collected for antibody characterization, and the cells were cryopreserved for subsequent experiments.

The cryopreserved hybridoma cells were thawed and cultured under standard conditions (37°C, 5% CO_2), with the medium being changed every two days. Once the cells reached confluence, they were harvested and injected intraperitoneally into pristine-primed BALB/c mice (7-10 weeks old). Ascitic fluid was collected after 10

days. For antibody purification, the ascites was clarified and a 10 mL aliquot was mixed with an equal volume of phosphate-buffered saline (PBS). The mixture was applied twice to a Protein A affinity chromatography column. The column was washed extensively with PBS, and bound monoclonal antibodies were eluted using a 0.2 M glycine solution (pH 2.0). The purified antibodies were neutralized, and stored at -20°C for future use.

XRD pattern of the AuDTNB@Ag nanoparticles

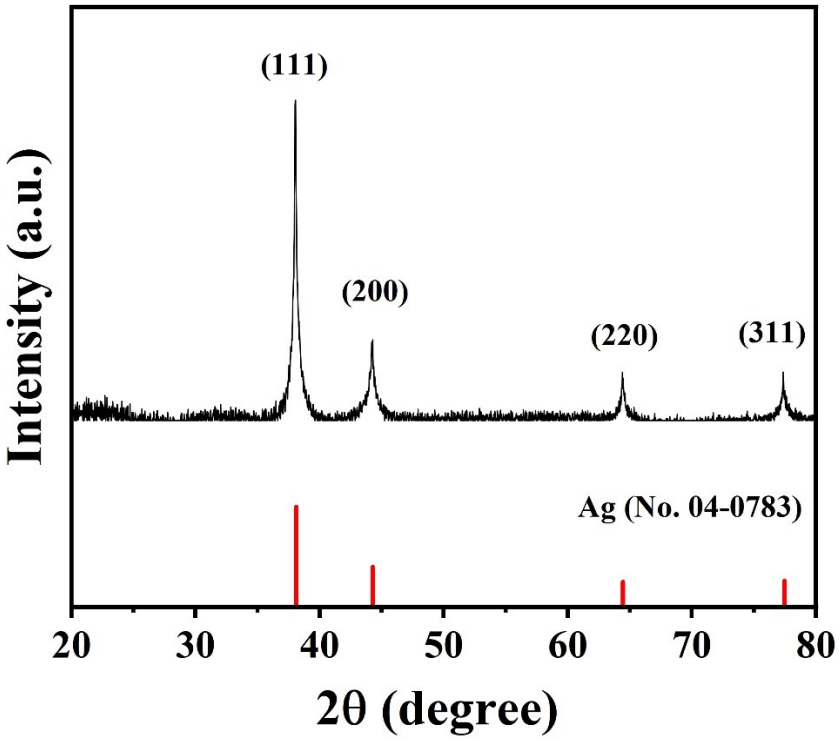

Fig. S1 XRD pattern of the Au^{DTNB}@Ag nanoparticles

Synthesis of Au@Ag^{DTNB} NPs

To 10 mL of AuNPs, 200 μL of a 1% trisodium citrate solution and 800 μL of ascorbic acid (10 mM) were added and mixed thoroughly. Under continuous stirring, 600 μL of a silver nitrate solution (1 mM) was then added dropwise. Stirring was continued for 40 min. The mixture was centrifuged at 10,000 rpm for 10 min, and the pellet was redispersed in 10 mL of ultrapure water. Subsequently, 100 μL of a DTNB solution (1 mM) was added to 10 mL of the above Au@Ag solution, and the reaction was allowed to proceed for 2.5 h at room temperature under light-protected conditions with stirring. The product was then centrifuged at 10,000 rpm for 15 min, and the pellet was redispersed in 10 mL of ultrapure water. This washing step was repeated twice to obtain the final Au@Ag^{DTNB} dispersion.

Establishment of standard and calibration curves for indirect enzyme-linked immunosorbent assay

The silk fibroin was serially diluted (0.01, 0.1, 1, 3, 5, 7, 10, 30, 50, 70, 100, 200, 400, 600, 800, and 1000 ng/mL) in carbonate-buffered saline (CB, pH 9.6). A 100 μL aliquot of each dilution was added to individual wells of a 96-well microplate and stored overnight at 4°C. The following day, the liquid in the plate was discarded, and each well was washed three times with 200 μL of PBS buffer under gentle shaking. After washing, the wells were blocked with 200 μL of 1% BSA solution and incubated at 37°C for 60 minutes. Following another three washes with 200 μL of PBS, 100 μL of the anti-silk fibroin monoclonal antibody was added to each well, and the plate was incubated at 37°C for 60 minutes. The plate was washed three more times, and then 100 μL of the enzyme-labeled goat anti-mouse secondary antibody was added, followed by incubation at 37°C for 60 minutes. After five additional washes with PBS, 100 μL of TMB substrate solution was added to each well to initiate the enzymatic reaction. The color development was allowed to proceed in the dark for 10 minutes and was subsequently stopped by adding 100 μL of 0.05 M H_2SO_4 solution. The optical density (OD) at 450 nm was immediately measured using a microplate reader. A calibration curve for silk fibroin detection was established, demonstrating a linear range of 10–600 ng/mL and a detection limit of 5.49 ng/mL.

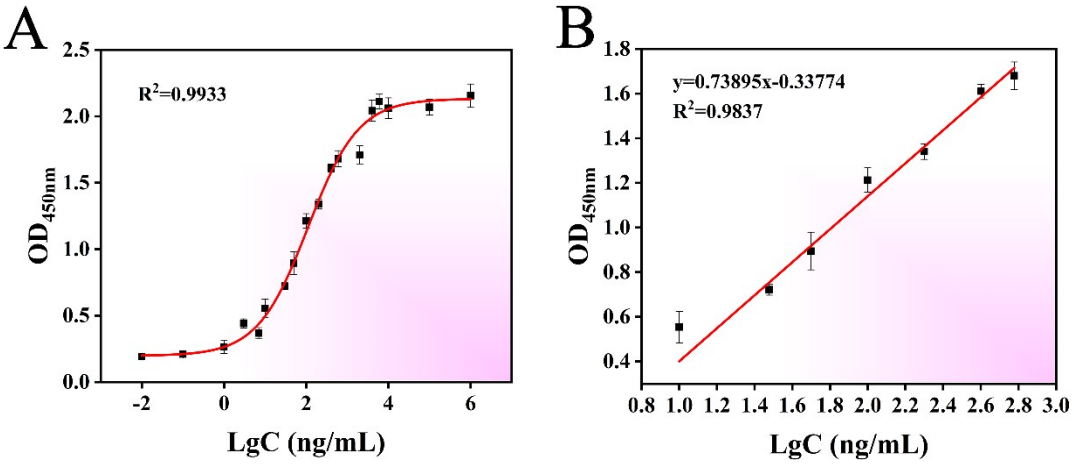

Fig. S2 Detection of silk fibroin by ELISA: (A) standard curve of silk fibroin at different concentrations; (B) calibration curve of silk fibroin at different concentrations.

Optimization of experimental conditions for the SERS-ICA strips

The detection sensitivity of the SERS-ICA strips was significantly improved through systematic optimization of several key parameters, including the concentration of the coating antigen on the T line, the amount of conjugated antibody, the volume of the SERS immunoprobe, and the reaction time. First, the optimal concentration of the coating antigen on the T line was determined. As shown in Fig. S3(A), the SERS signal intensity at the T line increased with higher SF concentrations, whereas the signal at the C line decreased correspondingly. At a concentration of 0.15 mg/mL, the signal intensities of the T and C lines became comparable, indicating an optimal balance for competitive immunoreactions. Consequently, 0.15 mg/mL was selected as the optimal coating concentration. Second, the amount of conjugated antibody (anti-SF mAb) was optimized. Appropriate antibody concentrations could provide sufficient Raman signals, whereas an excess of antibody was found to reduce the sensitivity of the competitive reaction. As presented in Fig. S3(B), the SERS signal intensity increased with the antibody amount until a plateau was reached beyond 6 μL per mL of Au^{DTNB}@Ag nanoparticles, suggesting saturation of the available binding sites on the nanoparticle surface. Accordingly, 6 μL/mL was chosen as the optimal antibody amount. Third, the volume of the applied SERS immunoprobe was investigated. Based on the results in Fig. S3(C), the signal intensities of both T and C lines increased with the probe volume and stabilized at 4 μL, indicating that sufficient probe had been delivered to the strip. Thus, 4 μL was established as the optimal probe volume. Finally, the reaction time was examined. As shown in Fig. S3(D), the SERS signal reached a steady state after 10 min, with no significant further enhancement upon prolonged incubation. Therefore, a reaction time of 10 min was adopted.

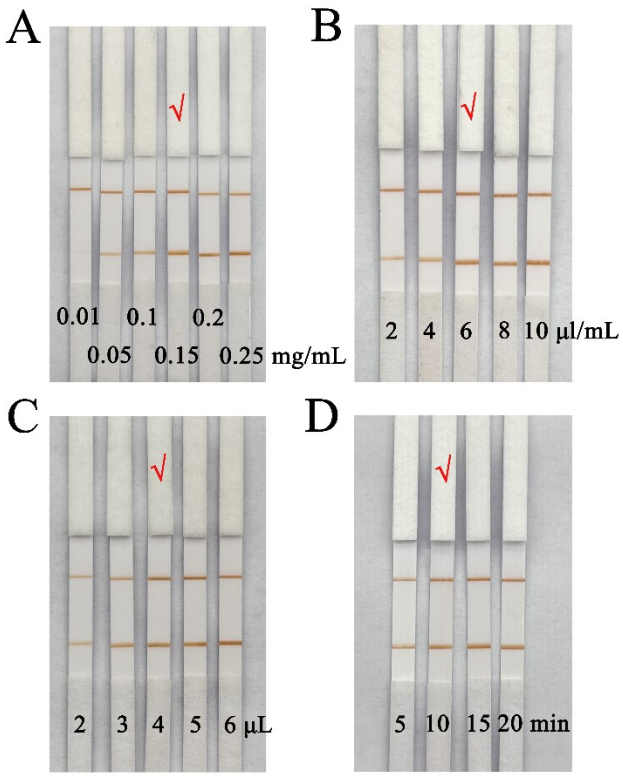

Fig. S3 Optimization of the SERS-ICA strips: (A) concentration of SF; (B) conjugation amount of anti-SF mAb; (C) volume of the Au^{DTNB}@Ag-mAb immunoprobe; (D) detection time.