Electronic Supplementary Material (ESI) for Analytical Methods.

Ultrasensitive dual enhanced electrochemical immunosensor to detect ancient wool relics

Boyi Chen[a], Zhongyuan Wang[a], Junsen Wang[a], Hailing Zheng[b], Junyi Zhou[a], Xushi Chen[a], Bing Wang[a,*], Yang Zhou[b,*], Zhiqin Peng[a]

a. School of Materials Science & Engineering, Zhejiang Sci-Tech University, Hangzhou 310018, China

b. Key Scientific Research Base of Textile Conservation, State Administration for Cultural Heritage, China National Silk Museum, Hangzhou 310002, China

*E-mail: wbing388@163.com (B. Wang) and cnsmzhouyang@126.com (Y Zhou)

Telephone and fax: +86-571-86843867

Preparation of the tailored antibody

An anti-wool keratin polyclonal antibody was tailored through the following steps. First, an antigen emulsion was obtained by mixing 1 mg of wool keratin powder, 1 mL of saline and the same amount of Freund's complete adjuvant. Then, 100 μL of emulsion was injected into each rabbit using the subcutaneous multipoint and posterior thigh injection method. Primary immunization continued for 2 weeks, followed by booster immunization. In the booster immunization process, Freund's incomplete adjuvant was used. Each booster immunization phase lasted for 2 weeks. Next, the antibody titer in the blood of the rabbit ear was tested by indirect ELISA after 3 rounds of enhanced treatment. If the antibody titer was sufficient, the blood of the entire rabbit was removed to a superclean bench. After the blood was completely coagulated, it was placed in a 37 ℃ thermostat for 2 h and subsequently placed in a 4 ℃ refrigerator overnight to fully shrink the blood clot until the antiserum was completely precipitated. Finally, the antiserum was collected and centrifuged at 4 ℃ for 10 min. The separated supernatant was aliquoted and stored at -20 ℃ for later use.

The antiserum was purified by immunoaffinity chromatography technology. First, the synthesized hapten (polypeptide "KKYEEEIALRC") and chromatography filler sulfo-link-gel were cross-linked to block the N-end of "KKYEEEIALRC". The column was pretreated with 20 mL of 50 mM PBS 7.4 at a flow rate of 60 mL h^{-1}. Then, 15 mL of antiserum with 50 mM PBS 7.4 was diluted to 20 mL and the loading was repeated. Then, the column with 30 mL of 50 mM PBS 7.4 was washed at a flow rate of 60 mL h^{-1}. The antibody was eluted with glycine-HCl at a pH of 3.0 and a concentration of 1

M. After elution, the peptide column was washed with 50 mM PBS 7.4 containing 0.02% thimerosal sodium, and the purified antibody was stored at -20 ℃ for later use.

All animal experiments were performed according to the national standard GB14925-2001 and the guidelines issued by the Ethical Committee of Zhejiang Sci-Tec University.

Optimization of the experimental Parameters

To achieve better electrochemical activity of immunosensors, it is essential to optimize the experimental conditions such as the concentration of antibody and immunoprobes. The volume ratio of AFIL to GO was an important parameter for the performance of the immunosensor. Figure S1A shows that the ideal volume ratio of AFIL to GO was 1:100. The coating amount of GO-IL was another parameter that affected the electrochemical activity of immunosensors. Figure S1B illustrates the DPV response of electrodes coated with different volumes of GO-AFIL. The peak current increased with increasing GO-AFIL modification amount from 1 to 3 μL. However, with an increase in coating volume from 4 to 5 μL, the DPV response gradually decreased. These results proved that within a certain range, GO-AFIL could increase the electrochemical performance of the immunosensor. However, at higher coating amounts, the efficiency of electronic transmission may decrease. Thus, the ideal coating amount of GO-AFIL was 3 μL.

Furthermore, the coating amount of AuNPs and antibody concentration were optimized. As shown in Figure S1C, the peak current increased when the amount of AuNPs increased from 1 to 4 μL. However, with an increase in coating volume from 4

to 5 μL, the DPV response gradually decreased. Thus, 4 μL was chosen to be the ideal coating amount. In addition, the peak current increased when the concentration of antibody decreased, which indicates a lower coating amount of antibody on the electrode surface. Therefore, an initial antibody concentration of 0.35 mg mL^{-1} was selected for subsequent experiments (Figure S1D).

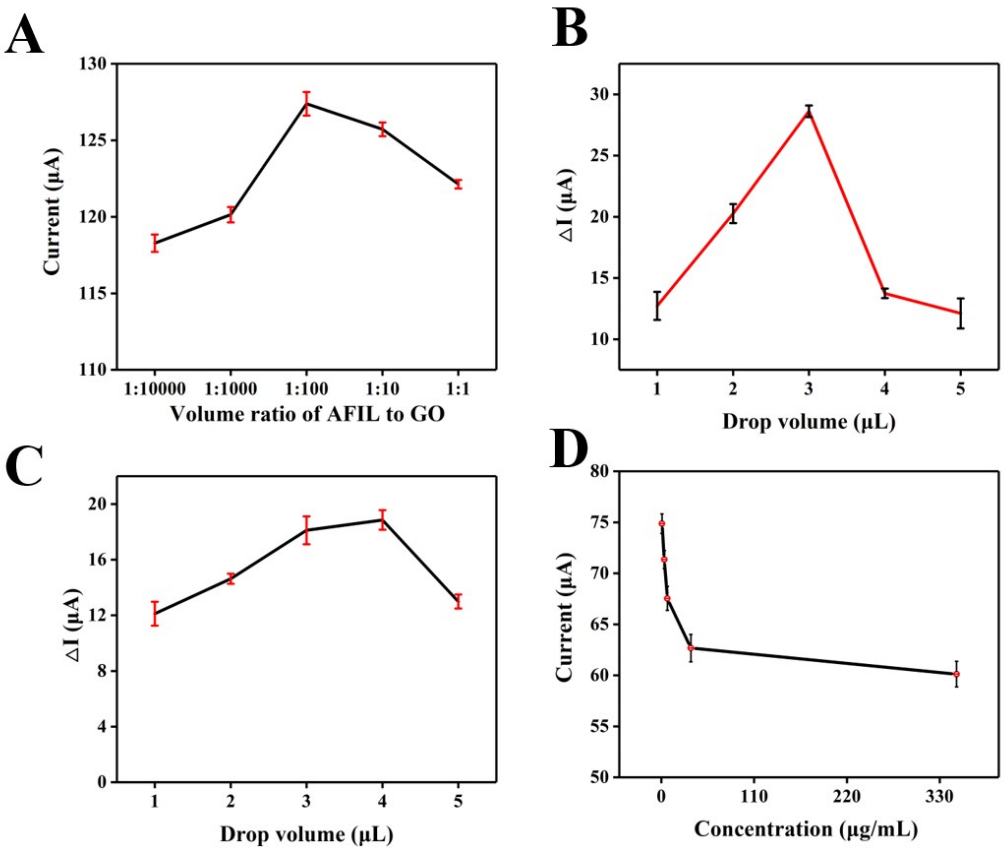

Figure S1. Optimization of the experimental parameters. (A) Volume ratio of IL to GO. (B) Volume of GO-IL solution and (C) AuNPs solution. (D) Concentration of antibody.

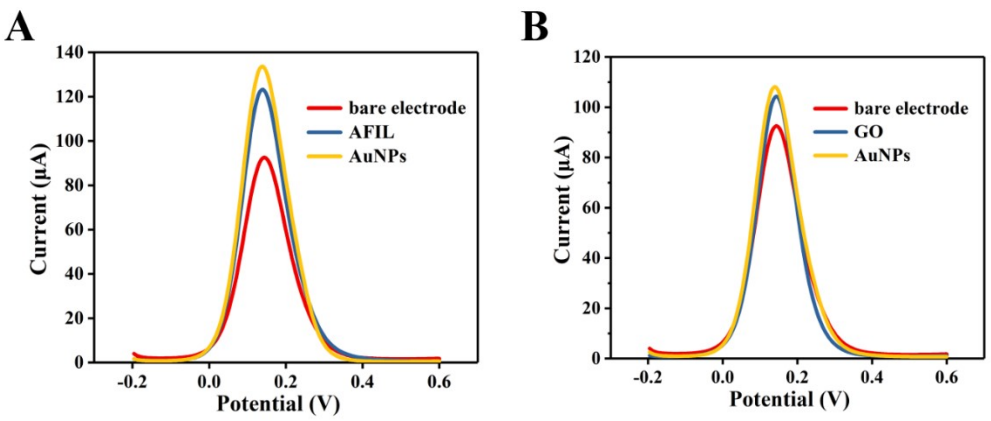

Figure S2. DPV curves of different immunosensors. (A) AFIL and AuNP electrode modification; (B) GO and AuNP electrode modification.

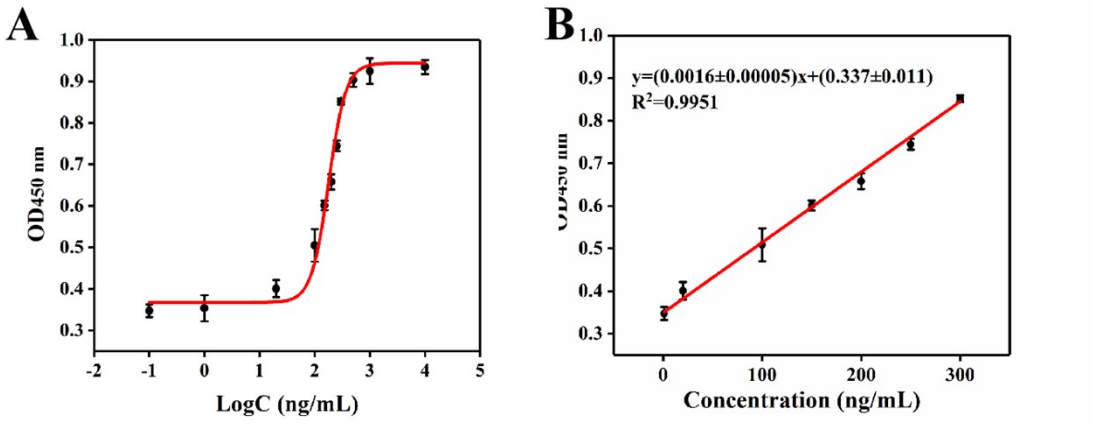

Figure S3. (A) Standard curve and (B) calibration curve of ELISA for different concentrations of keratin.

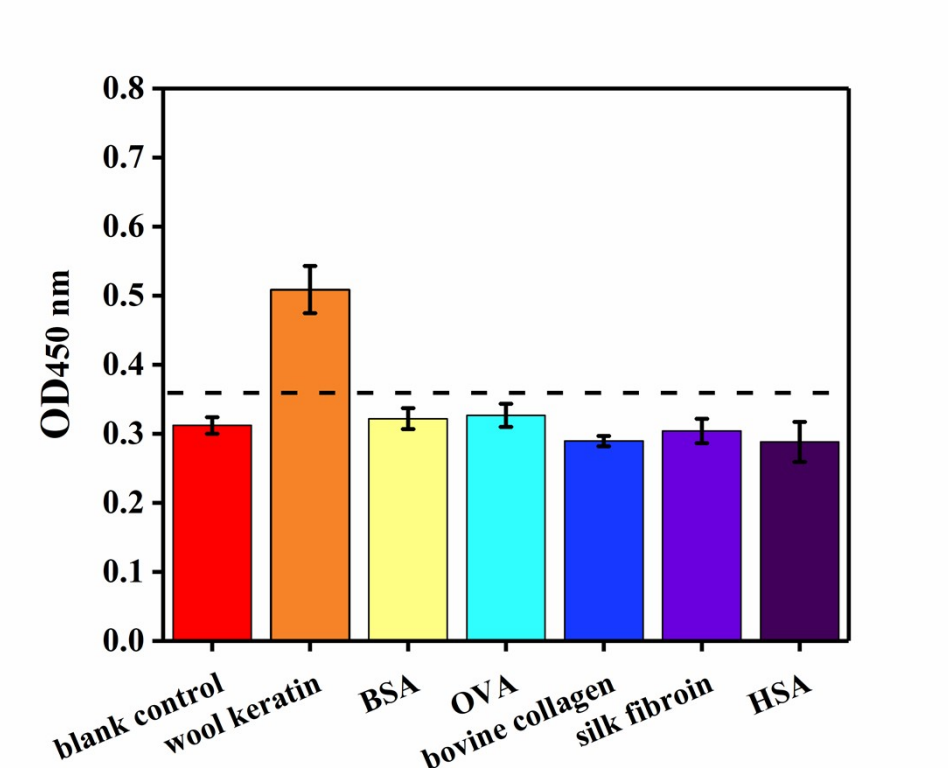

Figure S4. Specificity of ELISA. The concentration of each control protein was 100 ng mL^{-1}. (A) Keratin; (B) BSA; (C) OVA; (D) bovine collagen; (E) silk fibroin; (F) HSA.

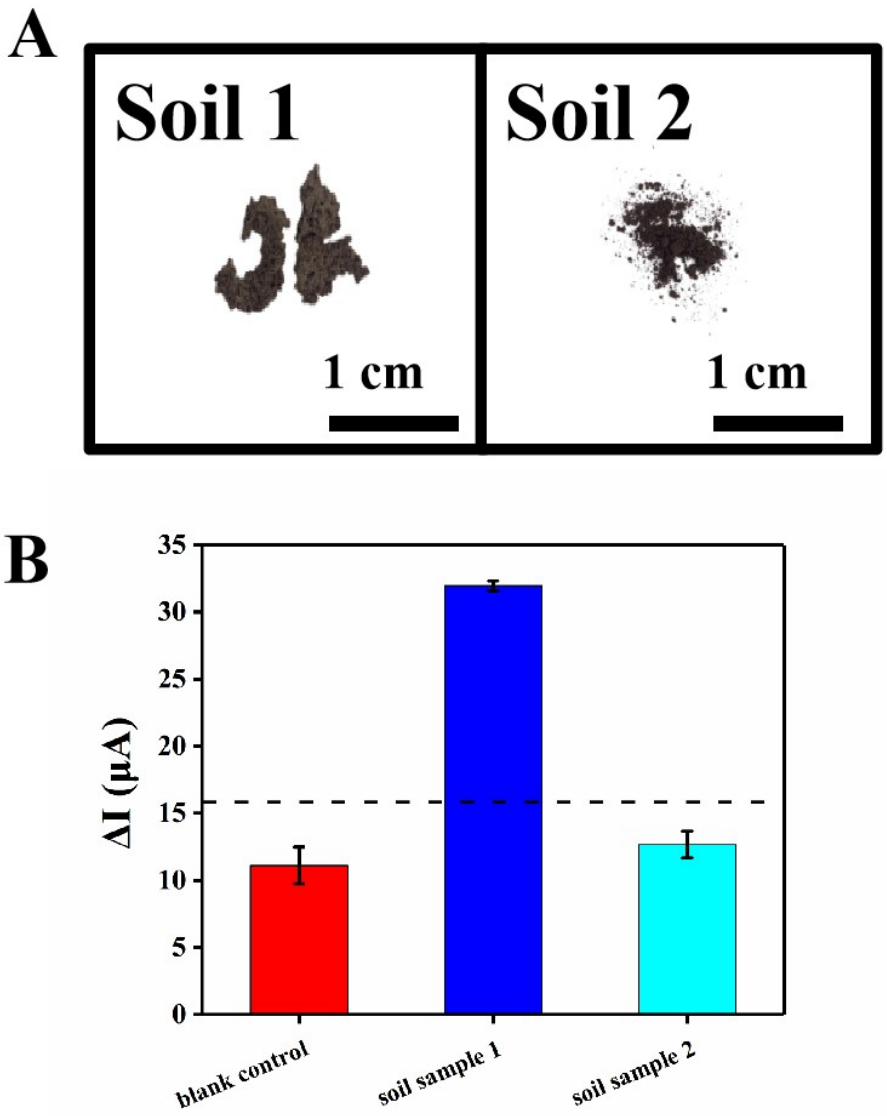

Figure S5. (A) Original aspect of ancient soil samples. (B) Immunosensor results for archaeological soil samples.

Table S1. Description of the Ancient Fabric samples

Sample	Age	Site location	Description
S1	Warring States period (475~211 BC)	Zada County, Tibet, China	Dark brown fabrics, no weaving trace can be seen, harsh handfeel
S2	Three Kingdoms period (220~280 AD)	Kazakhstan	Flaxen fabrics, a little weaving trace can be seen in its rolled part, soft handfeel
S3	Three Kingdoms period (220~280 AD)	Kazakhstan	long strips of red fabrics, no clearly weaving trace can be seen, soft handfeel
S4, S5, S6, S7, S8	Bronze Age (1979~1379 BC)	Xiaohe, Xinjiang, China	S4: Canary fabrics, no weaving trace can be seen, soft handfeel, partial pulverization S5: brown slag shape fabrics, no weaving trace can be seen, harsh handfeel, easy crushed by finger press S6: Dark brown fabrics, clearly weaving trace can be seen, alternate horizontal and vertical weaving ,harsh handfeel, S7 and S8: long strips of red and yellow fabrics, no clearly weaving trace can be seen, soft handfeel
S9	Three Kingdoms period (220~280 AD)	Luopu, Xinjiang, China	Canary fabrics, no weaving trace can be seen, soft handfeel, partial pulverization
S10	Northern Wei Dynasty (386~534 AD)	inner Mongolia, China	fragile black sheet fabric, no weaving trace can be seen, harsh handfeel, easy crushed by finger press, the quality is very light