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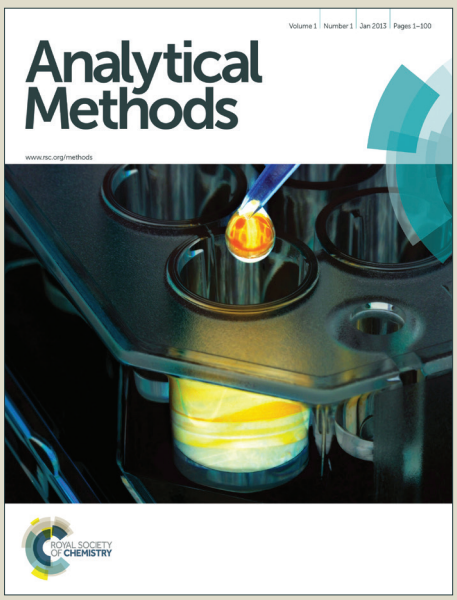

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ARTICLE

Development of a gold-based immunochromatographic strip assay for the detection of ancient silk

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The origin of silks remains one of the most puzzling mysteries. However, the on-site identification of ancient silks usually becomes particularly difficult after thousands of years due to degradation and sample contamination. In this paper, a portable immunochromatographic strip (ICS) technique using colloidal gold-labelled polyclonal antibody was well-designed and developed. This technique requires only 10 min to complete the qualitative detection of silks. The strip exhibits good sensitivity, specificity and thermal stability. The detection limit of the strip is 1.5 μg/mL, and no cross-reactions with other possible interference antigens were noted. Moreover, the strip can maintain the effectiveness after treated at 60°C for 30 days. These characteristics make the ICS assay to be especially suitable for the on-site detection in archaeological field.

Introduction

Silk is one of the oldest proteinaceous fibers used to produce clothing and textiles. Given that silk is a material used in magnificent works of art and artefacts of precious cultural heritage, accurate assessment of silks is necessary to deeply understand ancient civilization and design suitable conservation methods for further display and research.[1] However, its protein composition makes silk easily deteriorated by light, humidity, heat and microorganisms.[2-4] The identification of ancient silks becomes particularly difficult when they have suffered thousands of years of erosion.

Numerous conventional methods, including optical microscopy,[5] nuclear magnetic resonance,[6, 7] capillary electrophoresis mass spectrometry,[8] and Fourier-transform infrared spectroscopy,[9] have been employed for the determination of ancient silk. Despite the meaningful information that can be achieved in some cases by these methods, the drawback that should never be ignored is that they are not suitable for the detection and distinguishment of ancient silk with contamination, severe degradation or homogenous proteins from different species. Moreover, these analytical methods usually require expensive and sophisticated instruments and skilled manipulation techniques, which limits their wide application in routine measurements and create increasing difficulties for archaeologists.

Compared with these traditional methods, immunoassays with high sensitivity and specificity are portable, rapid and cost-effective.[10, 11] In recent decades, immunoassays have become popular and are increasingly considered as complementary or even alternative methods to residue analysis.[12-14] Several immunoassays based on ELISA, immunofluorescence microscopy (IFM), immunodotting, immunochromatography and similar techniques have been employed to identify milk residues as well as species of archaeological bone and proteins in ancient binding materials.[10, 15] Positive results have been obtained in such studies, and all of the studies suggest that the immunoassays provide sensitive and specific identification of ancient proteinaceous samples. However, the repeated coating and washing steps, long reaction time and need for highly skilled personnel confine these techniques to the laboratory environment and analytical devices. Thus, a rapid, inexpensive, portable and on-site detection system for archaeological samples is urgently needed.

The ICS assay is an on-site qualitative detection method that has been widely adopted for the rapid identification of various targets,[16-19] such as the assessment of bacteria,[20-23] mycoplasmas,[24] viruses[25-27] and heavy metal ions in water.[28] In recent years, ICS has received considerable attention due to its excellent portability, short assay time, low sample consumption and reduced overall cost.[29-32] The possibility of observing antigen and antibody reactions by the naked eye arises from the use of test strip labelling substances, such as highly coloured colloidal gold nanoparticles,[33] carbon nanoparticles,[34] fluorescent particles,[35-37] and quantum dot nanobeads,[38] to create a change in colour density, which typically reflects the changes at the test line within a few minutes.

In our previous work, ELISA was successfully used to detect collagen-I and ovalbumin in ancient silk.[39] Recently, we report the preparation of one polyclonal antibody and its use for the identification of unearthed ancient silk relics.[40] However, the technique for the on-site detection of ancient silk has not yet

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been established. In this paper, a simple, rapid and cost-effective colloidal gold-based immunochromatographic strip assay has been well-designed and developed as an alternative method for silk detection. Three archaeological samples derived from the Kazakhstan tomb (sample I), the Chu tomb (sample II) and the Song tomb (sample III) were analyzed. The results indicated that ICS has the potential to become a routine analytical tool at archaeological sites and in conservation science laboratories.

Experimental

Archaeological Samples

Three precious archaeological fabric samples were chosen for immunodetection. The original condition of the samples is presented in Fig. 1. Sample I (Fig. 1A) was excavated from Kazakhstan. Sample II (Fig. 1B) was unearthed from the Chu tomb (approximately 2300 years old), Anji County, Zhejiang Province. Sample III (Fig. 1C) was unearthed from the Song tomb (approximately 760 years old), Ningbo City, Zhejiang Province. However, the unearthed fabrics appear to be fragile and contaminated, even seriously concreted, which makes them difficult to distinguish.

Reagents

Goat anti-rabbit IgG alkaline phosphatase (AP)-conjugated antibody (500 μg at 2 mg/mL) and goat anti-rabbit antibody (500 μg at 1 mg/mL) were supplied by Hangzhou Hua'an Biotechnology Co., Ltd. Bovine serum albumin (BSA), human serum albumin (HSA), ovalbumin and collagen Ⅰ were purchased from Sigma-Aldrich. Natural silks were obtained from Zhejiang Misai Silk Co., Ltd. Trisodium citrate, H_2O_2, $HAuCl_4$, K_2CO_3, borate-buffered saline and hydrogen peroxide were supplied by Tianjin Gaojing Fine Chemical Co., Ltd.

Phosphate-buffered saline (PBS) solution at pH 7.4 was used for the washing steps. PBS solution at pH 9.6 was used as the diluent for ELISA antigens. Further, 1% BSA in PBS (pH 7.4) was used to dilute antibody and block the unbound sites. All other reagents were of analytical grade and were used as received. The water used in all experiments was purified by a TPM Ultrapure water system.

All the silk fibroin, sericin, keratin, cotton fiber and hemp fiber were extracted in advance. The extraction of silk fibroin and sericin can be summarized as follows. Silk was firstly boiled in 0.5% (w/w) Na_2CO_3 solution at a bath ratio of 1:100 for 30 min. Then, the insoluble fibroin was removed and the remaining solution was dialyzed with a molecular weight cut-off of 3500 for 3 days. Sericin solution was thus obtained. Meanwhile, the insoluble silk fibroin was washed with water and dried at 60 °C overnight. Next, dried silk fibroin was dissolved in the extracting solution (the molar ratio of calcium chloride–water–ethanol was 1:8:2) at 72 °C for 1 h. After filtering, silk fibroin solution was dialyzed with a molecular weight cut-off of 10000 for 3 days to remove calcium and chloride ions. Finally, both silk fibroin and sericin solutions were freeze-dried and the pale yellow silk fibroin and sericin powders were obtained. Keratin was extracted in another way. First, 2% (w/w) sodium hydroxide and 0.3% (w/w) hydrogen peroxide solutions were prepared. Next, shredded wool fabrics were completely immersed in alkali solutions at 50 °C at a bath ratio of 1:50. Nitrogen was continuously replenished in the reactor. A clear liquid was obtained when the wool fabrics were completely dissolved by mechanical stirring at 200 r/min for 4 h. Next, the solutions were dialyzed for 72 h with a molecular weight cut-off of 3500 to ensure that contaminating ions were completely removed. Pure keratin solutions were freeze-dried, and keratin powders were obtained. Cotton fiber and hemp fiber were exacted as described before.[40]

Preparation of Primary Antibody

Polyclonal antibody against silk fibroin was produced by immunization as follows. For the initial immunization, three New Zealand white rabbits (14-16 weeks old) were injected subcutaneously at multiple points with silk fibroin immunogen (500 μg in PBS, mixed with an equal volume of Freund's complete adjuvant to form an emulsion). Then, Freund's incomplete adjuvant was substituted for Freund's complete adjuvant as an emulsifier every 2 weeks. Ten days after the third and fourth booster immunization, the titre of the antiserum was measured by drawing venous blood. Sera were collected 1 week after the last injection. The resultant anti-SF antibody was further purified using a Protein A column and stored (3.15 mg/mL) at -20 °C before use.

Indirect Competitive ELISA Procedures

Assays were performed in 96-well microplates in an indirect competitive format. Each well of the microplates was coated with 100 μL of silk fibroin solution (10 μg/mL in PBS, pH 9.6) and incubated at 4 °C overnight. Then, the coating antigens were removed, and the wells were washed thrice with PBS (pH 7.4). Then, 200 μL of BSA blocking solution was added to each well and incubated at 37 °C for 2 h. The solutions were then removed, and the wells were washed thrice with PBS (pH 7.4). Next, standard solutions (silk fibroin) or sample solutions (50 μL) and 50 μL of primary antibodies were added to each well and incubated at 37 ^{o}C for 1 h. For positive control, standard solutions or sample solutions were replaced by 1% BSA in PBS (pH 7.4); for negative control, primary antibody was replaced by PBS (pH 7.4). The solutions were removed, and the wells were washed thrice with PBS (pH 7.4). Next, 100 μL of goat anti-rabbit IgG AP-conjugated antibodies were added to each well and incubated at 37 °C for 1 h. The solutions were removed, and the wells were washed trice with PBS (pH 7.4). Next, 100 μL of substrate solution (TMB colour system) was added to each well in a dark environment at room temperature for 10 min. Finally, 100 μL of 2 mol/L H_2SO_4 was added to terminate the colour reaction. The sample absorbance at λ=450 nm was measured using a microplate reader (Model 550, Bio Rad) and denoted as OD_{450}.

Sensitivity was evaluated using the IC_{50} values, which represented the concentration of silk fibroin that produced 50% inhibition rate. The inhibition rate was calculated according to the following formula.

$$\text{Inhibition rate} = [\text{OD}_{450}(\text{positive control}) - \text{OD}_{450}(\text{sample})]/[\text{OD}_{450}(\text{positive control}) - \text{OD}_{450}(\text{negative control})] \times 100\%$$

The detection limit of was defined as the concentration that produced 10% inhibition rate,[41] and cross-reactivity was calculated with the formula below.

$$\text{Cross} - \text{reactivity} = \text{IC}_{50}(\text{silk fibroin})/\text{IC}_{50}(\text{interference antigens}) \times 100\%$$

Preparation of Colloidal Gold-Labelled Polyclonal Antibody

Colloidal gold with an average diameter of 30 nm was produced by the reduction of $HAuCl_4$. Briefly, 100 mL of 0.01% $HAuCl_4$ was heated to boiling under constant stirring, and then 1.4 mL of 1% trisodium citrate (w/v) was added quickly and stirred for 15 min until the colour of the solution became a transparent wine red. The quality of the colloidal gold was characterized via ultraviolet (UV) spectra.

The colloidal gold-labelled anti-SF polyclonal antibody was prepared as follows. The colloidal gold solution was adjusted to pH 8.3 with 0.1 M K_2CO_3. Afterwards, the minimum amount of protein concentration for labelling was employed in the following steps. Suitable anti-SF polyclonal antibody solutions were diluted with 5 mM borate-buffered saline (BBS) (pH 9.0) to gradient concentrations of 5 to 50 μg/mL. Then, 1 mL of colloidal gold solution was added to 1 mL of each dilution and incubated for 5 min at room temperature. Next, 100 μL of 10% NaCl solution was added to the mixture and placed at room temperature for 2 h. The minimum amount of anti-SF polyclonal antibody was evaluated by a colour change from reddish to blue. The minimum concentration of the solution that did not change colour with a 20% increment was believed to be the most suitable quantity of anti-SF antibody. Then, 19 μL of anti-SF antibody (3.15 mg/mL) was mixed with 15 mL of colloidal gold for 1 h with rapid stirring. Next, 1.5 mL of 1% BSA was added to block the unbound colloidal gold sites for an additional 5 min. The solutions were centrifuged for 15 min at 8500 rpm, and the precipitates were washed thrice with 0.02 M PBS (pH 7.4) containing 1% trehalose, 0.5% BSA and 0.1% tween 20. Finally, the resultant colloidal gold-labelled polyclonal antibody was stored at 4 °C for use.

Preparation of Immunochromatographic Strip

The immunochromatographic strip consists of four parts: sample pad, conjugate pad, absorbent pad and nitrocellulose membrane. The colloidal gold-labelled antibody was sprayed onto a glass fiber pad at 1 μL/cm by a metal spraying machine and then dried at 37°C for 1 h. Meanwhile, silk fibroin (4.8 mg/mL) and goat anti-rabbit IgG (1.5 mg/mL) were sprayed onto a nitrocellulose membrane at 1 μL/cm in the lines that would become the test line and control line, respectively. Similarly, the membrane was dried at 37°C for 1 h. All the four parts were assembled together as previously described.[42] Finally, the strip was cut into 3 mm-wide and housed in a plastic case.

Pretreatment of Archaeological Samples

The archaeological samples were separately extracted. The extraction of ancient wool sample (Sample I) can be summarized as follows. 2 mg of sample was added into 100 μL of extracting solutions (including 2% (w/w) sodium hydroxide and 0.3% (w/w) hydrogen peroxide) and incubated for 1 h. The extraction of the ancient silk samples depends on their preserved condition. In general, well preserved silks (Sample II) were extracted similarly to that of silk fibroin. 2mg of sample was added into 100 μL of extracting solutions (the molar ratio of calcium chloride–water–ethanol was 1:8:2) and incubated at 72 °C for 1 h. The poor preserved ancient silks (Sample III) usually become partially soluble in aqueous solution. As a result, they can be extracted directly through incubating in PBS (pH 7.4) or water for 1 h (without heating). Finally, the supernatant of each extracting solution was collected and transferred individually into centrifuge tube for the ICS detection.

Results and discussion

Indirect competitive ELISA

To ensure the sensitivity of the anti-SF polyclonal antibody, the indirect competitive ELISA format was investigated.[42] The optimized antibody dilution (v/v) was obtained by panel titrations in advance. Antibody dilutions of 1:3000 for the primary antibody and 1:5000 for the secondary antibody were chosen given their higher sensitivity and specificity. Then, the ELISA calibration curve for silk fibroin was established using silk fibroin concentrations ranging from 0.1 to 10^9 ng/mL and shown in Fig. 2. IC_{50}, which is the concentration of standard solution causing 50% inhibition of binding between the primary antibody and the immobilised silk fibroin, is 19.952 μg/mL. This result indicates that the anti-SF antibody exhibited high sensitivity. Moreover, the detection limit, which is defined as the concentration of standard solution corresponding to 10% inhibition rate, is 1.435 μg/mL. In addition, the inhibition rate and logarithm of the silk fibroin concentration clearly exhibited a good linear relationship (R^2=0.974) in silk fibroin concentrations range from 10^3 to 10^5 ng/mL, which ensures quantitative detection in this concentration range. It is believed that this method has the potential to become an accurate detection method for ancient silk.

Table 1 presents the cross-reactivity of the anti-SF antibody to possible interference antigens for ELISA. Clearly, minimal cross-reactivity ($<$0.196%) was observed in our study. This result indicates that other possible interference antigens do not interfere with silk detection. It is not surprising that negligible reactivity was detected with all the proteins and fibers, given that the element types, molecular structure and matter state of the compounds clearly differ.

Sensitivity, thermo stability, and specificity of ICS

The detection of silk is based on competitive immunoreactions. As shown in Fig. 3A**,** goat anti-rabbit IgG antibody and silk fibroin at appropriate concentrations were immobilized on the nitrocellulose membranes as the control and test lines, respectively. The sample solutions could flow chromatographically along the nitrocellulose

strip, first passing the pad containing gold-labelled anti-SF antibody and then the test line and control line before entering the absorption pad. The entire reaction process requires only 10 min. If silk fibroin in a sample is above a certain threshold (detection limit), it combines with the gold-labelled anti-SF antibody prior to the immobilized capture reagent (silk fibroin) at the test line. Thus, no red colour appears at the test zone. In contrast, if the silk fibroin in the sample is below the detection limit, a visible red colour appears at the test line. However, no matter silk fibroin presents in the sample or not, gold-labelled anti-SF antibody will continue flowing to the control line and combine with secondary antibody (Goat anti-rabbit IgG). The control line thus exhibits red colour. In other words, if only the control line becomes red, it illustrates that the silk fibroin is above the detection limit; if both the test line and the control line become red, it indicates the silk fibroin is below the detection limit. The more silk fibroin presents in the sample, the weaker red colour appears at the test line.[43]

The main purpose of the strip in this study is the on-site qualitative detection of silk fibroin in ancient silk. To develop a sensitive ICS, various parameters, such as size and uniformity of the colloidal gold nanoparticles, pH of solutions used, concentration of coating solutions sprayed on the test and control line, were considered and optimized prior to the assay. Then, the sensitivity, stability and specificity of the strip were evaluated. Fig. 3B presents the detection limit of the ICS, which was defined as the lowest concentration of silk fibroin that caused the disappearance of the red colour at the test line. The results were obtained by identifying and comparing the colour of test line with the standard colour card for ICS within 10 min after the reaction started. According to Fig. 3B, the detection limit of the ICS for silk fibroin is approximately 1.5 μg/mL. Even though the sensitivity of ICS is similar to that of the ELISA technique, the strips are considerably more convenient because they can be used by archaeologists for rapid on-site detection without special equipments. Fig. 3C presents the detection limit of ICS treated at 60°C for 30 days. Approximately detection limit (1.5 μg/mL) of strip was detected, which revealed no differences in the colour intensity of the immunoreactive bands before and after thermal treatment. These results indicated that the strip exhibited high thermo stability, suggesting that it can be stored for extended periods of time at room temperature.[21, 25]

To evaluate the strip specificity, a number of possible interference antigens were prepared and used for detection. Table 2 presents the cross-reactivity of anti-SF antibody with various antigens for the ICS. Both the test line and the control line of the strip exhibited clear red colour at various concentrations of the possible interfering antigens. This result indicates that the ICS exhibited a high specificity for silk fibroin without cross-reactivity with other proteins or fibers, which is highly consistent with the ELISA results.

Archaeological Sample Analysis

Three archaeological samples were analyzed using the ICS method. PBS and silk fibroin solutions (10 μg/mL) were used as negative and positive controls, respectively. As shown in Fig. 4, for the PBS solutions and Sample I extract (Fig. 4A), red colour appeared at both the test line and the control line of the strip, whereas only the control line exhibited red colour for sample II extract (Fig.4B), sample III extract (Fig. 4C) and silk fibroin. These results are consistent with the ELISA results, also conforming the validity and accuracy of the ICS method.

In addition, compared with other immunoassays, such as ELISA and IFM, ICS method has its unique advantages in the on-site detection of archaeological silks. First, a little amount of sample or a drop of sample solution can meet the demand of real-time detection by ICS and the results can be obtained within 10 min. However, for ELISA and IFM, continuous repeated incubating and washing steps make them difficult for the rapid detection and it often takes several hours to complete the tests. All the three immunological techniques show high sensitivity and specificity, and they only cost less than five dollars for the detection of each sample. The detection limit of ICS is approximately 1.5 μg/mL and it requires samples to be aqueous soluble, similarly to those of ELISA. Meanwhile, although a small piece of silk sample can be observed by IFM, it requires samples to be insoluble in aqueous solution. These characteristics make IFM not quite applicable for the detection of seriously degraded silks. In a word, ICS is especially suitable for the identification of poor preserved ancient silks (severely degraded) or even the silk traces, which is also the key challenge in archaeological site.

Conclusions

In summary**,** an on-site cost-effective and rapid lateral flow ICS method was developed to detect archaeological silk at archaeological sites and in conservation laboratories. The generated signal (red colour at the test line of the strip) can be unambiguously read by the naked eyes in only 10 min. The sensitivity, thermo stability and specificity of the ICS were further evaluated. Despite the sensitivity of ICS is similar to that of ELISA, the simple operation, rapid response and portable features make it especially suitable for the on-site detection of ancient silk specimens that might have suffered thousands of years of erosion. Moreover, this strip system is versatile and can be easily extended to the analysis of other archaeological proteinaceous materials by utilizing other gold-based antibodies. In short, ICS assay has the potential to be a powerful tool for the on-site detection in archaeological field.

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Notes and references

1. Z. Wu, D. Huang, Z. Hu, Y. Zhou, F. Zhao, Z. Peng and B. Wang, *Fibers and Polymers*, 2014, **15**, 1146-1152.

2. X. Zhang, I. V. Berghe and P. Wyeth, *Journal of Cultural Heritage*, 2011, **12**, 408-411.
3. J. F. Ming, Y. X. Jiang and B. Q. Zuo, *Advanced Materials Research*, 2011, **332-334**, 3-10.
4. M.-Y. Li, Y. Zhao, T. Tong, X.-H. Hou, B.-S. Fang, S.-Q. Wu, X.-Y. Shen and H. Tong, *Polymer Degradation and Stability*, 2013, **98**, 727-735.
5. M. Wilding, *Optical microscopy for textile fibre identification. In Identification of textile fibers*, Woodhead Publishing, Cambridge, , United Kingdom, 2009.
6. Z. Zhu, D. Gong, L. Liu and Y. Wang, *Analytical and bioanalytical chemistry*, 2014, **406**, 2709-2718.
7. S. Lahiri, M. Maiti and K. Gangopadhyay, *Journal of Radioanalytical and Nuclear Chemistry*, 2015, DOI: 10.1007/s10967-014-3922-3.
8. M. Moini, K. Klauenberg and M. Ballard, *Analytical chemistry*, 2011, **83**, 7577-7581.
9. X. Zhang and P. Wyeth, *Science China Chemistry*, 2010, **53**, 626-631.
10. M. Palmieri, M. Vagnini, L. Pitzurra, B. G. Brunetti and L. Cartechini, *Analytical and bioanalytical chemistry*, 2013, **405**, 6365-6371.
11. S. Zhang, X. Lai, X. Liu, Y. Li, B. Li, X. Huang, Q. Zhang, W. Chen, L. Lin and G. Yang, *Journal of agricultural and food chemistry*, 2012, **60**, 3580-3585.
12. J. K. Biswal, S. Jena, J. K. Mohapatra, P. Bisht and B. Pattnaik, *Archives of virology*, 2014, **159**, 1641-1650.
13. S. Sivajothi, V. C. Rayulu, P. Malakondaiah and D. Sreenivasulu, *Journal of Parasitic Diseases*, 2014, DOI: 10.1007/s12639-014-0465-z.
14. Y. Zhang, C. Tan, J. Zhang, W. Sheng and S. Wang, *Food Analytical Methods*, 2013, **7**, 1305-1311.
15. M. Vagnini, L. Pitzurra, L. Cartechini, C. Miliani, B. G. Brunetti and A. Sgamellotti, *Analytical and bioanalytical chemistry*, 2008, **392**, 57-64.
16. Z. Mei, Y. Deng, H. Chu, F. Xue, Y. Zhong, J. Wu, H. Yang, Z. Wang, L. Zheng and W. Chen, *Microchimica Acta*, 2012, **180**, 279-285.
17. S. H. Paek, S. H. Lee, J. H. Cho and Y. S. Kim, *Methods*, 2000, **22**, 53-60.
18. Y. Xu, Z.-B. Huang, Q.-H. He, S.-Z. Deng, L.-S. Li and Y.-P. Li, *Food Chemistry*, 2010, **119**, 834-839.
19. S. F. Chou, *The Analyst*, 2013, **138**, 2620-2623.
20. S. Shukla, H. Leem and M. Kim, *Analytical and bioanalytical chemistry*, 2011, **401**, 2581-2590.
21. P. Sithigorngul, S. Rukpratanporn, N. Pecharaburanin, P. Suksawat, S. Longyant, P. Chaivisuthangkura and W. Sithigorngul, *Journal of microbiological methods*, 2007, **71**, 256-264.
22. M. Blazkova, B. Javurkova, L. Fukal and P. Rauch, *Biosensors & bioelectronics*, 2011, **26**, 2828-2834.
23. C. Pengsuk, P. Chaivisuthangkura, S. Longyant and P. Sithigorngul, *Biosensors & bioelectronics*, 2013, **42**, 229-235.
24. K. Meng, W. Sun, P. Zhao, L. Zhang, D. Cai, Z. Cheng, H. Guo, J. Liu, D. Yang, S. Wang and T. Chai, *Biosensors & bioelectronics*, 2014, **55**, 396-399.
25. P. Chaivisuthangkura, S. Senapin, P. Wangman, S. Longyant and P. Sithigorngul, *Archives of virology*, 2013, **158**, 1925-1930.
26. K. Omidfar, S. Kia, S. Kashanian, M. Paknejad, A. Besharatie, S. Kashanian and B. Larijani, *Applied biochemistry and biotechnology*, 2010, **160**, 843-855.
27. K. Maejima, M. Himeno, O. Netsu, K. Ishikawa, T. Yoshida, N. Fujita, M. Hashimoto, K. Komatsu, Y. Yamaji and S. Namba, *Journal of General Plant Pathology*, 2014, **80**, 176-183.
28. J. Chen, S. Zhou and J. Wen, *Analytical chemistry*, 2014, **86**, 3108-3114.
29. L. Chen, Z. Wang, M. Ferreri, J. Su and B. Han, *Journal of agricultural and food chemistry*, 2009, **57**, 4674-4679.
30. B. B. Dzantiev, N. A. Byzova, A. E. Urusov and A. V. Zherdev, *TrAC Trends in Analytical Chemistry*, 2014, **55**, 81-93.
31. Y. Tang, J. Xu, W. Wang, J. Xiang and H. Yang, *European Food Research and Technology*, 2010, **232**, 9-16.
32. L. Hu, J. Liu, Q. Wang, Y. Zhang, R. Jia, C. Cai, W. Wu, S.-F. Chen and P. He, *Journal of Applied Phycology*, 2013, **25**, 1091-1099.
33. B. Holubová-Mičková, M. Blažková, L. Fukal and P. Rauch, *European Food Research and Technology*, 2010, **231**, 467-473.
34. C. Suarez-Pantaleon, J. Wichers, A. Abad-Somovilla, A. van Amerongen and A. Abad-Fuentes, *Biosensors & bioelectronics*, 2013, **42**, 170-176.
35. X. Chen, Y. Xu, J. Yu, J. Li, X. Zhou, C. Wu, Q. Ji, Y. Ren, L. Wang, Z. Huang, H. Zhuang, L. Piao, R. Head, Y. Wang and J. Lou, *Analytica chimica acta*, 2014, **841**, 44-50.
36. D. Xu, X. Wu, B. Li, P. Li, X. Ming, T. Chen, H. Wei and F. Xu, *Food Science and Biotechnology*, 2013, **22**, 585-591.
37. F. Zhang, M. Zou, Y. Chen, J. Li, Y. Wang, X. Qi and Q. Xue, *Biosensors & bioelectronics*, 2014, **51**, 29-35.
38. X. Li, W. Li, Q. Yang, X. Gong, W. Guo, C. Dong, J. Liu, L. Xuan and J. Chang, *ACS applied materials & interfaces*, 2014, **6**, 6406-6414.
39. Y. Zhou, B. Wang, M. Sui, F. Zhao and Z. Hu, *Studies in Conservation*, DOI: 10.1179/2047058414Y.0000000150.
40. Q. Zheng, X. Wu, H. Zheng and Y. Zhou, *Analytical and bioanalytical chemistry*, 2015, **470**, 3861-3867.
41. S. Gandhi, N. Caplash, P. Sharma and C. Raman Suri, *Biosensors & bioelectronics*, 2009, **25**, 502-505.
42. J. Jiang, Z. Wang, H. Zhang, X. Zhang, X. Liu and S. Wang, *Journal of agricultural and food chemistry*, 2011, **59**, 9763-9769.
43. L. A. Masinde, W. Sheng, X. Xu, Y. Zhang, M. Yuan, I. R. Kennedy and S. Wang, *Microchimica Acta*, 2013, **180**, 921-928.

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Table 1 Cross-reactivity of anti-SF antibody to possible interference antigens for ELISA.

Interference antigens	IC_{50} (μg/mL)	Cross-reactivity
Silk fibroin	19.652	100%
Sericin	$>10^4$	<0.196%
Keratin	$>10^4$	<0.196%
Cotton fiber	$>10^4$	<0.196%
Hemp fiber	$>10^4$	<0.196%
Collagen	$>10^4$	<0.196%
HSA	$>10^4$	<0.196%
Ovalbumin	$>10^4$	<0.196%

Table 2 Cross-reactivity of anti-SF antibody to possible interference antigens for ICS.

Interference antigens		Concentration (μg/mL)					
		0	5	10	15	20	25
Silk fibroin	Test line	+	−	−	−	−	−
	Control line	+	+	+	+	+	+
Sericin	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+
Keratin	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+
Cotton fiber	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+
Hemp fiber	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+
Collagen	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+
HSA	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+
Ovalbumin	Test line	+	+	+	+	+	+
	Control line	+	+	+	+	+	+

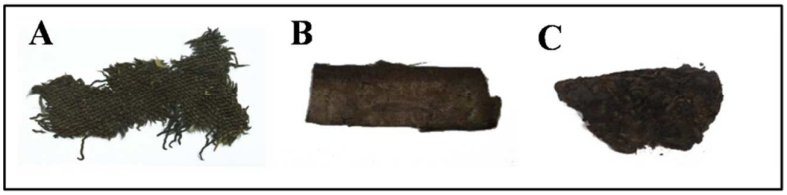
Figure captions

Fig. 1 Digital images of ancient textile fragments. (A) Wool fragment from Kazakhstan; (B) silk fragment from the Chu tomb in Anji County, Zhejiang Province; (C) silk fragment from the Song tomb in Yuyao City, Zhejiang Province.

Fig. 2 ELISA calibration curves obtained for silk fibroin at optimized primary and secondary antibody dilutions. The dilution ratio of primary and secondary antibody is 1:3000 and 1:5000, respectively.

Fig. 3 (A) Schematic diagram of the colloidal gold-based immunochromatographic assay. (B) Sensitivity test result for the ICS. (C) Stability test result for the ICS. All tests were performed at room temperature thrice. The labels (0.1 to 2.5) indicate the concentrations of silk fibroin (μg/mL).

Fig. 4 ICS detection results for PBS, silk fibroin and archaeological samples. (A) Wool fragment from Kazakhstan; (B) silk fragment from the Chu tomb in Anji County, Zhejiang Province; (C) silk fragment from the Song tomb in Yuyao City, Zhejiang Province.



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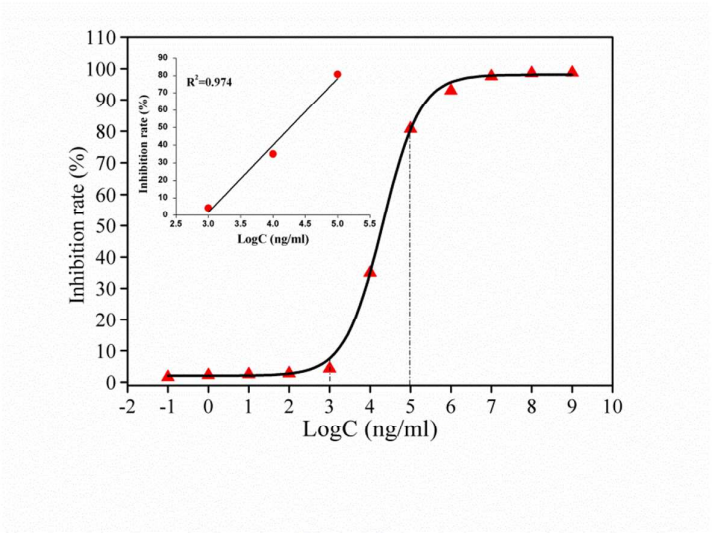

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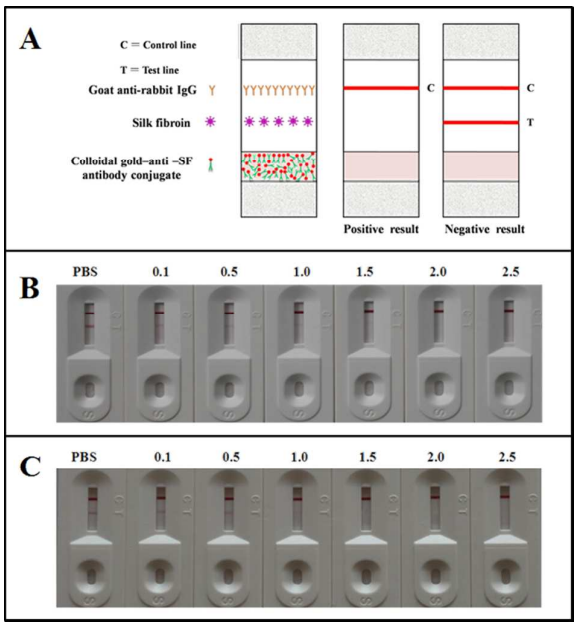

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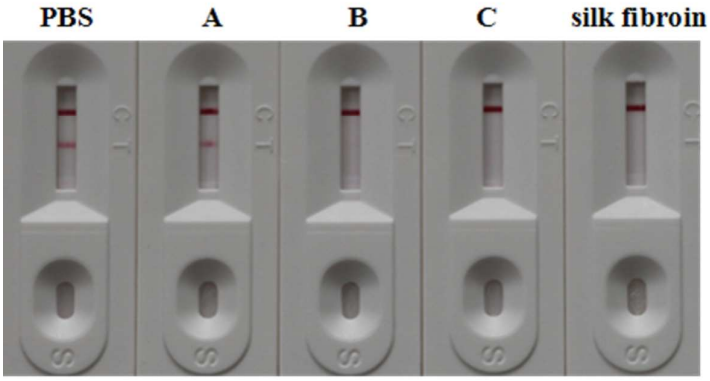

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A portable, rapid, sensitive and specific gold-based immunochromatographic strip assay for the on-site detection of ancient silks