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Research Article

Highly sensitive nanozyme strip: an effective tool for forensic material evidence identification

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ABSTRACT

During criminal case investigations, blood evidence tracing is critical for criminal investigation. However, the blood stains are often cleaned or covered up after the crime, resulting in trace residue and difficult tracking. Therefore, a highly sensitive and specific method for the rapid detection of human blood stains remains urgent. To solve this problem, we established a nanozyme-based strip for rapid detection of blood evidence with high sensitivity and specificity. To construct reliable nanozyme strips, we synthesized $CoFe_2O_4$ nanozymes with high peroxidase-like activity by scaling up to gram level, which can be supplied for six million tests, and conjugated antibody as a detection probe in nanozyme strip. The developed $CoFe_2O_4$ nanozyme strip can detect human hemoglobin (HGB) at a concentration as low as 1 ng/mL, which is 100 times lower than the commercially available colloidal gold strips (100 ng/mL). Moreover, this $CoFe_2O_4$ nanozyme strip showed high generality on 12 substrates and high specificity to human HGB among 13 animal blood samples. Finally, we applied the developed $CoFe_2O_4$ nanozyme strip to successfully detect blood stains in three real cases, where the current commercial colloidal gold strip failed to do. The results suggest that the $CoFe_2O_4$ nanozyme strip can be used as an effective on-scene detection method for human blood stains, and can further be used as a long-term preserved material evidence for traceability inquiry.

KEYWORDS

$CoFe_2O_4$ nanozyme, nanozyme strip, peroxidase-like activity, human hemoglobin, blood evidence

1 Introduction

Blood stain is one of the common important traces in the crime scene, and it is the inevitable product during the process of human casualties [1]. The identification of blood traces plays a very important role in the scene analysis and reconstruction, especially in the cases of major homicides [2–4]. However, with the suspects' anti-reconnaissance awareness increasing, the blood marks in the scene were often wiped off or covered up artificially by a large amount of animal blood to hide their residual biological evidences, thus increasing the complexity of the investigations [5]. Therefore, an accurate determination of the blood spot marks, i.e. whether there was human blood present, will provide important evidence for the investigations [6]. The current standard method for human blood identification in China is the colloid gold test strip method [7]. Despite its ease to operate characteristic, the colloid gold test strip often leads to false positive results due to lacking specificity [8]. In additional, it can also give false negative results due to its low sensitivity (100 ng/mL), or human blood material degradations [9]. Therefore, there remains urgent demands for the development of an accurate detection method for human blood marks with high sensitivity and specificity.

Nanozymes, as a new generation of artificial enzymes [10–13], have been widely used in disease diagnosis [14–17], biological molecule detection [18–21] and environmental analysis [22–25], particularly in sensing field. This is due to their intrinsic characteristics including high catalytic activity, stability, low cost and reusability. We also previously developed a series of rapid detection technologies based on nanozymes, such as chromogenic nanozyme-strip [18], chemiluminescence nanozyme strip [26] and nanozyme-strip for nucleic acid detection [27], but these are just conceptual verifications, and have not really been put into production.

In this work, we prepared $CoFe_2O_4$ nanozymes with high peroxidase-like catalytic activity through the strategy of doping cobalt (Co) into Fe_3O_4 nanoparticles, and developed a $CoFe_2O_4$ nanozyme-based strips. With various optimized factors such as paired antibodies and nanozymes amount, the developed $CoFe_2O_4$

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nanozyme strips can detect human hemoglobin (HGB) as low as 1 ng/mL, much lower than the current colloid gold test strip, and simultaneously is highly specific to human blood samples, in the case of various materials or even blood from other animals. Besides, the whole detection process, upon sample collection, only takes around 20 min. The successful detection of the blood marks from actual cases, where the recommended commercial colloid gold strip fails to do, further demonstrated that we developed a rapid, highly sensitive and specific strip products for human blood spot detection. As the 3,3'-diaminobenzidine (DAB) is used as the catalytic substrate of nanozymes, the formation of brown DAB precipitation band can not only greatly enhance the detection signal, but also can be used as the material evidence for traceability and long-term preservation.

2 Experimental

2.1 Chemicals and reagents

A $FeCl_3{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $MnCl_2{\cdot}4H_2O$, poly (acrylic acid), DAB, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 3,3',5,5'-tetramethylbenzidine (TMB), bovine serum albumin (BSA), and ethylene glycol were purchased from Sigma-Aldrich Co., LLC. (Shanghai, China). Hydrogen peroxide (H_2O_2), ethanol, anhydrous sodium acetate, and acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). DAB and TMB chromogenic kit were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd (Beijing, China). Fiberglass pads, sample pads, absorbent pads and polyvinyl chloride (PVC) plastic boards were purchased from Shanghai Kinbio Tech. Co., Ltd (Shanghai, China). Nitrocellulose membrane was purchased from Sartorius (Germany). Goat anti-mouse IgG antibodies, human HGB and mouse anti-HGB antibodies (detection antibodies and capture antibodies) were purchased from Wuhan Aoke Botai Biotechnology Co., Ltd (Wuhan, China). The standard commercial colloidal gold strip products, human blood, all kinds of animal blood and physical evidence were provided by the Institute of Forensic Science, Ministry of Public Security of the People's Republic of China.

2.2 Preparation and characterization of $CoFe_2O_4$ nanozymes

Carboxyl-modified $CoFe_2O_4$ nanozymes were prepared by the liquid-phase hydrothermal method [28], similar to our previous synthesis method of Fe_3O_4 nanozymes [18] and made some modifications. Briefly, following addition of 3.17 g $CoCl_2{\cdot}6H_2O$ and 7.2 g $FeCl_3{\cdot}6H_2O$ (the molar ratio of Co to Fe is 1:2) into 400 mL ethylene glycol under rapid stirring, 30 g of anhydrous sodium acetate and 3 g of poly(acrylic acid) were added. It should be pointed out that when Fe_3O_4, $MnFe_2O_4$ and $CuFe_2O_4$ were synthesized, $CoCl_2{\cdot}6H_2O$ needs to be replaced by $FeCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$ and $MnCl_2{\cdot}4H_2O$ with the same molar mass. After fast stirring for 30 min, the reaction mixture was then placed in a 200 °C autoclave reactor for 12–14 h [29, 30]. The products were washed several times with ethanol and dried at 60 °C. With a small amount of the nanozymes dispersed in either ethanol or aqueous solution, the size and morphology of the prepared nanozymes were characterized by JEOL 2000FX 200 kV transmission electron microscopy (TEM). The hydrate particle size of nanozymes was measured using a 271-DPN dynamic light scattering (DLS) instrument. The UV–Vis absorbance spectra were scanned using a U-3900 absorption spectrophotometer (Hitachi, Japan).

2.3 Peroxidase activity and kinetic assay of $CoFe_2O_4$ nanozymes

The peroxidase-like activity of $CoFe_2O_4$ nanozymes was tested using the similar method as before [18, 26, 31]. For example, the reaction mixture was prepared by mixing 20 μg/mL nanozymes with 1 M H_2O_2 in 0.2 M acetate/sodium acetate buffer, pH 3.6, using TMB as a co-substrate. The absorption intensity changes at 652 nm were recorded at 37 °C. The specific activity of the nanozymes was calculated according to the following conversion formula (Eq. (1)).

$$a_{\text{nanozyme}} = V \div (\varepsilon \times l) \times (\Delta A \div \Delta t) \div [m] \quad (1)$$

Where a_{nanozyme} is the specific activity (U/mg) of the nanozymes; V is the volume of the reaction mixture; ε is the molar extinction coefficient of TMB; l is the pathlength for the holding cuvette; $\Delta A/\Delta t$ is the initial rate of changes in absorbance at 652 nm per min; $[m]$ is the nanozyme weight (mg) of each assay.

2.4 Preparation of $CoFe_2O_4$ nanozyme probes

The $CoFe_2O_4$ nanozyme probes were prepared as previously described [7, 18]. Briefly, 5 mg $CoFe_2O_4$ nanozymes were functionalized by addition of 5 mg EDC and NHS, then incubated at room temperature for 30 min. The functionalized nanozymes were mixed with 100 μg/mL mouse anti-HGB detection antibodies by ultrasonic dispersion, then incubated for 2–3 h at room temperature. After washing with phosphate buffered saline (PBS) buffer (pH 7.0) twice, 50 mM Tris-HCl buffer (pH 7.4) once, incubation with 5% BSA-PBS buffer for 30 min and freeze-drying treatment, the prepared nanozyme detection probes solution could be stored at room temperature for more than half a year without affecting its performance.

2.5 Preparation of $CoFe_2O_4$ nanozyme strip

The $CoFe_2O_4$ nanozyme-strip consists of a PVC plastic board, a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad, and the whole preparation process was according to the description of our previous study with some modifications [7, 18]. First, the $CoFe_2O_4$ nanozyme probes labeled by mouse anti-HGB detection antibodies were dispensed on the pretreated fiberglass pad using an IsoFlow™ Dispenser (Imagene Technology, New Hampshire, USA) to form the conjugate pad. Then the paired capture antibody of mouse anti-HGB was immobilized on a nitrocellulose membrane at 1.2 mg/mL (in 5 mM borate buffer, pH 8.8) to form the test line (T-line), as well as the goat anti-mouse antibody (1.5 mg/mL in the same borate buffer) as the control line (C-line). The sample pad was treated with 10 mM PBS (pH 7.4), containing 5% BSA and 0.1% Tween-20, then dried at 60 °C for 1 h. Next, the prepared above sample pad, conjugate pad, nitrocellulose membrane and absorbing pad were assembled on the PVC backing plate and followed by complete drying at 37 °C for 1–2 h. The paper boards were cut into the same size test strips (25 mm × 5 mm) by a strip cutter (Economic Cutter ZQ2000, Shanghai Kinbio Tech Co., Ltd., Shanghai, China). The prepared strips were stored in sealed bags under dry conditions at room temperature.

2.6 $CoFe_2O_4$ nanozyme strip test

Briefly, 80 μL sample solution (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP40 (v/v), 1% BSA (w/v)) containing human HGB or blood stain was dropped on the sample pad of the $CoFe_2O_4$ nanozyme strip. Following a chromatography step of 15 min, the nanozyme substrates buffer (DAB and H_2O_2) were added onto the detection membrane. The color development was stopped with deionized water after 5 min, and the detection of the samples can

be either visualized qualitatively by eyes or measured quantitatively by using a color reader. For the sensitivity detection: Three healthy human blood samples were diluted to 1×10^4–1 ng/mL, vortexed for 20 s, then incubated at room temperature for about 10 min. The samples were spun at 3000 rpm for 5 min, and the supernatant was extracted for commercial colloidal gold strip and $CoFe_2O_4$ nanozyme strip detections. For the specificity detection: human blood and common animal blood (cattle, sheep, horse, rabbit, rabbit, chicken, duck, goose, dog, cat, fish, rat and mouse) were diluted 100 times, following the same treatment as above, then the supernatants were collected for the $CoFe_2O_4$ nanozyme strip detections. Also, with blank cotton swabs as the control group, human blood was dropped on different common substrates (fiber, cotton cloth, glass, ceramics, sand, nails, blades, latex gloves, plastic clips, toothpick, plastic gun heads, and feathers), which might appear in criminal scene. After sampling with cotton swabs, human HGB was detected with $CoFe_2O_4$ nanozyme-strip.

2.7 $CoFe_2O_4$ nanozyme-strip for real-life criminal case applications

The following real cases are for the same scenarios where in the blood stain detection process of case investigation, the blood stains on the murder weapons used by the suspect were not detected by the commercial colloidal gold strip, which are commonly used by the police, and the $CoFe_2O_4$ nanozyme-strip was used for detections. In case 1: Li, in a county in Hebei province, was killed by a hard-headed plastic crutch smashing his skull. The crutch was thrown into a ditch, soaking in the mud for several days before the police found it. The trace spots were collected by the two-step dry and wet method. Following the extraction for the supernatant, the human HGB was detected by colloid gold test strip and $CoFe_2O_4$ nanozyme-strip respectively. In case 2: A homicide and corpse division case occurred in a city in Zhejiang province, China. The case handling unit was required to check whether there was the victim's blood on the crime tool kitchen knife. Since the kitchen knife has been cleaned, it was not detected on the scene. The trace spots were collected by the two-step dry and wet method. After soaking with water, the supernatant was obtained by centrifugation, and the human HGB was detected by colloid gold test strip and $CoFe_2O_4$ nanozyme-strip separately. In case 3: A taxi driver, in a county of China, was murdered and his car was also robbed. After investigation, a possible blood-stained paper was discovered. The suspect spot was collected by the two-step dry and wet method. The supernatant was examined by colloid gold test strip and $CoFe_2O_4$ nanozyme-strip separately to check whether there was blood present.

2.8 Statistical analysis

All data are presented as mean ± standard deviation (n = 3) and analyzed using GraphPad Prism v. 8.0.2. Error bars shown represent standard error were derived from three independent measurements.

3 Results and discussion

3.1 Synthesize and characterization of $CoFe_2O_4$ nanozymes

Figure 1 shows the particle size of the synthesized $CoFe_2O_4$ nanozyme and the characterization of its catalytic activity. The $CoFe_2O_4$ nanozymes were synthesized using the liquid-phase hydrothermal method and characterized using various methods. As revealed by TEM images (Fig. 1(a)), the synthesized $CoFe_2O_4$ nanozymes showed a spherical shape with an average particle size of around 110 nm, and the particle size of different batches is homogeneous and consistent (Figs. S1(a) and S1(b), and Fig. S2 in the Electronic Supplementary Material (ESM)). From EDS mapping, the Co, Fe, and O elements were uniformly distributed in the as-synthesized nanozyme (Figs. S1(c)–S1(f) in the ESM). The X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) confirmed that the synthesized material was $CoFe_2O_4$ and the surface was successfully modified with poly(acrylic acid) (PAA, Figs. S3 and S4 in the ESM). To further investigate the metal ion valence states, we performed additional X-ray photoelectron spectroscopy (XPS) measurements for the $CoFe_2O_4$ nanozyme (Fig. S5 in the ESM). The Co 2p and Fe 2p spectra showed that both Co and Fe have two oxidation states of +2 and +3, suggesting its potential higher redox activity.

The DLS measurements were carried out to investigate the diameter distribution of $CoFe_2O_4$ nanozymes. The results showed that the average hydrated particle size of $CoFe_2O_4$ nanozymes was about 135 nm with good uniformity (polydispersity index (PDI) = 0.037) (Fig. 1(b) and Fig. S6 in the ESM). It has been shown that $CoFe_2O_4$ nanozymes have the intrinsic peroxidase-like activity and can catalyze the oxidation of peroxidase substrates like TMB/DAB to produce colorimetric products [32]. We next quantitatively characterized the peroxidase-like activity of $CoFe_2O_4$ nanozymes by measuring the changes in absorption intensity during the $CoFe_2O_4$-catalyzed colorimetric reaction (Fig. 1(c)). As shown in Fig. 1(d), the specific activity of $CoFe_2O_4$ nanozymes is 9.90 U/mg, which is almost 2-fold higher than that of Fe_3O_4 nanozymes (5 U/mg) without Co doping (Fig. S7 in the ESM). At the same time, we also prepared Cu- and Mn-doped nanozymes, and the ratio of doped metal elements to Fe elements was 1:2. The results showed that the Co-doped nanozyme had the highest activity (Fig. S8 in the ESM), so the Co-doped nanozyme ($CoFe_2O_4$) was selected in this study. The experimental results indicate that the doped metal plays a key role in the peroxidase-like activities. The standard redox potential of the doped metals may have strong correlation with the level of catalytic activity, and the higher standard redox potentials facilitate the generation of more free radicals [33]. The $CoFe_2O_4$ nanozyme shows the highest peroxidase (POD)-like catalytic activity due to the highest standard redox potential of the doped metal (Co^{3+}/Co^{2+} = 1.808 V > Mn^{3+}/Mn^{2+} = 1.51 V > Fe^{3+}/Fe^{2+} = 0.77 V) among the M-doped MFe_2O_4 nanozymes (M = Co and Mn). Although $CuFe_2O_4$ showed a much lower redox potential (Cu^{2+}/Cu^{+} = 0.153 V), there was the report showing that $CuFe_2O_4$ nanozyme with comparable catalytic ability to that of $CoFe_2O_4$ [34]. For practical use, we further have realized the large-scale preparation of $CoFe_2O_4$ nanozymes, and the output of each batch can be supplied for 6 million test reagents (Fig. 1(e)).

3.2 $CoFe_2O_4$ nanozyme strip design and detection for human HGB

The $CoFe_2O_4$ nanozyme strip test is based on the principle of double antibody sandwich lateral flow immunoassay, and its design strategy is shown in the schematic diagram (Fig. 2(a) and Fig. S9 in the ESM). It is composed of a sample pad, a conjugate pad (pre-sprayed with $CoFe_2O_4$ nanozyme probes), a nitrocellulose membrane (pre-sprayed with test line antibodies and control line antibodies) and an absorbent pad which are sequentially assembled on a PVC backing plate. When the developed nanozyme strip is used for testing, the dried bloodstain needs a pretreatment by transferring into liquid sample. The liquid sample then flows through the conjugate pad, and then the target analytes HGB were recognized specifically by the detection antibodies on the $CoFe_2O_4$ nanozyme probes to form immunocomplexes. Along with lateral flow, the nanozyme

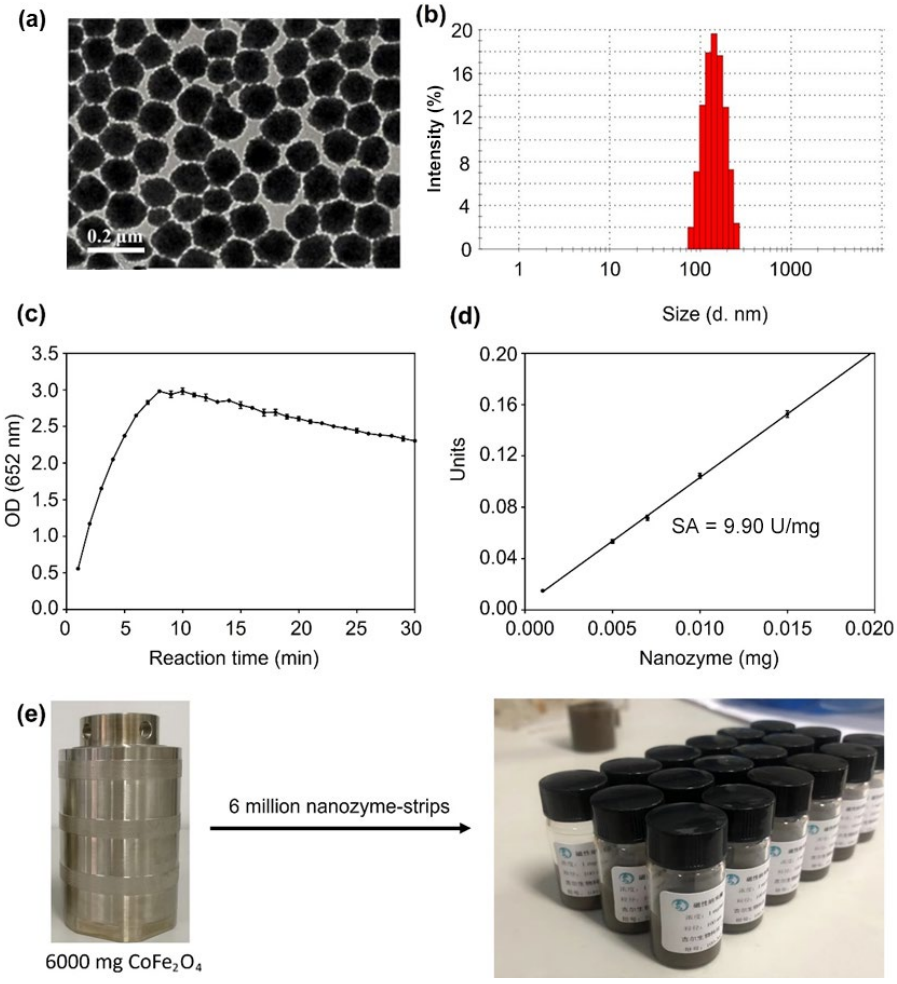

Figure 1 Synthesize and characterization of $CoFe_2O_4$ nanozymes. (a) TEM images of $CoFe_2O_4$ nanozymes, scale bar: 0.2 μm. (b) DLS analysis of $CoFe_2O_4$ nanozymes. (c) The reaction-time curves of TMB colorimetric reaction catalyzed by $CoFe_2O_4$ nanozymes. (d) The specific activities of $CoFe_2O_4$ nanozymes were calculated as 9.90 U/mg. (e) Scale up production of $CoFe_2O_4$ nanozymes for supplying 6 million test reagents.

complexes were captured by the paired capture antibodies on T-line, aggregated and formed the sandwich immunocomplexes. While $CoFe_2O_4$ nanozyme probes without HGB combination were found to bind with the goat anti-mouse antibodies at C-line. Due to the high peroxidase activity of the $CoFe_2O_4$ nanozyme probes, the presence of the human HGB can be visualized by the nanozyme-catalyzed color reaction induced by incubating the nanozyme-strip with DAB colorimetric substrates at the T-line after capturing HGB. However, with or without HGB, a colored control line will be produced when the $CoFe_2O_4$ nanozyme probes pass the C-line and bind to the immobilized goat anti-mouse antibodies. The signal intensity of the test line can be either visualized qualitatively by eyes or measured quantitatively by using a color reader.

Upon the assembly of the $CoFe_2O_4$ nanozyme-strip, we compared its performance with the commercially available colloidal gold strip. Both strips were able to detect the minimum concentration (1000 ng/mL) as required by public safety administration in China. However, the detection limit of human HGB for standard colloidal gold strip is around 100 ng/mL (Fig. 2(b)), while that for $CoFe_2O_4$ nanozyme-strip can reach as low as 1 ng/mL (Fig. 2(c)), showing the high detection sensitivity of the newly developed nanozyme-strip method.

Figure 3 shows the general applicability of $CoFe_2O_4$ nanozyme strip for blood evidence detection. Due to the complexity of crime scenes, we further determined whether the substrate materials where the blood spots reside on have impacts on the detection performance of the developed $CoFe_2O_4$ nanozyme-strip. We have selected 12 different common materials to simulate the physical evidence at the crime scene, including fiber, cotton, glass, ceramics, sand, nails, blades, latex gloves, plastic clips, toothpicks, pipette tips, and feathers, as blood potential retaining materials and blank cotton swaps as a negative control. As shown in Fig. 3(a), $CoFe_2O_4$ nanozyme strip clearly showed the trace amount of blood spot on various substrates, indicating that the developed $CoFe_2O_4$ nanozyme strip can detect human blood spots from a variety of materials.

In addition to wiping off the blood marks on the crime scene, the criminals also tend to use the bloods from other animals to cover the human blood trace. We thus further tested whether the developed nanozyme strips could specifically detect human HGB apart from animal blood. As shown in Fig. 4, we examined the common animal blood (such as cattle, sheep, horse, rabbit, chicken, duck, goose, dog, cat, fish, rat, and mouse) on $CoFe_2O_4$ nanozyme strip, and used human blood as a positive control. By analyzing the results, the brown band can appear at the test line only when human blood sample is added, indicating the high specificity of the developed $CoFe_2O_4$ nanozyme strip (Fig. 4).

Figure 5 shows the blood mark detections in three real criminal cases. As illustrated in the materials and methods section, in these cases, the blood spots have been either diluted, contaminated, or cleaned. In case 1, the blood marks were wiped off crutch joint. As shown in Fig. 5(a), the commercial colloid gold test strip method could not detect the trace amount of human HGB. By contrast, the developed $CoFe_2O_4$ nanozyme strip was able to detect the positive signals accurately, showing the important tracing

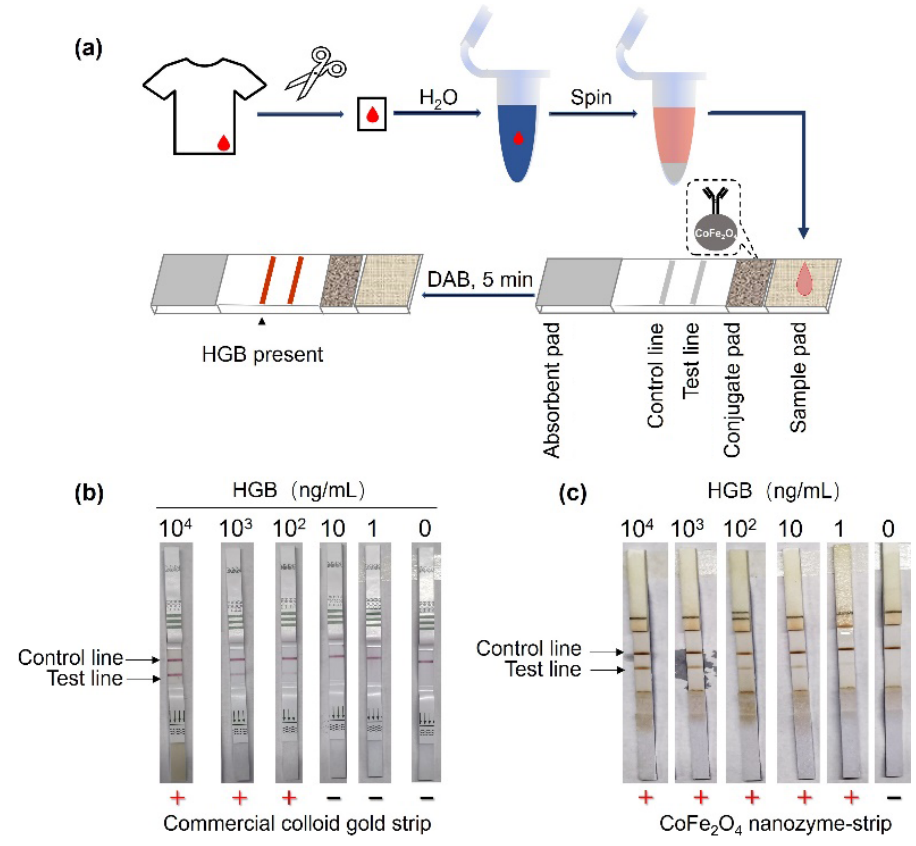

Figure 2 $CoFe_2O_4$ nanozyme strip method for detecting human HGB. (a) Conceptual nanozyme-strip technology and illustrating of the $CoFe_2O_4$ nanozyme-strip detection method. $CoFe_2O_4$ nanozymes catalyze the oxidation of a colorimetric substrate DAB to produce a color reaction at the test line after binding to human HGB. (b) Commercially available colloid gold strip detection for human HGB. (c) $CoFe_2O_4$ nanozyme-strip detection for human HGB.

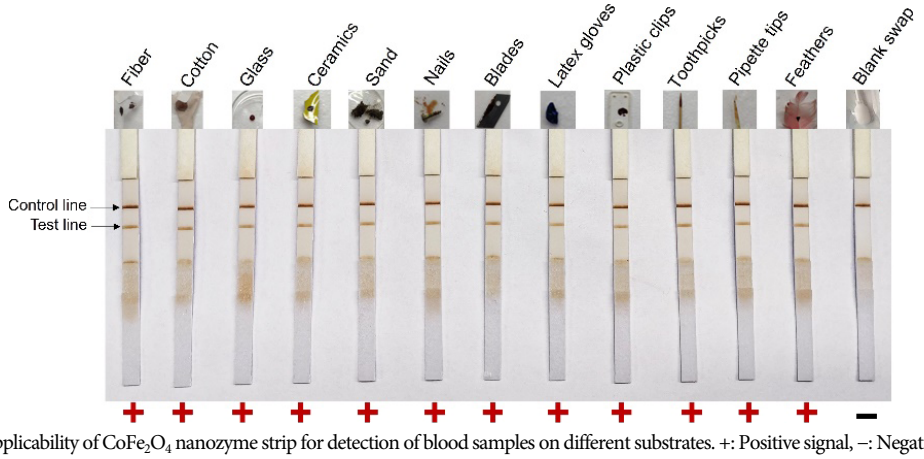

Figure 3 The general applicability of $CoFe_2O_4$ nanozyme strip for detection of blood samples on different substrates. +: Positive signal, −: Negative signal.

evidence. In case 2, due to the texture of knife and the subsequent cleaning, the colloid gold test strip still failed to show the conclusive results. In comparison, the developed $CoFe_2O_4$ nanozyme strip method showed strong positive signals, indicating the better performance of $CoFe_2O_4$ nanozyme strip (Fig. 5(b)). In case 3, the blood stained on the waste papers and the nanozyme strip could still visualize the correct positive signals. Because of the sensitivity and effectiveness of this test strip, the $CoFe_2O_4$ nanozyme strip products have selected into the Double Ten Program of the Ministry of Public Security of the People's Republic of China in 2019 for key promotion.

4 Conclusions

In this work, we synthesized $CoFe_2O_4$, $CuFe_2O_4$, $MnFe_2O_4$, Fe_3O_4 nanozymes by doping different metal elements (Co, Cu and Mn), and the results showed that the Co-doped nanozyme ($CoFe_2O_4$) had the highest activity. This may be because the doping of different metals into Fe_3O_4 nanoparticles changes the chemical valences of metal sites in the particle surfaces, which subsequently alters the affinity of the surfaces for chemical groups and regulates the peroxidase-like catalytic activities. We further prepared nanozyme detection probes based on $CoFe_2O_4$ nanozymes, developed the corresponding test strip products and applied it to the detection of human hemoglobin. Compared with the commercially available colloidal gold strip, this developed $CoFe_2O_4$ nanozyme strip showed higher sensitivity for human HGB with enhanced detection limit by at least 100 times. Moreover, nanozyme strip shows high specificity for human HGB among blood samples from other species or different staining materials. Upon sample collection, it can trace the human HGB presence in as low as 20 min. Importantly, the brown precipitated band formed by nanozyme-catalyzed DAB can not only enhance the detection signal, but also can be used as preservation material

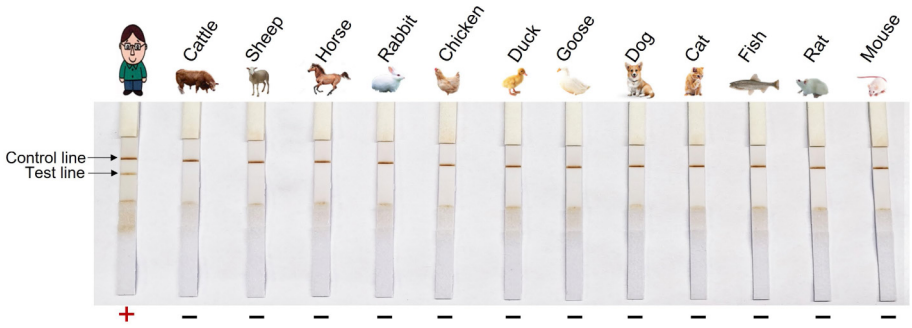

Figure 4 The specificity of $CoFe_2O_4$ nanozyme strip for the detection of human blood but not animal blood. +: Positive signal, −: Negative signal.

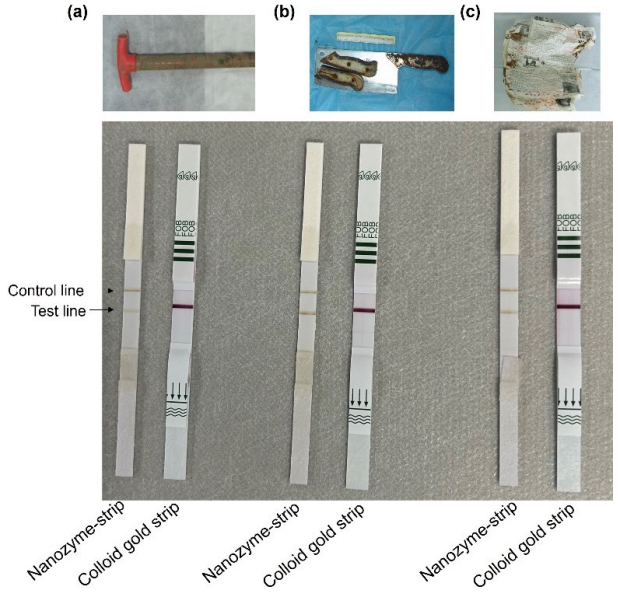

Figure 5 Comparison of the performance of $CoFe_2O_4$ nanozyme strip and the commercial colloid gold strip in real-life cases. (a) Case 1: Blood samples wiped from crutch joint and buried in mud for several days. (b) Case 2: Blood samples wiped from the knife. (c) Case 3: Blood samples wiped from waste papers.

evidence for traceability inquiry due to the long-term stability of DAB. In conclusion, we have developed a rapid, highly specific, and sensitive $CoFe_2O_4$ nanozyme strip for applications in a wide range of blood stain detection in the criminal investigation.

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Electronic Supplementary Material: Supplementary material (TEM, EDS, SEM, XRD, FT-IR, XPS, DLS characterizations of $CoFe_2O_4$ nanozyme; comparisons among the reaction kinetics of various nanozymes and detailed nanozyme strip detection presentations) is available in the online version of this article at https://doi.org/10.1007/s12274-023-6012-4.

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