Colloidal gold immunochromatographic strip for Detect AMI biomarker cTnI

Jun Xie, Yijun Lin, Hong Wang*

College of Life Science and technology, Jinan University, No. 601, Huangpu Avenue, Guangzhou 510632, China.

*Correspondence: wanghong@jnu.edu.cn

Abstract

Acute myocardial infarction (AMI) was a common fatal cardiovascular disease, causing millions of deaths worldwide every year. Cardiac troponin I (cTnI) was considered the 'gold standard' biomarker for AMI diagnosis. In this study, we developed a Colloidal gold immunochromatographic strip for cTnI detection. The linear detection range of the Colloidal gold immunochromatographic strip for cTnI was 15.63-250ng/mL. In addition, the Colloidal gold immunochromato- graphic strip showed high recovery (88.6%) for detection of cTnI clinical samples. These results indicate that the colloidal gold immunochromatographic strip may be a valuable tool for preliminary screening of AMI.

Keywords

Acute myocardial infarction (AMI), Cardiac troponin I (cTnI), Colloidal gold immunochro matographic strip.

1. Introduction

Acute myocardial infarction is myocardial necrosis caused by acute and continuous is chemia and hypoxia of coronary arteries and it's the most severe manifestation of coronary artery disease.Acute myocardial infarction cause more than 2.4 million deaths in the USA, more than 4 million deaths in Europe and northern Asia, and morethan a third of deaths in developed nations annually[1, 2].In recent years, the incidence of myocardial infarction has risen significantly in China. It has now been discovered that at least 2 million patients and newly discovered patients each year are at least 500,000.In China, most AMI patients die within the acute stage (30 days after onset), and 75% die within the first 24 hours,also about 62% of AMI deaths occur before hospital arrival[3].Some patients started with mild symptoms and found no problems,when the symptoms were severe, they could no longer exercise their pump function, causing the patient to suddenly die. Therefore, early diagnosis of acute myocardial infarction is very important for its prevention and treatment[4].We need a simple and quick way to detect AMI, point-of-care testing(POCT) refers to a detection method carried out at the sampling site, using portable analytical instruments and supporting reagents to quickly obtain test results[5-7], and this is exactly what we need.

Cardiac troponin I (cTnI), myoglobin (Mb) and CK-MB are considered to be markers of myocardial infarction diagnosis[8-13].When the myocardium is damaged cTnI is present in the blood , appears early in time, has a long duration, and has high specificity,therefore, cTnI is considered as the "gold standard" for AMI diagnosis[14].

The common methods to detect cTnI include enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), and radioimmunoassay (RIA)[15-17]. However, these methods have obvious problems such as long detection time, multi-step processing, high total cost, skilled and professional operators,and need complicated instruments, so they are not suitable for use in remote areas and households[18],and not suitable for POCT[19-22].This article we develop a cTnI colloidal gold immunochromatographic strip, it's process simple, short time spent and the result can be seen directly without the complicated instruments,so it is suitable for point-of-care testing(POCT) for preliminary screening of AMI.

2. Materials and methods

2.1 Materials.

Nitrocellulose (NC) membrane (HFB13504), conjugation pad, sample pad, plastic backing, and absorbent pad from Shanghai Jiening Biotech (Shanghai, China); All other metal powders were purchased from Merck Chemicals (Darmstadt,Germany). Cardiac troponin I (cTnI) was obtained from HyTest (Finland). Goat anti-mouse antibody(Sigma,USA), mAb1 and mAb2 was a pair of monoclonal antibodies that specifically recognize cTnI and are produced by our laboratory.

2.2 Equipment.

A Philips transmission electron microscope (TEM, Philips,Holland), a configurable multi-mode microplate reader(Bio Tek, USA), and a Beckman centrifuge (Beckman,Germany) were used in this work. The platform contains amotion control element with the Biostrip dispenser HGS102and the programmable strip cutter HGS201 (purchased locallyin Shanghai, China.

2.3 Synthesis of colloidal gold (AuNPs).

The specific procedure for synthesizing 16 nm of colloidal gold using the trisodium citrate reduction method of Frens et al.wasas follows[23]. Briefly, the 100 mL conical flask was immersed in aqua regia and then siliconized and dried. 50 mL ultrapure water was added into a conical flask, heated and stirred, and 1 mL of 1% chloroauric acid (HAuCl4) and 2 mL of 1% sodium citrate were added to the boiled water, heated, and stirred for 15 min. The solution changed from colorless to brown/purple and eventually to red. Heat was removed and mixture was stirred at the same speed for 15 min. After cooling to room temperature, the volume was set to 50 mL and 200 μL was aliquoted for spectral scanning. Finally, AuNPs solution was stored at 4 degrees Celsius. After 24 h, the prepared colloidal gold particles were observed by scanning TEM.

2.4 Preparation of mAb2-AuNPs.

First, 60 μL of 250 mM K2CO3 was added to 10 mL of AuNPs and then mixed with 10 μL of 1 mg/mL mAb2. After 30 min incubation, 1 mL of 10% BSA was added. The solution was incubated for another 30 min and then centrifuged at 7000 rpm for 15 min. The supernatant was removed, and the precipitate was suspended in 1 mL PBS buffer (pH 7.4).

2.5 Assembly of colloidal gold immunochromatographic strip for cTnI.

For the sample pad, to reduce the non-specific adsorption of mAb2-AuNPs with antibodies on the T-line and to control the immunological reaction time, 1% BSA and 0.25% Tween-20 were added into the sample pad treatment solution. For the conjugation pad, a desired volume of mAb1-AuNPs was loaded onto the pad using a dispenser system and stored at 37 ℃. For the NC membrane, mAb1 containing 2 mg/mL or goat anti-mouse antibody were dispensed onto a specified area of the NC membrane as the test line (T-line) or the calibration line (C-line), respectively. Next, the sample pad, conjugation pad, NC membrane,and absorbent pad were pasted onto plastic backing, and each component had an overlap of 2 mm. Finally, the colloidal gold immunochromatographic strip was cut to a width of 4 mm using a programmable HGS201 strip cutter.

2.6 cTnI detection by colloidal gold immunochromatographic strip.

Aliguots of 100 μL of different concentrations of cTnI solutions (pH 7.4) were added to the sample pad. After 10 min, images of the colloidal gold immunochromatographic strip analyzed using image j.

2.7 Specificity of colloidal gold immunochromatographic strip for cTnI detection.

To evaluate the specificity of the colloidal gold immunochromato- graphic strip for cTnI detection, 100 μL of 500 ng/mL mycotoxins (CK-MB, Mb, or TNNI) was added to the sample pads. After 10 min, the results of the colloidal gold immunochromatographic strip were decided using image j.

2.8 Detection of cTnI in clinical samples by colloidal gold immunochromatographic strip

100 μL of clinical serum samples diluted twice with PBST were tested. cTnI concentrations of these solutions were detected by colloidal gold immunochromatographic strip.

3. Results and discussion

3.1 Principle of colloidal gold immunochromatographic strip.

The principle of colloidal gold immunochromatographic strip in Fig. 1. The conjugation pad was used to load mAbs-AuNPs; the NC membrane was coated with mAb1 and goat anti-mouse antibody as the T-line and C-line, respectively (Fig. 1a); the sample pad was used to apply liquid samples and an absorbent pad served as a liquid sink. When samples don't contain or low concentration of cTnI were applied, mAb2-AuNPs can't bind to the mAb1 at the T-line and the mAb2-AuNPs bind to the Goat anti-mouse antibody at the C-line, thus a single red color band will be shown at the C-line position(Fig. 1b). By contrast, when highly concentrated cTnI samples are applied, cTnI will bind to the mAb2-AuNPs then bind to the mAb1 formed a sandwich-like structure and produce a visible red band at the T-line position. The excess mAb2-AuNPs without cTnI will continue to migrate and finally be captured by goat anti-mouse antibody at the C-line position to form a mAb2-AuNPs-anti-mouse IgG complex and produce a second red color band at the C-line position.The red intensity of the T-line increases with the increased cTnI concentration[24].

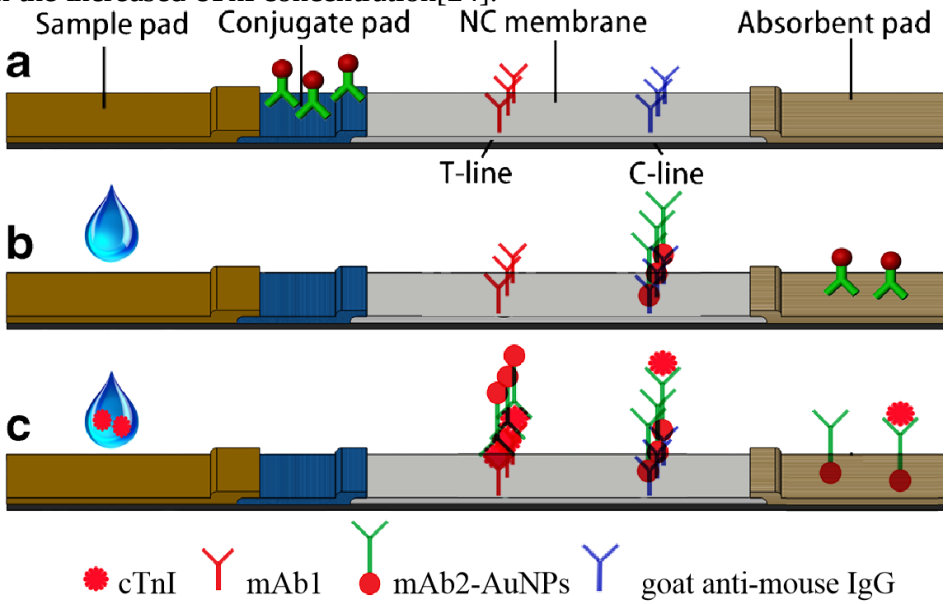

Fig. 1 Principle of colloidal gold immunochromatographic strip for cTnI detection. a Assembly of colloidal gold immunochromatographic strip. b Colloidal gold immunochromatographic strip for detection of no or low concentration cTnI. When no or low concentration cTnI samples are applied, the signa red color band shown at the C-line. c Colloidal gold immunochromatographic strip for detection of high concentration cTnI. When high concentration cTnI samples are applied, cTnI competitively binds mAb2-AuNPs then band the mAb1, and the signa red color band shown the T-line and the c-line.

3.2 Synthesis and characterization of AuNPs and mAb2-AuNPs

In order to prove the of synthesized AuNPs and Ab-AuNPs was successful, the size and morphology of the particles were observed under the transmission electron microscopy (TEM) and are shown in Fig. 2.In Fig. 2a, the 16 nm AuNPs diluted with PBST are small, uniform in size, well dispersed, quasi-spherical, and nonaggregated particles. As shown in Fig. 2b, the Ab-AuNPs diluted with PBST are also of uniform size, well dispersed, quasi-spherical, and nonaggregated particles, but they are larger than AuNPs in a. Fig. 2c was the UV–vis spectra of the solutions. The characteristic absorption peaks of the AuNPs solutions are 520 nm and the characteristic absorption peaks of the Ab-AuNPs

solutions are 522 nm, as we can seen from the TEM result this red-shifts was caused by the increased of the particle.

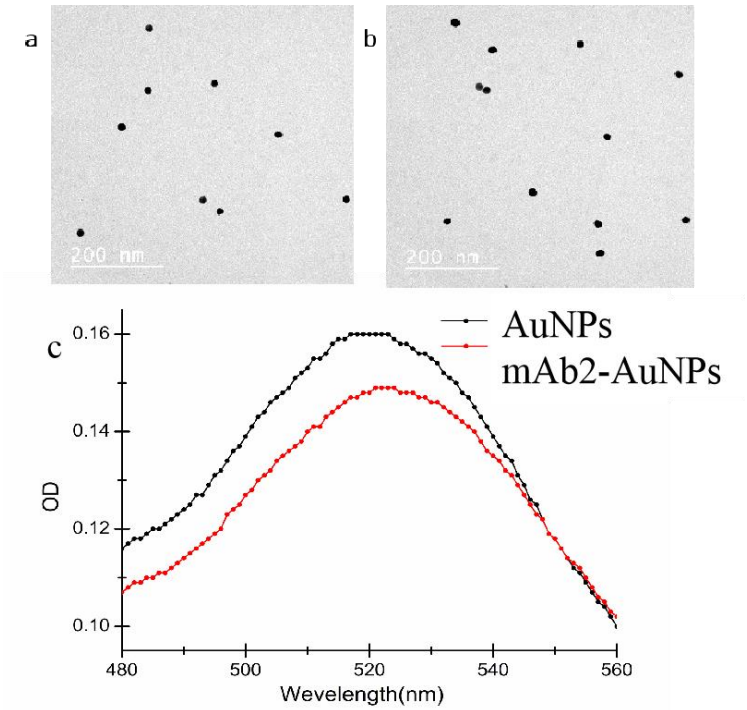

Fig. 2 Synthesis andcharacterization of AuNPs and mAb2-AuNPs. a-b The TEM image of AuNPs and mAb2-AuNPs. c UV–vis spectra of AuNPs and mAb2-AuNPs.

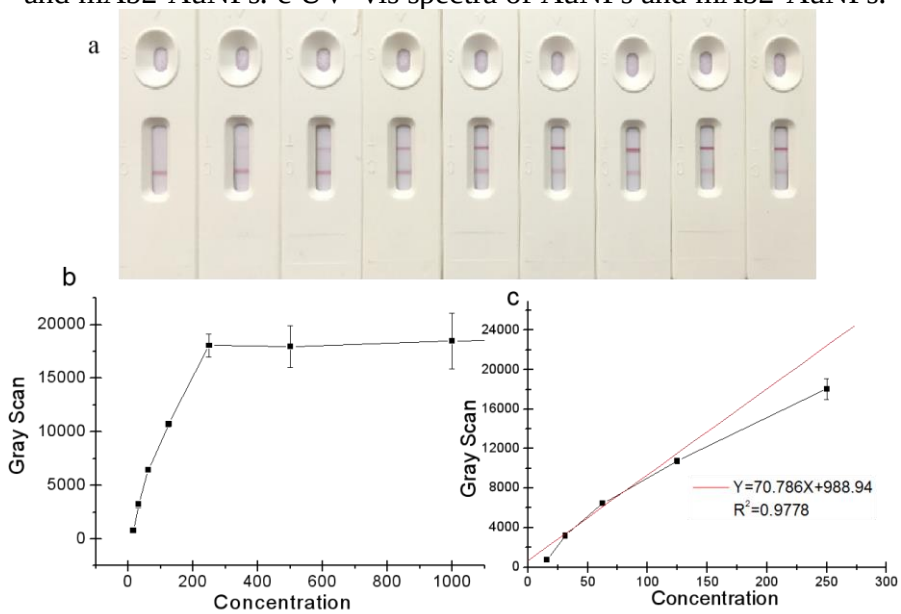

Fig.3 cTnI concentration-dependent Gray Scan of the colloidal gold immunochromatographic strip. a cTnI concentration-dependent T values of colloidal gold immunochromatographic strip. b Calibration curve of the colloidal gold immunochromatographic strip for cTnI detection. Each value represents the mean of three independent experiments (n = 3)

3.3 Performance of colloidal gold immunochromatographic strip for detection of cTnI

Under optimal conditions, the performance of the colloidal gold immunochromatographic strip was further evaluated by testing a series of cTnI concentrations in triplicate. The result of the colloidal gold immunochromatographic strip were analysed using image j. When 0 ng/mL cTnI was tested, the signal of the T-line was indiscernible (Fig. 3a), whereas the the signal of the T-line appeared when

the cTnI concentration reached 15.63 ng/mL, and the signal of the T-line was stronger as the cTnI concentration increased. Therefore, the sensitivity of the colloidal gold immunochromatographic strip was defined as 15.63 ng/mL. The results of the colloidal gold immunochromatographic strip were analyzed using image j(Fig. 3b). The signal of the colloidal gold immunochromatographic strip increased with increasing cTnI concentration (Fig. 3b). A calibration curve of the colloidal gold immunochromatographic strip was established by fitting cTnI concentration to the corresponding signal value (Fig.3c). The linear detection range of the fcolloidal gold immunochromatographic strip for cTnI detection was 15.63–250 ng/mL.

3.4 Specificity of colloidal gold immunochromatographic strip for detection of cTnI

To study the specificity of the colloidal gold immunochromatographic strip for detection, cTnI analogues (500 ng/mL) were detected colloidal gold immunochromatographic strip and the resulting T calculated using the cTnI standard curve. The T-line of colloidal gold immunochromatographic strip for cTnI appears as a bright line, whereas the T-line of colloidal gold immunochromatographic strip for TNNI, CK-MB, and Mb shown weak signals. The were shown in Fig. 5, suggesting that the developed colloidal gold immunochromatographic strip is highly specific.

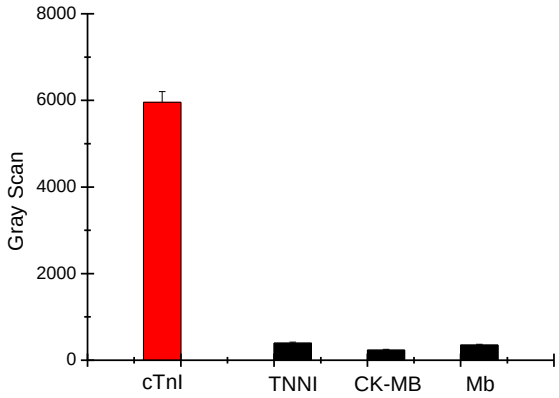

Fig. 4 Specificity of the colloidal gold immunochromatographic strip for cTnI detection.Each value represents the mean of three independent experiments (n = 3)

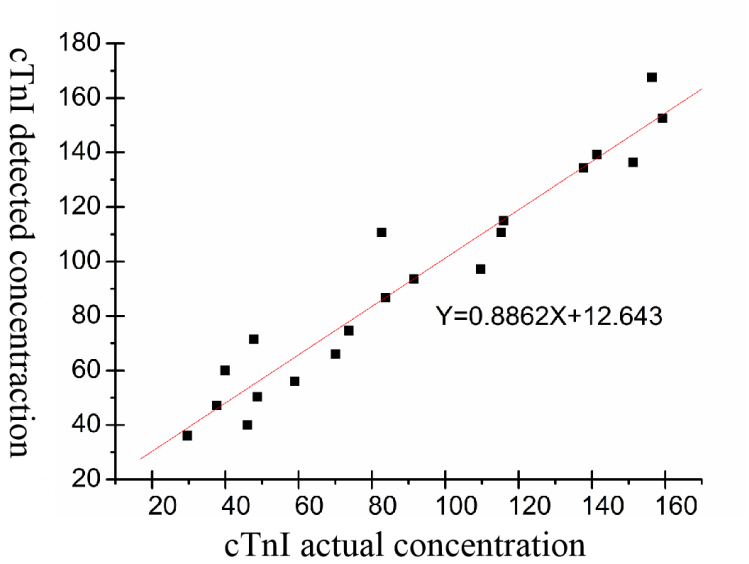

Fig. 5: Clinical samples detection.

3.5 Detection of cTnI in clinical samples by colloidal gold immunochromatographic strip

At last, we used colloidal gold immunochromatographic strip to detect 20 clinical samples the result was shown in Fig. 5. The cTnI concentrations in the clinical samples detected by colloidal gold

immunochromatographic strip had a good correlation with the hospital instrument test results(88.62%), indicating that the developed colloidal gold immunochromatographic strip is an effective tool for the detection of cTnI in clinical samples.

4. Conclusion

Here, we describe a colloidal gold immunochromatographic strip integrating AuNPs for the detection of cTnI in clinical samples. The measured signal increased with increasing cTnI concentration. The linear detection range of the colloidal gold immunochromatographic strip was 15.63-500 ng/mL. Furthermore, the colloidal gold immunochromato- graphic strip showed high recovery for cTnI clinical samples. Therefore, we believe that the colloidal gold immunochromatographic strip described here could potentially be adapted to large-scale preliminary screening of AMI .

Acknowledgements

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