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Highly sensitive and selective lateral flow immunoassay based on magnetic nanoparticles for quantitative detection of carcinoembryonic antigen

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ABSTRACT

Carcinoembryonic antigen (CEA) is an important biomarker in cancer diagnosis. Here, we present an efficient, selective lateral-flow immunoassay (LFIA) based on magnetic nanoparticles (MNPs) for in situ sensitive and accurate point-of-care detection of CEA. Signal amplification mechanism involved linking of detection MNPs with signal MNPs through biotin-modified single-stranded DNA (ssDNA) and streptavidin. To verify the effectiveness of this modified LFIA system, the sensitivity and specificity were evaluated. Sensitivity evaluation showed a broad detection range of 0.25–1000 ng/ml for CEA protein by the modified LFIA, and the limit of detection (LOD) of the modified LFIA was 0.25 ng/ml, thus producing significant increase in detection threshold compared with the traditional LFIA. The modified LFIA could selectively recognize CEA in presence of several interfering proteins. In addition, this newly developed assay was applied for quantitative detection of CEA in human serum specimens collected from 10 randomly selected patients. The modified LFIA system detected minimum 0.27 ng/ml of CEA concentration in serum samples. The results were consistent with the clinical data obtained using commercial electrochemiluminescence immunoassay (ECLIA) ($p < 0.01$). In conclusion, the MNPs based LFIA system not only demonstrated enhanced signal to noise ratio, it also detected CEA with higher sensitivity and selectivity, and thus has great potential to be commercially applied as a sensitive tumor marker filtration system.

1. Introduction

Carcinoembryonic antigen (CEA), a type of oncofetal glycoprotein present in cell surfaces, may be crucial in metastatic dissemination of cancer cells [1,2]. Since CEA is produced in gastrointestinal tissues during embryonic development and stopped after birth, a low level of CEA is found in the blood of healthy adults. CEA has already been validated as a crucial tumor biomarker and ideal diagnostic adjuvant [3] for diagnosis of several types of cancer such as lung cancer [4], colorectal cancer [2], breast cancer [5], and bladder cancer [6], etc. Thus, development of a rapid, efficient method for detecting CEA is in great demand for clinical diagnosis and patient condition evaluation.

The lateral-flow immunoassay (LFIA) or immunochromatography, a rapid and convenient immunodiagnostics tool, has become a hotspot in the past decade. LFIA is based on a solid-phase thin layer that combines the principles of capillary phenomenon and immune recognition reaction [7]. Antibody-labeled nanoparticles, such as colloidal gold [8], superparamagnetic

Abbreviations: CEA, carcinoembryonic antigen; LFIA, lateral-flow immunoassay; MNPs, magnetic nanoparticles; ssDNA, single-stranded DNA; ECLIA, electrochemiluminescence immunoassay; GMBN, gold magnetic biofunctional nanobeads; MRI, magnetic resonance imaging; AuNPs, gold nanoparticles; LOD, limit of detection; NC membrane, nitrocellulose membrane; cTnI, cardiac troponin I; HFABP, heart fatty acid binding protein; CRP, C-reactive protein; SNR, signal to noise ratio

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nanoparticles [9], silica nanoparticles [10], quantum dots [11] or other composite materials like gold magnetic biofunctional nanobeads (GMBN) [12], have been used as color constituent. Among the nanoparticles mentioned above, colloidal gold has been widely adopted because of its excellent optical and chemical stability. However, colloidal gold is easily affected by circumstantial factors such as pH value of electrolyte solution. During labeling process of antibody or oligonucleotide probe, non-optimal conditions always cause irreversible coagulation of colloidal gold. In addition, the detection limit of nanogold-based LFIA is yet to be improved. Therefore, other alternatives have been developed overcoming such disadvantages.

To develop stable and sensitive diagnosis method with LFIA, magnetic nanoparticles (MNPs) have been explored as a new nano-biolabel material. MNPs have widely employed in different biomedical purposes such as protein and DNA separation [13], biosensing [14], bacterial detection [15], magnetic resonance imaging (MRI) [16], and drug delivery. The stable physical properties of MNPs make the label process independent of experimental factors such as pH value and salt ions strength. Additionally, it's harder for labeled MNPs to react with other substances, thus improving reproducibility. All of these factors together have widened applicability of MNPs in the biomedical domain. Apart from good stability and reproducibility, a sensing element should also possess high sensitivity. A few efforts have been made to enhance sensitivity of MNPs-based LFIA. Liu et al. [17] prepared magnetic Fe_3O_4 particle aggregates by cross-linking carbonyl mediated Fe_3O_4 nanoparticles with poly-L-lysine for the detection of paraoxon methyl and achieved an optimized detection limit of almost 40-fold lower than that of the mother Fe_3O_4 particles. For more efficient detection in LFIA, other agents, such as composite of MNPs with gold nanoparticles have also been tested to develop a novel immunochromatographic test strip [12]. However, little has been done in the direction of signal enhancement for MNPs-based LFIA.

Our group has already contributed in improving the detection limit of LFIA system. In our previous study [18], we proposed a signal enhancement mechanism for gold nanoparticles (AuNPs) based LFIA, where biotinylated single-stranded DNA (ss DNA) was used to link two AuNPs and streptavidin-labeled AuNPs were used as amplifier. As a result, we not only reached ultrasensitive detection limit but also achieved simultaneous detection of high-sensitivity cardiac troponin I (cTnI) and myoglobin.

In this paper, we present a highly sensitive MNPs based LFIA, combining the amplification strategy described above with the advantages of MNPs. To achieve signal enhancement, we linked secondary MNPs (carboxy group modified) with detection MNPs through biotin-streptavidin interaction. We evaluated the specificity of the new LFIA as well as demonstrated its efficacy by testing clinical samples (human serum samples). In addition, we compared the detection results of serum samples obtained from our newly developed LFIA with commercial electrochemiluminescence immunoassay (ECLIA).

2. Materials and methods

2.1. Reagents

Carboxy group modified MNPs (average size 200 nm) were purchased from Xintong Biotechnology Co., Ltd (Shanghai, China). Anti-CEA mAb_1 and Anti-CEA mAb_2 were purchased from Medix Biochemica (Kauniainen, Finland), and CEA protein was purchased from Fitzgerald (Luminairs, UK). Tumor Marker Plus Control was purchased from Bio-Rad (California, USA). Anti-mouse IgG, bovine serum albumin (BSA), trehalose and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) were purchased from Sigma-Aldrich (St. Louis, USA). Streptavidin was purchased from Invitrogen (Camarillo, USA). The biotin-modified oligonucleotide probes were synthesized by Takara Bio Inc. (Shiga, Japan). The sample pad, conjugate pad, absorbant pad, nitrocellulose (NC) membrane and baseboard were all provided by JieYi Biotechnology Co., Ltd. (Shanghai, China). Tween-20, sodium chloride (NaCl) and sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydrochloric acid and boric acid were bought from Ling Feng Chemical Reagent Co., Ltd (Shanghai, China). Sucrose was purchased from DingGuo Biotechnology Co., Ltd. (Shanghai, China). In addition, Tris-EDTA (TE) buffer was purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

2.2. Assay principles of modified LFIA

To enhance the signal intensity of MNPs based LFIA assay, the following approach was adopted. The secondary MNPs were linked to the primary detection MNPs of same sizes through biotin-modified ssDNA. Fig. 1 illustrates a schematic of point-of-care detection of CEA protein. Firstly, the carboxy modified MNPs were labeled with anti-CEA mAb_1 through covalent binding with amino group. Embracing a similar approach, the oligonucleotide probe modified with biotin at 5′ end was bound with MNPs through amino group at 3′ end. Signal MNPs were coated with streptavidin. These diversely modified MNPs were then coated on two pieces of conjugate pad, respectively (Fig. 1A).

The sensing process is as follows: typically, a solution containing CEA protein is dropped on the sample pad, and the solution rehydrates streptavidin/MNPs and biotin/ssDNA/MNPs/anti-CEA mAb_1 complexes in turn. Those complexes migrate along NC membrane by capillary action. When the solution reaches the test line containing immobilized anti-CEA mAb_2, CEA protein is finally identified and captured by anti-CEA mAb_1 and anti-CEA mAb_2 via immune responses, forming biotin/ssDNA/MNPs/anti-CEA mAb_1/CEA/anti-CEA mAb_2 compounds. With the addition of signal MNPs, MNPs/streptavidin/biotin/ssDNA/MNPs/anti-CEA mAb_1/CEA/anti-CEA mAb_2 compounds are finally generated and fixed on the test line (Fig. 1B). The rest of MNPs/streptavidin/biotin/ssDNA/MNPs/anti-CEA mAb_1 complexes reach control lines where coated anti-mouse IgG is captured via unspecific conjugation between monoantibody and anti-mouse IgG. Thus, two brown bands can be observed as a result of MNPs accumulation. In absence of antigen in sample solution, only control line will be visible.

2.3. Optimizing LFIA system

2.3.1. Preparation of CEA specific MNP probe

To prepare the detection probe, 0.13 mg magnetic beads were first washed with 0.01 M MES solution, and then the nanoparticles were mixed with 65 μg EDC, 3.9 μg anti-CEA mAb_1, and 13 μl ssDNA (1 μM). After gently mixing, this MNP mixture was incubated at room temperature for 2 h.

To prepare the signal probe, 50 μg EDC and 3 μg streptavidin were added into 0.1 mg magnetic beads, and then the solution was mixed at room temperature for 2 h.

2.3.2. Preparation of sample pad, conjugate pad, and the MNPs based LFIA strips

The conjugate pad was made with treated glass fiber. It was soak in aqueous solution containing 0.003 M boric acid, 0.004 M sodium perborate, 10% (w/v) sucrose, 2% (w/v) trehalose and 0.05% (v/v) Tween-20 for 30 min. The sample pad, made from treated glass fiber, was obtained by steeping in aqueous solution containing 0.003 M boric acid, 0.004 M sodium perborate, 0.5% (w/v)