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Utilization of a lateral flow colloidal gold immunoassay strip based on surface-enhanced Raman spectroscopy for ultrasensitive detection of antibiotics in milk

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ABSTRACT

An ultrasensitive method for the detection of antibiotics in milk is developed based on inexpensive, simple, rapid and portable lateral flow immunoassay (LFI) strip, in combination with high sensitivity surface-enhanced Raman spectroscopy (SERS). In our strategy, an immunoprobe was prepared from colloidal gold (AuNPs) conjugated with both a monoclonal antibody against neomycin (NEO-mAb) and a Raman probe molecule 4-aminothiophenol (PATP). The competitive interaction with immunoprobe between free NEO and the coated antigen (NEO-OVA) resulted in the change of the amount of the immobilized immunoprobe on the paper substrate. The LFI procedure was completed within 15 min. The Raman intensity of PATP on the test line of the LFI strip was measured for the quantitative determination of NEO. The IC_{50} and the limit of detection (LOD) of this assay are 0.04 ng/mL and 0.216 pg/mL of NEO, respectively. There is no cross-reactivity (CR) of the assay with other compounds, showing high specificity of the assay. The recoveries for milk samples with added NEO are in the range of 89.7%–105.6% with the relative standard deviations (RSD) of 2.4%–5.3% ($n = 3$). The result reveals that this method possesses high specificity, sensitivity, reproducibility and stability, and can be used to detect a variety of antibiotic residues in milk samples.

1. Introduction

Aminoglycoside antibiotics, such as neomycin, gentamicin, amikacin and kanamycin are widely used in veterinary medicine to treat bacterial infections in animals due to their cost effective feature [1]. Among them, neomycin is classified as a broad spectrum antibiotic due to its growth inhibition of Gram-positive bacteria such as *Staphylococcus aureus* and Mycobacterium tuberculosis, and Gram-negative bacteria such as *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and Proteus vulgaris [2]. Other antibiotics are only used in the treatment of Gram-negative bacterial infections [3]. However, antibiotic residues in edible animal tissues enter the human body through the food chain, and might cause severe ototoxicity and nephrotoxicity [4]. Moreover, neomycin is extremely nephrotoxic compared to other aminoglycosides [5]. In the milk processing plant, antimicrobial residues may inhibit starter cultures for cheese and yoghurt production [6]. Thus, monitoring and control of aminoglycoside antibiotic residues in milk is important with regard to public health issues. Most importantly, the European Union, the United States, and China have set maximum residue limits (MRLs) for antibiotics. In addition, some countries have recently strengthened supervision and administration of antibiotics in food animals by revising the related rules and regulations [7].

Many different methods have been developed for the determination of NEO residues in food products including colorimetric [8–10], enzyme-linked immunosorbent assay (ELISA) [11–13], Resonance Rayleigh Scattering [14], fluorescence [15], liquid chromatographic method with charged aerosol detection (LC-CAD) [16], electrochemical [17], chromatography [18–19], UV [20], aptasensor [21], and lateral flow immunoassay (LFI) [11]. ELISA and LFI are the most commonly used methods and are the basis of a number of commercially available systems. The major drawback of ELISA is that it is time-consuming with multiple steps in the procedure. In contrast, the AuNPs based LFI strip only has a single step, and therefore is simple, fast, and low cost. Especially, it can provide on-site detection by unskilled personnel and has been used to monitor high molecular mass analytes such as bacteria [22], viruses [23–24], hormones [25], and parasite antigens [26–27],

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and many smaller drugs [28–29]. Despite widespread use of this method for fast detection, it still suffers from certain inadequacies, the most important of which is that the assay is semi-quantitative and has low sensitivity. Therefore it is highly desired to develop a novel LFI strip with high sensitivity of detection.

Surface-enhanced Raman spectroscopy (SERS) has attracted considerable interests in biodetection due to its nondestructive and ultrasensitive features. SERS provides a "molecular fingerprint" that can be used to identify a molecule or verify its presence with its intrinsic signals in a sample [30]. SERS can enhance the Raman signals of target species adsorbed on Ag or Au metallic nanostructures by as much as 6 to 14 orders of magnitude, which even allows the detection of single molecules [31–32]. With these excellent advantages, SERS has previously been applied in immunoassays [33]. In recent years, SERS based immunoassay (SERSIA) in sandwich format for macromolecules detecting [34–37], and in the competitive format for small molecular compounds determining [38–39] have been reported. SERSIA can combine the high sensitivity of SERS and the high specificity of immunosorbent assays for optimal detection. However, the preparation of SERS active metal substrates involves multiple immobilization steps, high cost and poor reproducibility of the SERS signal, making the detection of aminoglycoside antibiotics a challenging problem [40].

Usually, nitrocellulose (NC) membranes are widely used as substrate material in the immunochromatographic assay. The NC membrane has a strong adsorption and fixation capacity for the antibodies and a good three-dimensional pore structure allowing the smooth passage of AuNPs. Therefore, the NC membrane instead of gold film as the substrate is applied for detection. It concentrates the Raman signals on 150 μm width of the test line, which can help to signal acquisition and get better reproducibility. Meanwhile, the test strips are extremely cheap and convenient, holding much promise for wide application in SERS-LFI method.

So far there are no reports on the detection of antibiotics with monoclonal antibody using SERS-LFI technology, to the best of our knowledge. In this study, we developed an immunoprobe consisting of dual labeled AuNPs with NEO-mAb and PATP. With LFI, the immunoprobe can be highly concentrated in the test zone, giving well resolved Raman signals which are inversely proportional to the analyte concentration. Moreover, this method also shows high sensitivity for neomycin detection and satisfactory recoveries from spiked milk samples. Notably, it can also be used for the analysis of other antibiotics following similar procedures.

2. Materials and Methods

2.1. Materials

Neomycin (NEO, ≥98%), bovine serum albumin (BSA) and Tween-20 were purchased from Sigma (St Louis, MO, USA). 4-Aminothiophenol (PATP) was purchased from Aladdin China Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4$) and trisodium citrate were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The artificial antigen (NEO-BSA and NEO-OVA) and the anti-neomycin monoclonal antibody (NEO-mAb) were produced in the Key Laboratory of Animal Immunology of the Chinese Ministry of Agriculture. The peroxidase-conjugated goat anti-mouse IgG (GaMIgG-HRP) was purchased from Solarbio science and technology Co., Ltd. (Beijing, China). Nitrocellulose membrane, glass fiber, and absorbent pad were purchased from Millipore, other reagents and solvents were of analytical grade or higher. Milli-Q purified water was used throughout.

2.2. Apparatus

UV–vis spectra were measured using UV–vis spectrophotometer (UV2600, Shimadzu). Spectrophotometric microtiter reader (MULTISKAN FC, Thermo Co.) was used for absorbance measurement. Transmission electron microscopy (TEM) images were obtained on an FEI Technai G2 S-Twin at an accelerating voltage of 200 kV. The XYZ Biostrip Dispenser and CM 4000 Cutter (Bio-Dot, Irvine, CA, USA) were used to assemble strip. Raman spectra were obtained using a confocal microprobe Raman system (HR 800) equipped with a holographic notch filter and a CCD detector. A long working distance 50× objective was used to collect the Raman scattering signal. And the size of the laser spot is 1.7 μm. The excitation wavelength was 632.8 nm from a He-Ne laser.

2.3. Production and Characterization of Monoclonal Antibody

Six BALB/c female mice (7–8 weeks old) were immunized with NEO-BSA by subcutaneous injections at multiple points. After the fourth injection, the mice that showed the highest titer and sensitivity were selected to be spleen donors for hybridoma production. The obtained monoclonal antibody (NEO-mAb) was purified from murine ascite fluid by caprylic acid-ammonium sulfate method and stored at −80 °C until use. A competitive ELISA for the detection of NEO using this

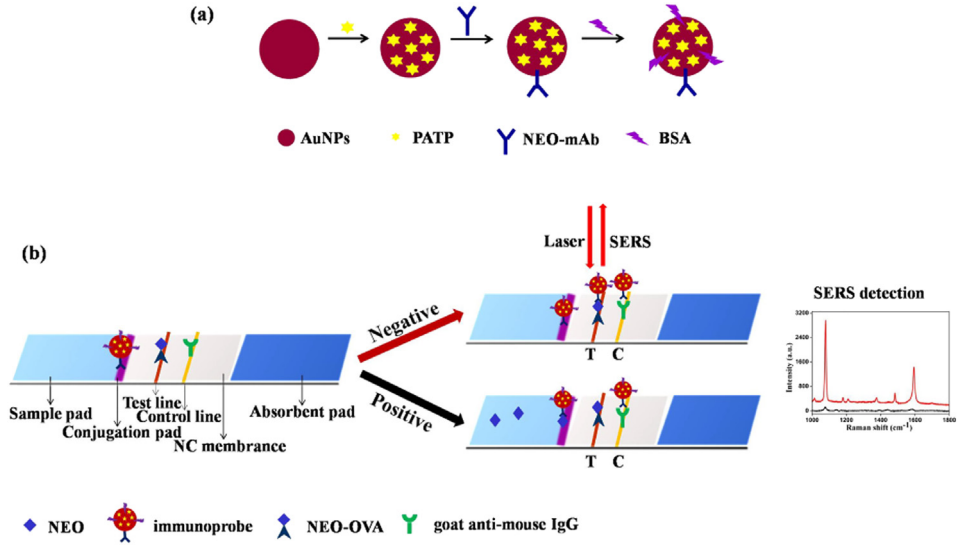

Fig. 1. (a) Schematic illustration showing preparation of immunoprobe; (b) Assembly of the LFI strip and the schematic diagram of the SERS-LFI strip for NEO detection.

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