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Quantitative detection of multiplex cardiac biomarkers with encoded SERS nanotags on a single T line in lateral flow assay

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ABSTRACT

Quick and accurate quantification of multiple biomarkers is quite a challenge in high throughput detection. Herein, we developed a novel lateral flow assay (LFA) based on Raman dyes (RDs) encoded core-shell surface enhanced Raman scattering (SERS) nanotags for rapid quantification of three cardiac biomarkers on a single test (T) line for early diagnosis of acute myocardial infarction (AMI). Owing to the signal amplification of encoded SERS nanotags and high surface area to volume ratio (SVR) of nitrocellulose (NC) membrane, rapid quantification of CK-MB, cTnI, and Myo with ultra-high sensitivity and wide linear dynamic range (LDR) was realized. We envision this method to find wide applications in *in vitro* diagnostics (IVD).

1. Introduction

Precision medicine depends on how much health information can be obtained and how well it can be interrogated and monitored. More and more data are needed for precision medicine and human health except for these from hospitals. Thus, point of care testing (POCT) technology, which aims to the facile and quick acquirement of health information at the point of care, becomes one key point of current health monitoring. In the last decades, POCT devices as well as quantitative and high throughput technologies are springing up, which contributes greatly to the big health data acquisition [1–4].

Lateral flow assay (LFA) is one of the most widely used POCT test owing to its specific, affordable, robust, and deliverable to end users [5]. With the increasing numbers of biomarkers discovered, there is often a need to detect several biomarkers simultaneously to generate meaningful or conclusive information so as to ensure precise diagnostics and mitigate patient's risk [6]. Multiplex detection is becoming indispensable in contemporary clinical diagnoses. The common ways to realize multiplex detection in LFA are setting parallel test (T) lines [7–9] or T dots array [10,11] on nitrocellulose (NC) membrane with one line detecting one biomarker. On the other hand, due to the limited quantification ability of gold colloids labels [12] for its colorimetric signal readout, quantitative LFAs utilizing fluorescence [13] or Raman labels [7,14,15] have attracted much more attentions and become the trend of future development. For fluorescence labels-based multiplex detection LFA, the quantitative results can be obtained from one image of all the T lines or T dots and then analyzed respectively. There are also reports that use fluorescence labels to realize the detection of two biomarkers on a single T line to reduce assay time and cost [16]. However, the fluorescence labels are amenable to bleaching and the sensitivity still need to be improved [17–19]. Besides, the FWHM (full-width half-maximum) of fluorescence spectrum is relatively wide, which leads to the overlap of the characteristic fluorescence peaks and limits its encoding capacity. In comparison, surface enhanced Raman scattering (SERS) nanotags based LFA (SERS LFA) has proved to have better performance for its high stability and sensitivity, as well as large encoding capacity. It has been used by Choo [7] and our group [20] for quantitative and high sensitive detection of multiplex biomarkers through setting multiple parallel T lines on the NC membrane. Nevertheless, the quantitative Raman signal is always acquired through

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Raman mapping of the T line one by one, which poses a great demand on precision and cost of the Raman instrument and requires longer signal acquisition time. The resulting problem is serious since sometimes the analysis time is crucial for life. Longer time will result in higher death rate. For example, in the diagnosis of acute myocardial infarction (AMI) [21], quick quantification of three cardiac biomarkers creatine kinase-MB isoenzymes (CK-MB), cardiac troponin I (cTnI), and myoglobin (Myo) are prerequisite to avoid deaths [22]. The therapy initiated within 30–60 min after onset of symptoms can greatly decrease mortality [21]. The time for AMI diagnosis with SERS LFA holds three T lines we developed before is about 28 min including 7 min immunoreaction and 21 min signal acquisition of three T lines, which still need to be improved for prompt AMI diagnosis [20]. If the three cardiac biomarkers are detected on a single T line in SERS LFA, the detection results can be obtained through single signal acquisition so that the signal acquisition time can be decreased by at least three times. In addition, the Raman instrument can be simplified with low cost and reduced design difficulty. Meantime, the reagent consumption and cost, preparation time of SERS LFA as well as manual operation can be decreased.

Except for high sensitivity got from the rational design of metallic structure [23,24], SERS nanotags have been proved to have the coding capacity by absorbing different Raman dyes (RDs) on the metal surface [25]. It has been used for multiplex detection of biomarkers on bead-based system [17,26] and high-throughput quantitative analysis was achieved with less sample consumption, rapid detection time as well as low cost. Herein, we construct a multiplex LFA with RDs encoded core-shell SERS nanotags as labels for quantitative and rapid detection of CK-MB, cTnI, and Myo on a single T line. As shown in Fig. 1, silver core and gold shell bimetallic nanoparticles (NPs) with Methylene blue (MB), Nile blue A (NBA), and Rhodamine 6 G (R6 G) encapsulated in the interface (Ag^{MB}@Au, Ag^{NBA}@Au, and Ag^{R6G}@Au) to form SERS nanotags. Detection antibodies against CK-MB, cTnI, and Myo are connected with SERS nanotags respectively and put in the conjugate pad. Crucially, one single T line composed of capture antibodies against CK-MB, cTnI, and Myo is constructed on the NC membrane for multiple analysis. Taking advantage of the encoded SERS nanotags, after the immunoreaction, Raman signal on the T line is collected for instant quantification of three cardiac biomarkers simultaneously. As far as we know, this is the first time three cardiac biomarkers are quantitatively detected on a single T line in SERS LFA.

2. Experimental section

2.1. Reagents and materials

Methylene blue (MB), Nile blue A (NBA), Rhodamine 6 G (R6 G), sucrose, sulfo-*N*-hydroxysuccinimide (NHS), tween 20, and ethyl dimethylaminopropyl carbodiimide (EDC) were received from Alfa Aesar. Thiolated-carboxylated PEG (HS-PEG−COOH, MW ~5 kDa) and thiolated PEG (PEG-SH, MW ~5 kDa) were bought from Laysan Bio, Inc. (USA). Silver nitrate ($AgNO_3$), tetrachloroaurate (III) trihydrate ($HAuCl_4{\cdot}3H_2O$), trisodium citrate ($Na_3C_6H_5O_7$), sodium borohydride ($NaBH_4$), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$), bovine serum albumin (BSA) were purchased from Sigma-Aldrich. A pair of monoclonal antibodies against CK-MB, a pair of monoclonal antibodies against cTnI, a pair of monoclonal antibodies against Myo, goat anti-mouse IgG antibody, CK-MB, cTnI, and Myo were provided by GeteinBiotech (China). Conjugate pad and sample pad were commercially available from PALL Corporation (USA). Backing pad and absorption pad were bought from Shanghai Goldbio Tech Co., Ltd (China). NC membrane was purchased from Whatman-GE Healthcare Company (UK). 0.01 M Phosphate buffer (pH 7.0) are home-made. Clinical serum samples were collected from Zhongda Hospital Southeast University and approved by its Institutional Ethics Committee.

2.2. Synthesis of silver-gold core-shell nanoparticles

The synthesis of Ag^{MB}@Au and Ag^{R6G}@Au NPs are the same with Ag^{NBA}@Au which have been reported before [20]. Here, MB (6.0 μM, 4 mL), NBA (6.0 μM, 4 mL), and R6 G (12.0 μM, 4 mL) were added drop by drop to silver NPs (36 mL) and reacted for 20 min under vigorously stir, respectively. Then, Ag@MB, Ag@NBA, and Ag@R6 G were used as seeds respectively to develop Au shell on them based on the reduction reaction between $NH_2OH{\cdot}HCl$ and $HAuCl_4$. To 40 mL of Ag@MB, Ag@NBA, and Ag@R6 G colloid, 4.65 mM $HAuCl_4$ (2 mL) and 65.2 mM $NH_2OH{\cdot}HCl$ (2 mL) were added drop by drop by two separate pipets at 2 mL min^{-1} under vigorous stirring and continued for 45 min. Then,

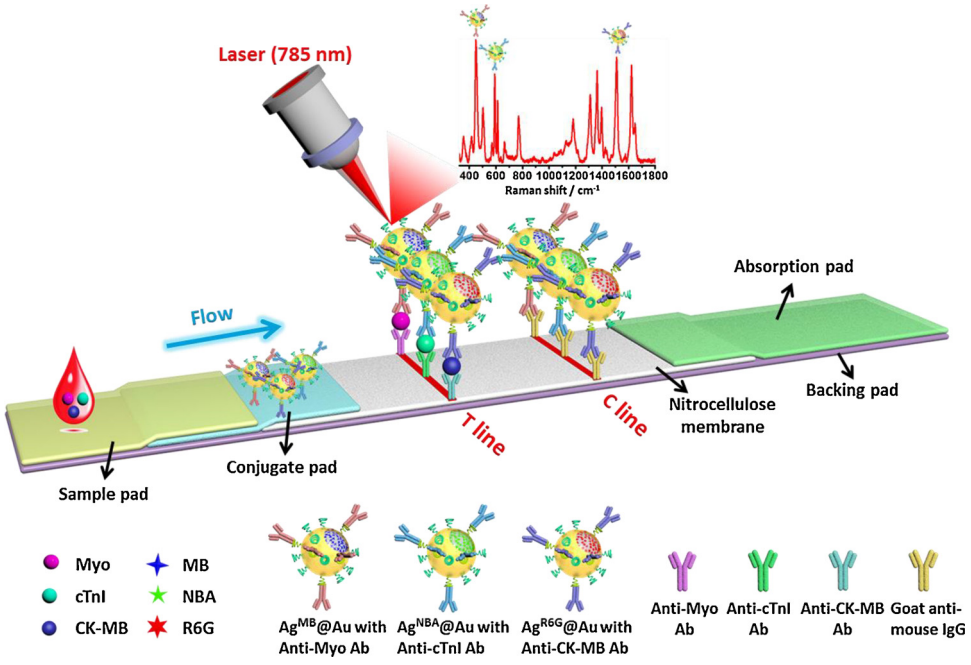

Fig. 1. Schematic illustration of quantitative LFA for cardiac biomarkers detection on a single T line with RDs encoded core-shell SERS nanotags.