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A colorimetric and electrochemical immunosensor for point-of-care detection of enterovirus 71

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ABSTRACT

Point-of-care detection of human enterovirus 71 (EV71), the major pathogen that causes hand, foot, and mouth disease (HFMD) among children, is urgently needed for early diagnosis and control of related epidemics. A colorimetric and electrochemical immunosensor for point-of-care detection of EV71 has been developed based on dual-labeled magnetic nanobeads amplification. The dual-labeled magnetic nanobeads (DL-MBs) are fabricated by simultaneous immobilization of EV71 monoclonal antibody (mAb) and horseradish peroxidase (HRP) on magnetic nanobeads. By capturing EV71 virions in 20 μL sample on mAb modified AuNPs-coated ITO electrode and subsequently binding with DL-MBs, with the addition of TMB and H_2O_2, colorimetric signals corresponding to EV71 with a concentration of 1.0 ng mL^{-1} can be directly read out by naked eyes, making it possible towards point-of-care detection of the virus. Furthermore, on the reduction of oxidized TMB on the electrode, electrochemical signal can be detected in the same detection cell without solution transfer, with a detection limit of 0.01 ng mL^{-1}. Validated with clinical samples, the colorimetric and electrochemical immunosensor shows a complete consistency with reverse transcription-polymerase chain reaction (RT-PCR) results. So far as we know, this is the first report on EV71 detection using electrochemical method. The merits of this assay, including high sensitivity, ability for colorimetric detection and easy to operation, ensure a promising future in point-of-care diagnostics of virus related diseases.

1. Introduction

Hand, foot and mouth disease (HFMD) is one of the most frequently infectious diseases among children, which has been recognized as a severe public health threat (Jiang et al., 2012; Ooi et al., 2010; Solomon et al., 2010). The major causative pathogen (Mao et al., 2016; Wang et al., 2012), enterovirus 71 (EV71), a non-enveloped single-stranded RNA virus, usually causes children aged under 5 a high incidence of severe neurological and cardiopulmonary complications, which may ultimately lead to death (Wong et al., 2010). Therefore, it is urgently needed to establish sensitive point-of-care EV71 detection methods for early diagnosis of HFMD. Conventional methods for virus detection mainly include virus isolation by cell culture (Terletskaia-Ladwig et al., 2008), polymerase chain reaction (PCR) (Iturriza-Gómara et al., 2006), and enzyme-linked immunosorbent assays (ELISAs) (Lequin, 2005; Wang et al., 2004). However, it remains difficult to detect EV71 sensitively, conveniently, and robustly in clinical samples.

Some novel approaches for EV71 detection have also been developed. Chen et al. developed homogeneous fluorescence “turn on” immunoassays for EV71 detection based on the quenching of quantum dots with $Ru(bpy)_2(mcbpy\text{-}O\text{-}Su\text{-}ester)(PF6)_2$-antibody complex (Chen et al., 2011) and graphene oxide (Chen et al., 2012), respectively. The corresponding limit of detection reached 0.64 ng mL^{-1} and 0.42 ng mL^{-1}. Liu et al. (2013) developed an improved ELISA for colorimetric detection of EV71 based on acetylcholinesterase (AChE)-catalyzed hydrolysis reaction and aggregation of gold nanoparticles (AuNPs). Without any pretreatment, the sensitivity is about one order of magnitude lower than that of RT-PCR. However, no quantitative analysis was shown by the method. In 2015, our group proposed a method to capture EV71 with antibody-labeled magnetic nanobeads and detect with quantum dots-labeled antibody, with a detection limit of 858 copies/500 μL (Wang et al., 2015a). However, specific instrument or enrichment with immuno-labeled magnetic nanobeads are required, hampering its usage in resource-constrained settings.

Colorimetric immunoassays enable naked-eye readout detection of targets (Liu et al., 2013), thus possess potential in point-of-care detection.

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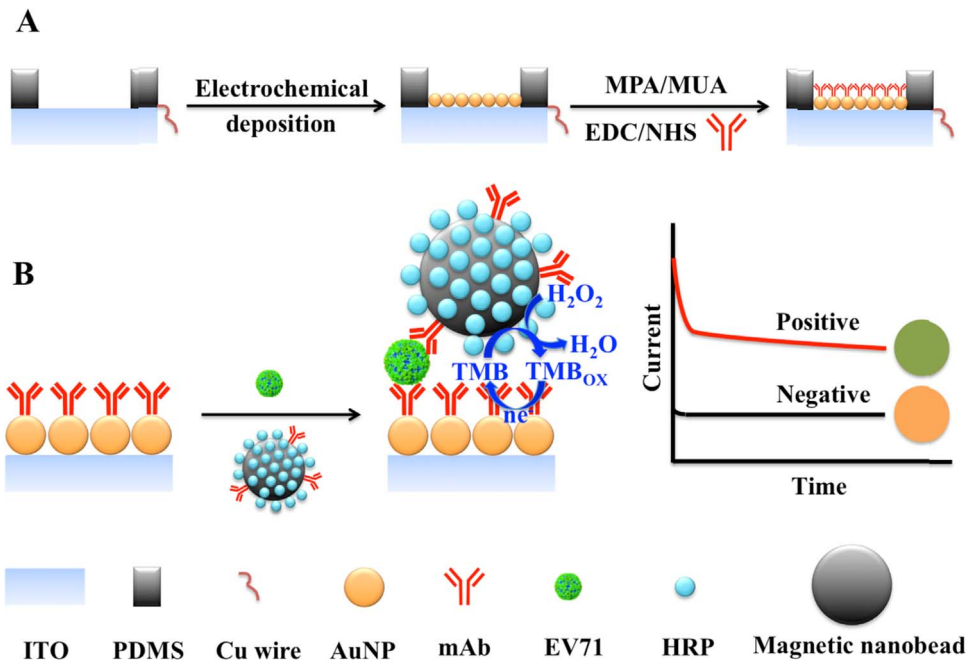

Scheme 1. Schematic illustration of (A) fabrication of the EV71 monoclonal antibody (mAb) modified AuNPs/ITO electrodes and (B) the colorimetric and electrochemical immunosensing for EV71 detection based on dual-labeled magnetic nanobeads (DL-MBs) amplification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

However, relatively poor quantitative detection, narrow linear range, and low sensitivity of colorimetric immunoassay restricts its further development. With the help of highly sensitive electrochemical detection, the most promising method for automatic and rapid on-site detection (Wang et al., 2015b), these limitations of colorimetric analysis could be remedied. Moreover, compared with other nanomaterials as the carriers to prepare signal tags, magnetic nanobeads for immunoassay facilitate manipulation and separation. Therefore, magnetic nanobeads have been widely used as candidates to further improve sensitivity of the colorimetric and/or electrochemical immunoassay (Gehring et al., 1996; Hayat et al., 2011; Wu et al., 2015; Leonardo et al., 2017; Reverté et al., 2013). However, so far as we know, separation processes are generally needed in magnetic nanobeads-based colorimetric and electrochemical immunoassays.

Herein, a pretreatment-free colorimetric and electrochemical immunosensor for point-of-care detection of EV71 has been developed with enzyme catalytic reaction-based amplification. As illustrated in Scheme 1, AuNPs are electrodeposited onto ITO electrode and subsequently functionalized with EV71 monoclonal antibody (mAb). Dual-labeled magnetic nanobeads (DL-MBs) are prepared by simultaneous immobilization of mAb and horseradish peroxidase (HRP) on magnetic nanobeads. By capturing EV71 virions in 20 μL sample on the mAb modified AuNPs-coated ITO electrodes and subsequently incubation with the DL-MBs, a specific sandwich-type format formed. In the presence of TMB/H_2O_2 substrate, large amount of colored oxidative products are produced by HRP-catalyzed redox reaction. Thus colorimetric analysis is performed in the homemade detection cell. Furthermore, with the oxidized TMB being reduced on the electrode, responses corresponding with virus concentrations can be detected by chronoamperometry. Thus highly-sensitive point-of-care detection of EV71 can be conveniently achieved based on this method. We believe the proposed colorimetric and electrochemical immunosensor is promising in clinical analysis.

2. Experimental section

2.1. Materials and reagents

Inactivated EV71 samples and clinical samples were provided by Hubei Provincial Center for Disease Control and Prevention. EV71 mAb was purchased from Abnova (Taipei, Taiwan). Super paramagnetic nanobeads (200 nm) were purchased from Ademtech SA (Pessac, France). Mercaptopropionic acid (MPA), 11-mercaptoundecanoic acid (MUA), N-(3-Dimethylaminopropyl)-N′ ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino) ethane sulfonic acid (MES), horseradish peroxidase (HRP), and Cy3-labeled secondary antibody were purchased from Sigma-Aldrich (St. Louis, USA). Poly(dimethylsiloxane) (PDMS) and curing agent were obtained from GE Toshiba Silicones Co., Ltd. (Tokyo, Japan). Substrate 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2 were commercially available from G-Biosciences (St. Louis, USA). All other chemical reagents were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water obtained with a Milli-Q water purification system was used in all experiments.

2.2. Instruments

Scanning electron microscopic (SEM) images were taken by field-emission scanning microscopy (FE-SEM, SIGMA). UV–vis spectra were recorded with a UV-2550 spectrophotometer (Shimadzu, Tokyo, Japan). Zetasizer Nano ZS90 (Malvern Instruments, UK) was used to detect the hydrated particle size and the surface charge. Immunofluorescence images were obtained with an ECLIPSE TE2000-U microscope (Nikon, Japan). All electrochemical experiments were performed on CHI 660a electrochemical workstation (CH Instruments, USA).

2.3. Preparation of dual-labeled magnetic nanobeads (DL-MBs)

Simultaneous immobilization of mAb and HRP on super paramagnetic nanobeads was achieved according to the literature (Mani et al., 2009; Yu et al., 2006). Briefly, commercial carboxylfunctionalized magnetic nanobeads (150 μg) were dispersed in 200 μL of MES (pH 6.0, 0.1 M) buffer and washed thrice. Then 100 μL of PBS containing 0.0125 μM mAb and 1 μM HRP was mixed with the magnetic nanobeads preactivated with EDC/NHS and incubated overnight. The resulting mixture was washed with PBS buffer until the supernatant couldn't make TMB/H_2O_2 substrate turn blue in 30 min. After blocking of nonspecific binding sites with 2% BSA and 0.05% Tween-20 in PBS