Biosensors and Bioelectronics 66 (2015) 95–102

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Indirect immunofluorescence detection of *E. coli* O157:H7 with fluorescent silica nanoparticles

Ze-Zhong Chen [a,b], Li Cai [a], Min-Yan Chen [a], Yi Lin [a], Dai-Wen Pang [a], Hong-Wu Tang [a,*]

[a] Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, State Key Laboratory of Virology, and Wuhan Institute of Biotechnology, Wuhan University, Wuhan 430072, PR China
[b] College of Chemistry and Environmental Engineering, Shanxi Datong University, Datong 037009, PR China

ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form
4 November 2014
Accepted 6 November 2014
Available online 8 November 2014

Keywords:
Fluorescent nanoparticles
Water-in-oil microemulsion
Bioassay
Bacteria
Flow cytometry

ABSTRACT

A method of fluorescent nanoparticle-based indirect immunofluorescence assay using either fluorescence microscopy or flow cytometry for the rapid detection of pathogenic *Escherichia coli* O157:H7 was developed. The dye-doped silica nanoparticles (NPs) were synthesized using W/O microemulsion methods with the combination of 3-aminopropyltriethoxysilane (APTES) and fluorescein isothiocyanate (FITC) and polymerization reaction with carboxyethylsilanetriol sodium salt (CEOS). Protein A was immobilized at the surface of the NPs by covalent binding to the carboxyl linkers and the surface coverage of Protein A on NPs was determined by the Bradford method. Rabbit anti-*E. Coli* O157:H7 antibody was used as primary antibody to recognize *E. coli* O157:H7 and then antibody binding protein (Protein A) labeled with FITC-doped silica NPs (FSiNPs) was used to generate fluorescent signal. With this method, *E. Coli* O157:H7 in buffer and bacterial mixture was detected. In addition, *E. coli* O157:H7 in several spiked background beef samples were measured with satisfactory results. Therefore, the FSiNPs are applicable in signal-amplified bioassay of pathogens due to their excellent capabilities such as brighter fluorescence and higher photostability than the direct use of conventional fluorescent dyes.

1. Introduction

The analysis of pathogenic bacteria is of vital importance in clinical diagnosis, infectious diseases prevention as well as public health and safety. Enterohemorrhagic *Escherichia coli* of serotype O157:H7 is one of the most dangerous agents of food-borne diseases because it has the ability to produce one or more toxins called Shiga-like toxins or Vero cytotoxins (O'Brien et al., 1983; Strockbine et al., 1986; Boyce et al., 1995; Tarr and Neill, 2001; Anderson et al., 2013). These toxins may cause serious gastrointestinal infections. *Escherichia coli* O157:H7 have been implicated in outbreaks of food-borne illness world-wide associated with the consumption of a variety of contaminated foods (Bell et al., 1994; Borczyk et al., 1987). Rapid and sensitive approaches used to identify this harmful bacterium are needed to prevent the occurrence of widespread outbreaks. Traditional methods for the detection of pathogenic bacteria involve following basic steps: preenrichment, selective enrichment, biochemical screening and serological confirmation (March and Ratnam, 1986; Liu and Li, 2001; Framer and Davis, 1985). This process is laborious and time-consuming. Many methods have been developed for rapid detection of pathogenic bacteria, including polymerase chain reaction (PCR) (Anderson et al., 2000; Fitzmaurice et al., 2004; Ibekwe and Grieve, 2003; Deng et al., 2014), enzyme-linked immunosorbent assays (ELISA) (Sunwoo et al., 2006; Bennet et al., 1996; Lee, et al., 2012), fluorescent labeling (Tison, 1990; Regnault et al., 2000; Han et al., 2000; Zhao et al., 2004b; Hahn et al., 2005; Wu et al., 2013; Carrión and Simonet, 2011; Amin and Elfeky, 2013) and amperometric immunosensor (Li et al., 2012), etc. Among them, fluorescent labeling is a very common method to visualize biological cells and various techniques used to detect the presence of bacterial pathogens have been performed on the basis of this method using fluorophores such as fluorescein isothiocyanate (FITC). However, conventional organic fluorescent dye-based probes are not suitable due to their poor photostability. Advances in nanoparticle technology have yielded fluorescent probes that are more photostable and sensitive than conventional fluorescent dyes (Li et al., 2008; Shen et al., 2011, 2012). Dye-doped silica NPs, exhibiting important advantages such as high luminescence and photostability compared to conventional fluorescent dyes, have been widely applied in biological imaging and ultrasensitive bioanalysis, including cell staining, DNA detection, cell surface receptor targeting, and ultrasensitive detection of bacteria (Cai et al., 2013; Chen et al., 2012; Ye et al., 2004; Wang et al., 2007; He

* Corresponding author. Fax: +86 27 68754067.
E-mail address: hwtang@whu.edu.cn (H.-W. Tang).

http://dx.doi.org/10.1016/j.bios.2014.11.007
0956-5663/

et al., 2009, 2008; Shi et al., 2010; Santra et al., 2001; Quentin et al., 2007). Each nanoparticle encapsulates thousands of fluorescent dye molecules in a protective silica matrix, providing a highly amplified and reproducible signal for fluorescence-based bioanalysis (Wang et al., 2006; Zhao et al., 2004a,b). Compared with conventional immunoassays, where one antibody molecule is linked with only a few dye molecules, the bioconjugated NPs enable significant amplification of the analytical signal because a large number of dye molecules are encapsulated in the individual NPs.

In this paper, we present fluorescent labeling and detection of intact bacterial cells with functionalized dye-doped silica NPs. We report the use of Protein A-conjugated FITC-doped silica nanoparticles (PFSiNPs) as sensitive, specific and stable fluorometric labels for pathogenic *Escherichia coli* O157:H7. Protein A was used as an affinitive adsorber to avoid direct attachment of antibody to NPs since direct covalent immobilization of antibodies onto solid substrates always causes the loss of biological activity of the antibodies (Lu et al., 1996). To keep full antibody activity, *E. coli* O157:H7 was first recognized with the specific antibody and then signaled by PFSiNPs. By using the microscope imaging and flow cytometry, the separation-free detection of *E. coli* O157:H7 was carried out in buffer and bacterial mixture, respectively. Meanwhile, signal intensity and photostability of FITC-doped silica NPs (FSiNPs) were compared with that of conventional fluorescent dye FITC. Considering the antibodies for various bacteria, this method might be promising for rapid and sensitive detection of other bacteria.

2. Experimental

2.1. Chemicals and materials

Triton X-100, cyclohexane, n-hexanol, ammonium hydroxide (NH_4OH, 28–30 wt%), tetraethyl orthosilicate (TEOS), fluorescein isothiocyanate (FITC), N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), 3-aminopropyltriethoxysilane (APTES), carboxyethylsilanetriol sodium salt (CEOS), 2-(N-morpholino) ethanesulfonic acid (MES), Protein A from *Staphylococcus aureus* were purchased from Sigma-Aldrich Chemical Co. Purified rabbit anti-*E. coli* O157:H7 IgG, purified rabbit anti-CEA IgG, FITC-conjugated goat anti-rabbit IgG was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, glycine and sodium azide (NaN_3) of analytical grade were obtained from China National Medicines Group Shanghai Chemical Reagents Company (Shanghai, China). Coomassie Brilliant Blue G-250 was obtained from National Medicine Group Chemical Reagent Co., Ltd. The prepared protein reagent contains 0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid in 100 mL buffer. Bovine Serum Albumin (BSA) was purchased from Shanghai Ruji Biological Technology Development Co., Ltd. Distilled deionized water was used for the preparation of all aqueous solutions.

2.2. Bacteria

E. coli strain O157:H7 and *E. coli* strain DH5α were obtained from Microbial Culture Collection Center of Guangdong Institute of Microbiology (Guangdong, China). Cultures were maintained on tryptic soy agar (TSA) slants at 7 °C and activated by culturing through three consecutive 24 h transfers in trypticase soy broth (TBS). Cultures for inoculation were grown in TSB for 18 h in a shaker incubator (100 rpm) at 37 °C and 1.0 mL decimally diluted in 9.0 mL sterile 0.01 M phosphate buffered saline (PBS, pH 7.4) to achieve about 100 and 1000 cfu/mL. The bacterial suspensions were counted in a Petroff–Hausser chamber, and the concentrations of bacteria were adjusted for use in experiments.

2.3. Instrumentation

The morphology and uniformity of FSiNPs and PFSiNPs were measured with a transmission electron microscope (JEOL, JEM100CXII, Japan). A spectrofluorometer (Hitachi, F-4600, Japan) equipped with a 150 W xenon lamp was used to record excitation and emission spectra. Fluorescence images were recorded with a Nikon ECLIPSE TE2000-U inverted fluorescence microscope equipped with a Nikon INTENSILIGHT C-HGFI lamp and Q-IMAGING RETIGA 2000R CCD. An infra-red spectrometer (FT-IR, Nicolet-5700, USA) was used to confirm the modified carboxyl on the surface of NPs. All dynamic light scattering and zeta-potential measurements were carried out using a Zetasizer (Nano-ZS, Malvern Instruments, Malvern, UK). UV–vis absorption spectra of FITC, FSiNPs and PFSiNPs were recorded with a UV–vis spectrophotometer (UV-2550, Shimadzu, Japan). Determination of protein concentration according to the Bradford method was done with the same UV–vis spectrophotometer.

2.4. Synthesis of fluorescent–silica precursor

The isothiocyanate group of FITC easily couples with the amino group of APTES to form a stable fluorescent conjugate. In this conjugate FITC is covalently attached to the APTES silane compound by a stable thiourea linkage. Briefly, 35 μL APTS and 3 mg FITC are combined together in 0.5 mL of absolute ethanol under dry nitrogen atmosphere and stirring magnetically for 12 h. The FITC–APTES conjugate solution is protected from light during reaction and storage to prevent photobleaching, and this conjugate will be used as the fluorescent silane reagent.

2.5. Synthesis of carboxyl-modified silica NPs

The carboxyl-modified FSiNPs were synthesized using the water-in-oil (W/O) reverse microemulsion method. The microemulsion consisted of a mixture of 1.77 mL of Triton X-100, 7.5 mL of cyclohexane, 1.6 mL of n-hexanol, 50 μL FITC–APTS conjugate, 340 μL water and 100 μL of TEOS that was stirred for 30 min at room temperature, and then 60 μL of NH_4OH was added. The aqueous ammonia served as both a reactant (H_2O) and a catalyst (NH_3) for the hydrolysis of TEOS. The mixture was allowed to be stirred for 24 h, followed by the addition of 50 μL of TEOS, 50 μL of CEOS for particle post-coating and surface modification. The mixture was further reacted for 24 h, and the silica particles were released from the microemulsion by the addition of acetone. The NPs were separated from the reaction mixture by centrifugation at 6000 rpm for 5 min and washed 2 times with acetone, 2 times with ethanol and 2 times with water, respectively. For re-dispersing NPs, each centrifugation step was followed by vortex and sonication.

2.6. Dye leaking experiment

After washing steps, FSiNPs were checked if there was any dye leaking. Aqueous suspension of FSiNPs (10 mg/mL) were first sonicated for about 5 min and then centrifuged at 10,000 rpm for 5 min. The supernatant was clear. The fluorescence of the supernatant was measured using a spectrofluorometer. The fluorescence intensity of the supernatant was close to that of the background signal. This experiment was repeated 3 times. There was no noticeable change in the supernatant fluorescence intensity in