Biomaterials 35 (2014) 1618–1626

Contents lists available at ScienceDirect

Biomaterials

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

Polyion complex micellar nanoparticles for integrated fluorometric detection and bacteria inhibition in aqueous media

Yamin Li, Xianglong Hu, Sidan Tian, Yang Li, Guoqing Zhang, Guoying Zhang**, Shiyong Liu*

CAS Key Laboratory of Soft Matter Chemistry, Hefei National Laboratory for Physical Sciences at the Microscale, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China

ARTICLE INFO

Article history:
Received 21 September 2013
Accepted 27 October 2013
Available online 13 November 2013

Keywords:
Polyion complex micelles
Aggregation-induced emission
Bacteria detection
Macromolecular antimicrobials
Biosensors

ABSTRACT

The development of portable and inexpensive detection methods can significantly contribute to the prevention of water-borne infectious diseases caused by pathogenic bacteria. Here we designed a nanosystem capable of both bacterial detection and inhibition, where polyion complex (PIC) micelles are constructed from negatively-charged tetraphenylethylene (TPE) sulfonate derivatives, which exhibit the aggregation-induced emission (AIE) feature, and cationic diblock copolymers, poly(ethylene oxide)-*b*-quaternized poly(2-(dimethylamino)ethyl methacrylate) (PEO-*b*-PQDMA). Upon contacting with bacteria, the PIC nanosystem disintegrates presumably due to competitive binding of polycation blocks with negatively-charged bacterial surfaces. This process is accompanied by a conspicuous quenching of TPE fluorescence emission, serving as a real-time module for microbial detection. Furthermore, the sharp decrease in CFU is indicative of prominent anti-microbial activities. Thus, PIC micelles possess dual functions of fluorometric detection and inhibition for bacteria in aqueous media. By tuning the charge density of TPE sulfonate derivatives and chain length of cationic PQDMA blocks, optimal performance against Gram-negative *Escherichia coli* has been achieved with a detection limit of 5.5×10^4 CFU/mL and minimum inhibitory concentration (MIC) of 19.7 μg/mL. Tests against Gram-positive *Staphylococcus aureus* were also conducted to demonstrate versatility of the nanosystem.

1. Introduction

One perpetual challenge to human health is the constant threat from existing and emerging infectious diseases [1], amongst which many water-borne pathogens have recently become more prevalent as a result of global climate change and environmental pollution [2]. For example, both extreme drought and excessive precipitation can result in poor water-body sanitation; therefore more susceptible individuals can be exposed to pathogen-contaminated water, particularly in developing countries. Therefore, facile detection for water-borne pathogens can play a crucial role in helping disease prevention, a considerably less costly practice than cure. Currently there exist various methods for bacteria detection, mainly including specimen culturing, polymerase chain reaction (PCR), target-specific immunoassays, and electrochemical techniques [3–7]. But they either require long-waiting time (days) for culture to grow or involve expert biochemical preparations including cell lyses, purification, and extraction. Consequently, the need for rapid, simple and inexpensive bacteria detection is tremendous.

Ideally, analysis on whole-bacterium without going through biochemical preparation is highly preferred. Through antibody–antigen recognitions or other specific ligand–receptor interactions, detection techniques without the necessity of extensive preparation steps based on localized surface plasmon resonance (LSPR), surface enhanced Raman scattering (SERS), diagnostic magnetic resonance (DMR), colorimetric, and fluorescent approaches have been developed [8–16]. However, in many scenarios, microbes to be detected are unknown or may not be previously identified, so that probes grafted with recognizing ligands such as carbohydrates or antibodies might not be effective. Thus a more universal means of detection must be designed in order to expand the scope of bacterial detection means. Given that most bacteria possess charged surfaces, more commonly negative [17], it is thus conceivable to develop fluorescent probes that are responsive to negatively charged surfaces. In this aspect, Rotello and coworkers [18] constructed an enzyme-gold nanoparticle hybrid system for

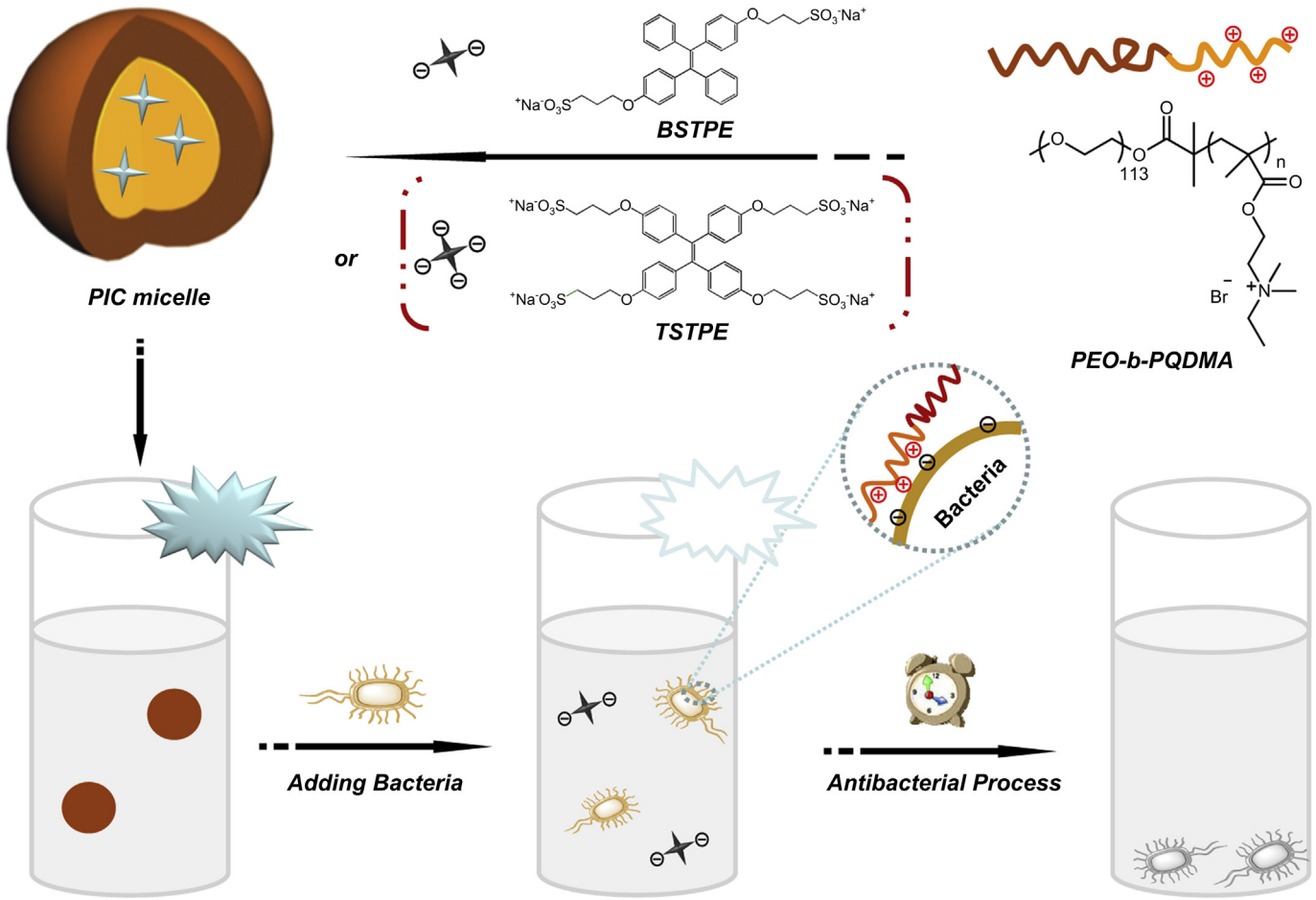
* Corresponding author. Tel./fax: +86 551 63607348.
** Corresponding author.
E-mail addresses: gyzhang@ustc.edu.cn (G. Zhang), sliu@ustc.edu.cn (S. Liu).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.10.077

the detection of microbial contamination by exploiting negatively-charged nature of bacterial surfaces, in which the activity of β-galactosidase (β-Gal) was initially inhibited by electrostatically bound cationic gold nanoparticles (AuNPs). When contacting with bacteria, the binding of anionic bacteria surface to the cationic AuNPs frees β-Gal molecules and restores its catalytic activity, providing an easy colorimetric readout. Wang et al. [19] developed a fluorescence resonance energy transfer (FRET) system using quaternized conjugated polymer donor and fluorescein acceptor to test the antimicrobial susceptibility and screen potent antibiotics. In addition, Bunz and Rotello et al. [20] utilized non-covalent conjugates of anionic poly(para-phenyleneethynylene) (PPE) with hydrophobic ammonium-functionalized gold nanoparticles for fluorescent bacterial detection in aqueous media.

In the context of antibacterial agents, many classes of small-molecule antibiotics have been heavily used in the battle against pathogenic bacteria. Positively charged polymers or polycations belong to another large category of anti-microbial agents [21–26]. Compared to small-molecular antibiotics, polymeric ones can be implemented on utensil as non-vanishing and protective coatings, leading to sustained inhibitory effects [27]. Furthermore, it is unlikely that mutated resistant strains can evade from being inhibited by polycations through such a general type of non-covalent interactions, i.e., mainly electrostatic interactions. Whitten et al. [28] reported that cationic PPEs can be employed for light-induced inactivation of pathogenic bacteria and Lorenzo et al. [29] developed poly(hexamethylene biguanide) functionalized core–shell paramagnetic nanoparticles for dual functions of antibacterial activity and magnetic manipulation of bacteria cells. In both cases, supramolecular interactions between functional biomaterials and negatively-charged bacterial surface were involved. If synthetic cationic polymers can simultaneously combine functions of bacteria detection and inhibition, it will be highly desirable considering their potential applications in aqueous environments. Nevertheless, polycations that both exhibit responsive fluorescence properties towards bacterial surface and possess antibacterial activities have not been reported yet.

In the current work, we attempt to fabricate polyion complex (PIC) micelles possessing dual functions of both bacteria sensing and inhibiting. As shown in Scheme 1, PIC micelles with PEO coronas can be fabricated by mixing neutral-cationic diblock polyelectrolytes, poly(ethylene oxide)-*b*-quaternized poly(2-(dimethylamino)ethyl methacrylate) (PEO-*b*-PQDMA), with tetraphenylethylene (TPE) sulfonate derivatives (BSTPE or TSTPE). Within micellar cores, positively charged $QDMA^+$ moieties of PQDMA block will be physically caged and shielded by negatively charged sulfonic acid functionalities of TSTPE or BSTPE via electrostatic interactions. In addition, TPE sulfonates within PIC micelles will be rendered highly fluorescent owing to the well-documented aggregation-induced emission (AIE) effect originally reported by Tang and coworkers [30–33]. We hypothesized that in the presence of bacteria, competitive binding of negatively charged



Scheme 1. Schematic illustration of the fabrication of polyion complex (PIC) micelles from TPE sulfonate derivatives (BSTPE or TSTPE) with neutral-cationic diblock polyelectrolyte, poly(ethylene oxide)-*b*-quaternized poly(2-(dimethylamino)ethyl methacrylate), PEO-*b*-PQDMA. By taking advantage of the AIE feature of TPE sulfonate derivatives, highly fluorescent PIC micelles can quantitatively detect the presence of bacteria in aqueous environment and also serve as physically caged polymeric antibacterial agent.