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# Rapid fluorometric bacteria detection assay and photothermal effect by fluorescent polymer of coated surfaces and aqueous state

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ABSTRACT

A fluorescent dye and a photothermal agent were grafted onto a cationic polymer for rapid and simple bacteria detection in liquid and solid phase based fluorescence on/off. The integrated poly(vinylpyrrolidone) (PVP) backbone with catechol and bromoethane moieties possesses unique optical properties due to the presence of boron dipyrromethane (BODIPY) and near infared NIR-responsive IR825 (F-PVP). The cationic segments showed distinct fluorescence quenching patterns after interaction with gram-positive and gram-negative bacteria via polyion complex interactions. Fluorescence quenching depended on direct interaction of the bacterial cell membrane, as confirmed using SEM and confocal imaging. The detection limit was 1 mg/mL for the liquid-phase assay and the minimal detectable concentration of bacteria using the solid-phase assay was $10^6$ CFU/mL. After bacterial detection in contaminated area, our system can directly kill bacteria via the photothermal conversion ability of the IR825 substituent using NIR exposure by polymer solution and limited in coated PP. Finally, the proposed biosensor is capable as potential material for detection of bacteria in simple liquid and solid phase assay.

## 1. Introduction

Bacterial contamination in medical devices is a major public health concern, which has attracted global attention, as it can cause fatal diseases, resulting in an increased death rate of patients and increased medical costs (Fauci and Morens, 2012; Kang et al., 2014; Khan et al., 2009). Various techniques have been used to detect microbial infections, including specimen culturing, polymerase chain reaction (PCR), and target-specific immunoassays (Lazcka et al., 2007; Valloran et al., 2016; Wang et al., 2015). PCR is the most commonly used method due to its efficiency and specificity, but it requires sophisticated instruments and costly dye-labeled primers for whole step detection (Gebert et al., 2008; Wilson et al., 1995). Fluorescent quantum dots have been reported to be useful for sensing of multiple pathogens through antibody conjugation. However, challenges remain for fluorescence-based detection; the major problem is development of a method for large-scale and reproducibility of quantum dots (QDs) with thin, biocompatible coatings (Ray et al., 2012; Zhao et al., 2009). Therefore, further research is required to develop rapid, simple, and inexpensive methods for responsive bacterial detection.

Considering interaction mechanism between cell and material, which are many groups in bacterial cell outer membranes, which can be deprotonated to produce negative charges, there may be many ways to improve current techniques. We therefore attempted to develop an advanced method for bacterial detection by exploiting the negative charge on bacteria. Cationic conjugated polymers are a promising platform to interact with the negative charge on the surface of bacteria without extensive biochemical preparation, understanding single electrostatic interaction can be used to design a simple and rapid detection technique (Bandyopadhyay et al., 2015; Miranda et al., 2011; Wilson et al., 2001). A few reports have discussed bacterial detection based on fluorescence on/off system. Cationic poly(phenylene vinylene) polymers have been reported to be effective for detection and discrimination of single bacteria in aqueous media based on the intensity of the fluorescence signal; polyion complex micellar nanoparticles showed fluorescence quenching after competitive binding with bacteria, and aggregation of the cationic CdTe QDs on the surface of bacteria was reported to result in quenching of the fluorescence of the CdTe QDs (Y.Q. Li et al., 2014; Y. Li et al., 2014; Yang et al., 2015; Yuan et al., 2014). Along these examples, the interaction between negatively

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charged bacteria and synthetic material–based fluorescence quenching generated different patterns for gram-negative and gram-positive bacteria; most of the gram-positive bacteria showed only a slight quenching effect, owing to the difficulty of the chemical interaction between the negative charges in the long chain technoic acid with the positively charged bacterial surface (Birdsell et al., 1975). A recent work, reported only a limited capacity for bacterial detection in aqueous phase and no reports have been published on emission-based bacterial detection in solid phase assay (Chen et al., 2014; Chung et al., 2013; Y.Q. Li et al., 2014; Y. Li et al., 2014; Ning et al., 2011). The aim of the present work was to design a polymer for both detection and killing of bacteria, not only in solution but also in a solid-phase assay. This proposed system offers a novel, simple, and rapid method for detection of bacteria.

The developed materials were prepared using Boron dipyrromethane (BODIPY) dye and IR825 conjugated to poly(vinylpyrrolidone) (PVP) using a quarternization method and the cationic moieties were obtained from excess conjugated bromoethane in the polymer system. For solid detection, the catechol moieties were used to fabricate adhesive properties so that it can be applied to propylene (PP) films. The combined polymer, with positively charged amine groups, possesses the tunable fluorescence of BODIPY and the excellent heat conversion of near infrared (NIR)-responsive IR825. The interaction of the prepared polymer with bacteria was expected to result in fluorescence quenching, for use in bacterial detection. Coating of PP films with the prepared polymer produced a detection pattern similar to that observed in liquid media, confirming that the material shows promise for use in biosensors in the fields of microbiology and medicine. After detection, this biosensor can directly kill bacteria using the photothermal conversion ability of the IR825 substituent. This platform shows excellent potential for development of materials that can detect bacteria within a second, followed by direct killing of bacteria.

## 2. Material and methods

### 2.1. Materials and characterization

Poly(vinylpyrrolidone) (PVP, 40,000 MW), 2-chloro-3′,4′-dihydroxyacetophenone (CCDP), bromoethane ethanol, nitrogen, deionized water, phosphate-buffer saline (PBS), diethyl ether, MRS, lysogeny broth (LB), deuterium oxide ($D_2O$), dimethyl sulfoxide (DMSO), propylene (PP), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and agar were purchased from Sigma-Aldrich, Yongin City, Kyunggi-Do, South Korea. Propidium iodide and SYTO 9 were purchased from Molecular Probe, Life Technologies (Invitrogen). Boron dipyrromethane (BODIPY) with benzyl chloride on the meso position was prepared as previously described, and the heptamethine dye IR825 was synthesized using a previously reported protocol (Chae and Baek, 2012; Cheng et al., 2013).

$^1$H NMR spectra was recorded using (Bruker AVANCE 400 spectrometer operating at 400 MHz) with DMSO as the solvent. The absorption spectra of the sample were recorded using a UV–vis absorption spectrometer (UV–vis, Optizen 2120 UV spectrophotometer, Mecasys, Yuseong-gu Daejeon, South Korea). The fluorescent properties were measured using a L550B luminescence spectrometer from Perkin Elmer. The NIR laser was set at 808 nm (PSU-III-LRD, CNI Optoelectronics Tech. Co. LTD, China). Photothermal heating curves were examined using an infrared camera (NEC Avio, Thermo Tracer TH9100). Field-emission scanning electron microscopy (FE-SEM) micrographs were obtained using a SEM/EDX, JSM-6700F, JEOL, Musashino, Akishima, Tokyo, Japan. X-ray photoelectron spectra (XPS) were acquired using an ESCALAB apparatus (Omicrometer, Taunusstein, Germany) and a system (PHI Quantera-II, Ulvac-PHI, Chigasaki, Kanagawa, Japan). Live/dead bacterial analysis was performed using a LSM510 confocal laser scanning microscope (CLSM, Carl Zeiss, Germany) using 405, 488, and 543 nm emission filters with 20× magnification. Particle size was measured with dynamic light scattering (DLS; Zetasizer Nano, Malvern-Germany). Static water contact angles were measured with a DO3210 instrument (KRUSS Ltd., Germany).

### 2.2. Synthesis of CCDP/IR825/BODIPY/ethyl bromide quaternized PVP (F-PVP)

The CCDP/IR825/BODIPY/ethyl bromide–conjugated PVP was synthesized using the quaternization reaction as previously described (Kang et al., 2016). In detail, PVP (2 g) and CCDP (0.3032 g) were dissolved in 60 mL of anhydrous ethanol in a 250-mL then stirred for 12 h at 70 °C under a nitrogen atmosphere. At the end of the reaction, solvent was evaporated and precipitated using diethyl ether. The resulting CCDP quaternized PVP (C-PVP) was dried in a vacuum oven and used for the next step. The BODIPY (0.0746 g), IR825 (0.3 g) and an excess of bromoethane (1.12 mL) as cationic moieties were quaternized with C-PVP (4 g) using the same method above but conducted for 48 h under a nitrogen atmosphere. Un-reacted precursors were removed on a rotary evaporator then precipitated using diethyl ether, and the residues were processed in a vacuum oven to obtain a dried sample.

The conjugated polymer was characterized using $^1$H NMR spectroscopy (Fig. 1). $^1$H NMR (400 MHz, d6-DMSO, δ): 0.81–1.25 (2H, $-CH_2$ of PVP), 2.12–2.23 (2H, $-CH_2$ of VP ring), 2.24–2.32 (2H, $-CH_2$ of VP ring), 3.15–3.28 (2H, $-CH_2$ of VP ring), 3.47–3.66 (H, –CH of PVP), 6.67–7.53 (aromatic protons of catechol), 0.94 (3H, $-CH_3$–$CH_2$ of BODIPY), 1,3 (3H, $-CH_3$ of BODIPY), and 7.9–8.3 (2H, aromatic proton of BODIPY and IR825).

### 2.3. Preparation of coated PP

PP-coated F-PVP was obtained using a dip-coating method, using catechol chemistry in PBS (pH 8.5) (Lee et al., 2008). PP film were cut into small pieces (5 cm) and rinsed with ethanol and deionized water followed dried using dryer pump. These PP slides were immersed in a F-PVP solution (10 mg/mL, PBS pH 8.5) for 24 h and then dried in air. In the end, we named this treated PP as coated PP.

### 2.4. The liquid and solid-phase detection assay using F-PVP with bacteria

For liquid-phase detection assay, the solutions of *S. aureus* (gram-positive, strain ATCC 25323) and *E. coli* (gram-negative, strain ATCC 25922) were prepared in LB and MRS broth (50 mL) media, respectively, and incubated at 37 °C for 24 h. Then F-PVP solution (1 mg/mL) was added to the bacterial solution ($10^8$ cells/mL) and incubated for 1 h. At the end of incubation period, the solution was centrifuged followed by washing with PBS to remove the unreacted F-PVP. To verify the capacity of the F-PVP for detection of single bacteria, the bacterial pellet labeled with F-PVP was added with PBS (pH 7.4) solution and then the fluorescent properties were observed using a L550B luminescence spectrometer at an excitation wavelength of 520 nm and a LSM510 confocal laser scanning microscope (Scheme S1a).

For solid-phase detection assay, the coated PP was added to solutions of each bacterial strain at a concentration of $10^8$ cells/ mL and incubated for 1 h. After the incubation period, the coated PP was removed from the bacterial solution and washed with PBS. The coated PP was dried at room temperature and its fluorescent properties were measured using a LSM510 confocal laser scanning microscope as illustrated in Scheme S1b.

### 2.5. Scanning electron microscopy for bacterial cell imaging

For SEM imaging, the bacterial suspensions obtained using similar