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Photoelectrochemical Biosensors

Photoelectrochemical Strategy for Discrimination of Microbial Pathogens Using Conjugated Polymers

Xin Zhou,[a, b] Pengbo Zhang,[a] Fengting Lv,*[a] Libing Liu,[a] and Shu Wang*[a, b]

Abstract: A photoelectrochemical (PEC) biosensor for facile and sensitive identification of pathogenic microorganisms was developed. Cationic poly(phenylene vinylene) derivative (PPV) as photoelectrochemical active species was modified on the electrode. Under light irradiation, PPV could be excited and generate efficient photocurrent. PPV also had the ability to bind with negatively charged membrane of pathogenic microorganisms, which hindered the electron transfer between electrode and electrolyte. As a result, the photocurrent would decrease obviously. For *E. coli*, *B. subtilis* and *C. albicans*, the photocurrent density was reduced by 18, 33 and 59%, respectively. Based on the reduction degree of the photocurrent after capturing different types of species of pathogenic microorganisms, a PEC sensor for discrimination of pathogenic microorganisms was realized.

With the increasing of pathogen infections in clinical diagnosis, it becomes a serious public health issue which has aroused people's great attention. Pathogen infections caused by bacteria and fungi have led to grave consequences, including expensive healthcare bill, life-threatening diseases and increasing mortality of patients.[1] Rapid and reliable identification of pathogenic bacteria and fungi plays a crucial role in determining the primary causes of infectious diseases and providing basis for formulating effective anti-infection therapeutic regimen. In clinical diagnosis, the existing blood culture method is time-consuming and poorly accurate, which may delay the treatment time.[2] Recently, enormous attention has been devoted to exploring ingenious methods to identify pathogens such as DNA microarray,[3] polymerase chain reaction (PCR),[4] mass spectrometry[5] and surface plasmon resonance (SPR) technique.[6] However, the implementation of these methods depends on complicated instruments and operations, which confines their clinical applications in a certain extent. Thus, it is essential to develop new approaches.

Conjugated polymers (CPs) with delocalized conjugated backbones have good optical and electrical properties, such as broad absorption wavelength range, continuously tunable energy level and high charge carrier mobility.[7] With these properties, CPs have been extensively employed in field-effect transistors,[8] light-emitting diodes,[9] and photovoltaic cells.[10] The excellent light-harvesting capacity and optical signal amplification effects make CPs a promising candidate in chemical and biological detection.[11] Our previous work has constructed a timesaving and dependable fluorescence sensor based on the disassembly of supramolecular probe for in situ identification of pathogens. The fluorescence signal of PFP-NMe_3^+/cucurbit[7]uril complex changes before and after disassembly by amantadine on pathogen surface.[12] Besides, we have reported a cationic poly(*p*-phenylene vinylene) derivative (PPV-NMe_3^+) for simple and rapid identification of Gram-positive bacteria (*B. subtilis*), Gram-negative bacteria (*E. coli*) and the fungi (*C. albicans*) via monitoring the fluorescence intensity changes of PPV-NMe_3^+ in buffer solutions with different concentration. PPV-NMe_3^+ bearing quaternary ammonium group side chains could regulate the diverse interactions due to the different membrane structure between bacteria and fungi.[13] These fluorescence sensors are faced with the problem of photobleaching and photoquenching, which will reduce the reliability and stability of the test.

Electrochemical sensors have been widely used in pathogen detection due to their simplicity, convenience and high sensitivity.[14] At present, most of the electrochemical methods need the modification of biological recognition elements on electrodes, such as antibodies,[15–18] nucleic acids[19–22] and antimicrobial peptides.[23–26] These natural ligands perform their functions in stringent environmental conditions, otherwise they may be prone to inactivation or degradation. Hence, replacing these natural ligands with synthetic materials with good electrical conductivity, specific recognition performance and/or photoelectric property is an effective and considerable strategy. Photoelectrochemical (PEC) sensor which estimates the photocurrent change of photoelectrochemical active materials induced by analyte, is a potential detection technique in chemistry,

[a] X. Zhou, P. Zhang, Prof. F. Lv, Prof. L. Liu, Prof. S. Wang
Beijing National Laboratory for Molecular Sciences, Key Laboratory of Organic Solids, Institute of Chemistry
Chinese Academy of Sciences
Beijing 100190 (P. R. China)
E-mail: wangshu@iccas.ac.cn

[b] X. Zhou, Prof. S. Wang
College of Chemistry
University of Chinese Academy of Sciences
Beijing 100049 (P. R. China)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/asia.201800783.

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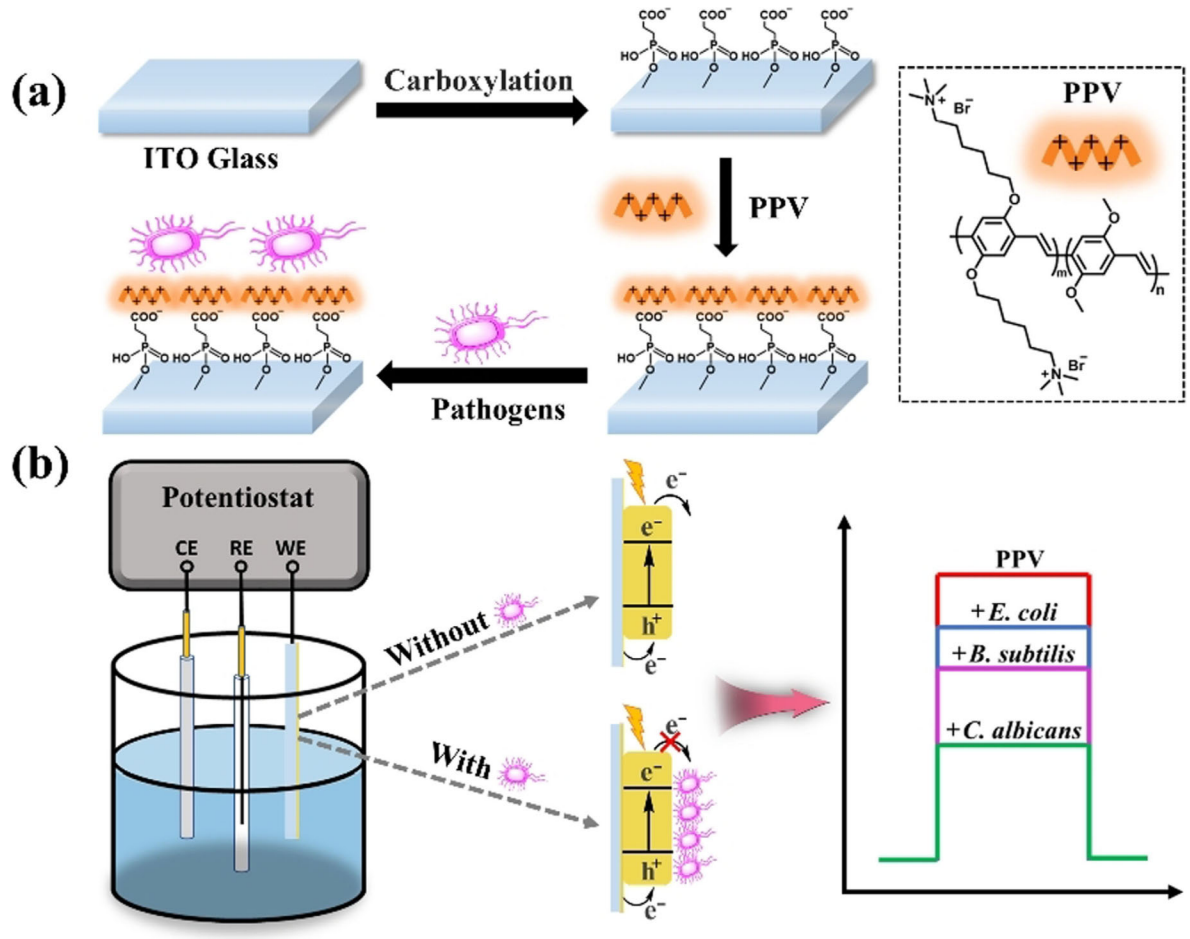

Scheme 1. (a) The fabrication process of PPV electrode and PPV/pathogens electrode. (b) The principle of photoelectric conversion with PPV electrode. In a standard three-electrode setup, the PPV electrode was used as working electrode (WE). Ag/AgCl electrode was used as reference electrode (RE) and platinum wire was used as counter electrode (CE). When pathogens were coated on PPV electrode, the electron transfer between electrode and electrolyte was hindered. As a result, the photocurrent decreased obviously. Various species of pathogens exhibited different degrees of photocurrent reduction.

biology, environment and food security.[27] Here, we developed a novel PEC biosensor based on PPV for fast, simple and sensitive identification of pathogenic bacteria and fungi (Scheme 1). In order to effectively deposit the positively charged PPV on ITO electrodes which were used as working electrode, 3-phosphonopropionic acid was self-assembled on ITO electrodes. Then the electrodes were functionalized with carboxyl group in neutral conditions (COOH-ITO electrode). PPV was subsequently electrostatically deposited on the electrodes (PPV-ITO electrode). PPV as photoelectrochemical active species could absorb visible light to generate electron-hole pairs and photocurrent subsequently. Simultaneously, PPV had the ability to bind with negatively charged membrane of pathogenic microorganisms, which hindered the electron transfer between electrode and electrolyte. As a result, the photocurrent would decrease obviously. Based on the reduction degree of photocurrent after capturing different species of pathogenic microorganisms, a PEC sensor of pathogenic microorganisms was proposed, which showed good analytical performance. PPV was synthesized according to the procedure in the literature.[13] The UV/Vis absorption spectrum and fluorescence spectrum were shown in Figure S1. To confirm that PPV was successfully deposited on ITO electrodes, we measured the fluorescence spectra of PPV-ITO electrode and COOH-ITO electrode. As shown in Figure 1, PPV-ITO electrode displayed a characteristic fluorescence peak of PPV at 550 nm which was consistent with the fluorescence spectra of PPV. Whereas, there was no fluorescence peak for COOH-ITO electrode. This observation confirms the coverage of PPV on ITO electrode.

To identify pathogens, the PPV-ITO electrode was immersed in *E. coli*, *B. subtilis* and *C. albicans* solution, respectively. The three kinds of pathogens could bind with PPV by electrostatic and hydrophobic interactions and coat on the surface of electrode.[13] The immobilization of PPV and different pathogens on ITO was characterized using scanning electron microscope (SEM). As displayed in Figure 2, the COOH-ITO electrode exhibited clean and smooth surface. After PPV modification, massive aggregation emerged which indicated the attachment of PPV on ITO electrode. Further addition of pathogens, a huge amount of *E. coli*, *B. subtilis* and *C. albicans* were coated on the surface. At the same concentration of pathogen, the number of *E.coli*, *B. subtilis* and *C. albicans* attached to the electrode

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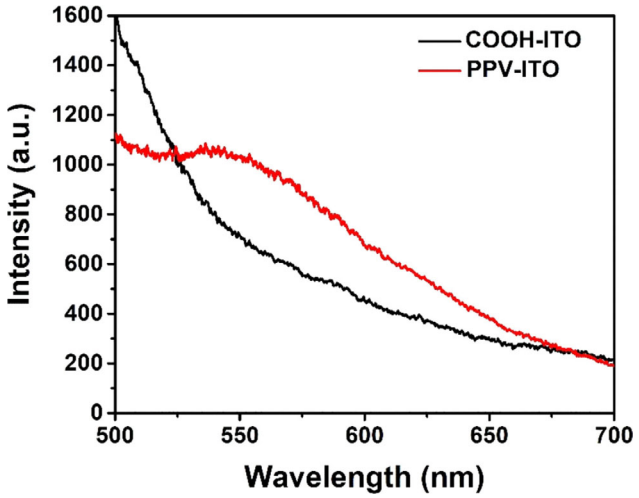

Figure 1. Fluorescence emission spectra of COOH-ITO electrode and PPV-ITO electrode with an excitation of 450 nm.

were 152, 111 and 136 respectively, which showed no significant difference. But the coverage of *E.coli, B. subtilis* and *C. albicans* on electrode was obvious distinct with each other. As pathogens had different volumes, they covered different surface area of electrode. *C. albicans* was the biggest among them, so the largest area surface of electrode was covered by *C. albicans*, followed by *B. subtilis* and *E. coli* least.

To assess the PEC performance of the modified ITO electrodes, photocurrent responses of COOH-ITO electrode and PPV-ITO electrode were measured under $\lambda = 450$ nm light irradiation. Platinum wire and Ag/AgCl were used as counter and reference electrodes, respectively. As shown in Figure 3a, no apparent photoelectric response was observed for COOH-ITO electrode. After the modification of PPV, it exhibited a cathode photocurrent density of $\approx 980\ nA\,cm^{-2}$. It indicated that the photocurrent was based on electron transfer from photoexcited PPV to oxygen in the electrolyte solutions.[28] The photocurrent density decreased remarkably after the pathogens coated on PPV-ITO electrodes. The decrease of photocurrent density was mainly because increased steric hindrance impeded the diffusion of electron and nonconducting pathogens hindered the photoinduced electron transfer between electrode and electrolyte. For *E. coli*, *B. subtilis* and *C. albicans*, the photocurrent density reduced by 18%, 33% and 59%, respectively. The current density decreased with increasing the size of pathogens. *C. albicans* covered the largest area surface, so the impedance was the biggest, leading to the greatest decline of current density. *E. coli* whose size was relatively small caused little variation of photocurrent density. The current density of *B. subtilis* coated PPV-ITO electrode was in the middle which was in conformity with the size of *B. subtills*. By increasing the volume of pathogen, the coverage ratio went up and the electron transfer resistance increased correspondingly. As a result, the photocurrent decreased in the order of *E.coli*, *B. subtilis* and *C. albicans.* Figure 3b showed that with increasing concentrations of the pathogens, the current density declined stepwise. As shown in Figure S2, the linear fitting results showed that the photocurrent density had a linear dependence relation with the pathogen concentration at the range of $OD_{600} = 0.5$ to 2. The linear equations were $I = 59.45OD - 416.01$ (the correlation coefficient $R^2 = 0.9999$) for *E. coli*, $I = 31.57OD - 327.53$ ($R^2 = 0.9921$) for *B. subtilis* and $I = 19.83OD - 210.26$ ($R^2 = 0.9868$) for *C. albicans.* And there was no change in the order of the current density at any concentrations. The number of pathogens coated on PPV-ITO electrode grew with the increasing concentrations, which caused the impedance increasing. As a result, the current density reduced. The pathogens volume was constant and displayed significant difference, hence the current density was in the fixed order and different obviously. This detection method exhibited high sensitivity and wide concentra-

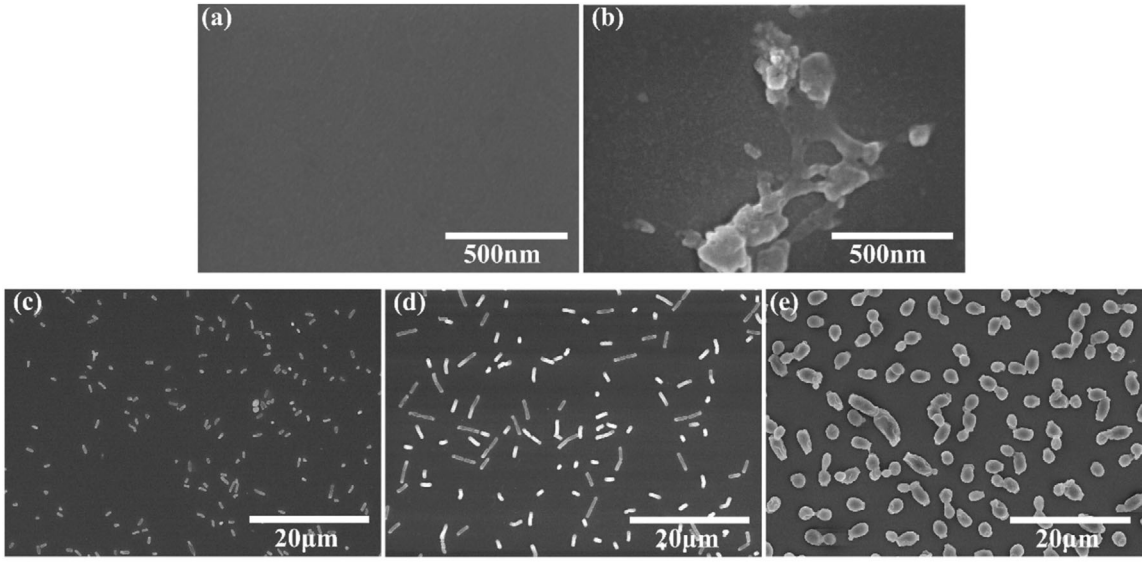

Figure 2. SEM images of the (a) COOH-ITO electrode, (b) PPV-ITO electrode. Scale bar is 500 nm; (c)–(e) *E. coli*, *B. subtilis* and *C. albicans* coated PPV-ITO electrodes, respectively. Scale bars are 20 μm.

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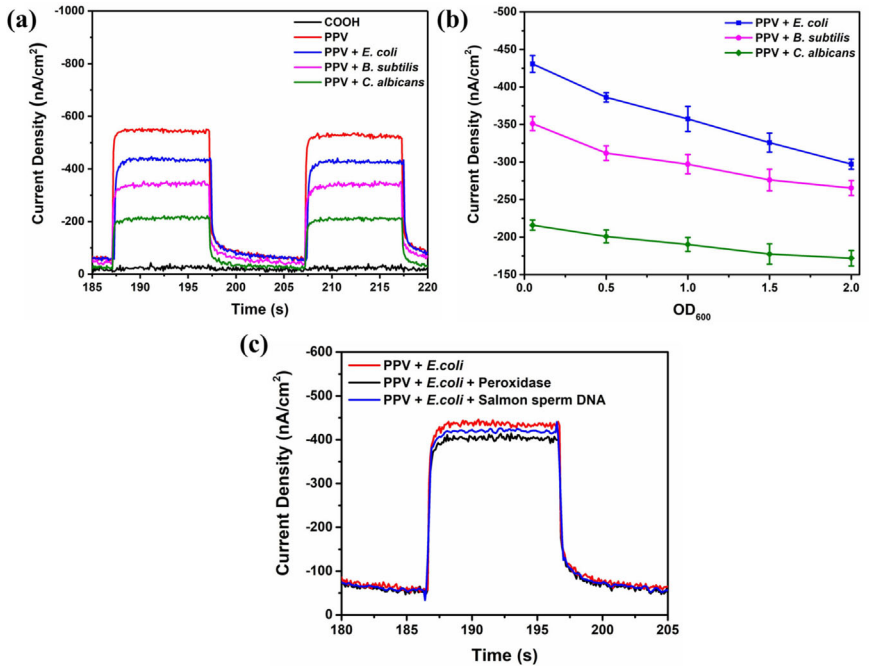

Figure 3. (a) Photocurrent density of COOH-ITO electrode, PPV-ITO electrode and *E. coli*, *B. subtilis* and *C. albicans* coated PPV-ITO electrodes. (b) Photocurrent density of *E. coli*, *B. subtilis* and *C. albicans* coated PPV-ITO electrodes at different OD_{600}. (c) Photocurrent density of *E. coli* coated PPV-ITO electrode in the presence of the interfering substances (peroxidase or salmon sperm DNA.)

tion range toward pathogens detection. To evaluate the interference of other negatively charged species such as DNA and proteins on the photocurrent, we added salmon sperm DNA (10 μM) or horseradish peroxidase (10 μM) into *E.coli* solution separately. Then the PPV-ITO electrodes were immersed in the solution and the photocurrent was measured. Figure 3c showed that the influence of salmon sperm DNA and horseradish peroxidase on the photocurrent of PPV/pathogens electrodes was not significant. As a result, other negatively charged species had no effect on the pathogens detection.

In conclusion, a simple and sensitive PEC sensor for pathogens detection was designed based on PPV without any signal amplification. PPV has excellent photoelectric conversion performance and can bind with pathogens through electrostatic and hydrophobic interactions. PPV could be excited under 450 nm light irradiation and transfer the electron to oxygen in electrolyte, generating strong photocurrent signal. After introducing pathogens, which generated steric hindrance and blocked the electron transfer, a signal-off PEC sensor was proposed. The pathogens have different size, so they exhibited different electron transfer resistance and lead to the photocurrent decline in different degree. According to the reduce degree, we could identify *E. coli*, *B. subtilis* and *C. albicans*. *E. coli* caused minimal decline, while *C. albicans* caused maximum decline. *B. subtilis* was between *E. coli* and *C. albicans*. We provided a novel and simple analysis strategy for pathogens identification. As all pathogens possess different volume, the method has broad prospective on expanding application to other pathogens.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: conjugated polymers · pathogenic microorganisms · PEC sensor · photocurrent density

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Xin Zhou, Pengbo Zhang, Fengting Lv, Libing Liu, Shu Wang**

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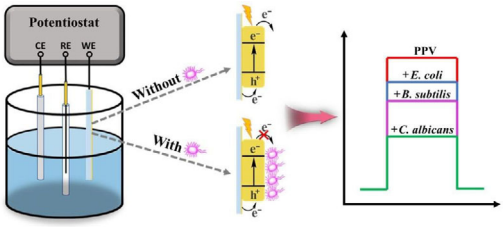

First in the PEC-ing order: A photoelectrochemical (PEC) biosensor for facile and sensitive identification of pathogenic microorganisms was developed. Cationic poly(phenylene vinylene) derivative (PPV) as photoelectrochemical active species was modified on the electrode. Under light irradiation, PPV could be excited and generate efficient photocurrent. PPV also had the ability to bind with negatively charged membrane of pathogenic microorganisms, which hindered the electron transfer between electrode and electrolyte. As a result, the photocurrent would decrease obviously. For *E. coli*, *B. subtilis* and *C. albicans*, the photocurrent density was reduced by 18, 33, and 59%, respectively. Based on the reduction degree of the photocurrent, a discrimination of pathogenic microorganisms was realized.

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