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Vancomycin-conjugated polythiophene for the detection and imaging of Gram-positive bacteria†

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Bacterial infections can cause serious health problems. The rapid identification of bacteria plays a vital role in the treatment of bacterial infection at an early stage of the disease. In this work, an active polythiophene derivative containing reactive pentafluorophenyl (PFP) ester pendant groups was prepared *via* Fe^{3+}-catalyzed oxidative polymerization. As far as we know, this is the first report of active polythiophene with reactive PFP ester moieties. The active polythiophene derivative was conjugated with vancomycin and α-methoxy-ω-amino poly(ethylene glycol) (mPEG-NH_2) *via* a reactive ester–amine reaction, resulting in the formation of water-soluble and fluorescent vancomycin-containing polythiophene (PTPVan). Since vancomycin can selectively interact with Gram-positive bacteria and kill them, the antibacterial properties of PTPVan were evaluated. The detection of Gram-positive bacteria was carried out by observing the color change and the fluorescence response of bacteria upon incubation with PTPVan through the naked eye and a fluorescence spectroscope, respectively. The staining of Gram-positive bacteria was observed using a confocal laser scanning microscope (CLSM).

Introduction

Bacterial infections are some of the most serious health problems and have led to the increased morbidity, mortality and cost of health care for patients.[1,2] In particular, infections resulting from Gram-positive bacteria can cause a variety of serious diseases including sepsis, bacteremia, pneumonia, osteomyelitis and endocarditis.[3,4] Bacterial identifications play vital roles in clinical diagnosis to determine the origins of infections and provide information for the selection of appropriate courses of treatment. Conventional methods are mainly based on recovering and culturing of bacteria from patients.[5–7] Advanced detection methods, including the polymerase chain reaction (PCR), NMR spectrometry, surface plasmon resonance (SPR) and immunoassays,[5,8–11] are also employed to detect the pathogenic bacteria. Although these detection technologies can meet the requirements for clinical diagnosis and have desirable sensitivity and selectivity, they are time-consuming or require sophisticated equipment.[12–16] Thus, it is still interesting to develop rapid and simple methods for the identification of microbes, especially Gram-positive bacteria.

Staining that can differentiate bacteria by the naked eye or fluorescence techniques is a powerful tool for bacterial detection.[17–22] Conjugated polymers are promising candidates for biological optical imaging, due to their reduced cytotoxicity and improved photostability in comparison to small molecular dyes.[23–27] Functional moieties on the side chains of conjugated polymers play an important role as they are indispensable for the conjugation of bioactive molecules.[28] In addition, the post-functionalization step is critical to impart water solubility/dispersibility to the conjugated polymers. The reactive pentafluorophenyl (PFP) ester-based ester–amine reaction is an efficient and versatile method for the post-functionalization of polymers, substrates, particles and graphene sheets.[29–35] The introduction of reactive PFP esters on the side chains of the conjugated polymer could offer an elegant approach for attaching bioactive molecules and improving its water solubility/dispersibility.

Herein, PFP thiophene-3-acetate (PFPTA) was synthesized and polymerized using $FeCl_3$ as an oxidant. As far as we know, this is the first report of an active thiophene monomer and polymer with PFP ester moieties. The resulting poly(PFPTA) (PPFPTA) polymer was then conjugated with vancomycin and α-methoxy-ω-amino poly(ethylene glycol) (mPEG-NH_2) *via* a reactive ester–amine reaction, leading to the formation of vancomycin-conjugated polythiophene (PTPVan) (Scheme 1). Vancomycin is a glycopeptide antibiotic that can bind to D-alanyl–D-alanine (D-Ala–D-Ala) moieties on the cell wall of Gram-positive bacteria.[4,11,36–44] This binding could interfere the transglycosylase step of peptidoglycan biosynthesis, lower the rigidity of the cell wall, and ultimately cause bacterial death.[3,45,46] PEG is a non-toxic and non-immunogenic polymer, and PEGylation could increase the solubility and stability of conjugated polymers under physiological conditions.[47,48] The attachment of vancomycin and PEG to the side

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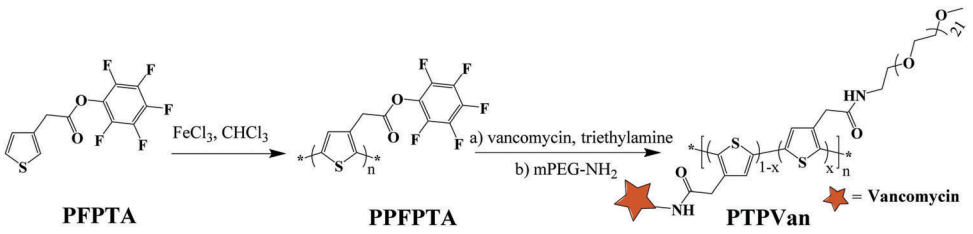

Scheme 1 Synthetic route of PPFPTA and PTPVan.

chains of polythiophene could endow it with good solubility as well as selective interaction with Gram-positive bacteria. The antibacterial properties of PTPVan against both Gram-positive and Gram-negative bacteria were studied. The identification of Gram-positive bacteria was carried out by staining the bacteria with PTPVan and observing the phenomenon with the naked eye, a fluorescence spectroscope and a confocal laser scanning microscope (CLSM).

Experimental section

Materials

Pentafluorophenyl trifluoroacetate (98%) and 3-thiopheneacetic acid (98%) were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). Methoxypolyethylene glycol amine (mPEG-amine, M.W. = 1000) was purchased from Alfa Aesar Co. Ltd (Tianjin, China). Vancomycin hydrochloride (USP grade) was purchased from Aladdin Reagent Co. (Shanghai, China). *Escherichia coli* (*E. coli*, ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), *Staphylococcus aureus* (*S. aureus*, ATCC 25923 and 43300), methicillin-resistant *S. aureus* (MRSA, ATCC 33592) and *Staphylococcus epidermidis* (*S. epidermidis*, ATCC 12228) were obtained from the American Type Culture Collection. All other regents were purchased from J&K Scientific or Aladdin Reagent Co., and were used without further purification.

Synthesis of PFPTA

Tetrahydrofuran (150 mL) and 3-thiopheneacetic acid (5.0 g, 35.2 mmol) were added to a 250 mL round bottom flask. The solution was cooled to 0 °C in an ice bath, followed by the successive addition of triethylamine (19.6 mL, 140.6 mmol) and pentafluorophenyl trifluoroacetate (12.1 mL, 70.4 mmol). After that, the reaction mixture was stirred at room temperature for 1.5 h. The solvent was removed by rotary evaporation, and the residue was diluted with 200 mL of ethyl acetate. The solution was washed with 200 mL of saturated $NaHCO_3$ aqueous solution twice. The separated organic phase was dried with anhydrous Na_2SO_4 overnight, and the solvent was removed by rotary evaporation. The obtained crude product was purified by column chromatography using ethyl acetate and hexane (v/v = 1 : 10) as eluents. ^{1}H NMR (400 MHz, $CDCl_3$-*d*, δ, ppm): 7.34 (thiophene–H, 1H), 7.25 (thiophene–H, 1H), 7.10 (thiophene–H, 1H) and 4.00 (thiophene–CH_2–, 2H). ESI-MS: *m*/*z* 309.29 ([M + H$^+$], calcd 309.23).

Preparation of poly(PFPTA) (PPFPTA)

Dry $FeCl_3$ (1.94 g, 12.0 mmol) and anhydrous chloroform (30 mL) were added to a 100 mL three-neck round bottom flask. The mixture was stirred at room temperature under an argon atmosphere for 30 min, giving a dark brown solution. To this suspension, PFPTA (0.92 g, 3.0 mmol) dissolved in anhydrous chloroform (10 mL) was added dropwise within 15 min. After stirring at room temperature under argon for 24 h, the solution was poured into 500 mL of methanol. The precipitate was collected by filtration, soxhlet extracted with methanol for 3 days and dried under reduced pressure for 24 h.

Preparation of vancomycin-conjugated polythiophene (PTPVan)

PPFPTA (50.0 mg, 0.16 mmol of repeat units) was dissolved in 20 mL of *N*,*N*-dimethylformamide. The mixture was stirred at 50 °C for 30 min, followed by the successive addition of vancomycin hydrochloride (89.1 mg, 0.06 mmol) and triethylamine (22.3 μL, 0.16 mmol). After stirring at 50 °C for 24 h, mPEG-amine (160 mg, 0.16 mmol) in 5 mL of deionized water was added dropwise to the solution through a syringe needle within 30 min. The reaction mixture was stirred at 50 °C for another 24 h, followed by the dialysis with deionized water (molecular weight cutoff = 5000 Da) for 3 days. The solution was further passed through a 0.22 μm filter membrane and lyophilized. The resulting yellow brown powder was referred to as PTPVan1. The PTPVan sample prepared by the addition of vancomycin hydrochloride (133.7 mg, 0.09 mmol) was referred to as PTPVan2.

Determination of the antimicrobial properties of PTPVan

E. coli, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were cultured according to the ATCC protocols/specifications. The bacterial suspensions were then dispersed in phosphate-buffered saline (PBS, pH 7.4) solution to give rise to a final concentration of 1×10^5 bacterial cells per mL. Stock solution of PTPVan1 (or PTPVan2) was prepared in PBS solution at concentrations of 0.5 and 1.0 mg mL^{-1}. The bacterial suspension was then mixed with the PTPVan stock solution at a volume ratio of 1 : 2. After culturing at 37 °C for 2 h, 100 μL of the solution was introduced into 2 mL of tryptic soy broth (TSB). The mixture was vortexed for 10 s and 100 μL of the mixture was added to each well of a 96-well plate. The plate was incubated at 37 °C, and the time-dependent optical density (OD) of each well at 600 nm was recorded on a microplate reader (BioTek Instruments ELX800, USA).

Controlled experiment was carried out by using PBS solution to culture with the bacteria for 2 h.

Conjugation of PTPVan with bacteria measured by fluorescence spectroscopy

The bacteria were suspended in PBS solution to reach a concentration of 1×10^9 bacterial cells per mL. Four hundred microliter of the PTPVan1 stock solution (1 mg mL^{-1}) was mixed with 800 μL of the bacterial suspension. After culturing at 37 °C for 30 min, the bacterial suspension was centrifuged at 11 000 rpm for 5 min. The bacterial cakes were re-dispersed in 1 mL of PBS solution and the bacterial suspension was centrifuged again. Three hundred microliter of Buffer BS (TaKaRa Bio Inc., Catalog No. 9763-4) and 50 μL of lysozyme solution (TaKaRa Bio Inc., Catalog No. 9763-2) were added to the bacterial cake in a 2 mL PCR tube. The mixture was vortexed and incubated at 37 °C for 1 h. During incubation, the PCR tube was taken out from the water bath and vortexed for 10 s after every 20 min. After lysis, the sample was further sonicated for 10 min and measured using a fluorescence spectrophotometer *via* excitation at 458 nm. Controlled experiment was carried out using PBS solution to culture with the bacteria for 30 min.

Conjugation of PTPVan with bacteria measured by confocal laser scanning microscopy (CLSM)

The bacteria were dispersed in PBS solution to achieve a concentration of 1×10^7 bacterial cells per mL, and 1.5 mL of the bacterial suspension was added into a glass bottom cell culture dish (Titan Technology, Catalog No. 02036560) at 37 °C for 4 h. The solution was then aspirated and replaced with 0.5 mL of the PTPVan2 stock solution (1 mg mL^{-1}). The cells were cultured for 30 min, and then washed with deionized water exhaustively to remove the free and loosely attached PTPVan2. The bacteria were then observed on a LSM800 confocal microscope (Carl Zeiss, Germany). Controlled experiment was carried out by using PBS solution to culture with the bacteria for 30 min.

Characterization

The chemical structures were characterized using ^{1}H NMR spectroscopy on a Bruker DRX 400 MHz spectrometer. The FT-IR spectroscopy measurements were carried out on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. The UV-visible absorption spectra were obtained from a Shimadzu UV-2550 spectrophotometer. The fluorescence spectra were measured on a Shimadzu RF-5301PC spectrophotometer. Gel permeation chromatography (GPC) was performed on a Waters GPC system, equipped with a Waters 1515 isocratic HPLC pump, a Waters 717 plus Autosampler injector and a Waters 2414 refractive index detector, using tetrahydrofuran as an eluent at 35 °C and at a flow rate of 1.0 mL min^{-1}.

Results and discussion

PFPTA was polymerized by chemical oxidation with anhydrous $FeCl_3$ in chloroform, resulting in the formation of an active polythiophene derivative with reactive PFP ester groups (PPFPTA). The crude product was purified by soxhlet extraction. The as-obtained PPFPTA has a number-average molecular weight (M_n) of 23 000 g mol^{-1} and a polydispersity index (PDI) of 1.68. The degree of polymerization of PPFPTA is about 74. PPFPTA was reacted with vancomycin and mPEG-NH_2 *via* a reactive ester–amine reaction in the presence of triethylamine. The FT-IR spectrum of PPFPTA (Fig. 1a) exhibits two absorption bands at 1785 cm^{-1} and 1514 cm^{-1}, attributable to the stretches of the activated carbonyl (C═O) group and aromatic fluorine, respectively.[49] After substitution with vancomycin and PEG, the strong absorption bands at 1785 cm^{-1} and 1514 cm^{-1} are barely discernible and the amide C═O stretch is appeared in the FT-IR spectra of PTPVan1 and PTPVan2 (Fig. 1b and c). This result indicates that the reactive PFP ester moieties have been successfully converted into amide groups. Fig. S3 (ESI†) shows the ^{1}H NMR spectra of PTPVan1 and PTPVan2 in D_2O. The appearance of characteristic proton signals of PEG and vancomycin indicates that this post-functionalization step has been successfully carried out. The molar ratios of PEG to vancomycin are calculated based on the integrated area ratios of methylene protons in PEG and methyl (CH_3–CH(R)–) protons in vancomycin. The PEG to vancomycin molar ratios are determined to be 2.1 : 1 and 1.4 : 1, respectively, for PTPVan1 and PTPVan2. Since the degree of polymerization of PPFPTA is about 74, the respective degrees of substitution of vancomycin in PTPVan1 and PTPVan2 are around 23 and 30.

The presence of vancomycin in the side chains of polythiophene could endow it with antimicrobial properties. This hypothesis was verified using the growth inhibition assay. PTPVan (or free vancomycin) and bacteria (*E. coli*, MRSA, *P. aeruginosa*, *S. aureus* or *S. epidermidis*) were cultured for 2 h, followed by mixing with TSB and measuring OD at 600 nm. The growth curves of *E. coli*, MRSA, *P. aeruginosa*, *S. aureus* and *S. epidermidis* treated with PTPVan1 and free vancomycin are shown in Fig. 2. Both Gram-negative and Gram-positive bacteria grow in similar manners but with differences in turbidity in the controlled experiments (incubated with PBS solution). The growths of Gram-negative bacteria (*E. coli* and *P. aeruginosa* strains) are not influenced in the presence of PTPVan1 and free vancomycin. Upon the addition of PTPVan1 (0.5 mg mL^{-1}), the growth rates of Gram-positive bacteria

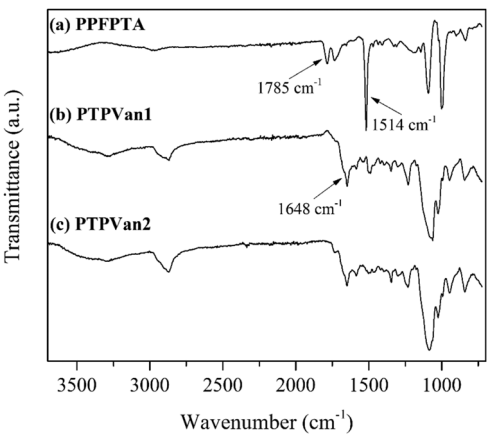

Fig. 1 FT-IR spectra of (a) PPFPTA, (b) PTPVan1 and (c) PTPVan2.

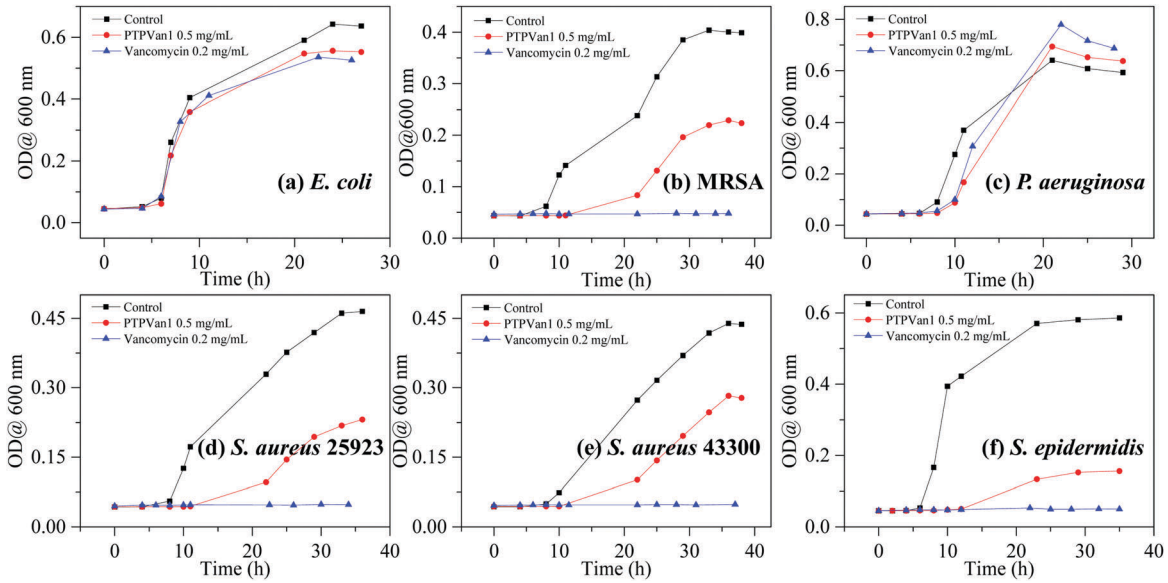

Fig. 2 Growth curves of (a) *E. coli*, (b) MRSA, (c) *P. aeruginosa*, (d) *S. aureus* 25923, (e) *S. aureus* 43300 and (f) *S. epidermidis* treated with PTPVan1 (0.5 mg mL^{-1}) and free vancomycin (0.2 mg mL^{-1}).

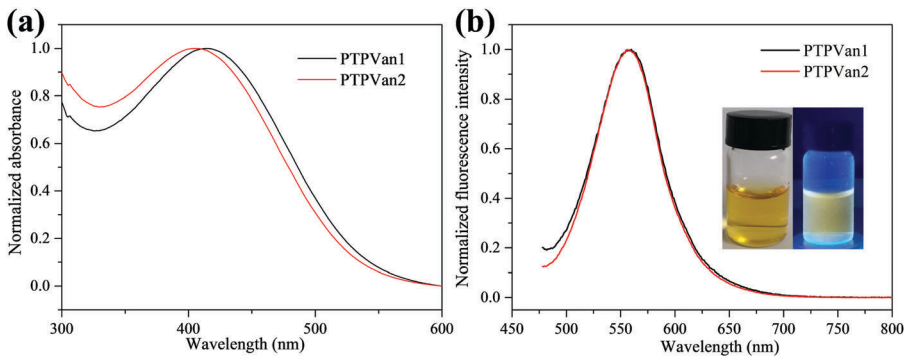

Fig. 3 (a) UV-visible absorption and (b) fluorescence spectra of PTPVan1 and PTPVan2 in deionized water. Inset: Photograph of PTPVan2 aqueous solution under ambient light (left) and a 365 nm UV lamp (right).

(MRSA, *S. aureus* and *S. epidermidis* strains) are affected. However, the growths of Gram-positive bacteria treated with free vancomycin (0.2 mg mL^{-1}) can be completely inhibited within the incubation time. These results indicate that vancomycin conjugated on the side chains of polythiophene *via* amidation has weaken its antibacterial activity against Gram-positive bacteria. Free vancomycin has MIC values of 250, >1000, 0.98, 0.98, 0.98 and 0.49 μg mL^{-1}, respectively, for *E. coli*, *P. aeruginosa*, MRSA, *S. aureus* 25923, *S. aureus* 43300 and *S. epidermidis* strains. Unfortunately, the MIC values of PTPVan against all of the bacterial strains are larger than 1000 μg mL^{-1}. The significantly reduced antibacterial properties of PTPVan suggest that they could not be used as antibacterial agents.

The optical properties of PTPVan were then investigated using UV-visible absorption and fluorescence spectroscopies. The UV-visible absorption spectra of PTPVan1 and PTPVan2 (Fig. 3a) in deionized water show absorption maxima at 403 and 413 nm, respectively. The absorption peak originates from the π–π* transition of the polythiophene backbone.[50,51] The photoluminescence (PL) spectra of PTPVan1 and PTPVan2 in

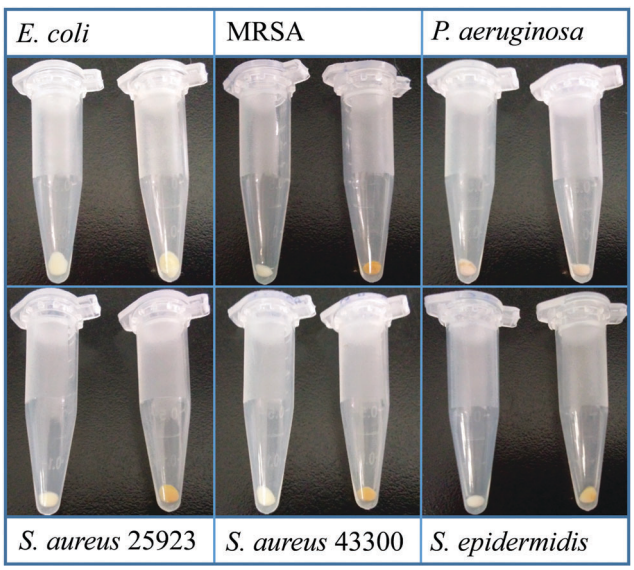

Fig. 4 Photographs of the blank (left) and stained (right) bacterial cakes after incubation with PBS and PTPVan1 solutions, respectively.

deionized water (Fig. 3b) exhibit emission peaks at about 558 nm with an excitation wavelength of 458 nm. Under 365 nm UV illumination, the PTPVan2 aqueous solution can emit fluorescence (inset of Fig. 3b). The fluorescence quantum yields of PTPVan1 and PTPVan2 in deionized water are determined to be 0.076 and 0.078, respectively, as measured using rhodamine 6G as the standard. These results suggest that the polythiophene derivatives still exhibit their intrinsic optical properties after the post-functionalization process and the fluorescent PTPVan can be used to detect bacteria *via* fluorescence techniques.

The identification of bacteria was carried out by staining the bacteria with PTPVan. After incubation with PTPVan1 for 30 min at 37 °C, the bacteria were centrifuged, washed with PBS solution and centrifuged again. The blank bacterial samples were obtained by incubation of the bacteria with PBS solution. The collected bacterial cakes are shown in Fig. 4. Except *P. aeruginosa*, all other blank bacteria cakes are white in color. After incubation with PTPVan1, the color of Gram-positive bacteria (MRSA, *S. aureus* 25923, *S. aureus* 43300 and *S. epidermidis* strains) changes to brown. However, there is no obvious color change for Gram-negative

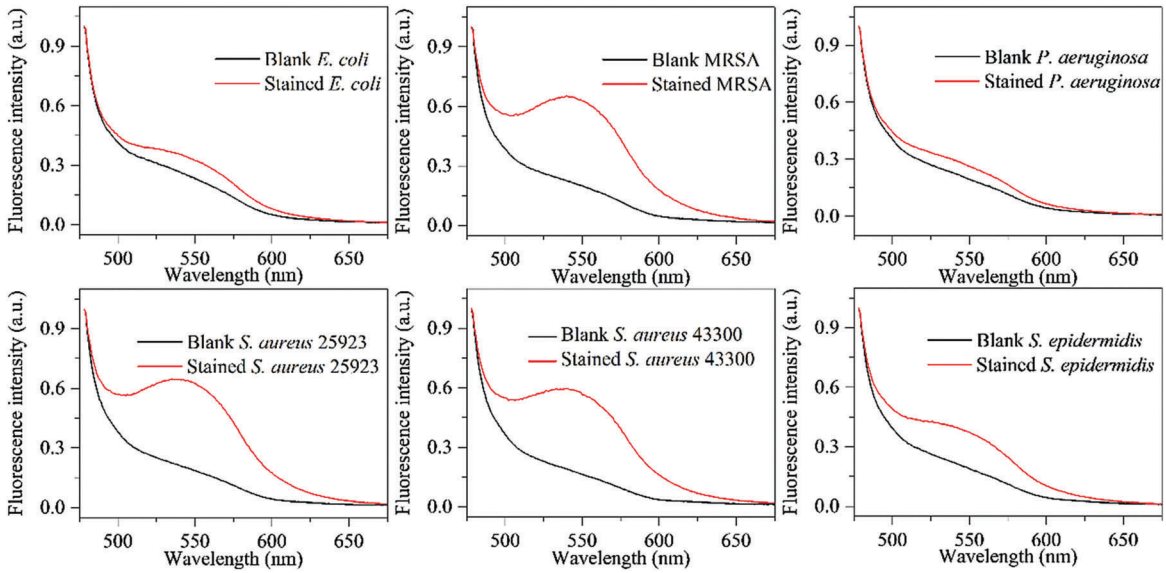

Fig. 5 Fluorescence spectra of blank and stained bacteria after lysis with lysosome *via* excitation at 458 nm.

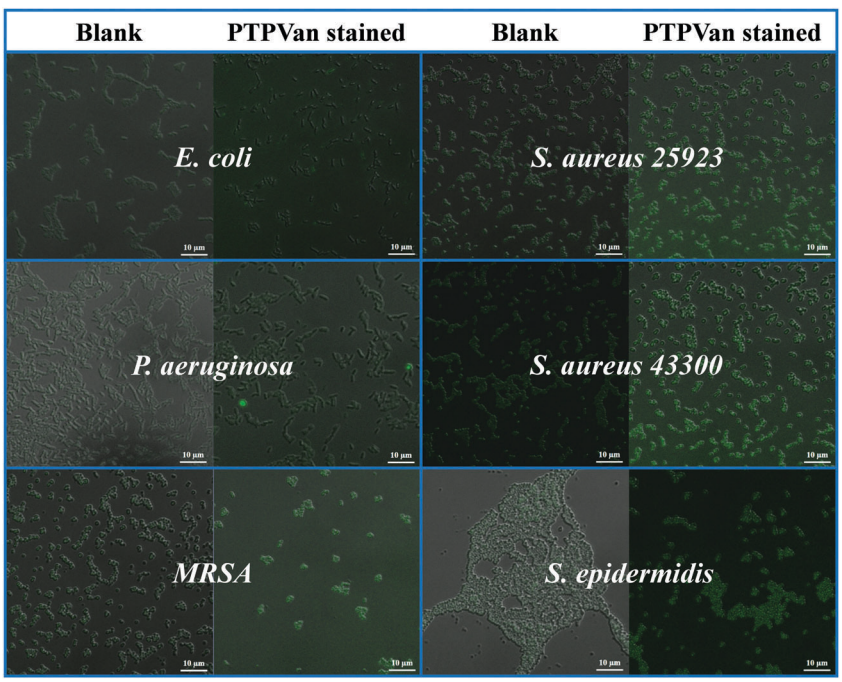

Fig. 6 Merged fluorescent and bright-field CLSM images of *E. coli*, MRSA, *P. aeruginosa*, *S. aureus* 25923, *S. aureus* 43300 and *S. epidermidis* before and after incubation with PTPVan2 for 30 min.

bacteria (*E. coli* and *P. aeruginosa* strains) after exposure to PTPVan1. Thus, Gram-positive bacteria could be distinguished through observing the color change of bacterial cakes by the naked eye.

To verify the feasibility of identification of the Gram-positive bacteria using fluorescence techniques, the PL spectra of bacteria before and after incubation with PTPVan were examined. Fig. 5 show the fluorescence responses of *E. coli*, MRSA, *P. aeruginosa*, *S. aureus* 25923, *S. aureus* 43300 and *S. epidermidis*. Upon incubation with PTPVan1, MRSA and *S. aureus* strains show significantly enhanced fluorescence intensities centred at about 535 nm, and *S. epidermidis* strain also shows increased fluorescence intensity. However, the Gram-negative bacteria (*E. coli* and *P. aeruginosa* strains) exhibit a negligible increase in the fluorescence intensity after interaction with PTPVan1. The increase in the fluorescence intensity of Gram-positive bacteria may be due to its binding capacity toward vancomycin moieties in the side chains of PTPVan1 and the subsequent enrichment of fluorescent polythiophene derivatives on the bacterial membranes. These results indicate that the fluorescence spectroscopy could be used to identify the Gram-positive bacteria with the aid of fluorescent targeting molecules.

A CLSM was also used to visualize and identify the Gram-positive bacteria. After incubation with PTPVan2 for 30 min, the bacteria were observed using the CLSM in the FITC channel under the excitation of 488 nm (Fig. 6). In comparison with the blank bacterial samples, the stained MRSA, *S. aureus* 25923, *S. aureus* 43300 and *S. epidermidis* exhibit enhanced fluorescence intensities. However, only a negligible fluorescence increase is observed on the Gram-negative bacteria (*E. coli* and *P. aeruginosa* strains) upon incubation with the PTPVan2 aqueous solution. These results suggest that there is a strong affinity between Gram-positive bacteria and vancomycin-functionalized PTPVan2 and the fluorescence image technique can be used to identify the Gram-positive bacteria in the presence of suitable fluorescent targets.

Conclusions

The active polythiophene derivative has been successfully conjugated with PEG and vancomycin *via* a reactive ester–amine reaction. The reactive ester–amine reaction offers an efficient method for the conjugation of vancomycin on the side chains of the conjugated polymer. This method can be easily extended to immobilize other bioactive molecules. The resulting vancomycin-conjugated polythiophene (PTPVan) is brown in color and can be used to distinguish Gram-positive bacteria by observing the color changes of centrifuged bacterial cakes. In addition, PTPVan is fluorescent and can be utilized to identify Gram-positive bacteria by measuring the fluorescence response or observing the fluorescent images of the stained bacterial samples. The combination of vancomycin and fluorescent polythiophene provides a simple and rapid approach for the detection of Gram-positive bacteria, and PTPVan has a potential application in the clinical diagnosis as a visual diagnostic material.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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References

1 M. van Oosten, T. Schäfer, J. A. C. Gazendam, K. Ohlsen, E. Tsompanidou, M. C. de Goffau, H. J. M. Harmsen, L. M. A. Crane, E. Lim, K. P. Francis, L. Cheung, M. Olive, V. Ntziachristos, J. M. van Dijl and G. M. van Dam, *Nat. Commun.*, 2013, **4**, 2584.
2 J. Zhou, G.-B. Qi and H. Wang, *J. Mater. Chem. B*, 2016, **4**, 4855–4861.
3 H. J. Chung, T. Reiner, G. Budin, C. Min, M. Liong, D. Issadore, H. Lee and R. Weissleder, *ACS Nano*, 2011, **5**, 8834–8841.
4 S. K. Choi, A. Myc, J. E. Silpe, M. Sumit, P. T. Wong, K. McCarthy, A. M. Desai, T. P. Thomas, A. Kotlyar, M. M. B. Holl, B. G. Orr and J. R. Baker, *ACS Nano*, 2013, **7**, 214–228.
5 V. Hoerr, L. Zbytnuik, C. Leger, P. P. C. Tam, P. Kubes and H. J. Vogel, *J. Proteome Res.*, 2012, **11**, 3231–3245.
6 H. Bai, H. Chen, R. Hu, M. Li, F. Lv, L. Liu and S. Wang, *ACS Appl. Mater. Interfaces*, 2016, **8**, 31550–31557.
7 Y. Wu, Q. Chen, Q. Li, H. Lu, X. Wu, J. Ma and H. Gao, *J. Mater. Chem. B*, 2016, **4**, 6350–6357.
8 A. A. Bergwerff and F. Van Knapen, *J. AOAC Int.*, 2006, **89**, 826–831.
9 S. Gebert, D. Siegel and N. Wellinghausen, *J. Infect.*, 2008, **57**, 307–316.
10 C. R. Bertozzi and M. D. Bednarski, *J. Am. Chem. Soc.*, 1992, **114**, 2242–2245.
11 Y. Wan, L. B. Zheng, Y. Sun and D. Zhang, *J. Mater. Chem. B*, 2014, **2**, 4818–4825.
12 A. J. Kell, G. Stewart, S. Ryan, R. Peytavi, M. Boissinot, A. Huletsky, M. G. Bergeron and B. Simard, *ACS Nano*, 2008, **2**, 1777–1788.
13 W. Kong, J. Xiong, H. Yue and Z. Fu, *Anal. Chem.*, 2015, **87**, 9864–9868.
14 Z. A. Islamy Mazrad, I. In, K.-D. Lee and S. Y. Park, *Biosens. Bioelectron.*, 2017, **89**, 1026–1033.
15 X. Yang, X. Zhou, M. Zhu and D. Xing, *Biosens. Bioelectron.*, 2017, **91**, 238–245.
16 M. Yu, H. Wang, F. Fu, L. Li, J. Li, G. Li, Y. Song, M. T. Swihart and E. Song, *Anal. Chem.*, 2017, **89**, 4085–4090.
17 Y. Wang, H. Chen, M. Li, R. Hu, F. Lv, L. Liu and S. Wang, *Polym. Chem.*, 2016, **7**, 6699–6702.

18 Y. Zhang, C. Li, Y. Fan, C. Wang, R. Yang, X. Liu and L. Zhou, *Nanoscale*, 2016, **8**, 19744–19753.
19 G. Feng, Y. Yuan, H. Fang, R. Zhang, B. Xing, G. Zhang, D. Zhang and B. Liu, *Chem. Commun.*, 2015, **51**, 12490–12493.
20 Y. Li, H. Yu, Y. Qian, J. Hu and S. Liu, *Adv. Mater.*, 2014, **26**, 6734–6741.
21 C. Zhu, Q. Yang, L. Liu, F. Lv, S. Li, G. Yang and S. Wang, *Adv. Mater.*, 2011, **23**, 4805–4810.
22 M. D. Disney, J. Zheng, T. M. Swager and P. H. Seeberger, *J. Am. Chem. Soc.*, 2004, **126**, 13343–13346.
23 C.-G. Qian, S. Zhu, P.-J. Feng, Y.-L. Chen, J.-C. Yu, X. Tang, Y. Liu and Q.-D. Shen, *ACS Appl. Mater. Interfaces*, 2015, **7**, 18581–18589.
24 G. Feng, D. Ding and B. Liu, *Nanoscale*, 2012, **4**, 6150–6165.
25 H. Xu, L. Wang and C. Fan, *Functional Nanoparticles for Bioanalysis, Nanomedicine, and Bioelectronic Devices Volume 1*, American Chemical Society, 2012, vol. 1112, pp. 81–117.
26 H. Yuan, Z. Liu, L. Liu, F. Lv, Y. Wang and S. Wang, *Adv. Mater.*, 2014, **26**, 4333–4338.
27 X. Zhang, Q. Zhao, Y. Li, X. Duan and Y. Tang, *Anal. Chem.*, 2017, **89**, 5503–5510.
28 M. Peters, N. Zaquen, L. D'Olieslaeger, H. Bové, D. Vanderzande, N. Hellings, T. Junkers and A. Ethirajan, *Biomacromolecules*, 2016, **17**, 2562–2571.
29 J. Lawrence and T. Emrick, *ACS Appl. Mater. Interfaces*, 2016, **8**, 2393–2398.
30 H. Jo, N. Haberkorn, J.-A. Pan, M. Vakili, K. Nielsch and P. Theato, *Langmuir*, 2016, **32**, 6437–6444.
31 S. Lin, A. Das and P. Theato, *Polym. Chem.*, 2017, **8**, 1206–1216.
32 K. A. Gunay and H.-A. Klok, *Polym. Chem.*, 2016, **7**, 970–978.
33 R. M. Arnold, C. D. McNitt, V. V. Popik and J. Locklin, *Chem. Commun.*, 2014, **50**, 5307–5309.
34 L. Q. Xu, B. Zhang, Y. Chen, K.-G. Neoh, E.-T. Kang and G. D. Fu, *Macromol. Rapid Commun.*, 2013, **34**, 234–238.
35 A. Das and P. Theato, *Chem. Rev.*, 2016, **116**, 1434–1495.
36 L.-L. Li, H.-L. Ma, G.-B. Qi, D. Zhang, F. Yu, Z. Hu and H. Wang, *Adv. Mater.*, 2016, **28**, 254–262.
37 J. Rao, J. Lahiri, L. Isaacs, R. M. Weis and G. M. Whitesides, *Science*, 1998, **280**, 708–711.
38 D. Zhong, Y. Zhuo, Y. Feng and X. Yang, *Biosens. Bioelectron.*, 2015, **74**, 546–553.
39 B. Xing, T. Jiang, W. Bi, Y. Yang, L. Li, M. Ma, C.-K. Chang, B. Xu and E. K. L. Yeow, *Chem. Commun.*, 2011, **47**, 1601–1603.
40 G. Qi, L. Li, F. Yu and H. Wang, *ACS Appl. Mater. Interfaces*, 2013, **5**, 10874–10881.
41 C. Ren, H. Wang, X. Zhang, D. Ding, L. Wang and Z. Yang, *Chem. Commun.*, 2014, **50**, 3473–3475.
42 C. Yang, C. Ren, J. Zhou, J. Liu, Y. Zhang, F. Huang, D. Ding, B. Xu and J. Liu, *Angew. Chem., Int. Ed.*, 2017, **56**, 2356–2360.
43 J. C. Sun, J. J. Li, H. Fan and S. Y. Ai, *J. Mater. Chem. B*, 2013, **1**, 5436–5442.
44 Y. A. Nor, H. W. Zhang, S. Purwajanti, H. Song, A. K. Meka, Y. Wang, N. Mitter, D. Mahony and C. Z. Yu, *J. Mater. Chem. B*, 2016, **4**, 7014–7021.
45 V. L. Healy, I. A. D. Lessard, D. I. Roper, J. R. Knox and C. T. Walsh, *Chem. Biol.*, 2000, **7**, 109–119.
46 J. F. Fisher and S. Mobashery, *J. Med. Chem.*, 2010, **53**, 4813–4829.
47 F. M. Veronese and A. Mero, *BioDrugs*, 2008, **22**, 315–329.
48 J. Liu, D. Ding, J. Geng and B. Liu, *Polym. Chem.*, 2012, **3**, 1567–1575.
49 L. Q. Xu, H. Jiang, K. G. Neoh, E. T. Kang and G. D. Fu, *Polym. Chem.*, 2012, **3**, 920–927.
50 M. Shao, Y. He, K. Hong, C. M. Rouleau, D. B. Geohegan and K. Xiao, *Polym. Chem.*, 2013, **4**, 5270–5274.
51 S. S. Balamurugan, G. B. Bantchev, Y. Yang and R. L. McCarley, *Angew. Chem.*, 2005, **117**, 4950–4954.