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Polypyrrole Actuator with a Bioadhesive Surface for Accumulating Bacteria from Physiological Media

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ABSTRACT A gold/polypyrrole (Au/PPy) bilayer actuator was fabricated by electrochemical deposition, and its surface was modified with a bioadhesive polymer, polydopamine. The actuator exhibited high performances of actuation in physiological media. Furthermore, the surface of the actuator is sticky in water and thus can seize bacteria from their aqueous solutions. Actuation greatly increased the efficiency of adhering bacteria on the actuator surface, and this technique provides a cheap and convenient approach for accumulating bacteria from physiological media.

KEYWORDS: bilayer actuator • bioadhesive surface • bacteria seizing • polypyrrole • polydopamine

INTRODUCTION

The accumulation of bacteria from physiological media is important for bacteria quantification, identification, and application (1). Typically, a bacterial culture may need complicated procedures and requires substantial investment in equipment and personnel training. The demand for quicker and possibly less accurate tests than a cell culture has generated interest in rapid bacterial screens and quantification. At present, various rapid-screen methods have been developed (2); however, most of them involve complicated operations and need expensive instruments. On the other hand, electrochemical soft actuators made of conducting polymers such as polypyrrole (PPy) and polyaniline have been studied extensively in recent years (3−12). Especially, PPy actuators can work in physiological media and, thus, have potential applications in cell biology and biomedicine (13−15). PPy actuators with nanotubular surfaces were tested to be adhesive to polystyrene nanoparticles (3). However, their ability of seizing particles with diameters on the micrometer scale is low (3). Furthermore, they have to be fabricated through a complicated template-guided growth procedure using expensive nanoporous membranes as the template (3, 4). Here, we report a convenient and cheap PPy actuator with a bioadhesive surface for efficiently accumulating bacteria with sizes of several micrometers from their aqueous solutions.

The adhesion forces of the usual adhesives were reduced dramatically or even lost in water. However, adhesive proteins secreted by mussel can be firmly coated on various wet surfaces (16−22). These proteins were found to be rich in 3,4-dihydroxy-L-phenylalanine (dopamine) moieties. A bioinspired adhesive surface also has been fabricated by simply immersing the substrate in a dilute aqueous solution of dopamine, and the pH of the solution was buffered to a typical value of the marine environment. In that case, a thin adherent polymer film, polydopamine, was coated on the substrate and its surface was reported to have adhesive properties in water (23). Therefore, we fabricated a Au/PPy bilayer actuator and modified its two surfaces by polydopamine to make the artificial "finger" sticky in water. This actuator exhibited high actuation performance. More interestingly, it is able to efficiently seize *Escherichia coli* bacterial cells from their culture media.

EXPERIMENTAL SECTION

Chemicals. Pyrrole (99%, Hongyu Chemical Co., Beijing, China) was used after distillation under reduced pressure. Dodecylbenzenesulfonic acid (DBSA; >99%, Acros), anhydrous lithium perchlorate (>99%, Jinke Institute of Fine Chemicals, Tianjin, China), sodium sulfite anhydrous (Beijing Chemical Factory, Beijing, China), potassium citrate (Beijing Chemical Factory, Beijing, China), Tris base (Beijing Chemical Factory, Beijing, China), chloroauric acid tetrahydrate (Sinopharm Chemical Reagent Co. Ltd.), and dopamine hydrochloride (99%, Alfa) were used as received.

Fabrication of the Au/PPy Bilayer Actuator. All of the electrosyntheses and electrochemical actuations were performed at room temperature in a one-compartment cell by the use of a model 273 potentiostat−galvanostat (EG&G Princeton Applied Research) under computer control. The working electrode was a conductive indium−tin oxide (ITO) glass sheet. The counter electrode was a stainless steel sheet (AISI 304), and it was placed 1.0 cm from the working electrode. All potentials were referred to a saturated calomel electrode (SCE) that was immersed directly in the solution. Electrolyte solution **1** was an aqueous solution of 0.02 mol L^{-1} chloroauric acid tetrahydrate, 1.2 mol L^{-1} sodium sulfite, and 0.25 mol L^{-1} potassium citrate; its pH value was 9. Electrolyte solution **2** was an aqueous solution containing 0.1 mol L^{-1} pyrrole and 0.1 mol L^{-1} DBSA.

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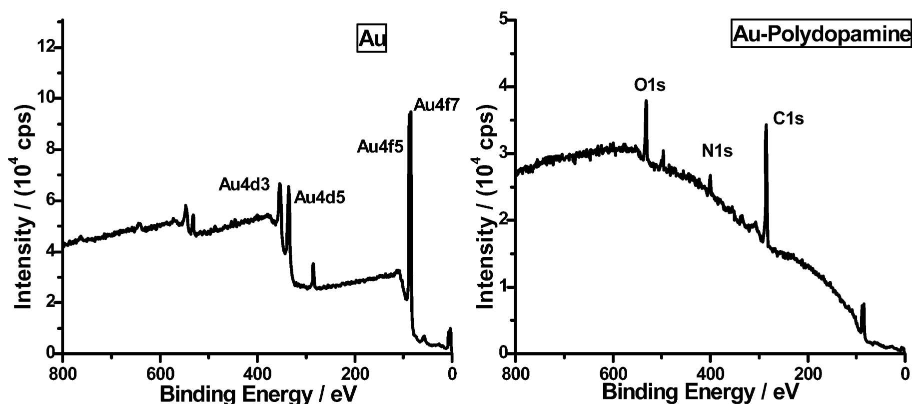

FIGURE 1. XPS spectra of the Au surface of a Au/PPy bilayer actuator before (left) and after (right) coating with a polydopamine film.

First, a thin Au layer was electrochemically deposited on the conductive surface of ITO glass by the direct reduction of 0.02 mol L^{-1} chloroauric acid in electrolyte solution **1** galvanostatically at 1 mA cm^{-2} for 1000 s. Successively, a PPy film doped by DBS^- anions, PPy(DBS), was deposited on the surface of the Au layer by the direct oxidation of 0.1 mol L^{-1} pyrrole in electrolyte solution **2** galvanostatically at 2 mA cm^{-2} for 600 s. Finally, the ITO electrode coated with the bilayer film was immersed in a 1 mol L^{-1} HCl aqueous solution for 8 h to destroy the conductive oxide layer of ITO. As a result, the Au/PPy bilayer film can be easily peeled off from the substrate electrode into a free-standing state. The thicknesses of the Au layer and PPy layer were measured by a scanning electron microscope to be 600 nm and 5 μm, respectively.

Chemical Deposition of Polydopamine Films on Actuator Surfaces. The deposition of polydopamine films followed the procedures reported by Messersmith's group (23). Dopamine (2 mg mL^{-1}) was dissolved in a 10 mmol L^{-1} Tris-HCl (pH 8.5) solution, and the Au/PPy bilayer actuator beams were vertically dipped into the solution for 6 h. pH-induced oxidation changed the solution color from transparent to dark brown. It should be noted here that a vertical sample orientation was necessary to prevent the nonspecific deposition of microparticles on surfaces. The as-modified surfaces were rinsed with ultrapure water and dried with N_2 gas before use. The polymer formed in this process is called "polydopamine" for simplification according to the literature (23), although its structure is still unclear. The repeating unit of this polymer is suggested to be 5,6-dihydroxyindole, which consists of dopamine—catechol residues.

Actuation of the Actuators. The cyclic voltammograms (CVs) of the actuators were carried out in an aqueous solution of 1% NaCl. The working electrode was a Au/PPy bilayer beam (20 × 3 mm); the counter electrode was a stainless steel sheet (AISI 304), and it was placed 1.0 cm from the working electrode. All potentials were referred to a SCE that was immersed directly in the solution.

Seizing Bacteria by Electrochemical Actuation. A model bacterium, *E. coli* DH5α, was cultured at 20 °C in an aqueous solution of 1% NaCl, 1% tryptone, and 0.5% yeast extract. Cell growth was monitored by optical density at 600 nm (OD_{600}) using a UV—visible spectrophotometer. The cell density was also evaluated from OD_{600}. A bilayer beam (20 × 3 mm) was immersed in the culture solution containing a certain amount of *E. coli* cells and actuated by cyclic voltammetric scanning. Other actuation conditions were the same as those described in the last section.

Characterizations. X-ray photoelectron spectroscopy (XPS) was performed on a PHI 550 EACA/SAM photoelectron spectrometer (Perkin-Elmer PHI) using Al Kα (1486.6 eV) radiation. Scanning electron microscopy (SEM) micrographs were recorded by using a Sirion-200 scanning electron microscope (FEI). The bacteria adhered to the actuator surface were fixed with 2.5% glutaraldehyde and sputtered with a thin layer of Au for SEM study.

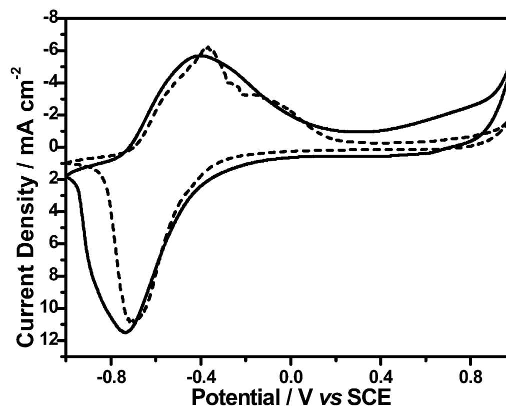

FIGURE 2. CVs of a Au/PPy bilayer actuator with (solid line) or without (dashed line) modification by polydopamine in an aqueous solution of 1% NaCl at a scan rate of 30 mV s^{-1}.

RESULTS AND DISCUSSION

XPS Analysis of the Au Surface of a Au/PPy Bilayer Actuator Modified by Polydopamine. A polydopamine film has been successfully coated on the surface of the actuator by chemical oxidation of dopamine, which was confirmed by XPS. The XPS spectra of the Au surface of a Au/PPy bilayer actuator before and after treatment with a dopamine solution are shown in Figure 1. By a comparison of these two spectra, it is clear that the characteristic XPS signals of the Au element (e.g., peaks at 84 and 88 eV, Au $4f^5$ and Au $4f^7$) for a pristine Au surface were completely suppressed after polydopamine coating (24). Instead, carbon (~285 eV), nitrogen (~399.5 eV), and oxygen (~532.5 eV) photoelectron peaks were observed. The disappearance of the signals specific to the Au element and the appearance of carbon, nitrogen, and oxygen peaks indicated the formation of a polydopamine coating thicker than 10 nm (23).

Bending Movements of the Actuator in a Physiological Solution. Figure 2 shows the stable CVs of an as-grown Au/PPy bilayer beam (20 × 3 mm) with or without polydopamine coating in a physiological solution of 1% NaCl at a potential scan rate of 30 mV s^{-1}. The CV of the bilayer actuator modified by polydopamine shows a couple of

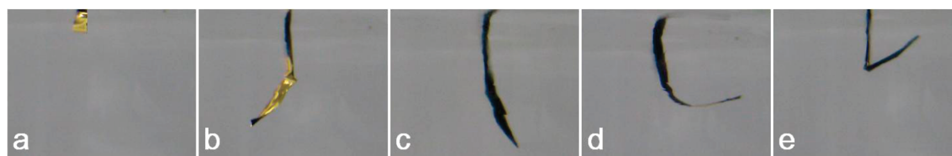

FIGURE 3. Optical images recorded during the processes of actuator bending in an aqueous solution of 1% NaCl at various potentials. The actuators were held with their Au side in the right: (a) oxidation at 0 V; (b) oxidation at −0.4 V; (c) original state; (d) reduction at −0.7 V; (d) reduction at −1.0 V.

strong and broad redox waves at approximately −0.40 (oxidation) and −0.70 V (reduction). They are attributed to the doping and dedoping of PPy. The CV of a polydopamine-modified Au foil (not shown here) also has a weak reduction wave related to dopamine−catechol residues at around −0.2 V (25), and it is probably overlapped with the oxidation wave of PPy.

On the other hand, the CV of the bilayer actuator with bare Au and PPy surfaces has a sharper reduction wave at around −0.65 V, while it has two oxidation waves at around −0.16 and −0.38 V. This is possibly due to the fact that PPy can be doped by both DBS^- (in the polymer matrix) and Cl^- (from the electrolyte) anions during the oxidation process. On the basis of the CV results, it is reasonable to conclude that the Au/PPy actuator kept the electroactivity of PPy after modification with polydopamine. Furthermore, the CVs of both actuators can be cycled repeatedly for over 100 cycles between the conducting (oxidized) and insulating (neutral) states without apparent decomposition, indicating a good electrochemical stability.

The as-grown PPy film doped with DBS^- anions is in the oxidized state, and therefore, it is a cation-driven (or cation-swelling) film. When the film was reduced, the large DBS anions (immobile counterions) could not be ejected from the film. For the first several cycles, in order to maintain the overall charge neutrality, the electrolyte cations (Na^+ ions) and water molecules from the electrolyte were incorporated into the film, and the film swelled (26, 27). After that, the Cl^- anions in the electrolyte could also participate in the ionic motion. Thus, the electrochemical process was dominated by cation driving, while being slightly affected by the incorporation of Cl^-. Thus, during the reduction process, the actuator bent to its Au side direction. On the other hand, when the film was oxidized, smaller cations were released from PPy matrix, which caused the polymer layer to contract and the actuator to bend to its polymer side direction. As shown in Figure 3, the actuator can bend close to 90° at a potential of −0.7 V, and it helically screwed for several cycles at lower potentials (e.g., −1.0 V), indicating that the equilibrium bending angles are much larger than 360°. During the oxidation process, the actuator bent to the opposite side, and it also screwed at higher potentials (e.g., 0 V).

Accumulating Bacterial Cells by Electrochemical Actuation. The surface of the polydopamine coating has a bioadhesive property in water. Thus, it is possible to apply the polydopmaine-modified Au/PPy bilayer actuator for seizing of bacterial cells from their culture medium. Parts a and b of Figure 4 show the SEM images of the Au surfaces of a Au/PPy bilayer actuator after and before polydopamine modification, respectively. The images indicate that both surfaces are relatively clean and compact. However, after

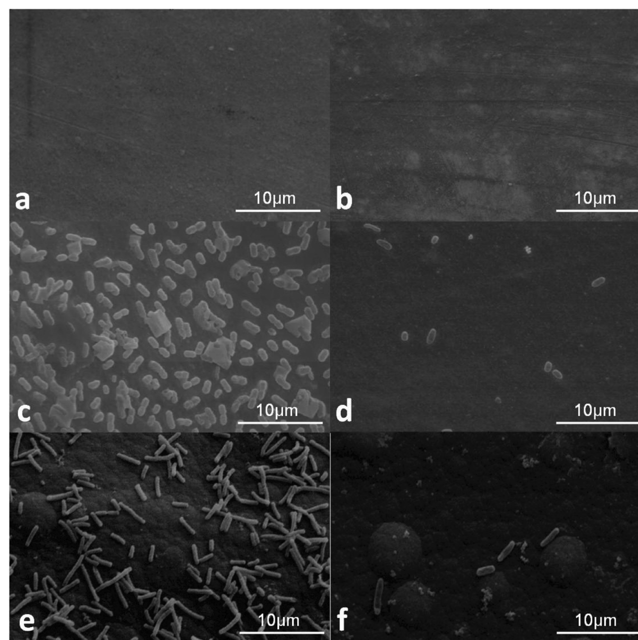

FIGURE 4. SEM images of the Au surface of a Au/PPy bilayer actuator modified with polydopamine before (a) and after actuation (c) and the bare Au surface without polydopamine coating before (b) and after actuation (d) and of the PPy surface of the actuator modified with polydopamine (e) and the bare PPy surface without polydopamine coating (f) after actuation in the bacterial culture solution. The solution contained 1×10^8 CFU mL^{-1} of *E. coli* DH5α, 1% NaCl, 1% tryptone, and 0.5% yeast extract, and the actuator was actuated by cyclic voltammetric scanning for 20 cycles in the potential range of −1.0 to +1.0 V (vs SCE) at a scan rate of 30 mV s^{-1}.

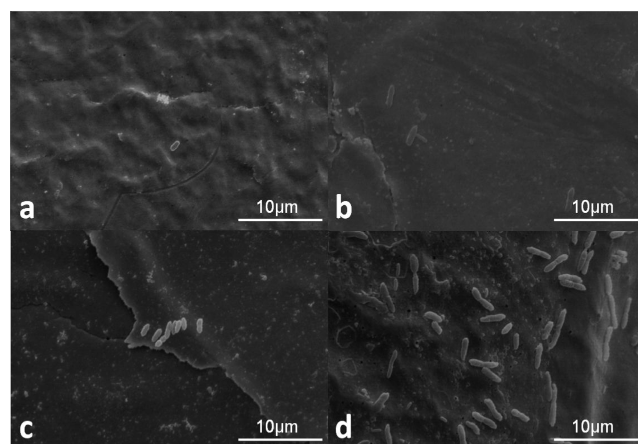

FIGURE 5. SEM images of the Au surface of the actuator modified with polydopamine after actuation in the bacterial culture with bacterial concentrations of 1×10^4 (a), 1×10^5 (b), 1×10^6 (c), and 1×10^7 (d) CFU mL^{-1} *E. coli* DH5α, respectively. The actuation conditions were the same as those of Figure 4.

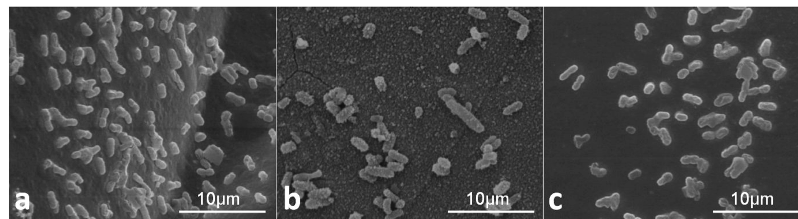

FIGURE 6. SEM images of the surface of the actuator with polydopamine coating after actuation (a) and dipping (b) in the bacterial culture with a bacterial concentration of 10^8 CFU mL^{-1} *E. coli* DH5α. The actuation conditions were the same as those of Figure 4. SEM image of a polydopamine-modified Au foil after immersion in the same bacterial solution and stirring for 10 min at a rate of 15 turns min^{-1} (c).

actuation in a bacterial culture solution containing 1×10^8 CFU (colony-forming unit) mL^{-1} of *E. coli* DH5α by cyclic voltammetric scanning for 20 cycles in the potential range of −1.0 to +1.0 V (vs SCE) at a scan rate of 30 mV s^{-1} and then rinsing by distilled water, many bacterial cells adhered to both the polydopamine-modified Au and PPy surfaces of the actuator. In a 100 μm^2 area, there were 30 ± 4 bacterial cells on the Au surface (Figure 4c; the standard deviations of average values were calculated from nine sets of data of three samples with three images each) and 31 ± 3 bacterial cells (Figure 4e) on the PPy surface. In comparison, only 2 ± 2 and 1 ± 1 bacterial cells were adhered to in a 100 μm^2 area on the corresponding bare Au (Figure 4d) and PPy surfaces (Figure 4f) of the actuator. These results indicate that the polydopamine-modified surfaces of the actuator are "sticky" (28−30); thus, they can efficiently seize bacteria such as *E. coli* from solutions. The capturing ability increased over 10 times after the surface of the bilayer actuator was modified with polydopamine. Because the bacterial capturing abilities of both surfaces of the Au/ PPy bilayer beam are almost the same as those shown in Figure 4, we focused on the modified Au surface in the following experiments for comparison.

The amount of the bacteria cells adhered to the actuator surface depends on the bacterial concentration, as shown in Figures 4c and 5. When the bacterial concentration was set as 1×10^8, 1×10^7, and 1×10^6 CFU mL^{-1}, the average numbers of bacteria captured on the actuator Au surface modified with polydopamine were measured to be 30 ± 4, 8 ± 2, and 2 ± 1 per 100 μm^2, respectively. When the bacteria concentration was changed to be lower than 1×10^5 CFU mL^{-1}, the average number of bacteria adhered to the actuator surface was tested to be less than 1 CFU mL^{-1} in an area of 100 μm^2. This is mainly due to the fact that a decease of the bacteria concentration reduced the possibility of bacterial contact with the actuator surface during the actuation process. However, if the surface area was enlarged to 600 μm^2 for counting of the bacteria, the average numbers of bacteria adhered to the actuator surface were measured to be 3 ± 1 and 1 ± 1 by using solutions containing 1×10^5 and 1×10^4 CFU mL^{-1} *E. coli* cells, respectively. Therefore, the low limit of detection of *E. coli* bacteria is about 1×10^5 CFU mL^{-1}. This value is close to that reported by using a multiplex fluorogenic polymerase chain reaction technique ($\geq 3.4 \times 10^4$) (31).

To further investigate the mechanism of accumulating bacteria on polydopamine-modified Au/PPy actuator surfaces, two control experiments were carried out. The first experiment compares actuation with simple dipping. Parts a and b of Figure 6 show the images of two actuators after capturing *E. coli* cells from the culture solution containing 10^8 CFU mL^{-1} of bacterial cells. Panel a was recorded after actuation of the actuator by cyclic voltammetric scanning for 20 cycles in the potential scale of +1 to −1 V at a scan rate of 30 mV s^{-1}, and panel b was recorded after just dipping the actuator in the bacterial solution. It is clear from this figure that, in a 100 μm^2 area, there are 30 ± 4 bacterial cells in panel a, while those in panel b are only 9 ± 3 bacterial cells. These results indicate that the repeated bending movements of the bilayer actuator with a bioadhesive surface driven by voltammetric cycling greatly improved its ability to seize or adhere bacterial cells compared to simple dipping. The second experiment compares the actuator with a polydopamine-modified Au foil. The Au foil was immersed in the same bacterial solution and mechanically stirred for 10 min at a rate of 15 turns min^{-1}. After this treatment, only 20 ± 3 per 100 μm^2 bacterial cells were observed on the surface of the Au foil (panel c). This value is much smaller than that using a Au/PPy actuator (30 ± 4). The explanation of this phenomenon is that the stirring effect of the actuator driven by cyclic voltammetric cycling is more sufficient and uniform throughout the bilayer beam. Furthermore, in the aqueous medium with a pH of around 7, the surface of the *E. coli* cells brings negative surface charges, so the bacterial cells can be electrostatically attracted and finally adhered to the surface of the actuator under positive potentials during cyclic voltammetric cycles.

CONCLUSIONS

In conclusion, the surface of the Au/PPy bilayer actuator was successively coated by a thin polydopamine film. The actuators kept their good actuation performances after modification. Furthermore, the surface of the polydopamine-modified actuator is sticky in water and can capture *E. coli* cells from their culture solution. Repeated bending movements driven by cyclic voltammetric scanning enhanced the bacterial seizing ability of the actuator. This work developed a convenient and cheap technique for accumulating bacterial cells from their aqueous solutions.

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REFERENCES AND NOTES

(1) Hug, T.; Gujer, W.; Siegrist, H. *Water Res.* **2005**, *39*, 3837.
(2) Leroy, C.; Delbarre-Ladrat, C.; Ghillebaert, F.; Rochet, M. J.; Compere, C.; Combes, D. *Lett. Appl. Microbiol.* **2007**, *44*, 372.
(3) He, X. M.; Li, C.; Chen, F. E.; Shi, G. Q. *Adv. Funct. Mater.* **2007**, *17*, 2911.
(4) Berdichevsky, Y.; Lo, Y.-H. *Adv. Mater.* **2006**, *18*, 122.
(5) Jager, E. W. H.; Smela, E.; Inganäs, O. *Science* **2000**, *290*, 1540.
(6) Williams, R. L.; Doherty, P. J. *J. Mater. Sci.: Mater. Med.* **1994**, *5*, 429.
(7) Schmidt, C. E.; Shastri, V. R.; Vacanti, J. P.; Langer, R. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8948.
(8) Wong, J. Y.; Langer, R.; Ingber, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3201.
(9) Aoki, T.; Tanino, M.; Sanui, K.; Ogata, N.; Kumakura, K. *Biomaterials* **1996**, *17*, 1971.
(10) John, R.; Spencer, M.; Wallace, G. G.; Smyth, M. R. *Anal. Chim. Acta* **1991**, *249*, 381.
(11) Pernaut, J.-M.; Reynolds, J. R. *J. Phys. Chem. B* **2000**, *104*, 4080.
(12) Smela, E. *Adv. Mater.* **2003**, *15*, 481.
(13) Xu, H.; Wang, C.; Wang, C. L.; Zoval, J.; Madou, M. *Biosens. Bioelectron.* **2006**, *21*, 2094.
(14) Immerstrand, C.; Holmgran-Peterson, K.; Magnusson, K.-E.; Jager, E. W. H.; Krogh, M.; Skoglund, M.; Selbing, A.; Inganäs, O. *MRS Bull.* 2002.
(15) Pelah, A.; Seemann, R.; Jovin, T. M. *J. Am. Chem. Soc.* **2007**, *129*, 468.
(16) Waite, J. H. *CHEMTECH* **1987**, *17*, 692.
(17) Waite, J. H. *Integr. Comp. Biol.* **2002**, *42*, 1172.
(18) Papov, V. V.; Diamond, T. V.; Biemann, K.; Waite, J. H. *J. Biol. Chem.* **1995**, *270*, 20183.
(19) Waite, J. H.; Qin, X. X. *Biochemistry* **2001**, *40*, 2887.
(20) Yu, M.; Deming, T. J. *Macromolecules* **1998**, *31*, 4739.
(21) Podsiadlo, P.; Liu, Z.; Paterson, D.; Messersmith, P. B.; Kotov, N. A. *Adv. Mater.* **2007**, *19*, 949.
(22) Lee, H. S.; Lee, B. P.; Messersmith, P. B. *Nature (London)* **2007**, *448*, 338.
(23) Lee, H. S.; Dellatore, M.; Miller, W. M.; Messersmith, P. B. *Science* **2007**, *318*, 426.
(24) He, Y. H.; Yuan, J. Y.; Su, F. Y.; Xing, X. H.; Shi, G. Q. *J. Phys. Chem. B* **2006**, *110*, 17813.
(25) Degrand, C.; Miller, L. L. *J. Am. Chem. Soc.* **1980**, *102* (18), 5728.
(26) He, X. M.; Shi, G. Q. *Sens. Actuators B* **2006**, *115*, 488.
(27) Gandhi, M. R.; Murray, P.; Spinks, G. M.; Wallace, G. G. *Synth. Met.* **1995**, *73*, 247.
(28) Lee, H.; Scherer, N. F.; Messersmith, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12999.
(29) Hwang, D. S.; Sim, B. S.; Cha, H. J. *Biomaterials* **2006**, *28*, 4039.
(30) Yu, M.; Hwang, J. H.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 5825.
(31) Ibekwe, A. M.; Watt, P. M.; Grieve, C. M.; Sharma, V. K.; Lyons, S. R. *Appl. Environ. Microbiol.* **2002**, *68*, 4853.

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