ANALYTICAL SCIENCES APRIL 2011, VOL. 27

A Glucose Sensor Fabricated by Piezoelectric Inkjet Printing of Conducting Polymers and Bienzymes

Yeon Hee YUN,*1 Byung Kook LEE,*1 Ji Suk CHOI,*1 Sungwon KIM,*2 Bongyoung YOO,*3 Yong Shin KIM,*4 Kinam PARK,*2 and Yong Woo CHO*1†

*1 *Department of Chemical Engineering and Department of Bionanotechnology, Hanyang University, Ansan, Gyeonggi-do 426-791, Republic of Korea*
*2 *Departments of Pharmaceutics and Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA*
*3 *Department of Materials Engineering, Hanyang University, Ansan, Gyeonggi-do 426-791, Republic of Korea*
*4 *Department of Applied Chemistry, Hanyang University, Ansan, Gyeonggi-do 426-791, Republic of Korea*

Piezoelectric inkjet printing of polymers and proteins holds great promise for fabrication of miniaturized bioelectronic devices, such as biochips and biosensors. In this study, a bienzymatic glucose biosensor prototype based on poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonic acid) (PEDOT-PSS), glucose oxidase (GOD), and horseradish peroxidase (HRP) was fabricated by a piezoelectric inkjet printer. An aqueous bioelectrical ink containing PEDOT-PSS, GOD, and HRP was prepared and printed on an indium-tin-oxide (ITO)-coated poly(ethylene terephthalate) (PET) film. The PEDOT-PSS/GOD/HRP sensor was covered with a cellulose acetate membrane. The use of bienzymatic sensing combined with conducting polymers *via* piezoelectric inkjet printing showed a synergistic effect resulting in significant amplification of the response signal. The glucose sensor reached steady-state current density within 3 s, indicating a fast response time, and exhibited a linear dose-dependent electrochemical response with high sensitivity. The overall result demonstrates that a glucose sensor with high sensitivity could be readily fabricated by a piezoelectric inkjet printing system.

(Received September 10, 2010; Accepted February 1, 2011; Published April 10, 2011)

Introduction

Biosensors are required to measure small amounts of physiological elements in complicated samples. For fabricating miniaturized, multifunctional devices, the technology should laminate several patterns in a small space, consume little solution, and ensure a short processing time. Several technologies are currently available, including inkjet printing, screen printing, and photolithography. They have the advantages of precision, speed, and low cost. A screen printer uses paste, which is forced through a mesh screen, to print an image on a surface. There is a resolution limitation when printing a small, precise device using screen printing because of the high viscosity of the paste. Current lithographical techniques have much better pattern accuracy and resolution than inkjet printing. Nonetheless, the simplicity, arbitrary geometries, low cost, and flexibility of inkjet printing make it highly attractive for fabrication of miniaturized biosensors. Two commonly used inkjet systems are thermal and piezoelectric. In both systems, an acoustic pulse ejects ink droplets through a nozzle. Recently, glucose sensors fabricated by thermal inkjet printing were reported.[1,2] In a thermal inkjet printer, a vapor bubble that ejects an ink droplet is formed by local heating at a temperature around 300°C. Piezoelectric inkjet printing has an advantage with respect to biological inks because the temperature inside and surrounding the nozzle can be easily controlled. The transformation of piezoelectric material causes volume variation and, therefore, generates an acoustic pulse. Any enzyme in water or other solvents can be used for ink material. Piezoelectric inkjet printing can eject a small droplet of only a few pico-liters. The ink viscosity is much lower than that of the screen paste. Polymers can be deposited in a desired location on any surface, such as film, glass, and metal. With the aid of computational topology design (CTD), inkjet printing of polymers can fabricate complex shapes with features in the micron ranges.[3-5] Indeed, inkjet printing is a powerful non-contact, non-destructive rapid prototyping technique. Inkjet printing has been extensively studied for fabrication of electronic devices, such as all-polymer transistors[6,7] and organic light emitting diodes (OLEDs).[4] Recently, inkjet printing has been applied to various biomedical fields, such as cell patterning, tissue engineering, biosensors and biochips.[8-11]

Glucose sensing is one of the most well-known biosensor applications and is an active area of research because of its importance for diagnosis and medical care of diabetes, which is the most common chronic disease, accounting for 10% of the world's population of sick people. Electrochemical glucose sensors have attracted continuous interest due to their intrinsic high sensitivity.[12-15] Typically, they are based on oxidase enzymes which generate hydrogen peroxide. Glucose

† To whom correspondence should be addressed.
E-mail: ywcho7@hanyang.ac.kr

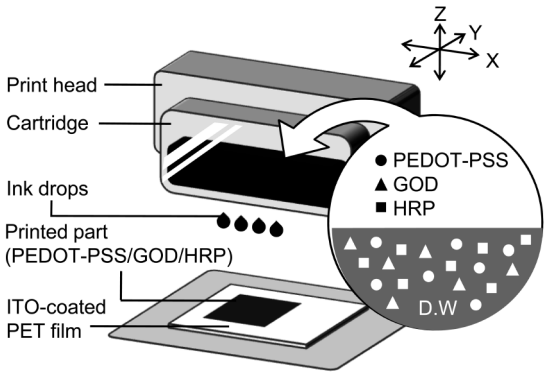

Fig. 1 Schematic representation of the piezoelectric inkjet printing system for fabrication of a bienzymatic glucose sensor prototype. Print head, 11 × 5 cm; cartridge, 8 × 6 cm.

Table 1 Components and basic properties of the bioelectrical ink

Ink component		Basic property (at 20°C)	
GOD	6.25 U/mL	Viscosity	4.6 cps
HRP	2.15 U/mL	Surface tension	71 mN/m
PEDOT-PSS	6.5 mg/mL	Contact angle	89°
Solvent	Water		

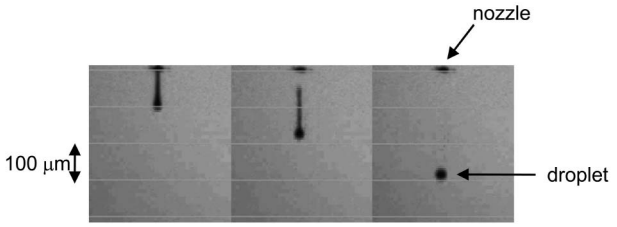

Fig. 2 Series of pictures showing ink droplet ejection from a nozzle of the piezoelectric inkjet printer.

quantification is generally performed by measuring a change in current, associated with the oxidation of hydrogen peroxide. Recently, bienzymatic (oxidase and peroxidase) glucose sensors have been developed, exhibiting improved characteristics.[16,17] In such a system, hydrogen peroxide is subsequently reduced by peroxidase.

Conducting polymers have attracted much interest not only as a suitable matrix for the entrapment of enzymes, but also as an efficient electron transfer medium. They are known to be compatible with biological enzymes in aqueous milieu and to have the ability to efficiently transfer electric charge produced by the electrochemical reaction to an electric circuit. Moreover, a conducting polymer can be deposited over defined areas of electrodes. The unique properties of conducting polymers have been exploited for the fabrication of electrochemical biosensors.[18]

In this study, by integrating the advantageous characteristics of piezoelectric inkjet printing, bienzymatic systems, and conducting polymers, a glucose sensor prototype was constructed. The bioelectrical ink containing glucose oxidase (GOD), horseradish peroxidase (HRP), and poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonic acid) (PEDOT-PSS) was inkjet-printed on an indium-tin-oxide (ITO) coated poly(ethylene terephthalate) (PET) film. Responses to different glucose concentrations were investigated by cyclic voltammetry and chronoamperometry.

Experimental

Materials

An aqueous dispersion of PEDOT-PSS (1.3 g/dL) was purchased from H. C. Stark GmbH (Goslar, Germany). A GOD/HRP reagent kit (Product Code G 3660), ITO-coated PET film (surface resistivity 35 Ω/cm^2), ferrocenemethanol (FcMeOH), glucose (dextrose anhydrous), cellulose acetate (CA, M_r 29000), were purchased from Sigma (St. Louis, MO). Other chemicals were of analytical grade and were used as received.

Bioelectrical ink preparation

Five hyndred units of GOD and 100 units of HRP were dissolved in 40 mL of deionized water. One milliliter of enzyme solution was mixed with 1 mL PEDOT-PSS dispersion in water. The mixture (PEDOT-PSS/GOD/HRP) was passed through a 0.2-μm syringe filter to remove dust. The bioelectrical ink was stored in a refrigerator and used within a week.

Glucose sensor fabrication

Printing was conducted on a piezoelectric drop-on-demand (DOD) inkjet printer (Dimatix Materials Printer DMP-2800, Dimatix Inc., Santa Clara, CA). The PEDOT-PSS/GOD/HRP ink was charged in a cartridge that has 16 nozzles linearly spaced at 254 μm with a typical drop volume of 10 pL. The temperature of the head and plate was set to 28°C. The firing voltage and frequency varied over a range of 25 – 40 V and 3 kHz, respectively. The inkjet-printed glucose sensors were dip-coated in a 3 g/dL cellulose acetate solution in THF/acetone (60/40, v/v).

Electrochemical measurements

Electrochemical measurements were performed with a VersaSTAT 3 potentiostat/galvanostat (Princeton Applied Research, Oak Ridge, TN) interfaced with a personal computer. A three electrode cell geometry was used in cyclic voltammetric and chronoamperometric experiments. The counter electrode was a Pt wire, the reference electrode was Ag/AgCl, and the printed glucose sensor was used as the working electrode. The responses of the working electrode were measured by cyclic voltammetry, dipping the electrode in 50 mL of a stirred PBS (0.1 M) in the presence of 1.08 mg FcMeOH (0.1 mM) at applied potentials ranging from –0.20 to +0.80 V, with a scan rate of 0.05 V/s. Before the addition of glucose solution, the background current was allowed to decay to a steady-state value. Chronoamperometry was conducted at an applied potential of +0.30 V.

Results and Discussion

Glucose sensor fabrication by piezoelectric inkjet printing

The essential parameters in bioelectrical ink formulation for piezoelectric inkjet printing are solvent, ink materials, and their composition. Ink materials are selected based on the function of the device. In this study, bienzymes, GOD and HRP were selected as functional ink materials for glucose sensing. PEDOT-PSS, a conducting polymer with excellent redox properties, was added to the bio-ink formulation as a matrix entrapping GOD and HRP. To maintain the enzymatic activity, water was used as a solvent for the bioelectrical ink. Figure 1

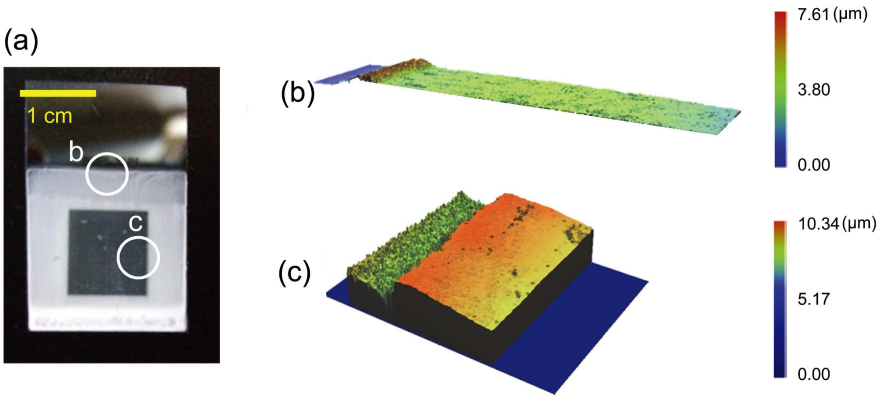

Fig. 3 (a) Macroscopic appearance of an inkjet-printed glucose sensor. (b), (c) Three-dimensional optical photographs taken by a surface profiler.

schematically represents a diagram of the present piezoelectric inkjet printing system. Table 1 shows components and physical properties of the bioelectrical ink. The inkjet printability is determined by a number of physical properties, such as viscosity, surface tension, and contact angle.[3,10,19,20] Ink viscosity should be below 20 cps, ideally 10 cps, in a piezoelectric inkjet printing system because the jetting force generated by an inkjet printer is limited.[21] The viscosity of PEDOT-PSS/GOD/HRP ink was 4.6 cps, low enough for inkjet printing. Surface tension is responsible for spherical liquid drops emerging from nozzles, which should be high enough to prevent dripping of the ink from the nozzle. The minimum surface tension is about 30 mN/m.[3,19] The surface tension of the bioelectrical ink was 71 mN/m, high enough for inkjet printing. The contact angle value of PEDOT-PSS/GOD/HRP ink on an ITO-coated PET film was 89°, indicating that the ink has appropriate wettability for the substrate. Drop ejection from a nozzle was monitored by a CCD camera equipped with an LED light, as shown in Fig. 2. The appropriate firing voltage and frequency were optimized to be 25 – 40 V and 3 kHz, respectively, through systematic studies under various conditions. Nozzles were maintained at 28°C.

In an aqueous environment, a partial dissolution of PEDOT-PSS/GOD/HRP may occur, making the device not suitable for measurements or practical use. Therefore, a water-resistant, selectively permeable membrane was used to cover the whole device, by means of dip-coating the inkjet printed area in a cellulose acetate solution. The cellulose acetate membrane formed a semi-permeable layer to allow the diffusion of FcMeOH into the printed glucose sensor and to keep the GOD and HRP inside the printed glucose sensor. A cellulose acetate membrane with adequate porosity has been widely used as a protective or selective coating material for a number of biological applications. The cellulose acetate coating was effective as a protective layer for GOD-based glucose sensors.[1,2,22]

Figure 3(a) shows a photograph of the glucose sensor fabricated by a piezoelectric inkjet printer. The dark square is the sensor, which was printed with the PEDOT-PSS/GOD/HRP ink and dip-coated with a cellulose acetate solution. Figure 3(b) shows the surface of the ITO-coated PET film, which was covered with a cellulose acetate membrane. The cellulose acetate-coated surface was quite homogeneous. Figure 3(c) shows the inkjet-printed PEDOT-PSS/GOD/HRP surface, which was also covered with a cellulose acetate membrane. The height difference between the two regions in Fig. 3(c) indicates that the thickness of the printed PEDOT-PSS/GOD/HRP is about 2.0 μm.

Electrochemical characterization

The substrate glucose and the natural co-substrate O_2 react with GOD, which produces gluconolacton and H_2O_2, where GOD_{ox} and GOD_{red} are the oxidized and reduced forms of GOD.[17,23]

$$\text{Glucose} + \text{GOD}_{ox} \longrightarrow \text{Gluconolacton} + \text{GOD}_{red} \quad (1)$$

$$\text{GOD}_{red} + \text{O}_2 \longrightarrow \text{GOD}_{ox} + \text{H}_2\text{O}_2 \quad (2)$$

The hydrogen peroxide then serves as substrate for HRP, which is, in turn, reduced by the redox mediator FcMeOH,[24] where HRP_{ox} and HRP_{red} are the oxidized and reduced forms of HRP.

$$\text{H}_2\text{O}_2 + \text{HRP}_{red} \longrightarrow 2\text{H}_2\text{O} + \text{HRP}_{ox} \quad (3)$$

$$\text{HRP}_{ox} + \text{FcMeOH} \longrightarrow \text{HRP}_{red} + 2[\text{FcMeOH}]^+ \quad (4)$$

Then the oxidized mediator $[FcMeOH]^+$ is electrochemically reduced at the electrode surface.

$$2[\text{FcMeOH}]^+ + 2e^- \longrightarrow 2\text{FcMeOH} \quad (5)$$

Two different glucose sensors were prepared, with or without PEDOT-PSS, and their electrochemical characteristics were investigated by cyclic voltammetry, as shown in Fig. 4. The cyclic voltammograms of the glucose sensors were obtained in a potential range of –0.2 to +0.8 V, in a range of glucose concentration of 0 to 1.55 mM, at a scan rate of 0.05 V s^{-1}. In a GOD/HRP printed sensor, FcMeOH serves as an electron mediator to shuttle electrons from the redox center of HRP to the working electrode. The anodic peak and the cathode peak appeared at about +0.50 V and +0.00 V, respectively (Fig. 4(a)). As can be clearly seen, the corresponding peak current density values increased steadily according to glucose concentration. The peak current densities at 1.55 mM glucose were around +9 and –11 μA/cm^2, respectively. PEDOT-PSS has a number of favorable electrochemical properties, including high electrical conductivity and low ionization potential, as well as direct and easy deposition on a sensor electrode.[25,26] Figure 4(b) shows that the PEDOT-PSS/GOD/HRP printed sensor exhibited better bioelectrical response to glucose concentration. The peak

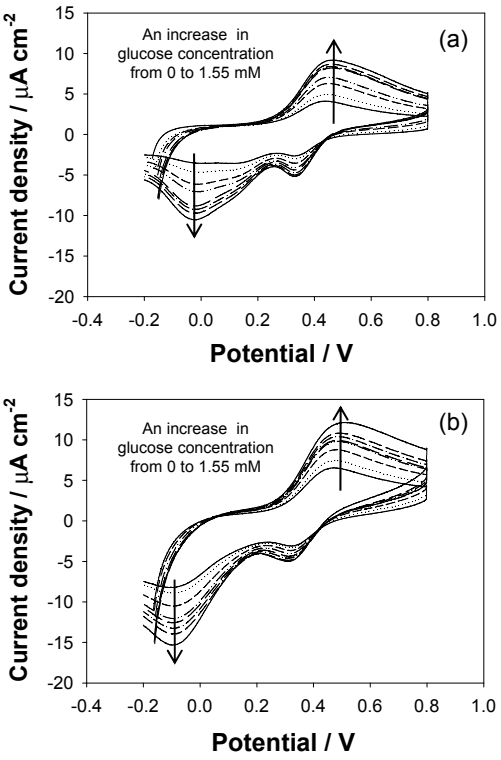

Fig. 4 Cyclic voltammograms of (a) GOD/HRP printed glucose sensor, (b) PEDOT-PSS/GOD/HRP printed glucose sensor. The glucose concentration increased from 0 to 1.55 mM (0, 0.16, 0.31, 0.59, 0.86, 1.10, 1.33, 1.55 mM) in PBS containing 0.1 mM FcMeOH. Scan rate: 0.05 V s^{-1}.

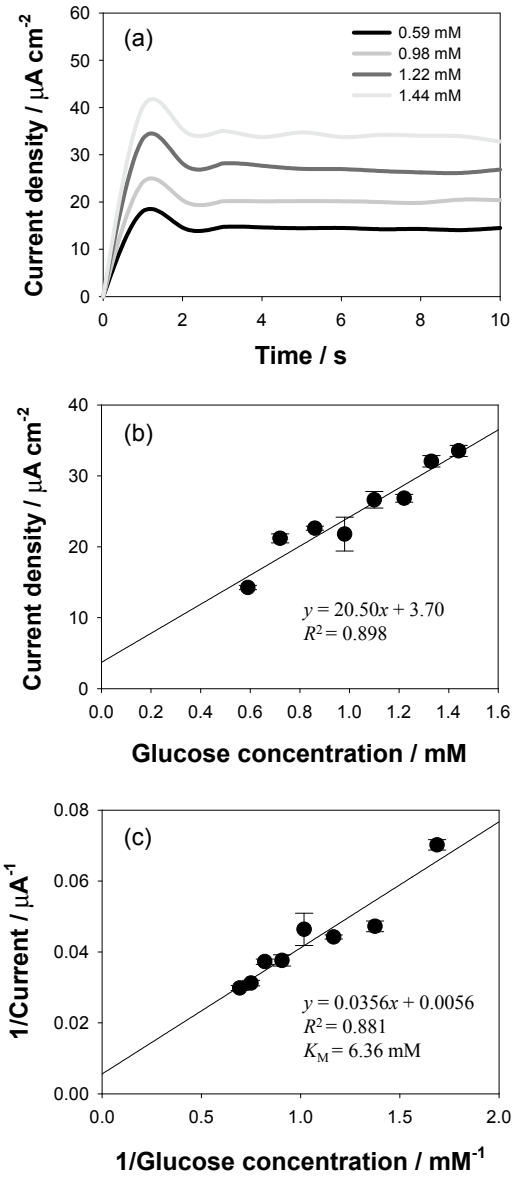

Fig. 5 (a) Chronoamperometric graphs of a PEDOT-PSS/GOD/HRP printed glucose sensor with the addition of glucose at the potential of +0.3 V in PBS. (b) Calibration curve. (c) Lineweaver–Burk plot.

current response to glucose was approximately 1.5 times more sensitive than the GOD/HRP printed sensor without the conducting polymer. These observations suggest that the PEDOT-PSS could facilitate electron transfer between the enzymes and the electrode surface. Second reduction peaks observed in both figures at the range of 0.3 to 0.4 V might represent the reduction reaction on pure ITO surface, because the printed PEDOT-PSS/GOD/HRP layers had porous structures allowing the penetration of electrolytes to contact the ITO directly.

Responses of the PEDOT-PSS/GOD/HRP printed sensor to glucose were studied by chronoamperometry. Figure 5(a) shows a typical response curve on injection of glucose to stirring PBS in the presence of FcMeOH at +0.30 V of applied potential. The PEDOT-PSS/GOD/HRP printed sensor responded rapidly to the addition of glucose. The sensor reached steady-state current density within 3 s. The calibration curve in Fig. 5(b) shows a linear range for the concentration of glucose from 0.59 M to 1.44 mM with a correlation coefficient (R^2) of 0.898. The detection limit was calculated to be 270 μM when the signal to noise ratio was 3. The sensitivity calculated from the calibration curve was 20.5 μA cm^{-2} mM^{-1}, which is an about 38-fold augmentation over the HRP-based, thermal inkjet printed glucose sensor (0.54 μA cm^{-2} mM^{-1}).[2] The higher sensitivity, compared to the glucose sensors fabricated by other methods,[1,27-29] may be attributed to the combination of the advantageous features of bienzymatic sensing and conducting polymers with piezoelectric inkjet printing. Bienzymatic glucose sensing systems have shown much higher sensitivity when compared to monoenzymatic ones.[16,17,30] Conducting polymers have improved the sensitivity of a wide variety of sensors due to their excellent electrical conductivity or charge transfer properties.[25] Piezoelectric inkjet printing, which can work at mild temperatures, has a critical advantage for fabrication of biosensors, because most biological macromolecules are highly susceptible to changes in environmental conditions, such as temperature. The apparent Michaelis–Menten constant (K_M) was calculated according to the method suggested in the literature.[30,31] The Lineweaver–Burk plot, the relationship between 1/current and 1/glucose concentration, is shown in Fig. 5(c). The K_M value of the PEDOT-PSS/GOD/HRP sensor was 6.36 mM. Because the Machaelis–Menten constant is inversely proportional to the affinity of the enzyme to the substrate, the low K_M value indicates that the PEDOT-PSS/GOD/HRP printed sensor possesses a biological affinity to glucose.

Conclusions

This study has explored the feasibility of piezoelectric inkjet printing for fabrication of bienzymatic glucose sensors. The aqueous bioink containing PEDOT-PSS, GOD, and HRP was prepared and printed on ITO-coated PET films. The inkjet processing variables were optimized through systematic studies under various conditions. The inkjet-printed glucose sensor responded quickly, within 3 s, and exhibited a linear dose-dependent electrochemical response with high sensitivity to the addition of glucose. Overall results imply that the glucose sensor with high sensitivity could be readily printed by a piezoelectric drop-on-demand (DOD) inkjet system. The methodology adopted in the present study could have broad utility in biosensors, particularly those requiring high-density integration and miniaturization.

Acknowledgements

This work was supported by the Engineering Research Center Program (Grant No. R11-2008-044-02001-0) and the Pioneer Research Center Program (Grant No. 2010-0002231) through the National Research Foundation (NRF), funded by the Ministry of Education, Science and Technology (MEST), Republic of Korea. This work was also supported by the Human Resources Development of the Korea Institute of Energy Technology Evaluation and Planning (Grant No. 20104010100620) grant funded by the Ministry of Knowledge Economy, Republic of Korea.

References

1. L. Setti, A. Fraleoni-Morgera, B. Ballarin, A. Filippini, D. Frascaro, and C. Piana, *Biosens. Bioelectron*., **2005**, *20*, 2019.
2. L. Setti, A. Fraleoni-Morgera, I. Mencarelli, A. Fillippini, B. Ballarin, and M. D. Biase, *Sens. Actuators, B*, **2007**, *126*, 252.
3. P. Calvert, *Chem. Mater*., **2001**, *13*, 3299.
4. B.-J. de Gans, P. C. Duineveld, and U. S. Schubert, *Adv. Mater*., **2004**, *16*, 203.
5. E. Tekin, P. J. Smith, and U. S. Schubert, *Soft Matter*, **2008**, *4*, 703.
6. C. J. Drury, C. M. J. Mutsaers, C. M. Hart, M. Matters, and D. M. de Leeuw, *Appl. Phys. Lett*., **1998**, *73*, 108.
7. T. Kawase, T. Shimoda, C. Newsome, H. Sirringhaus, and R. H. Friend, *Thin Solid Films*, **2003**, *438 - 439*, 279.
8. J. D. Kim, J. S. Choi, B. S. Kim, Y. C. Choi, and Y. W. Cho, *Polymer*, **2010**, *51*, 2147.
9. T. Boland, T. Xu, B. Damon, and X. Cui, *Biotechnol. J*., **2006**, *1*, 910.
10. J. Sumerel, J. Lewis, A. Doraiswamy, L. F. Deravi, S. L. Sewell, A. E. Gerdon, D. W. Wright, and R. J. Narayan, *Biotechnol. J*., **2006**, *1*, 976.
11. J. T. Delaney Jr, P. J. Smith, and U. S. Schubbert, *Soft Matter*, **2009**, *5*, 4866.
12. M. Tsujimoto, T. Yabutani, A. Sano, Y. Tani, H. Murotani, Y. Mishima, K. Maruyama, M. Yasuzawa, and J. Motonaka, *Anal. Sci*., **2007**, *23*, 59.
13. A. P. F. Turner, C. Beining, and S. A. Piletsky, *Clin. Chem*., **1999**, *45*, 1596.
14. R. Wilson and A. P. F. Tuner, *Biosens. Bioelectron*., **1992**, *7*, 165.
15. S. Park, T. D. Chung, S. K. Kang, R. Jeong, H. Boo, and H. C. Kim, *Anal. Sci*., **2004**, *20*, 1635.
16. K. M. Manesh, P. Santhosh, S. Uthayakumar, A. I. Gopalan, and K.-P. Lee, *Biosens. Bioelectron*., **2010**, *25*, 1579.
17. D. R. S. Jeykumari and S. S. Narayanan, *Analyst*, **2009**, *134*, 1618.
18. M. A. Rahman, P. Kumar, D.-S. Park, and Y. B. Shim, *Sensors*, **2008**, *8*, 118.
19. Y. H. Yun, J. D. Kim, B. K. Lee, B. Yoo, J.-H. Lee, and Y. W. Cho, *Polym.—Plast. Technol. Eng*., **2009**, *48*, 1318.
20. Y. H. Yun, J. D. Kim, B. K. Lee, Y. W. Cho, and H. Y. Lee, *Macromol. Res*., **2009**, *17*, 197.
21. S. D. Risio and N. Yan, *Macromol. Rapid Commun*., **2007**, *28*, 1934.
22. J. Liu, M. Agarwal, and K. Varahramyan, *Sens. Actuators, B*, **2008**, *135*, 195.
23. V. K. Gade, D. J. Shirale, P. D. Gaikwad, P. A. Savale, K. P. Kakde, H. J. Kharat, and M. D. Shirsat, *React. Funct. Polym*., **2006**, *66*, 1420.
24. R. Epton, M. E. Hobson, and G. Marr, *J. Organomet. Chem*., **1978**, *149*, 231.
25. N. K. Guimard, N. Gomez, and C. E. Schmidt, *Prog. Polym. Sci*., **2007**, *32*, 876.
26. J. Park, H. K. Kim, and Y. Son, *Sens. Actuators, B*, **2008**, *133*, 244.
27. B. Piro, L. A. Dang, M. C. Pham, S. Fabiano, and C. Tran-Minh, *J. Electroanal. Chem*., **2001**, *512*, 101.
28. D. J. Macaya, M. Nikolou, S. Takamatsu, J. T. Mabeck, R. M. Owens, and G. G. Malliaras, *Sens. Actuators, B*, **2007**, *123*, 374.
29. P. Santhosh, K. M. Manesh, S. Uthayakumar, S. Komathi, A. I. Gopalan, and K.-P. Lee, *Bioelectrochemistry*, **2009**, *75*, 61.
30. L. Zhu, R. Yang, J. Zhai, and C. Tian, *Biosens. Bioelectron*., **2007**, *23*, 528.
31. R. A. Kamin and G. S. Wilson, *Anal. Chem*., **1980**, *52*, 1198.