Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Fabrication of Biomemory Device Composed of Myoglobin on DTSSP Layer

Yong-Ho Chung [a], Taek Lee [a], Junhong Min [b] & Jeong-Woo Choi [a]
[a] Department of Chemical & Biomolecular Engineering, Sogang University, Mapo-Gu, Seoul, Korea
[b] College of Bionanotechnology, Kyungwon University, Bokjung-Dong, Sujung-Gu, Seongnam, Korea

Version of record first published: 19 Apr 2010

To cite this article: Yong-Ho Chung, Taek Lee, Junhong Min & Jeong-Woo Choi (2010): Fabrication of Biomemory Device Composed of Myoglobin on DTSSP Layer, Molecular Crystals and Liquid Crystals, 519:1, 19-26

To link to this article: http://dx.doi.org/10.1080/15421400903579788

Mol. Cryst. Liq. Cryst., Vol. 519: pp. 19–26, 2010

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400903579788

Taylor & Francis
Taylor & Francis Group

Fabrication of Biomemory Device Composed of Myoglobin on DTSSP Layer

YONG-HO CHUNG,[1] TAEK LEE,[1] JUNHONG MIN,[2] AND JEONG-WOO CHOI[1]

[1]Department of Chemical & Biomolecular Engineering, Sogang University, Mapo-Gu, Seoul, Korea
[2]College of Bionanotechnology, Kyungwon University, Bokjung-Dong, Sujung-Gu, Seongnam, Korea

Thin film layer of protein was developed for biomemory device consisting of myoglobin which is a metalloprotein. DTSSP molecules was utilized to immobilize myoglobin on gold substrate by chemical bond between sulfo-NHS of DTSSP and amine group of protein and self-assembly process of thiol groups of DTSSP on gold substrate. After immobilization process, the increased thickness of gold substrate was confirmed by surface plasmon resonance (SPR) and the change of morphology was investigated with scanning tunneling microscopy (STM) as a complimentary of SPR. The redox property of immobilized myoglobin on gold surface was verified by cyclic voltammetry (CV). The chronoamperometry (CA) was used in order to verify memory characteristics. The fabricated device presents successfully memory functions such as 'write' and 'erase'. In these results, DTSSP modified myoglobin layer can be used to realize biomemory device.

Keywords 3,3′-Dithiobis(sulphosuccinimidyl propionate) (DTSSP); Chronoamperometry (CA); cyclic voltammetry (CV); myoglobin; scanning tunneling microscopy (STM); surface plasmon resonance (SPR)

1. Introduction

The current silicon-based memory device for information storage has been developed through miniaturization of an integrated circuit over the past several decades. This development was supported by photolithography using light source with shorter wavelength than conventional technique, and the electron beam lithography has recently been encountered the limit of miniaturization [1]. As the more patterns were required in the same area, various problems occurred, such as an increase of price due to the additional process, the fluctuation of electrical properties due to nonuniformity of line width and thickness and heat problem resulted from driving voltage. So, several concepts using various materials have been investigated for overcoming those limitations of current technology [2–6]. Results included magnetic, ferroelectric random access memories using inorganic compounds and capacitive, resistive memories composed of organic materials

Address correspondence to Prof. Jeong-Woo Choi, Department of Chemical & Biomolecular Engineering, Sogang University, 1 Shinsoo-Dong, Mapo-Gu, Seoul 121-742, Korea. Tel.: (+82)2-705-8480; Fax: (+82)2-3273-0331; E-mail: jwchoi@sogang.ac.kr

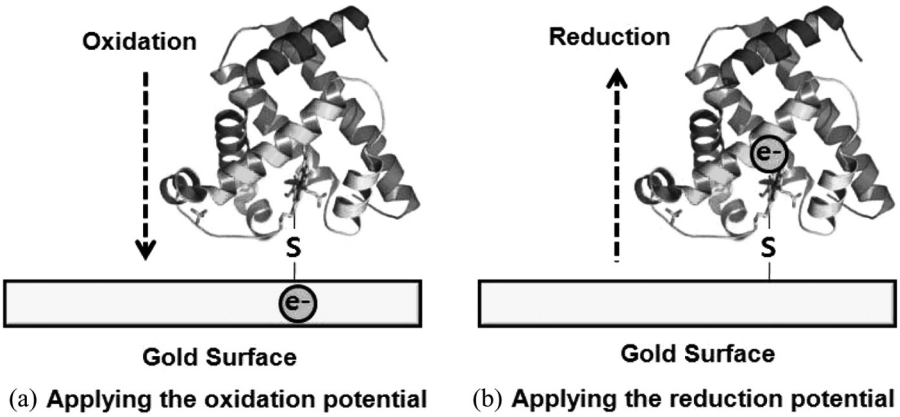


Figure 1. Schematic diagram of electron transfer between immobilized myoglobin and gold substrate (a) the oxidation state of myoglobin and (b) the reduction state of myoglobin.

[7–10]. The important functional parts of those devices were fabricated by inorganic and organic materials and each device has different merits and demerits [11].

Previously, our group attempted to fabricate bioelectronic device composed of biomolecules such as bio-photodiode and shift register memory [12–13]. However, the fundamental concept of this memory is based on biological materials in which information can be stored. There are so many bio-materials relating with electron transfer system in an organism and this process is more efficient than any other devices which was fabricated artificially. The states of those biomaterials were switched by oxidation and reduction and it could be directly applied to the function of memory.

Myoglobin has 153 amino acids and one heme prosthetic group containing iron ion which was related with electron transfer through the change of ferrous and ferric state. This method using oxidation and reduction was so efficient and fast that it was utilized in transfer process of materials such as oxygen in the biological system. If the redox property of myoglobin is controlled after immobilization on the metal substrate and the change of state is monitored with reliable tool, this electrochemical characteristic of myoglobin could be applied to the function of memory directly [14–16]. Therefore, the goal is to prove the memory function using myoglobin.

In this research, nanoscale film composed of myoglobin was fabricated for the application to the memory function. Figure 1 showed the schematic diagram about the electron transfer between myoglobin and gold substrate. SPR (surface plasmon resonance) and STM (scanning tunneling microscopy) measurement were conducted to verify immobilization of myoglobin using DTSSP fragment as a chemical linker on gold substrate. The redox property of immobilized myoglobin was verified via CV (cyclic voltammety) and the memory function of myoglobin was investigated with CA (chronoamperometry).

2. Experimental Details

2.1. *Materials*

Myoglobin extracted from *Horse heart* and DTSSP (3,3-dithiobis(sulphosuccinimidyl propionate)) were purchased (Sigma-Aldrich, USA). To prepare myoglobin and

DTSSP solution, 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (Sigma-Aldrich, USA) was used as a solvent. BK7 cover glass (18 mm × 18 mm, Superior, Germany) was purchased to fabricate Au substrate for SPR analysis. Distilled and deionized water (Milli-Q, >18M() was used in this experiment.

2.2. *Thin Film Fabrication*

The Au substrate consisting of Cr (2 nm) as an adhesion layer and Au (43 nm) was cleaned with piranha solution composed of 70 vol% H_2SO_4 (Daejung Chemical, Korea) and 30 vol% H_2O_2 (Duksan Pure Chemical, Korea) at 65°C for 5 min and then the cleaned substrate was rinsed several times with 99% ethanol and deionized water, and finally dried using nitrogen gas. 0.1 mg/ml myoglobin solution and 5 mM DTSSP solution were dissolved in 10 mM HEPES buffer and mixed to react between sulfo-NHS ester of DTSSP and primary amine of myoglobin. This reaction was operated in the 4°C for 3 hr and finished by adding 1 M Tris-HCl as a stop solution.

DTSSP has a disulfide bond at the center and sulfo-NHS esters which can react with amine groups in each side. 50 mM DTT (dithiothreitol) was added to cleave a disulfide bond. However, DTT should be removed in the mixture before self-assembly (SA) process [17–20] between gold surface and thiol groups of DTSSP fragments because it has also thiol groups. Since DTSSP fragment modified myoglobin is not dissolved in ethyl acetate in contrast to DTT, the extraction method using the difference of solvents was utilized to remove DTT. The cleaned Au substrate was immersed into the prepared solution for SA process for 12 hr and washed with deionized water, and finally dried using N_2 gas.

2.3. *Confirmation of Fabricated Myoglobin Layer Using Surface Plasmon Resonance (SPR)*

The thickness change of gold surface due to the immobilization was confirmed by SPR (Multiskop TM, Optrel GmbH, Germany) using He-Ne laser light source with a wavelength of 632.8 nm. This technique has been utilized to measure adsorption of various materials onto the flat metal because the degree of reflection angle depends on the thickness of adsorbed material [21]. All experiments of SPR analysis were operated at room temperature. The resolution of the angle was 0.01° and the range of incident angle was between 38° and 50°.

2.4. *Surface Analysis Measured by Scanning Tunneling Microscopy (STM)*

The morphology of Au surface before and after immobilization was validated with STM (Multimode SPM, Vecco, USA). When the thin and sharp probe approaches very near to the semiconducting or metallic materials, the phenomena of quantum tunneling due to applying bias happens and it allows to get image via measuring the difference in current. The parameters of STM measurement were scan size = 500 nm, Iset = 0.5 nA, scan rate = 1 Hz and $V_{bias} = 0.5$ V.

2.5. *Investigation of Electrochemical Properties*

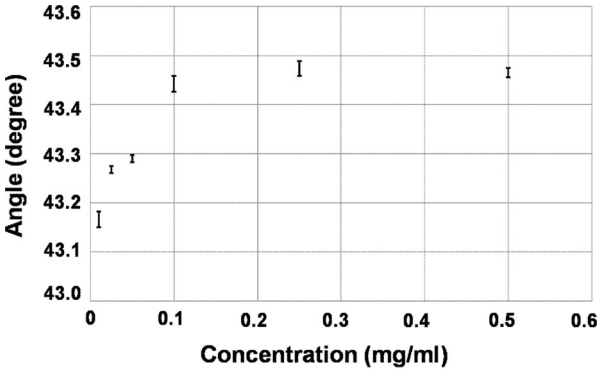
Cyclic voltammetry (CV) and chronoamperometry (CA) were operated using the electrochemical analyzer (CHI 660, USA). The volume of electrochemical cell

fabricated by quartz was 5 mL and the conventional three electrode system composed of working electrode (myoglobin-modified gold substrate), counter electrode (Pt wire), and counter electrode (Ag/AgCl in saturated KCl) was utilized to measure electrochemical properties. 10 mM HEPES buffer (pH 7.0) was used as electrolyte. These experiments were operated at room temperature.

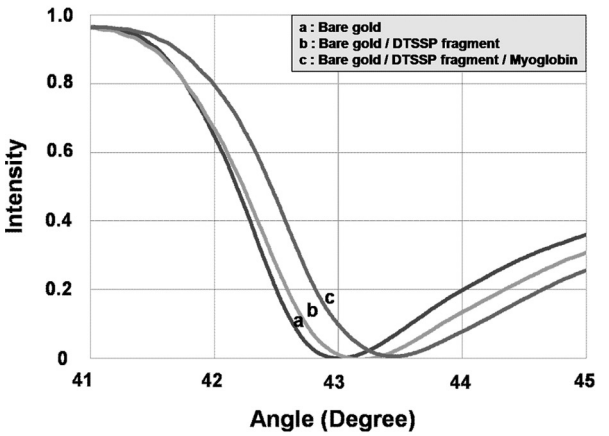
3. Results and Discussion

3.1. *Analysis of Increased Surface by SPR*

At first, the optimization of concentration was conducted for the immobilization of protein. Figure 2a described the angle shift which was induced by the immobilized myoglobin. The degree of resonance angle shift was increased in proportion to the concentration of myoglobin and finally saturated from near 0.1 mg/ml. It follows that 0.1 mg/ml was optimal concentration because the more amount of protein was not needed. After this process, all experiments in this study were operated with



(a)



(b)

Figure 2. Investigation of immobilization by SPR (a) optimization of concentration and (b) comparing angle shifts of (a) bare gold; (b) immobilized DTSSP fragment; and (c) immobilized myoglobin with DTSSP fragment.

this concentration. And then, SPR measurement for confirmation of the main immobilized materials was conducted. The bare gold, the immobilized DTSSP fragment without myoglobin, and the immobilized myoglobin with DTSSP fragment on the Au substrate were prepared. As shown in Figure 2b, the angle shift of single layer of DTSSP fragment and DTSSP fragment combined with myoglobin were 43.18° and 43.45°, respectively, compared to 43.00° of bare gold. This result indicated that the main material immobilized on gold surface was the myoglobin because the angle shift is in proportion to the amount of adsorbed materials on the metal surface, and DTSSP fragment was well conducted the function of chemical linker.

3.2. *The Investigation of Surface Structure by STM*

The surface was analyzed by STM for the complementary of SPR measurement. Figures 3a and 3b showed the image of bare gold and the myoglobin immobilized on gold surface with DTSSP fragment, respectively. The gold substrate made by sputtering had initially globular clusters of 60∼70 nm and irregular depth under

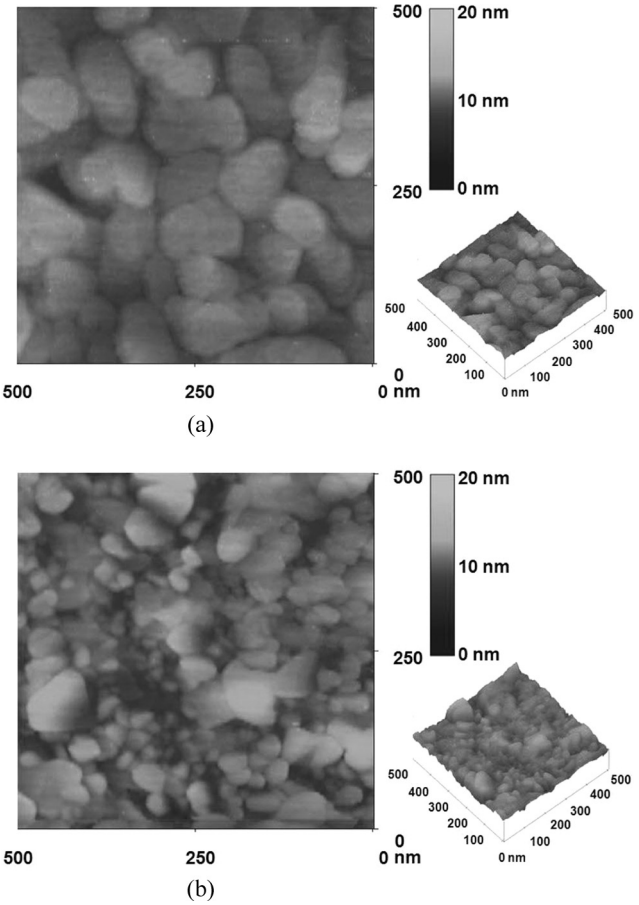
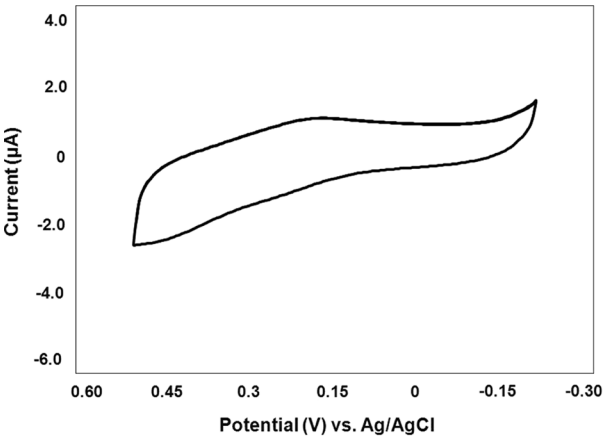


Figure 3. Confirmation of surface morphology by STM (a) bare gold and (b) immobilized myoglobin.

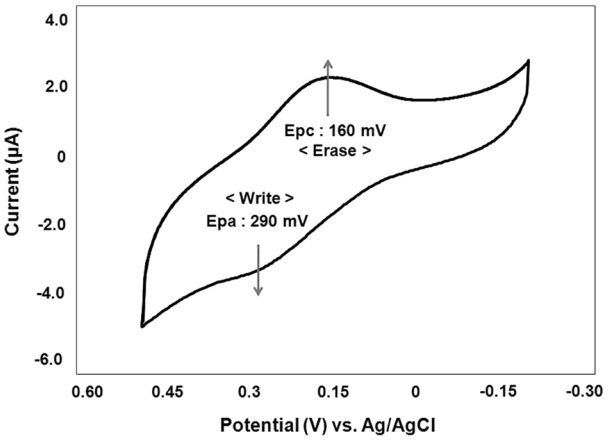
10 nm. After the immobilization, ball-shaped clusters of 20~30 nm were observed on the surface of gold substrate. The aggregated myoglobin on gold substrate, while the shape in solution is a typical, had much smaller clusters and fluctuation of the size than the bare gold. This morphology change of surface indicated that the myoglobin modified with DTSSP fragment was surely adsorbed on gold surface.

3.3. *Redox Property of Self-Assembled Myoglobin Layer by CV*

Cyclic voltammetry experiment was operated to confirm redox property of myoglobin immobilized on gold working electrode. Voltage was swept between 500 mV to −200 mV with scan sensitivity of 10^{-6} and scan rate of 50 mV/s. Figure 4b showed the voltammogram possessing two peaks indicating oxidation (290 mV) and reduction (160 mV) potentials compared to the bare gold (Fig. 4a). This result showed that electron transfer between immobilized myoglobin with DTSSP fragment and the gold substrate. And it meant myoglobin was well



(a)



(b)

Figure 4. Verifying redox property of (a) bare gold and (b) immobilized myoglobin by CV.

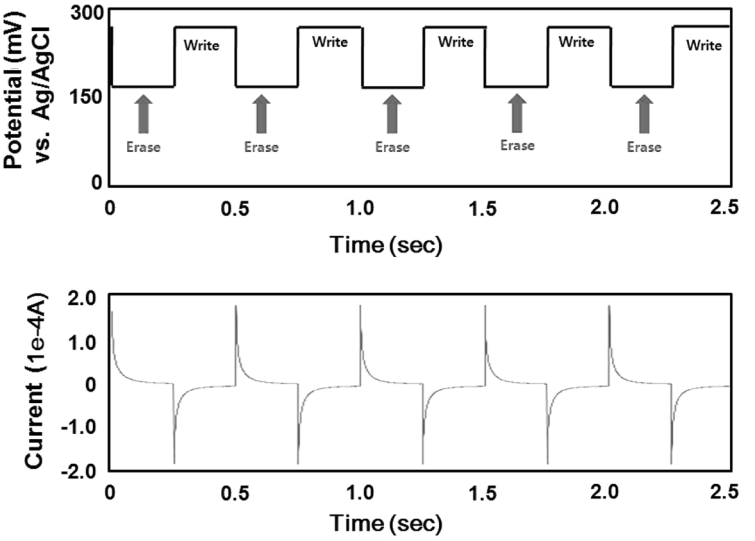


Figure 5. Confirming memory function of immobilized myoglobin by CA.

functionalized on gold substrate due to the fact that the immobilized myoglobin with DTSSP fragment does not lose its redox property after immobilization.

3.4. *Verifying the Memory Function by CA*

CA is an electrochemical technique to apply the stepped potential to the working electrode and detect the current of counter electrode as a function of time. In this study, the CA experiment was carried out to measure the behavior of charge flows. The myoglobin-immobilized gold substrate has two states which can be separated by applying different potential. When the working electrode was applied the oxidation potential (290 mV), the electrons flowed from immobilized myoglobin to the gold substrate and positive charges stored in myoglobin. Reduction step is a counter concept of oxidation step. Applying reduction potential (160 mV) to the oxidized myoglobin caused stored electrons in the gold substrate back to the myoglobin. These results can be used to memory function of 'write' and 'erase' according to inflow and outflow of electrons. Therefore, CA was utilized for demonstrating memory function. Figure 5b indicated the change of current due to the applied potential shown in Figure 5a. Pulse width and sensitivity of CA measurement were 0.25 s and 10^{-5} A, respectively. While the oxidation and reduction potential were applied to the working electrode in turns, the 'write' and 'erase' steps were equally repeated. It follows that this result can be adapted to a practical biomemory device if the method controlling the signal of single protein is developed.

4. Conclusion

In this study, well functionalized myoglobin layer using DTSSP on the gold substrate was fabricated for biomemory device. Myoglobin was modified by the chemical bond between amine group of myoglobin and sulfo-NHS ester of DTSSP to immobilize on gold surface. The SPR and STM measurements utilized for confirming

immobilization showed that the thin film consisting of myoglobin was fabricated well. Two peaks of oxidation (290 mV) and reduction (160 mV) potential were observed by CV, indicating that the redox property of immobilized myoglobin was retained well. CA experiment using this electrochemical property was conducted for verifying the memory functions. The electron transfer in myoglobin by oxidation and reduction potential can be directly adapted to 'write' and 'erase' steps and this result leads to the realization of memory composed of biomolecules.

Acknowledgments

This research was supported by the Nano/Bio Science & Technology Program (M10536090001-05N3609-00110) of the Ministry of Education, Science and Technology (MEST), by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea Government (MEST) (2006-05374), and by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (2009-0080860).

References

[1] Frank, D. J., Dennard, R. H., Nowak, E., Solomon, P. M., Taur, Y., & Wong, H.-S. P. (2001). *Proc. IEEE*, *89*, 259.
[2] Dong, Y., Yu, G., McAlpine, M. C., Lu, W., & Lieber, C. M. (2008). *Nano Lett.*, *8*, 386.
[3] Ichihashi, T., Fujita, J., Ishida, M., & Ochiai, Y. (2004). *Phys. Rev. Lett.*, *92*, 215702.
[4] Smits, J. H. A., Meskers, S. C. J., Janssen, R. A. J., Marsman, A. W., & de Leeuw, D. M. (2005). *Adv. Mater.*, *17*, 1169.
[5] Lee, M.-J., Han, S., Jeon, S. H., Park, B. H., Kang, B. S., Ahn, S.-E., Kim, K. H., Lee, C. B., Kim, C. J., Yoo, I.-K., Seo, D. H., Li, X.-S., Park, J.-B., Lee, J.-H., & Park, Y. (2009). *Nano Lett.*, *9*, 1476.
[6] Begtrup, G. E., Gannett, W., Yuzvinsky, T. D., Crespi, V. H., & Zettl, A. (2009). *Nano Lett.*, *9*, 1835.
[7] Oyamada, T., Matsushige, K., Sasabe, H., & Adachi, C. (2003). *Appl. Phys. Lett.*, *83*, 1252.
[8] Bandyopadhyay, A. & Pal, A. J. (2003). *Appl. Phys. Lett.*, *82*, 1215.
[9] Waser, R. & Aono, M. (2007). *Nat. Mater.*, *6*, 833.
[10] Lu, W. & Lieber, C. M. (2007). *Nat. Mater.*, *6*, 841.
[11] Weitz, R. T., Walter, A., Engl, R., Sezi, R., & Dehm, C. (2006). *Nano Lett.*, *6*, 2810.
[12] Choi, J.-W. & Fujihara, M. (2004). *Appl. Phys. Lett.*, *84*, 2187.
[13] Nam, Y. S., Choi, J.-W., & Lee, W. H. (2004). *Appl. Phys. Lett.*, *85*, 6275.
[14] Kim, S.-U., Kim, Y. J., Choi, J.-W., & Oh, B.-K. (2007). *Biochip J.*, *1*, 188.
[15] Lee, S. W., Chang, W. J., Bashir, R., & Koo, Y. M. (2007). *Biotechnol. Bioprocess Eng.*, *12*, 185.
[16] Lee, T., Kim, S.-U., Lee, J.-H., Min, J., & Choi, J.-W. (2009). *J. Nanosci. Nanotechnol.*, *9*, 1.
[17] Lee, W., Oh, B.-K., Bae, Y. M., Paek, S.-H., Lee, W. H., & Choi, J.-W. (2003). *Biosens. Bioelectron.*, *19*, 185.
[18] Choi, J.-W., Park, K.-W., Lee, D.-B., Lee, W., &. Lee, W. H. (2005). *Biosens. Bioelectron.*, *20*, 2300.
[19] Singh, R. P., Oh, B.-K., Koo, K.-K., Jyoung, J.-Y., Jeong, S., & Choi, J.-W. (2008). *Biochip J.*, *2*, 223.
[20] Kim, B.-S. & Choi, J.-W. (2007). *Biotechnol. Bioprocess Eng.*, *12*, 323.
[21] Oh, B.-K., Kim, Y.-K., Park, K. W., Lee, W. H., & Choi, J.-W. (2004). *Biosens. Bioelectron.*, *19*, 1497.