Biosensors and Bioelectronics 56 (2014) 91–96

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Study of glucose biosensor lifetime improvement in 37 °C serum based on PANI enzyme immobilization and PLGA biodegradable membrane

Lu Fang [a,b], Bo Liang [a,b], Guang Yang [a,b], Yichuan Hu [a,b], Qin Zhu [a,b], Xuesong Ye [a,b,*]

[a] Biosensor National Special Laboratory, Key Laboratory of Biomedical Engineering of Ministry of Education, College of Biomedical Engineering & Instrument Science, Zhejiang University, Hangzhou, China
[b] Cyrus Tang Center for Sensor Materials and Applications, Zhejiang University, Hangzhou, China

ARTICLE INFO

Article history:
Received 23 November 2013
Received in revised form
8 January 2014
Accepted 8 January 2014
Available online 18 January 2014

Keywords:
Glucose sensor
Bovine serum
PANI
PLGA

ABSTRACT

Glucose sensors with long life span were fabricated and evaluated in 37 °C serum to imitate *in vivo* conditions. Polyaniline nanofibers were electrodeposited on sensor electrodes for excessive glucose oxidase immobilization to extend the lifetime of sensors. The sensitivity of sensors stored in 37 °C bovine serum was stable for 20 days without apparent descents, and still remained 45% of its initial value after 44 days. In order to further improve the life span of sensors, a degradable polymer film, poly lactic-*co*-glycolic acid (PLGA), was used as an out coating layer of glucose sensors. Results showed that the PLGA coated glucose sensors left 80% of its initial sensitivity after 44 days' storage in 37 °C bovine serum. The sensors could be potentially used as long-term implantable devices for real time monitoring of glucose levels.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Implantable glucose sensors have played a leading role on the move towards blood glucose continuous monitoring (Block et al., 2008; Vaddiraju et al., 2010a). Numerous efforts have been devoted to the development of continuous glucose sensing devices (Edagawa and Yasuzawa, 2013; Liang et al., 2012; Wan et al., 2010). Life span of sensors is a key problem that hinders the development of the implantable glucose sensing devices. When a sensor is implanted, tissue is disrupted and capillaries are damaged, which generates biofouling in minutes or hours, acute inflammatory responses in several days and foreign-body capsule in weeks. Most of the implanted glucose sensors have function failures within weeks. The failure might be caused by two aspects. One is the biofouling and immune response, and the other is the instability of the sensors' own performance *in vivo* conditions. However, the full mechanism and explanations for the functional sensor damage are still not completely understood.

In the recent researches of lifetime of implantable glucose sensors, most of them have focused on the reduction of biofouling by developing a particular coating on the outer membrane surface that inhibits the protein adhesion to the surface, such as hydrophilic polyurethane (Han et al., 2007), epoxy-enhanced polyurethane membrane (Yu et al., 2006), Nafion coating (Wu et al., 2007), poly (2-hydroxyethyl methacrylate) (polyHEMA), poly(carboxybetaine) methacrylate (polyCBMA) (Yang et al., 2011) and poly(vinyl alcohol)/poly(lactic-*co*-glycolic acid) (PVA/PLGA) drug-eluting hydrogels coating (Vaddiraju et al., 2012). Yang et al. (2011) reported a glucose sensor coated with CBMA. The sensor was stored in 100% human blood serum at 4 °C between each measurement. The sensor did not show a decline in either sensitivity or linearity for the first 12 days, but after being immersed in blood serum for 21 days, a large decrease in the sensor response was observed. Han et al. (2007) reported a glucose sensor that had a lifetime in human serum for about 3 days at room temperature. An epoxy-enhanced polyurethane membrane, which included *ca.* 30–40% epoxy resin adhesive and 50–70% polyurethane, was developed and used as the outer protective membrane of the sensor. The sensor lost 60% of its sensitivity after being stored in bovine adult serum for 1 day (Yu et al., 2006). Vaddiraju et al. (2012) reported a glucose sensor which was developed by using macroscopic porosity to reduce sensitivity drifts resulting from biofouling and the glucose sensor kept 65% initial sensitivity after incubation in 37 °C serum for 30 days. These researches show that the lifetime of sensors can be partly improved by the low protein absorption surface coating. However, previous researches evaluated the sensor performances in serum at room temperature or even lower temperatures like 4 °C which were not sufficient to simulate *in vivo* conditions. Considering the temperature effect, 37 °C serum will be more appropriate for *in vitro* evaluation of implantable sensors.

Except for biofouling on the sensor surface, functional damages of sensors when implanted into the body are also contributors to the decrease of sensitivity for glucose sensors. The functional

* Corresponding author at: Zhejiang University, College of Biomedical Engineering & Instrument Science, Biosensor National Special Laboratory, Key Laboratory of Biomedical Engineering of Ministry of Education, Zheda Road 38, Hangzhou 310027, Zhejiang, China. Tel.: +86 571 87951090; fax: +86 571 87951676.
E-mail addresses: yexuesong@zju.edu.cn, yexs@mail.bme.zju.edu.cn (X. Ye).

0956-5663/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2014.01.017

damage is mainly caused by enzyme leakage from the out membrane (Vaddiraju et al., 2010a), enzymatic inhibition attributed to transition metal ions, like Zn^{2+} and Fe^{2+} (Binyamin et al., 2001) and low molecular weight serum components (Gerritsen et al., 2001). Excess enzyme loading is an effective method to minimize the effect of progressive loss of enzyme activity and maintain the response sensitivity (Sternberg et al., 1988; Yu et al., 2005). Another important reason for the functional damage is the degradation of the polyurethane outer membrane of the sensor (Anderson et al., 1998) or the extensive mineralization of Nafion membranes on the sensors (Mercado and Moussy, 1998). The formation of cracks and holes in the protective membranes of a biosensor will lead to changes in sensitivity, the loss of linearity, and the increased sensitivity caused by interferents. To date, some implantable glucose sensors have been reported, and they have been evaluated *in vivo* for more than one month (Yu et al., 2006), and even more than one year (Gough et al., 2010). However, as far as we know, only a few reported glucose sensors have been studied in 37 °C serum and achieved stable performances in a long period of time.

In this paper, we focus on the long-term stability of glucose sensors in 37 °C serum which is very close to the *in vivo* conditions. Two aspects are studied to improve the lifetime of glucose sensors. The first is excess and compatible enzyme immobilization by polyaniline (PANI) nanofibers. The second is poly lactic-*co*-glycolic acid (PLGA) biodegradable membrane based on the surface coating layer. Studies have shown that organic/polymer 1D-structure exhibits significant advantages over inorganic, since it can provide a compatible environment for the biomolecules-entrapment via electrostatic interaction and act as a mediator for electrochemical-based biosensor (Horng et al., 2009). Polyaniline (PANI) is a widely studied polymer and its nanofibers are applied in sensors and actuators. PANI nanofibers have excellent performances such as high surface area, porosity, and high conductivity (Zhang et al., 2009; Zhou et al., 2005). The porous structure of the PANI favors a high density immobilization of enzyme (Zhai et al., 2013) and the excess enzyme can maintain the sensor′s sensitivity. In this work, a Pt/PANI/GOx/PU electrode was prepared and its performances in PBS and serum were tested. The electrode was stored in bovine serum in 37 °C thermostat to imitate the *in vivo* conditions and evaluate its long time stability. PLGA out coating could degrade when immersed in PBS and a relay-released mechanism of glucose oxidize enzyme was formed during PLGA polymer degrading, as shown in our previous research (Fang et al., 2013). The latest released enzyme could maintain the sensor′s sensitivity by replacing enzyme of functional inactivation to accomplish the catalytic reaction, so the lifetime of glucose sensors could be increased. Herein, we coated a PLGA membrane on the outside of the prepared glucose electrode. Results showed that PLGA coating greatly maintained the sensitivity of the sensor when stored in 37 °C serum.

2. Experimental

2.1. Materials

Glucose oxidase (GOx) enzyme from aspergillus niger was purchased from Aladin Ltd. (Shanghai, China). Polyurethane (PU, product number 81367, Slectophore, an average mol. wt. of 100,000 (Yaseen et al., 2008)) was purchased from Sigma-Aldrich Co. Bovine blood serum (glucose concentration is about 3 mM) was purchased from Hangzhou Qizhenhu Bioscience Co., Ltd. PLGA (inherent viscosity: 0.05–0.15, copolymer ratio: 50:50, molecular weight: 6500) was purchased from Jinan Daigang Biomaterial Co., Ltd. Gold wire was purchased from China New Metal Materials Technology Co., Ltd. Bovine serum albumin (BSA), aniline monomer, glutaraldehyde (25%) and stetrahydrofunan (THF) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and they were reagent grade.

2.2. Apparatus

All electrochemical measurements were performed by using an electrochemical workstation (μAutolab III, Metrohm, Switzerland). Scanning electron microscopic images were collected on a field emission scanning electron microscope (SEM, SIRION FEI, The Netherlands).

2.3. Preparation of glucose biosensors

A needle type glucose biosensor electrode was prepared for testing. As shown in the schematic of the electrode in Fig. 1, the electrode contained four layers: electrodeposited Pt layer, PANI/GOx layer, PU layer and PLGA biodegradable layer. Gold wire (0.3 mm diameter, 30 mm length) was coated with a layer of insulating epoxy except for 10 mm length exposed at the end as a gold electrode. The electrodes were polished then immersed in a 10 mmol/dm^3 H_2PtCl_6/0.1 mol/dm^3 HCl solution and a bias of −100 mV (vs Ag/AgCl) was applied for ten minutes of platinization.

PANI thin film on the gold electrode was electropolymerized in 0.4 M aniline/0.1 M HCl solution at a current density of 0.1 mA/cm^2 for 10 min. Then, the electrodes with PANI film were soaked in ultrafiltered water to remove HCl. The enzyme solution containing 10 mg/ml (150 unit/ml) glucose oxidase and 30 mg/ml BSA was prepared in PBS (0.02 M, PH=7.0) for the immobilization of glucose oxidase. The electrode covered with PANI was dipped in the enzyme solution for 1 h to let enzyme absorb into the PANI fiber network. After that, the gold electrodes were taken out and rinsed with deionized ultrafiltered water, then dried at room temperature.

The GOx immobilized electrodes were held in a vertical position in a vial with 50 μl glutaraldehyde (25%). The electrodes were stored at 37 °C and GOx had been cross-linked for 3 h. After that,

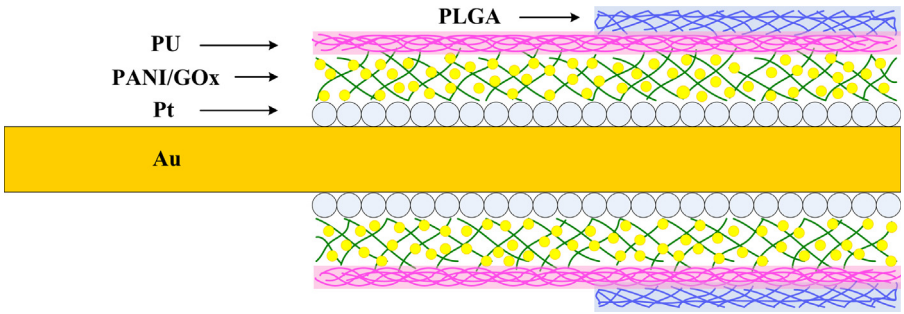

Fig. 1. Schematic diagram of the glucose biosensor electrode.

the electrodes were optionally dip-coated with polyurethane (PU) from a 3% (w/w) PU in 98% tetrahydrofuran (THF)/2% dimethylformamide (DMF) (w/w) solution (Chen et al., 2002; Qiang et al.; 2011). The polyurethane layer served as a barrier membrane to reduce the enzyme leaching and also served to limit the diffusion of glucose relative to oxygen, maximizing the sensor's dynamic response to glucose (Shin et al., 2004).

In order to further increase the lifetime of the sensor, PLGA biodegradable membrane was used as the out coating. Electrodes with and without PLGA biodegradable membrane were prepared to test the performance of the PLGA membrane. For the preparation of PLGA coated sensors, the electrodes were dipped in PLGA chloroform solution for a few seconds and dried overnight.

2.4. In vitro bovine serum experiments

Newly prepared glucose biosensors for long-time evaluations were stored in bovine serum in 37 °C thermostat to imitate the *in vivo* conditions and the stock solution was renewed every day. The electrodes' sensitivities were measured every 3–4 days in bovine serum with the glucose concentration ranging from 0 mM to 20 mM. All amperometric measurements were performed at room temperature (24 ± 1 °C, daytime) and the applied potential for amperometric measurements was 0.65 V vs. Ag/AgCl.

3. Results and discussions

3.1. Morphologies of the electrodes

The morphologies of PANI films electrodeposited on electrodes are examined by scanning electron microscopy (SEM). Fig. 2A shows the SEM images of PANI nanofibers obtained at 0.1 mA/cm^2 for 10 min in a solution containing 0.1 M aniline and 1.0 M HCl. It is clear to see that these PANI nanofibers are interconnected to form network structures, rather than isolated nanofibers. The diameters of the nanofibers range from 100 nm to 150 nm and the lengths are several microns. Such nanostructure of PANI is expected to provide adequate active-surface for the subsequent immobilization of enzyme.

The morphologies of PANI film after being dipped in enzyme solution and cross-linked by glutaraldehyde are shown in Fig. 2B. A uniform layer of thin membrane is formed and it wraps the PANI nanofibers as expected. This layer of membrane acts as a protection of PANI nanofibers and enzyme layer and prevents the leakage of enzyme.

3.2. Sensitivity and linearity of the sensor

Fig. 3 displays a typical amperometric response of the glucose sensor with the addition of successive aliquots of 1 mM glucose in PBS (Fig. 3A) and in bovine serum (Fig. 3B), under the constant potential at +0.65 V vs. Ag/AgCl at room temperature. The glucose sensor performs a rapid and sensitive response to each injection of glucose, which leads to a sharp rise in the current followed by a steady-state value within 10 s. The sensor exhibits a linear dynamic range from 0 mM to 14 mM in PBS and the correlation coefficient R is 0.998 (see insert of Fig. 3A). For glucose concentrations above 14 mM, the amperometric response of the sensor starts to deviate from linearity, owing to the limitation of enzymatic reaction (Williams et al., 1970) and oxygen dependence of GOx-based biosensors (Vaddiraju et al., 2010b). When the sensor is tested in bovine serum, it shows a little decrease in sensitivity from 4 μA/mM/cm in PBS to 3.5 μA/mM/cm in bovine serum as displayed in Fig. 3B. Protein cumulatively adhering on the outer limits the diffusion of glucose and oxygen through the membrane,

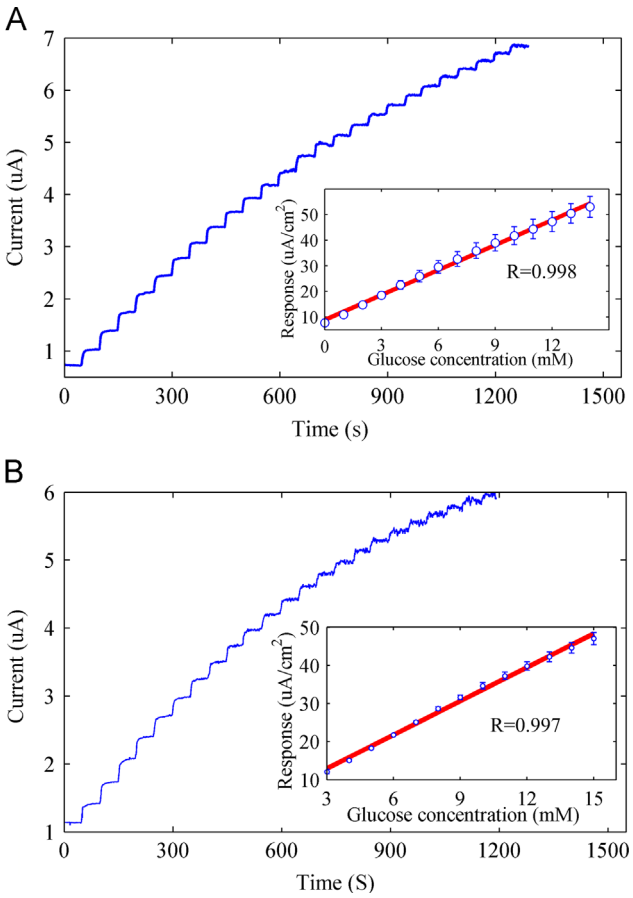

Fig. 3. Current response of glucose sensors as a function of glucose concentration in different media at room temperature: (A) in PBS and (B) in bovine serum.

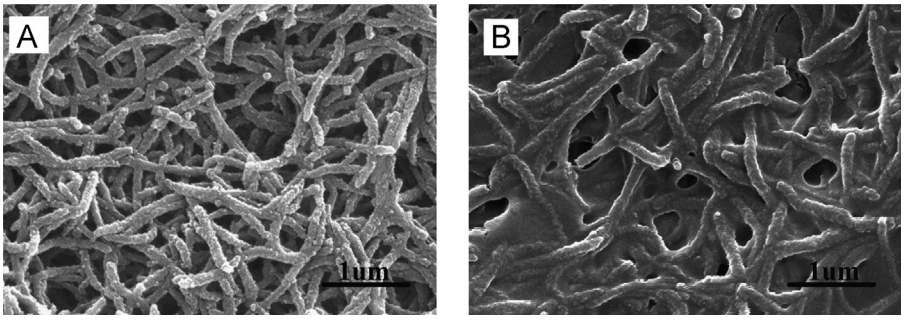

Fig. 2. SEM images of the surface morphologies of the sensor. (A) PANI nanofibers covered on the electrode. (B) PANI nanofibers and GOx enzyme crosslinked by glutaraldehyde.

which thus might cause these decreases. The linear dynamic range of the sensor in bovine serum is from 3 mM to 15 mM and the correlation coefficient R is 0.997. We also notice that the stabilization time in bovine serum is longer than that in PBS and it may be due to the relatively slow diffusion speed of glucose in bovine serum and protein adhering on the out membrane also plays a key role in slowing down the diffusion. The Michaelis-Menten equation is used to estimate the enzyme activity and the apparent Michaelis constant (K_m^{app}) is calculated. The K_m^{app} values of the sensors are 46 mM and 51 mM when respectively tested in PBS and in bovine serum which are higher than the Michaelis constant (K_m) value of free enzyme (Tang et al., 2004). The K_m^{app} value is a measure of the substrate concentration range over which the electrode response is approximately linear but the K_m value is a measure of the enzyme activity of free enzyme (Gregg and Heller ,1990). The obtained K_m^{app} is higher than the recent reported glucose biosensors (Uang and Chou, 2003; Zhao et al., 2007). The higher K_m^{app} means the wider linearity of the sensor and indicates larger glucose detection range (Qiang et al., 2011; Zang et al., 2007).

3.3. Long time stability of the sensor

Long time stability of the sensors was examined by storing sensors in bovine serum at 37 °C and testing the sensitivity changes every 3–4 days. The bovine serum was renewed every day to prevent possible bacterial growth and no antibiotic was added. The sensitivity changes were tested in bovine serum under the constant potential at +0.65 V vs. Ag/AgCl at room temperature. Fig. 4 shows sensitivity changes of the sensor during the 44 days experiment. Average sensitivity of 0–10 mM glucose concentration was calculated and relative sensitivity (calculated by $S_{test}/S_{initial}$ (S_{test}: sensitivity measured during the experiment, $S_{initial}$: sensitivity measured on the first day of the 44 days experiment)) was used to eliminate the influence of different initial sensitivities. As seen in Fig. 4, the sensor did not show a decline in sensitivity after being soaked in bovine serum over 20 days. Then, after the sensor has been immersed in bovine serum for 20 days, a decrease in sensor sensitivity was observed. After 44 days' immersion in 37 °C bovine serum, the sensor still remained 45% of the initial sensitivity.

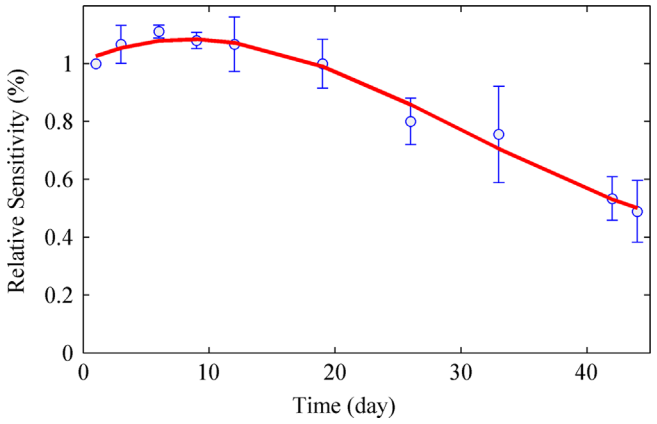

Fig. 4. Relative sensitivity changes of the Pt/PANI/GOx/PU sensor stored in 37 °C bovine serum and tested in bovine serum at room temperature.

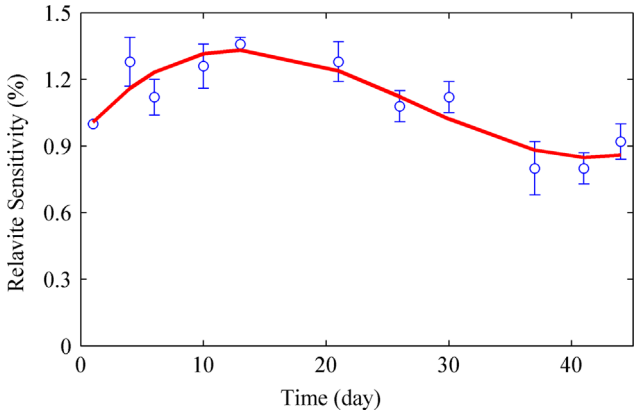

Fig. 6. Long time stability of the Pt/PANI/GOx/PU/PLGA sensors stored in 37 °C bovine serum and tested in bovine serum at room temperature.

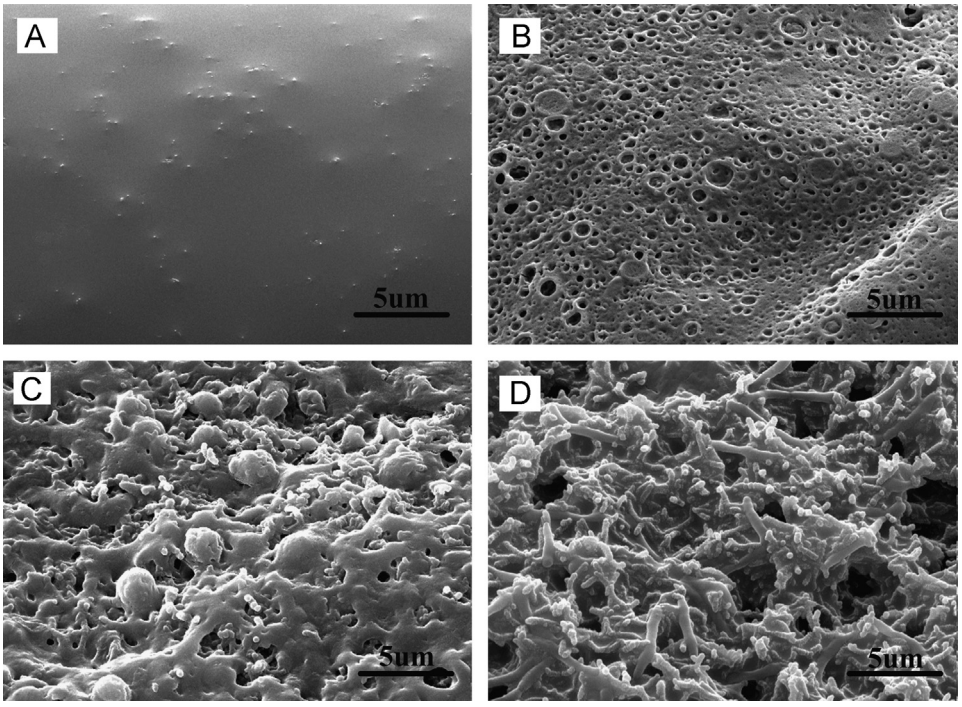

Fig. 5. Time dependent morphological changes of polymer scaffolds during PLGA polymers degrading. (a) 0 week, (b) 1 week, (c) 2 weeks and (d) 3 weeks.

Table 1
Comparison of sensors life time in serum.

Sensor structure	Life time in serum	Sensitivity left (%)	Condition	References
PVA/PLGA drug-eluting hydrogels coating	30 Days	65	Porcine serum at 37 °C	Vaddiraju et al. (2012)
Pt/GOx/CBMA	21 Days	50	Human blood serum at 4 °C	Yang et al. (2011)
Pt/pAP/HPU,PVAB	160 h	50	Human serum at room temperature	Han et al. (2007)
Pt–Ir/GOx/epoxy–PU	6 h	50	Bovine serum at room temperature	Yu et al. (2006)
Sensor with 3C-copolymer as outmost layer	70 h	50	Bovine serum at room temperature	Yang et al. (2000)
Pt/PANI/GOx/PU	44 Days	45	Bovine serum at 37 °C	This work
Pt/PANI/GOx/PU/PLGA	44 Days	80	Bovine serum at 37 °C	This work

3.4. Morphological changes of the PLGA coating sensor

The Pt/PANI/ GOx/PU/PLGA sensors were stored in 37 °C bovine serum to imitate an *in vivo* degradation condition for PLGA out coating. PLGA out coating was transparent and colorless in appearance and had a uniform and smooth surface for the newly prepared sensors. SEM micrographs in Fig. 5 illustrate the time dependent morphological changes of polymer scaffolds during PLGA polymers degrading in bovine serum. PLGA out coating is a thin and smooth membrane at the beginning as shown in Fig. 5 (A) and the small white particles on the surface are caused by the rise and fall of the PANI surface. After being immersed in bovine serum for one week, a porous structure is observed and this indicates the beginning of degrading (Fig. 5(B)). The porous structure expands further and the PNAI structure covered by PLGA begins to expose in Fig. 5(C) after degrading for two weeks. After three weeks′ degrading in bovine serum, PANI/enzyme layer covered with PLGA is completely exposed as shown in Fig. 5(D). PLGA polymers′ degradation time is affected by PLGA molecular weight, copolymer ratio, specimen size and environmental conditions. In this study, we choose the PLGA polymer containing a 50:50 ratio of lactic and glycolic acids because it hydrolyzes faster and it takes three weeks to degrade.

3.5. Long time stability of PLGA coating sensor

Fig. 6 displays the relative sensitivity changes of the Pt/PANI/GOx/PU/PLGA sensor as it was stored in 37 °C bovine serum. Sensitivity of the PLGA out coated sensor increases in the first two weeks and then begins to decrease. The increasing period of sensitivity is in line with the degradation period of PLGA discussed above. This indicates that during the PLGA degradation, the wrapped GOx enzyme layer is exposed slowly and takes part in the catalytic reaction. After 44 days′ immersion in bovine serum, the sensor still displays 80% of the initial sensitivity. This result is better than that of Pt/PANI/GOx/PU sensor which only left 45% of its initial sensitivity after 44 days′ storing in bovine serum. This result consists with Vaddiraju′s report in which sensitivities of the PLGA/PVA coated sensors increased during 30 days experiment because of the PLGA degradation and glucose permeability enhancement (Vaddiraju et al., 2010b). In our study, the sensors′ sensitivity increased in the first two weeks and then began to decease. The sensitivity change is associated with the PLGA degradation speed which could be controlled by adjusting the ratio of lactic and glycolic acids (Jain, 2000; Lü et al., 2009), the inherent viscosity (Shen and Burgess, 2012; Yeo and Park, 2004) and the membrane thickness.

Table 1 lists some of the published glucose sensors tested in serum and their performances are compared. Most of the sensors have a lifetime that is less than a week in serum at room temperature. The Pt/Gox/CBMA glucose sensor has kept its sensitivity for three weeks in human serum but it was stored in 4 °C. Sensors with PVA/PLGA drug-eluting hydrogels coating remained 65% of its initial sensitivity after being incubated in 37 °C serum for 30 days. Sensors presented in this work have the longest lifetime in 37 °C bovine serum.

We notice the sensitivity of Pt/PANI/GOx/PU/PLGA is not as stable as that of Pt/PANI/GOx/PU sensor for the first 20 days. The reason may be that during the degradation of PLGA, the permeability of the PLGA out layer was increased and the glucose diffusion was enhanced. This situation could be improved by matching the PLGA degrading speed and enzyme function failing speed. When the enhancement of the PLGA layer permeability compensates the enzyme of functional inactivation enzyme, the sensor′s performance will be stable and these will be our future researches.

4. Conclusion

This paper presents a Pt/PANI/GOx/PU glucose sensor aiming at long-term subcutaneous implantation. PANI nanofibers were introduced to provide a compatible environment and immobilize more GOx enzyme to maintain the sensor′s sensitivity. Sensors′ sensitivity and linearity were tested in PBS and bovine serum, respectively. The sensor did not show a decline of its sensitivity for 20 days and remained 45% of its initial sensitivity after 44 days′ storing in 37 °C bovine serum. Then PLGA polymers were introduced as a degradable out coating of the sensor to prolong the lifetime further. SEM studies indicate the macroscopic porosity changes during the degradation of PLGA out coating. The degradation increased the permeability of the out coating and enhanced the glucose diffusion. The Pt/PANI/GOx/PU/PLGA glucose sensor left 80% of its initial sensitivity after 44 days′ storing in 37 °C bovine serum. This work provides a compatible enzyme immobilization approach and a degradable member to improve the lifetime of implantable biosensors. Future work will aim to improve the sensors′ stability in serum under constant voltage bias and with additional protein and cells to simulate the *in vivo* conditions better.

Acknowledgment

This work was financially supported by the National Key Technology R&D Program of China (No. 2012BAI16B02) and the National Natural Science Foundation of China (Nos. 81171416, 60875078).

References

Anderson, J.M., Hiltner, A., Wiggins, M.J., Schubert, M.A., Collier, T.O., Kao, W.J., Mathur, A.B., 1998. Polym. Int. 46 (3), 163–171.
Binyamin, G., Chen, T., Heller, A., 2001. J. Electroanal. Chem. 500 (1–2), 604–611.
Block, C.D., Vertommen, J., Manuel-y-Keenoy, B., Gaal, L.V., 2008. Curr. Diabetes Rev. 4 (3), 159–168.
Chen, X., Hu, Y., Wilson, G.S., 2002. Biosens. Bioelectron. 17 (11–12), 1005–1013.
Edagawa, K., Yasuzawa, M., 2013. ECS Trans. 50 (12), 401–405.
Fang, L., Liang, B., Qin, Z., Bobo, H., Ye, X., 2013. IEEE Sens., 306–309

Gerritsen, M., Jansen, J., Kros, A., Vriezema, D., Sommerdijk, N., Nolte, R., Lutterman, J., Van Hövell, S., Van der Gaag, A., 2001. J. Biomed. Mater. Res. 54 (1), 69–75.
Gough, D.A., Kumosa, L.S., Routh, T.L., Lin, J.T., Lucisano, J.Y., 2010. Sci. Transl. Med. 2, 42.
Gregg, B.A., Heller, A., 1990. Anal. Chem. 62 (3), 258–263.
Han, J.H., Taylor, J.D., Kim, D.S., Kim, Y.S., Kim, Y.T., Cha, G.S., Nam, H., 2007. Sens. Actuators, B 123 (1), 384–390.
Horng, Y.-Y., Hsu, Y.-K., Ganguly, A., Chen, C.-C., Chen, L.-C., Chen, K.-H., 2009. Electrochem. Commun. 11 (4), 850–853.
Jain, R.A., 2000. Biomaterials 21 (23), 2475–2490.
Lü, J.-M., Wang, X., Marin-Muller, C., Wang, H., Lin, P.H., Yao, Q., Chen, C., 2009. Expert Rev. Mol. Diagn. 9 (4), 325–341.
Liang, B., Fang, L., Yang, G., Hu, Y., Guo, X., Ye, X., 2012. Biosens. Bioelectron. 43, 131–136.
Mercado, R.C., Moussy, F., 1998. Biosens. Bioelectron 13 (2), 133–145.
Qiang, L., Vaddiraju, S., Patel, D., Papadimitrakopoulos, F., 2011. Biosens. Bioelectron. 26 (9), 3755–3760.
Shen, J., Burgess, D.J., 2012. Int. J. Pharm. 422 (1), 341–348.
Shin, J.H., Marxer, S.M., Schoenfisch, M.H., 2004. Anal. Chem. 76 (15), 4543–4549.
Sternberg, R., Bindra, D.S., Wilson, G.S., Thevenot, D.R., 1988. Anal. Chem. 60 (24), 2781–2786.
Tang, H., Chen, J., Yao, S., Nie, L., Deng, G., Kuang, Y., 2004. Anal. Biochem. 331 (1), 89–97.
Uang, Y.-M., Chou, T.-C., 2003. Biosens. Bioelectron 19 (3), 141–147.
Vaddiraju, S., Burgess, D.J., Tomazos, I., Jain, F.C., Papadimitrakopoulos, F., 2010a. J. Diabetes Sci. Technol. 4 (6), 1540–1562.
Vaddiraju, S., Tomazos, I., Burgess, D.J., Jain, F.C., Papadimitrakopoulos, F., 2010b. Biosens. Bioelectron. 25 (7), 1553–1565.
Vaddiraju, S., Wang, Y., Qiang, L., Burgess, D., Papadimitrakopoulos, F., 2012. Anal. Chem. 84 (20), 8837–8845.
Wan, D., Yuan, S., Li, G., Neoh, K., Kang, E., 2010. ACS Appl. Mater. Interfaces 2 (11), 3083–3091.
Williams, D.L., Doig , A.R., Korosi, A., 1970. Anal. Chem. 42 (1), 118–121.
Wu, L., Zhang, X., Ju, H., 2007. Biosens. Bioelectron 23 (4), 479–484.
Yang, W., Xue, H., Carr, L.R., Wang, J., Jiang, S., 2011. Biosens. Bioelectron. 26 (5), 2454–2459.
Yang, Y., Zhang, S.F., Kingston, M.A., Jones, G., Wright, G., Spencer, S.A., 2000. Biosens. Bioelectron. 15 (5–6), 221–227.
Yaseen, M., Salacinski, H., Seifalian, A., Lu, J., 2008. Biomed. Mater. 3 (3), 034123.
Yeo, Y., Park, K., 2004. Pharm. Res. 27 (1), 1–12.
Yu, B., Long, N., Moussy, Y., Moussy, F., 2006. Biosens. Bioelectron 21 (12), 2275–2282.
Yu, B., Moussy, Y., Moussy, F., 2005. Front. Biosci. 10, 512–520.
Zang, J., Li, C.M., Cui, X., Wang, J., Sun, X., Dong, H., Sun, C.Q., 2007. Electroanalysis 19 (9), 1008–1014.
Zhai, D., Liu, B., Shi, Y., Pan, L., Wang, Y., Li, W., Zhang, R., Yu, G., 2013. ACS Nano 7 (4), 3540–3546.
Zhang, H., Wang, J., Wang, Z., Zhang, F., Wang, S., 2009. Synth. Met 159 (3), 277–281.
Zhao, Z., Chen, X., Tay, B., Chen, J., Han, Z., Khor, K., 2007. Biosens. Bioelectron. 23 (1), 135–139.
Zhou, H., Chen, H., Luo, S., Chen, J., Wei, W., Kuang, Y., 2005. Biosens. Bioelectron. 20 (7), 1305–1311.