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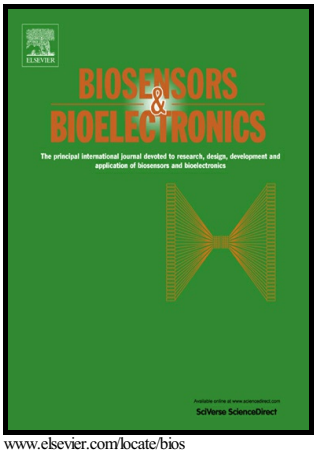

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A needle-type glucose biosensor based on PANI nanofibers and PU/E-PU membrane for long-term invasive continuous monitoring

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Abstract

A minimally invasive glucose biosensor capable of continuous monitoring of subcutaneous glucose has been developed in this study. This sensor was prepared using electropolymerized conductive polymer polyaniline (PANI) nanofibers as an enzyme immobilization material and polyurethane (PU)/ epoxy-enhanced polyurethane (E-PU) bilayer coating as a protective membrane. The sensor showed almost the same sensitivity (63 nA/mM) and linearity (0-20 mM with the correlation coefficient r^2 of 0.9997) in both PBS and bovine serum tests. When stored in 37 ℃ bovine serum, the sensor's sensitivity gradually increased about 30% of the initial value within the first 13 days and then remained stable for the rest of the study period of 53 days. In vivo implantation experiments using mice models showed real-time response to the variation of blood glucose with an average signal delay of about 8 minutes. Continuous monitoring showed that the sensor response increased for the first 12 days and then entered a stable period for 14 days. The sensor's baseline (530±10 nA) and the total response to 1 ml 50%

dextrose injection were almost the same (267±15 nA) in the stable period. The in vivo stable performances indicated that the sensor could be used as an implantable device for long-term invasive monitoring of blood glucose.

Keywords:

invasive; glucose sensor; continuous monitoring; polyaniline; epoxy-enhanced polyurethane

1. Introduction

Continuous blood glucose monitoring is widely used in diabetes mellitus to provide maximal information about changing blood glucose levels throughout the day, including the direction, magnitude, duration and frequency of such fluctuations, and trigger proper alarm in cases of hypo- and hyperglycemia(Guenther et al. 2013). However, minimally invasive sensors used in continuous blood glucose monitoring can only work reliably for a few days in the body. The durability of an implantable biosensor depends on many factors, such as the stability of the entrapped enzyme, the durability of the protective membrane and the severity of the inflammatory response. Of all these factors, the durability of the protective membrane is the most important factor that determines sensor lifetime(Yu et al. 2006). The implanted glucose sensors are subject to an acute inflammatory response in which the leukocytes migrate to the implanted device and adhere to the surface in an attempt to begin the process of phagocytosis(Anderson 1993). The phagocytosis will break the protective membrane and cause the enzyme leakage which could significantly reduce sensor stability. After the initial acute inflammatory response, chronic inflammation involves a sustained localized biological response at the sensor-tissue interface will form a fibrous encapsulation composed mainly of macrophages and collagen. The capsule

can alter local chemical concentration via metabolic reactions; for example, PH change around the implanted device (as low as 3.6) will affect the stability of the membrane and decrease the enzyme activity further(Zhao et al. 1993). So, improving the stability and biocompatibility of the protective membrane are the key to increase the durability of the implantable glucose sensors.

Biomolecules and polymers are two kinds of commonly used membrane materials coated on the implantable glucose sensors. Biomolecules, including collagen(Ju et al. 2008; Ju et al. 2010b), chitosan(Weng et al. 2008), cellulose(Cai and Kim 2010) and heparin(Sung et al. 2004), have been used to mitigate the foreign body response. Although they may be proved beneficial for in vivo use, they are capable of eliciting an immunogenic response and are often undesirably biodegradable. So, the biomolecules are not stable enough used as the protective membrane for implantable devices. Synthetic polymers have been used because of their ability to resist biofouling and their stable mechanical and chemical properties. Some of the synthetic polymers, such as Nafion (Chen et al. 2015), polyurethane (PU) (Yu et al. 2006), poly(carboxybetaine) methacrylate (polyCBMA) (Yang et al. 2011), poly-L-lactic acid (PLLA) (Koschwanez et al. 2008), expanded polytetrafluoroethylene (ePTFE) (Barbara et al. 2004) and poly (lactic-co-glycolic acid) (PLGA) (Ju et al. 2010a), have been proved to have certain effect on improving the lifetime of implantable glucose sensors. Of all these polymers, PU and its modified polymers have been used extensively as an outer membrane to act as a biocompatible interface with the surrounding host tissue. Wang et al. (Wang et al. 2013) reported coating PU electrospun fibers on implantable glucose sensors using a dynamic collector. The sensors were immersed in PBS pH 7.4 and incubated at 37 ℃. Repeated testing of sensor function showed that sensitivity typically increased up to 7 days and then remained stable for the rest of the study period of 84 days. Koh et al. (Koh et al. 2013) designed NO-releasing dendrimer-doped electrospun PU fibers as porous outermost glucose sensor

membrane and the biosensors remained functional for at least a week in serum at 37 ℃. Yu et al. (Yu et al. 2006) designed an epoxy-enhanced PU (E-PU) membrane in an effort to enhance the stability and biocompatibility of implanted glucose sensors. With such a membrane, eight sensors kept functioning in the rats for 10-56 days. However, the sensitivities of the sensors fluctuated obviously in their in vivo tests.

In our previous work (Fang et al. 2014), we introduced polyaniline (PANI) nanofibers as the carrier material to immobilize excessive glucose oxidase enzyme and degradable polymer Poly Lactic-co-Glycolic Acid (PLGA) as the protective membrane to prolong the lifetime of glucose sensors. The glucose sensors left 80% of its initial sensitivity after 44 days storage in 37 ℃ bovine serum. However, the stability of the sensor is still a challenge for long-term continuous invasive monitoring. In this work, we studied the effect of E-PU in improving the stability of implantable glucose sensors. Sensors with PANI nanofibers and E-PU membrane were fabricated and the long-time stability of the sensors were evaluated in 37 ℃ bovine serum in vitro and under the skin of dorsal cervical of rats in vivo. Results showed that PANI nanofibers combined with PU/E-PU membrane could greatly improve the stability of the implanted glucose sensor.

2. Experimental

2.1 Materials

Glucose oxidase (GOx) enzyme from aspergillus niger and was purchased from Aladin Ltd. (Shanghai, China). Polyurethane (PU) was purchased from Sigma-Aldrich Co., LLC. (Shanghai, China). Bovine blood serum was purchased from Hangzhou Qizhenhu Bioscience Co., Ltd. Gold wire was purchased from China New Metal Materials Technology Co., Ltd. Bovine serum albumin (BSA), aniline

monomer, glutaraldehyde (25%), tetrahydrofuran (THF) and dimethylformamide (DMF) were reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). ATACS5104 epoxy adhesive, polyethylene glycol dodecyl ether (Brij 30), Ascorbic acid (AA), acetaminophen (DA), and uric acid (UA) were purchased from Sigma-Aldrich Co., LLC. (Shanghai, China).

2.2 Apparatus

All electrochemical measurements were performed using an electrochemical workstation (μAutolab III, Metrohm, Switzerland). Blood glucose concentration from rat's tail vein was measured by a lab accurate glucose and lactate analyzer (Biosen C-Line, EKF, Germany). Scanning electron microscopic images were collected on a field emission scanning electron microscope (SEM, SIRION FEI, Netherlands).

2.3 Preparation of glucose biosensors

A needle type glucose biosensor electrode was prepared as described in our previous work (Fang et al. 2014). As shown in the insert of Fig.1(a), the working electrode of the glucose sensor contains four layers: electrodeposited Pt layer, PANI/GOx layer, PU layer and E-PU layer. The electrode fabrication process was as follows: Gold electrode was polished and 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. The PANI covered electrode was dipped in the enzyme solution (10 mg/ml) to let enzyme absorbed into nanofiber network. After that, the GOx immobilized electrodes were held in vertical position in a vial with 50 ml glutaraldehyde (25%). The electrodes were stored at 37 ℃ and GOx were cross-linked for 3 h. Different from the dip-coating method in our previous work, a metal wire with a circular ring in the end was used to coat PU membrane in this study

to form a membrane with uniform and controllable thickness. The circular ring with the diameter of 4-5 mm was dipped in the 3% (w/w) PU in 98% THF/2% DMF (w/w) solution to form a membrane of micron grade thickness in the middle of the ring. Then the electrode passed through the ring and a PU membrane was formed uniformly on the electrode surface. The PU coating step was repeated for 2-3 times to form a uniform barrier layer. The thickness of the applied membrane can be controlled from micro-meters to tens of micro-meters as desired by using this membrane coating method.

After being dried in the air at room temperature for 2 hours, an E-PU membrane was further coated on the electrode for 2 times with a circular ring by applying a 2.5% (w/v) E-PU THF solution containing ca. 40% epoxy adhesive and 10% surfactant agent (Brij 30) and ca. 50% PU (wt.)(Yu et al. 2006). The working electrode was assembled with a Pt counter electrode and an Ag/AgCl electrode to form a glucose sensor after completely dry (as shown in Fig.1(a)).

Some experimental parameters used in the electrode fabrication process, such as the voltage and time in platinization, the current and time in PANI electropolymerization and the solution concentration in the electrochemical process have been optimized in our experiments. Fig.S1shows the morphologies of Pt nanoparticles on Au electrode under different platinization voltage and Fig.S2 shows the Morphologies of PANI nanofibers covered on the electrode under different electropolymerization current.

2.4 In vitro bovine serum experiments

Five glucose biosensors for long-time evaluation were prepared and stored in bovine serum in 37 ℃ thermostat to imitate the in vivo condition and the stock solution was renewed every day. The electrodes sensitivities were measured every 3-4 days in bovine serum with the glucose concentration in the range

of 0-20 mM. All amperometric measurements were performed in 37 ℃ bovine serum and the applied potential for amperometric measurements was 0.6 V versus Ag/AgCl.

2.5 In vivo experiments

Implantation was conducted in male Sprague-Dawley rats. All devices were sterilized using gamma irradiation (16 Gy) before implantation. Glucose sensors were soaked in PBS for one day before implantation to stabilize the PU/E-PU membrane and shorten the stabilization period post-implantation. Pentobarbital sodium was injected into the abdominal cavity to anaesthetize rats. After anesthetization, the rats were positioned on the operating table using tape to avoid body movement. The glucose biosensors were expected to be implanted under the skin of dorsal cervical. Before implantation, the area on the dorsal cervical aspect of the rats was shaved and the exposed skin was surgically cleaned up with 2% chlorhexidine and alcohol. The skin was cut a small open of 3-5 mm wide and the sensors were implanted under the skin. The sensing electrodes with 4–6 mm of the connecting leads were located under the skin and the remaining 1–2 cm of the leads were exposed to the outside for connection to the potentiostat during tests. As shown in Fig.1(a), the glucose biosensor was composed of three electrodes fixed on a support by biocompatible glue. The working electrode is located between the counter electrode and the reference electrode. When the wound was stitched, the surgical sutures sew the skin between every two electrodes to immobilize the biosensors and prevent to be pulled out by the rats.

The in vivo performances of the sensors were tested by continuously recording the response signal of the implanted sensors for 2–4 h during pentobarbital sodium anesthesia every 4-5 days. The in vivo response of the sensors was assessed by abdominally injecting 1mL 50% dextrose after the signal was stable.

3. Results and discussion

3.1 Morphologies of sensor electrodes

The morphologies of the sensor electrodes were examined with scanning electron microscopy (SEM) in Fig.1(b) - Fig.1(d). Fig.1(b) shows the SEM image of PANI nanofibers after electropolymerized in 0.4 M aniline/0.1 M HCl solution at a current density of 0.1 mA/cm^2 for 10 min. The diameters of the PANI nanofibers are ranged from 80 nm to 110 nm and the length of the nanofibers is about 1 um. The PANI nanofibers uniformly covered the electrode and interconnected each other to form a network. The size of the nanopores is ranged from 10 nm to 250 nm and such nanostructure of PANI provides adequate active-surface for enzyme immobilization.

Fig.1(c) shows the morphology of the sensor electrode with PU membrane. The nanoporous structure of the PU membrane was formed due to the evaporation of solvent in the drying process. The size of the nanopores is ranged from 50 nm to 500 nm. The morphology of the sensor electrode coated with E-PU is shown in Fig.1(d). The surface of the electrode is smooth and uniform with E-PU membrane.

3.2 Response characteristics of the sensor

Fig.2(a) shows typical curves of the glucose sensor in response to step of 2 mM increases of glucose concentration in the range of 0 - 20 mM tested in PBS and bovine serum respectively under a constant potentialat of 0.6 V vs. Ag/AgCl at room temperature. When tested in PBS, the glucose sensor performs a rapid and sensitive response to each injection of glucose and the response time (90%) is about 30s. When tested in bovine serum, the sensor's response time increased to 50s which is caused by the protein adhering on the out membrane of the sensor and the slower diffusion speed of reactant in bovine serum. As shown in Fig.2(b), the sensors sensitivity is 63 nA/mM (1.34 uA/mM/cm^2) and the

correlation coefficient r^2 is 0.9997 within the glucose concentration of 0 - 20 mM in PBS which meets the requirement of physiological glucose measurements. Although the reaction speed is slowed, the sensor's sensitivity (62 nA/mM (1.34 uA/mM/cm^2)) and linearity (0 – 20 mM when correlation coefficient r^2 is 0.9996) have almost no change in bovine serum as shown in Fig.2(b). In this study, five glucose sensors were prepared and tested, and the sensitivity deviation of these sensors was less than 15%.

The Michaelis-Menten equation is used to estimate the enzyme activity and the apparent Michaelis constant (Km^{app}) is calculated. The higher Km^{app} means the wider linearity of the sensor and indicates larger glucose detection range. The Km^{app} of the glucose sensor in this study is calculated to be about 385 mM, which is much higher than our former reported sensor(Fang et al. 2014) and other reported glucose sensors(Chen et al. 2006; German et al. 2012; Ren et al. 2006).

The effects of interferons coexisting in blood such as l-ascorbic acid (AA), acetaminophen (AP) and uric acid (UA) were also tested. AA (0.11 mM), AP (0.17 mM) and UA (0.48 mM) were added into PBS respectively in every two steps of glucose concentration increasing as shown in Fig.3. The sensor's responses of these interferons relative to 1 mM glucose were 7.5% (AA), 9.5% (AP) and 4% (UA), respectively. The test results showed the combination of PU and E-PU membrane could achieve reliable permselectivity.

3.3 Performance in bovine serum

Four newly prepared glucose sensors were continuously applied with 0.6V voltage in 37 ℃ bovine serum to evaluate the in vitro stability of the sensors under constant voltage potential. The sensor responses were tested every 3-4 days in 37 ℃ bovine serum with the glucose concentration increased by 2mM during each step to 20mM final concentration. The initial averaged sensitivity ($S_{initial}$)

of each sensor was pre-measured in bovine serum before the sensor was continuously polarized. During the 66 days' experiment, a series of normalized averaged sensitivities (S_{test} / $S_{initial}$ ×100) were obtained from different sensors at different tested period.

As shown in Fig.4, the sensor sensitivity gradually increased about 30% of the initial value in the first 13 days and then started to remain stable (no statistical change) for the rest of the study period of 53 days. The initial increase was attributed to initial sensor polarization combined with the swelling of enzyme and mass-transport-limiting membrane layers. As studied by N. Wang(Wang et al. 2013) and J. A. Henry(Henry et al. 2007), the nanofiber diameter of the PU/E-PU out membrane gradually decreased when stored in PBS pH 7.4 at 37 ℃ owing to surface erosion. So, the hole size and the permeability of the PU/E-PU membrane increased in the joint effect of swelling and surface erosion. More reactant could pass through the protective membrane and the sensor sensitivity increased in the initial period of time. After 13 days, the hole size and the permeability of the PU/E-PU membrane tended to be stable and this made the sensor sensitivity stable.

We noticed that in other's serum experiments, the sensor sensitivity decreased with the increasing of diffusion resistance caused by proteins deposition(Han et al. 2007; Yang et al. 2011; Yang et al. 2000; Yu et al. 2006). However, in our serum experiments, the sensor sensitivity did not show significant decrease with the increasing of the soak period, suggesting that the serum constituents (such as proteins and grown factors) adsorption, might be less significant to sensor stability during long time continues glucose monitoring. Instead, the sensor out membrane seemed to play a more important role in maintaining sensor stability. The sensor stability varied with the change of the permeability of the out membrane rather than the amount of the proteins adsorption. The reason may be the amount of the protein adsorption at the membrane surface of our sensors is not as much as that in other's experiments

because of the good biocompatibility of the E-PU membrane. The remaining of the enzyme activity is another reason for the good sensor stability. On one hand, the PANI nanofibers provide a comfortable and biocompatible environment for the enzyme and this preserves the enzyme activity. Meanwhile, the PANI nanofibers immobilize glucose oxidize tightly inside the sensor (Zhai et al. 2013) and prevent it leaking out from the membrane. On the other hand, by introducing epoxy network into polymer, short polyurethane chains can be anticipated to combine tightly together, while the excellent adhesion property of epoxy adhesive also improve the connection stability between the protective membrane and the enzyme layer(Yu et al. 2006).

3.4 In vivo performance

Three glucose sensors were implanted in three rats respectively. The glucose sensor's in vivo performances were measured every 3–4 days from day 5 after implantation. When a 0.6V potential was applied on the sensor, the response current was large at the beginning and then reached a stable value after a run-in period of 60 minutes. In general, the stability process of the in vivo output signal is not significantly different from that of the in vitro response for the same sensor. However, the time needed for stability in vivo is much longer than that in bovine serum which is about 10 minutes in our experiments. Glucose diffusion in tissue fluid is much slower than in vitro because of the protein deposition on the sensor resulted from the acute inflammatory response and the reduced blood flow resulted from the damaged blood vessels (Dungel et al. 2008).

Blood glucose concentrations from tail vein were tested by a lab accurate glucose and lactate analyzer every 7-8 minutes and the results were compared with sensor's outputs. Fig.5(a) shows the sensor response on day 12. The response curve of the stabilization process is omitted. 1mL 50% dextrose was injected into the rat's abdominal cavity after the sensor stabilized at 0.6 V voltage for 65

minutes. As can be seen from fig.5(a), the sensor response current starts to rise 5 minutes after dextrose injecting and reaches a peak 20 minutes later, and then remains at the plateau value for about 10 minutes before falling toward the baseline. This follows well with the blood glucose concentration variation tested from tail vein. The average signal delay between the blood glucose concentration tested from tail vein and the value reported by the implanted sensor was about 8 minutes in our experiment. Of the signal delay, 1 minute is ascribable to the sensor itself, as determined from independent in vitro measurements, and an estimated 0.5 minute is ascribable to circulatory transport from the central venous infusion site to the implant site(Gough et al. 2010). The remainder of the average delay (7 minutes) is attributable to mass transfer and physiologic phenomena within the local tissues. This time delay value is consistent with other's studies (Boyne et al. 2003; Gough et al. 2010).

The implanted glucose sensor response variations according to 1 ml 50% dextrose during the 26 days experiment are shown in Fig.5(b). The sensor response increases for the first 12 days then enters a stable period which lasts for 14 days. The sensor was dragged out on day 32. The sensor sensitivity change process in vivo is consistent with that tested in the in vitro simulation environment. The increasing of the sensor sensitivity was attributed to the increased permeability of the PU/E-PU membrane on the one hand as described in chapter 3.3. On the other hand, the tissue and vascular injury caused by the implantation surgical operation in our experiment was much severer compared with the damage created by the implantation process of the commercial continues glucose monitoring sensors. This reduced the blood flow and lowered the glucose concentration around the sensor. The local edema and protein deposition during the acute inflammatory response process also limited glucose transport to the region around the sensors. Local glucose and oxygen consumption by metabolically active inflammatory cells lowered the glucose concentration further. So, the sensor

response was low for the first few days after implanting. New vascular grew with the healing of the wound and the reduction of inflammatory response, which increased the blood flow around the sensor and recovered the sensor response. After 12 days, the PU/E-PU membrane and the foreign body response both entered a stationary phase. The subcutaneous vascular density, capsule thickness and PU/E-PU membrane permeability started to change slowly and remain stable. So, the sensor response shows a relatively stable period during days 12-26.

Fig.5(c) shows the sensor response variation caused by 1 ml 50% dextrose injection at day 12, day 19 and day 26 respectively. The total blood glucose concentration change tested from tail vein according to 1 ml 50% dextrose is 8.7±2.4 mM. The rising and falling time of the blood glucose is depended on the degree of anesthesia and the metabolic rate of the rats. We can see that after stabilizing for 60 minutes, the sensor baseline (530±10 nA) and the total response to 1 ml 50% dextrose are almost the same (267±15 nA) at different test dates. These indicated that the Pt/PANI/GOx/PU/E-PU sensor performance could remain stable in vivo at least for 26 days.

Some of the reported implantable glucose sensors are listed in table 1 and their performances in vitro and in vivo are compared. The comparison of the sensitivity, linearity and selectivity of these sensors are listed in table S1. Most of the reported implantable sensors were evaluated in serum and had a lifetime less than one month with the sensitivity left less than 70% of the initial value (Chen et al. 2015; Vaddiraju et al. 2012; Yang et al. 2011; Yu et al. 2006). Some of the sensors were evaluated in vivo and had a lifetime less than one week (Chen et al. 2015; Kazuaki et al. 2014). The glucose sensors designed by Yu et al. successfully worked in vivo for 56 days but the sensor sensitivity in vivo had a large fluctuation (the in vivo sensitivity of the sensor was 1.78±0.71 nA/mM calculated from the published data). Sensors described in our previous work had a lifetime of 44 days in 37 ℃ bovine

serum with 80% sensitivity left. However, the biodegradability of PLGA changed the permeability of sensor outer membrane and caused a stability fluctuation. In this work, E-PU membrane was introduced to improve the sensor stability. The improved sensors have a stable response after implanted for 8 days as can be seen from Fig.5(b) and the in vivo sensitivity is 25±7.5nA/mM.

The good stability of our sensors is related to three reasons. First of all, PANI nanofibers provide a compatible environment to enzyme and the porosity of the nanostructure could immobilize excessive GOx enzyme and maintain the sensor sensitivity. Secondly, polyurethane is composed of short chain molecules and the resulting membrane has a relatively poor mechanical durability. Therefore, protective membranes based on polyurethane may not last long enough for implantable application. By introducing epoxy network into polymer, short polyurethane chains can be anticipated to combine tightly together and significantly improve stability of the protective layer. Finally, a membrane coating ring was used to coat the protective membrane instead of dipping or dripping. The PU/E-PU membrane formed by the coating ring is uniform and smooth with micron grade thickness. The uniform and smooth out coating could improve the biocompatibility and stability of the sensor. With the excellent stability, our sensor could be potentially used in continuous blood glucose monitoring the diabetes management.

4. Conclusion

This paper demonstrates that a minimally invasive glucose biosensor based on Pt/PANI/GOx/PU/E-PU structure is suitable for transcutaneous implantation. PANI nanofibers provide a compatible environment to enzyme and the porosity of the nanostructure could immobilize excessive GOx enzyme to maintain the sensor sensitivity. The PU membrane provide a balance between oxygen and glucose transport to the sensing layer while epoxy resins adhesive enhances the durability of PU

membrane and improves the stability of the protective layer. The sensors exhibited an excellent reproducibility when tested in PBS and bovine serum. Besides, the sensors worked stably for 66 days in 37 ℃ bovine serum, this indicated a good long time stability of the sensors. In vivo implantable experiments showed excellent response to the variation of blood glucose and the sensors worked continuously and steadily for 26 days. This work provides a compatible enzyme immobilization approach and a stable protective membrane to improve the lifetime of implantable biosensors. This sensor could be potentially used in long-term continuous blood glucose monitoring the diabetes management.

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Fig.1. The morphologies of the glucose sensors. (a) Schematic diagram of the glucose biosensor. (b) SEM image of PANI nanofibers covered on the electrode. (c) SEM image of PU membrane covered on PANI nanofibers. (d) SEM image of E-PU membrane covered on PU membrane.

Fig. 2. Characterization of the glucose sensors. (a) Current-time response curve of glucose sensor as a function of glucose concentration tested in PBS and in bovine serum at room temperature under a constant potentialat of 0.6 V vs. Ag/AgCl. (b) Glucose sensor sensitivity tested in PBS and in bovine serum.

Fig. 3. Current–time response curves in PBS with successive additions of 1 mM glucose (twice), 0.11 mM AA, 1 mM glucose (twice), 0.17 mM AP, 1 mM glucose (twice), 0.48 mM UA under 0.6 V vs. Ag/AgCl.

Fig. 4. Long time stability of the Pt/PANI/GOx/PU/E-PU sensors stored in 37 ℃ bovine serum.

Fig. 5. In vivo performances of the implanted glucose sensors. (a) The in vivo response (blue line) of the implanted sensor to glucose injection at day 12 compared with blood glucose values (red square) measured from the tail vein and tested by a lab accurate glucose and lactate analyzer.(b) The implanted glucose sensors' response changes to 1 ml 50% dextrose injection during the 26 days' experiment, blue square: sensors' response, red line: fitting curve of the sensors' response.(c) The sensor's response curve to 1 ml 50% dextrose at day 12 (blue line), day 19 (green line) and day 26 (red line) respectively.

Table 1. Comparison of sensors life times in vitro and in vivo.

Sensor structure	Lifetime in serum (sensitivity left)	Serum condition	Lifetime in vivo	Reference
Nafion/Gox/(PU/PDMS)/Heparin	no mention	no mention	7 days	(Kazuaki et al. 2014)
Pt–Ir/GOx/epoxy–PU	6 hours (50%)	Bovine serum at room temperature	56 days	(Yu et al. 2006)
Pt/GOx/CBMA	21 days (50%)	Human blood serum at 4 ℃	no mention	(Yang et al. 2011)
Pt/(PANI/Pt)/Gox/PVDF-Nafion	21 days (70%)	Bovine serum at 37 ℃	1 day	(Chen et al. 2015)
Pt/GOx/PU/(PVA/PLGA drug-eluting hydrogels coating)	30 days (65%)	Porcine serum at 37 ℃	no mention	(Vaddiraju et al. 2012)
Pt/PANI/GOx/PU	44 days (45%)	Bovine serum at 37 ℃	no mention	(Fang et al. 2014)
Pt/PANI/GOx/PU/PLGA	44 days (80%)	Bovine serum at 37 ℃	no mention	(Fang et al. 2014)
Pt/PANI/GOx/PU/E-PU	66 days (130%)	Bovine serum at 37 ℃	26 days	This paper

Highlights

- Excessive GOx enzyme was immobilized in PANI nanofibers to maintain the sensor's sensitivity.
- Layer by layer of PU/E-PU nanostructured protective membrane improved the biocompatibility and stability.
- Sensitivity and linearity remained constant in both PBS and bovine serum with high selectivity.
- The sensor's response follow the variation of blood glucose concentration in vivo in real time.
- The sensor has long-term stability in 37℃ bovine serum (66 days) and in vivo (26 days).

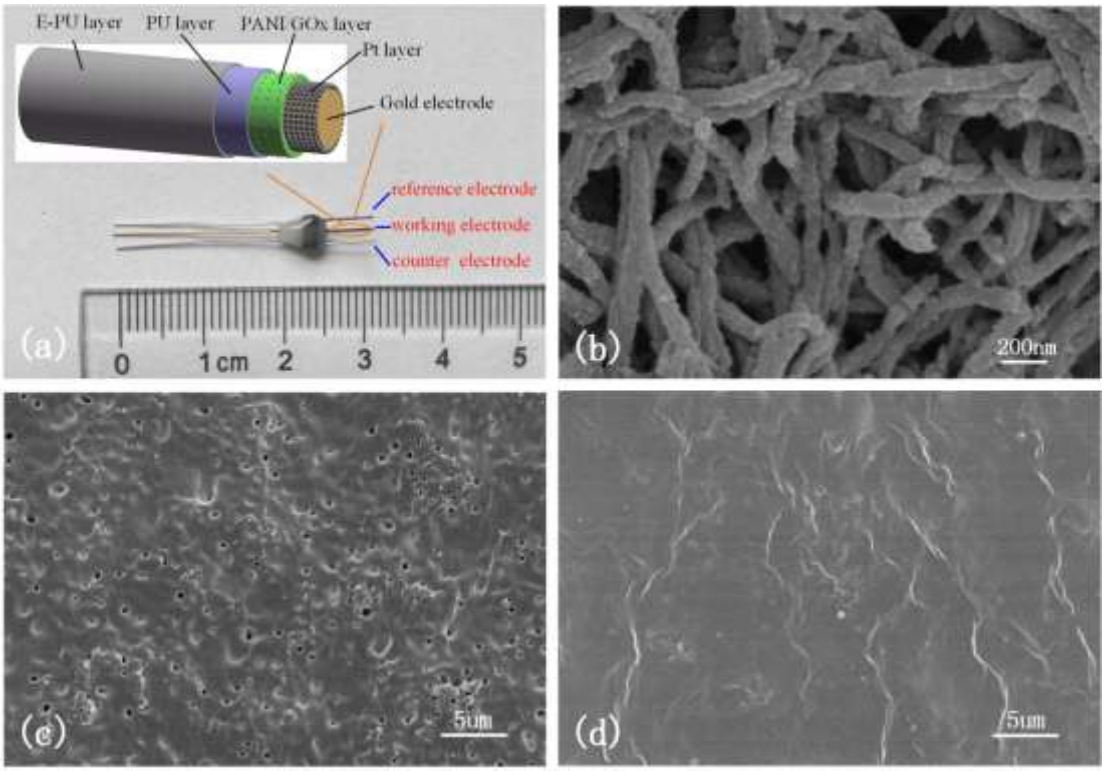

E-PU layer
PU layer
PANI GOx layer
Pt layer
Gold electrode
reference electrode
working electrode
counter electrode
(a)
0
1cm
2
3
4
5
(b)
200nm
(c)
5um
(d)
5um

Fig 1

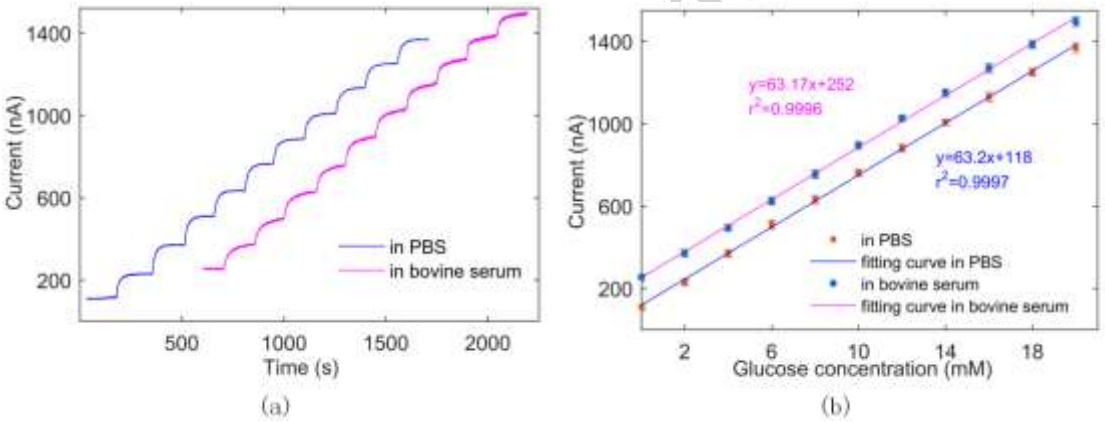

Current (nA)
Time (s)
in PBS
in bovine serum
(a)
y=63.17x+252
r²=0.9996
y=63.2x+118
r²=0.9997
in PBS
fitting curve in PBS
in bovine serum
fitting curve in bovine serum
Glucose concentration (mM)
(b)

Fig 2

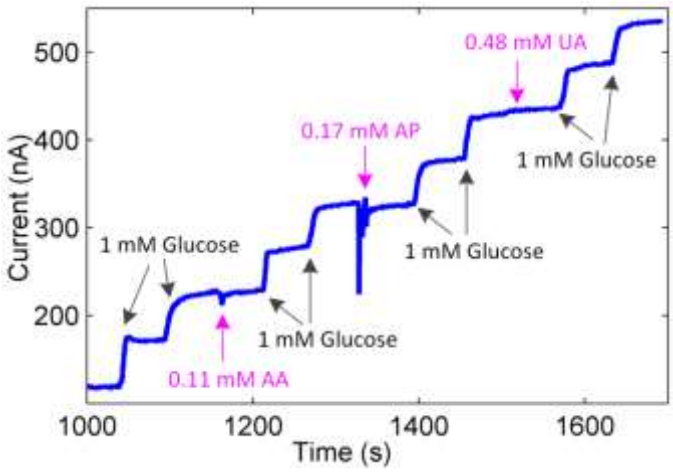


Fig 3

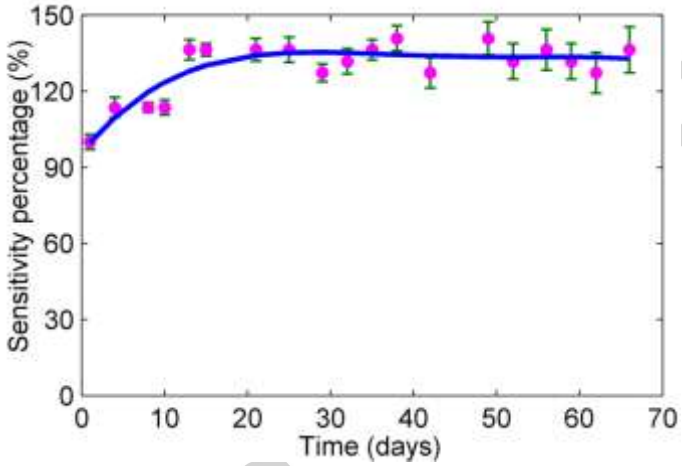
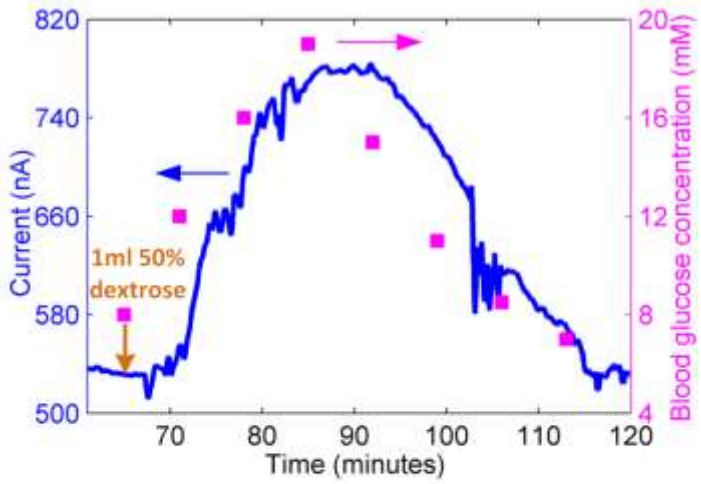
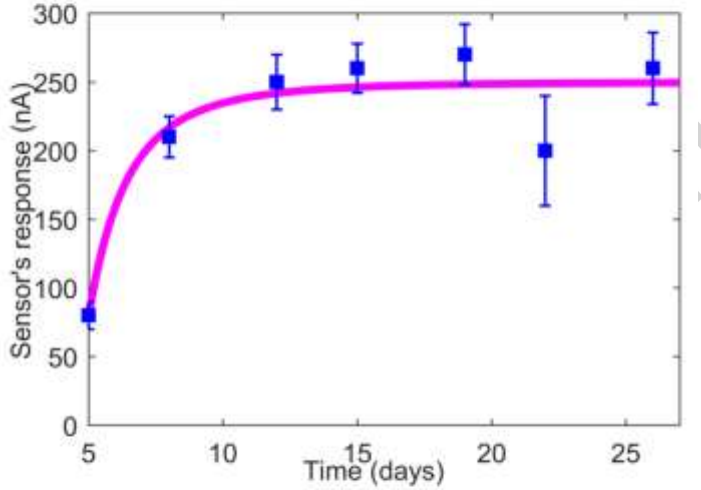


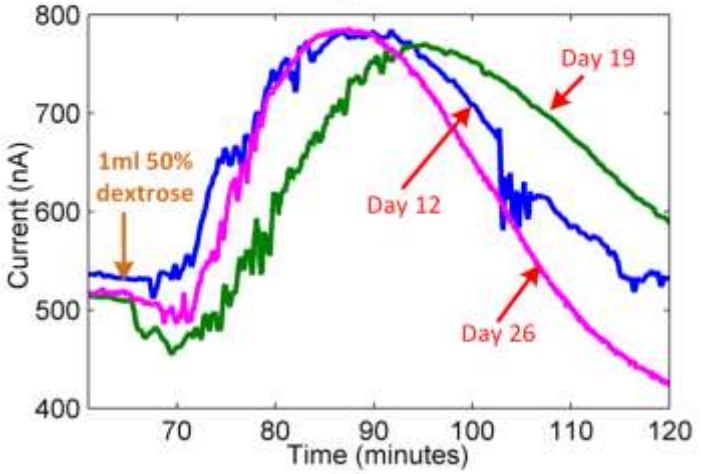
Fig 4


820
740
660
580
500
Current (nA)
1ml 50%
dextrose
20
16
12
8
4
Blood glucose concentration (mM)
70
80
90
100
110
120
Time (minutes)

(a)


300
250
200
150
100
50
0
Sensor's response (nA)
5
10
15
20
25
Time (days)

(b)


800
700
600
500
400
Current (nA)
1ml 50%
dextrose
Day 19
Day 12
Day 26
70
80
90
100
110
120
Time (minutes)

(c)

Fig 5