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Glucose affinity measurement by surface plasmon resonance with borate polymer binding

Dachao Li[a,*], Di Yang[a], Jia Yang[a], Yuan Lin[b], Yingjuan Sun[b], Haixia Yu[a], Kexin Xu[a]

[a] State Key Laboratory of Precision Measuring Technology and Instrument, Tianjin University, Tianjin, China
[b] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China

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ABSTRACT

A novel surface plasmon resonance (SPR) sensor bound to the borate polymer PAA-ran-PAAPBA through a layer-by-layer method was proposed for the determination of glucose concentration. In contrast to the enzyme electrode sensor, the use of optical refractive index sensing to detect glucose concentration eliminates the measurement drift caused by bioelectricity when the sensor is implanted into subcutaneous tissue; in addition, a borate polymer was used to replace the glucose oxidase (GOD) enzyme and it does not consume the glucose molecule during measurement through the affinity reaction between the polymer and glucose. In this study, the layer-by-layer self-assembly method was used to immobilize the borate polymer on the surface of the SPR sensor. The effects of the number of layers are discussed in the manuscript, and the regenerability, reproducibility, and stability of the SPR sensor were evaluated. Almost all of the studies are performed under specific alkaline conditions (usually close to or higher than the p*Ka* of PBA). And for the future application in vivo, we further investigated glucose detection at physiological conditions. The measurement resolution of the sensor bound to 12 polymer layers at physiological conditions was 1 mg/dL, and the R-squared value of the glucose concentration-ΔRU fitting curve within 1–1000 mg/dL was as high as 0.998, which indicates that this measurement may form the foundation of an implantable device for the continuous measurement of glucose concentration.

1. Introduction

Continuous blood glucose monitoring provides guidance for diagnosis and therapy [1]. The concentration of glucose in the blood is closely correlated to that in interstitial fluid [2–8]. Currently, the only technique available for continuous glucose monitoring in clinical applications involves the implantation of an enzyme electrode sensor under the skin to detect the concentration of glucose in interstitial fluid through an electrochemical method. However, since the enzyme electrode sensor detects the change of electric current caused by the reaction between the glucose oxidase (GOD) enzyme and the glucose, the bioelectricity of the human body may causes the drift during the measurement; thus, finger-prick blood collection is required to calibrate the sensor several times each day. In addition, the reaction between the enzyme electrode sensor and glucose is not reversible, which means that the consumption of glucose cannot be avoided during the measurement, and this effect cannot be ignored, particularly in cases of hypoglycemia [9].

A new glucose affinity measurement method based on a surface plasmon resonance (SPR) sensor with the borate polymer PAA-ran-PAAPBA [10] is proposed in this paper. The SPR sensor detects the differences in the refractive index of glucose solutions of different concentrations flowing over the surface of the sensor; therefore, this optical sensing method is not affected by the bioelectricity of the human body when the SPR sensor is implanted into subcutaneous tissue for continuous glucose monitoring. Moreover, the concentrations of any components in the interstitial fluid would affect the change in the refractive index, which means measurement result will be affected by all the components in the interstitial fluid. Therefore, the borate polymer PAA-ran-PAAPBA, which can specifically adsorb glucose molecules, is immobilized on the surface of the SPR sensor to replace GOD. The affinity reaction between the polymer and glucose molecules is reversible, which means that the molecules will bind to the polymer at high glucose concentrations and this bond will break at low glucose concentrations. This reversible binding to the borate polymer stands in contrast to the consumption of glucose observed during measurements with enzyme electrode sensors [11].

The binding materials that are most commonly used to specifically adsorb glucose to SPR sensors are concanavalin (Con A) [12,13]

* Corresponding author. Tel.: +86 022 27403916; fax: +86 022 27404209.
E-mail address: dchli@tju.edu.cn (D. Li).

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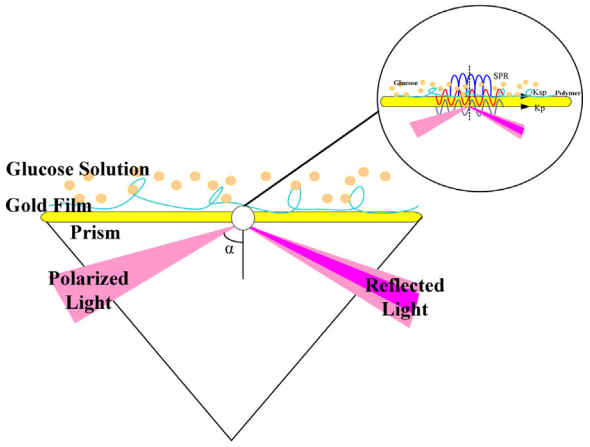

Fig. 1. Measurement principles underlying the use of the SPR sensor for the detection of glucose molecules.

and D-galactose/D-glucose binding protein (GGBP) [14–16]. Due to its immunogenicity and cytotoxicity, Con A is not suitable for implantable medical devices. The GGBP protein is inherently unstable, which makes it difficult for a sensor based on this protein to operate for a long time; furthermore, the binding of the protein via covalent bonds is complex. Indeed, the flow velocity, temperature, and preparation of the protein affect its binding properties [17,18] and only single layer of GGBP could be bound to the surface of the sensor. The borate polymer was introduced in the SPR sensing by our group, and this polymer has no immunogenicity or cytotoxicity, and its physical and chemical properties are stable [10], which extends the life of the SPR sensor based on this polymer. In this study, this borate polymer was bound to the surface of a sensor through layer-by-layer self-assembly method. This technology exhibits high reliability, making it possible to control the number of borate polymer layers and enabling the improvement of the measurement range and resolution of the SPR sensor.

2. Glucose measurement method by surface plasmon resonance

In this paper, the standard glucose solutions of different concentrations were used as standard to evaluate the accuracy and validation of the SPR sensor. Glucose solutions of known concentrations were sequentially injected through the surface of the SPR sensor without the borate polymer and the sensor bound with six or twelve layers of borate polymer. The effects of the number of layers are discussed in the manuscript, and the regenerability, reproducibility, and stability characteristics of the SPR sensor were evaluated. Almost all of the studies are performed under specific alkaline conditions (usually close to or higher than the p*Ka* of PBA). And for the future application in vivo, we further investigated glucose detection at physiological conditions.

2.1. Measurement principle of surface plasmon resonance

As shown in Fig. 1, the total internal reflection occurs when an incident beam of p-polarized light of a given wavelength strikes the interface between the prism and metal over the angle of total reflection through a prism. Under such conditions, part of the polarized light continues propagating in the form of an evanescent wave, which is parallel to the mental dielectric interface. When the wave vector of the evanescent wave (K_x) matches that of the surface plasmon wave (SPW) in the metallic film (K_{sp}), surface plasmon resonance occurs, and the associated optical electric field decays

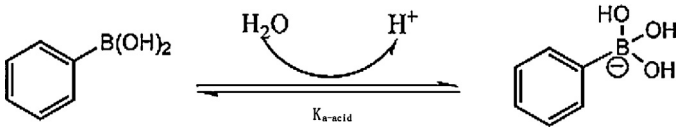

Fig. 2. Reaction of boric acid and water.

exponentially with distance from the surface. The incident angle at this moment is called the SPR angle (θ_{spr}).

The wave vector K_x of the horizontal component of the p-polarized light is

$$K_x = \frac{2\pi}{\lambda}\varepsilon_p \sin(\theta) \tag{1}$$

The wave vector K_{sp} of the surface plasmon resonance is

$$K_{sp} = \frac{2\pi}{\lambda}\sqrt{\frac{\varepsilon_n \varepsilon_o}{\varepsilon_m + \varepsilon_o}} \tag{2}$$

The SPR angle is

$$\theta_{spr} = \arcsin\left(Re\sqrt{\varepsilon_o\varepsilon_m/(\varepsilon_0 + \varepsilon_m)}/\sqrt{\varepsilon_p}\right) \tag{3}$$

where λ is the incident wavelength, and ε_p, ε_m, and ε_o represent the dielectric constants of the prism, gold film, and glucose solution, respectively [19,20].

According to the Maxwell equations, the velocity of light is

$$v = \frac{1}{\sqrt{\varepsilon\mu}} = \frac{1}{\sqrt{\varepsilon_0\mu_0}} \cdot \frac{1}{\sqrt{\varepsilon_r\mu_r}} = \frac{c}{\sqrt{\varepsilon_r\mu_r}} = \frac{c}{n} \tag{4}$$

Thus the relation between the refractive index and the dielectric constant is

$$n = \sqrt{\varepsilon_r\mu_r} \tag{5}$$

where c is the speed of light in vacuum, μ is the medium's permeability, ε is the medium's dielectric constant, μ_0 is the permeability in vacuum, ε_0 is the dielectric constant in vacuum, μ_r is the medium's relative permeability, and the ε_r is the medium's relative dielectric constant.

Based on the relationship between the refractive index and the glucose concentration, from Eq. (5) the glucose concentration can be obtained through the change in the dielectric constant, which can be calculated by the SPR angle [21]. In this study, 6 and 12 polymer layers were bound to a gold film, which could adsorb glucose molecules when a glucose solution was passed through the surface of the SPR sensor. Compared with the enzyme electrode sensor, which detects changes in electric current, the SPR sensor uses optical refractive index sensing to detect the glucose concentration, which eliminates the effects of bioelectricity when the sensor is implanted under the skin and results in high-precision measurements [19,20].

2.2. Affinity reaction between the borate polymer and glucose

As shown in Fig. 2, boric acid in aqueous exists in equilibrium between its uncharged normal state and its negatively charged dissociated state, and the percentage of the anionic state in the solution can be increased by increasing the solution pH because the p*Ka* of PBA is 8.8[22]. Fig. 3 shows the condensation reaction between the borate polymer and glucose. As shown, glucose will bind to the borate polymer to form boronate ester reversibly which depends on the concentration of glucose and the pH of the solution, because only tetrahedral anionic boric acid can form a stable complex with diols-containing targets [22]. Therefore, as the more glucose or the higher pH is present in the solution, the more PBA-glucose compounds form. In contrast, as the boronate ester bonding is reversible, PBA-glucose compounds disassociated

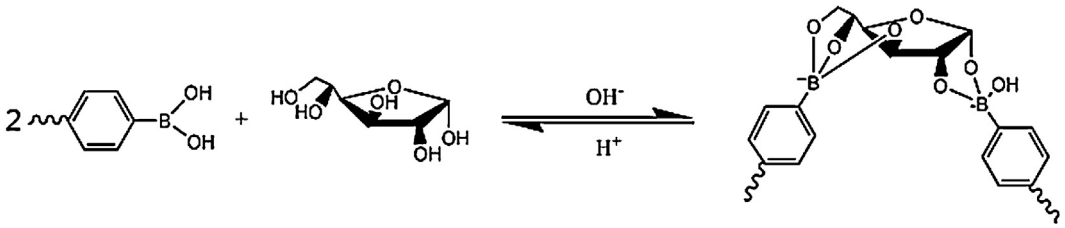

Fig. 3. Reaction of the borate polymer and glucose.

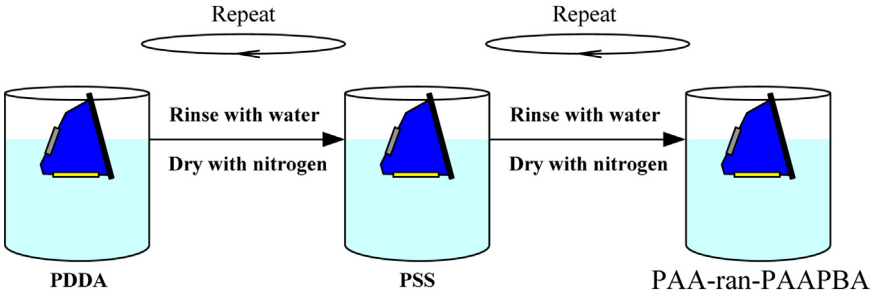

Fig. 4. Process used to bind the borate polymer.

through decreasing the glucose concentration or pH of solution. Thus, no glucose is consumed, and the SPR sensor exhibits good regenerability [17,18].

2.3. Binding process of the borate polymer

In this study, the borate polymer was bound to a gold surface through layer-by-layer self-assembly method, which uses weak interactions as the driving force for depositing multiple layers of film [23–25]. Compared to the use of a covalent bond for protein binding, electrostatic adsorption is easier to achieve. In particular, this method makes it possible to control the layers of the borate polymer, and as a result, the measurement range and resolution of the SPR sensor can be adjusted. The references [17–20] explain the details about the physics and chemistry behind the layer-by-layer self-assembly method. The reaction films were assembled as follows:

(1) Pretreatment
The gold surface of the SPR sensor was immersed in a solution (1:1:5 mixture of ammonia, H_2O_2, and H_2O) at 75 °C for 15 min, rinsed with excess ultrapure water, dried with nitrogen, and prepared for assembly.

(2) Reagents
The dipping concentration of the polyelectrolytes was 1 mg/mL for Poly (diallyldimethylammonium chloride) (PDDA, M_W = ~200,000–350,000 Da) and poly(sodium 4-styrene sulfonate) (PSS, M_W = 70,000 Da) were purchased from Sigma–Aldrich Co with 0.25 M NaCl for each. The borate polymer was synthesized as reported [10] was dissolved in a 0.01 M NaH_2PO_4 solution (pH 4.8), and the final concentration in the solution with 0.25 M NaCl was 0.1 mg/mL.

(3) Assembly
(a) The gold surface was dipped into the PDDA solution for 10 min, rinsed with ultrapure water to remove excess polycations and dried with nitrogen. (b) The sensor's gold surface was subsequently dipped into the PSS solution for 10 min, rinsed with ultrapure water, and dried with nitrogen as described above. Steps (a) and (b) were repeated until 2.5 bilayers, i.e., $(PDDA/PSS)_2PDDA$, which served as the basement membrane, were obtained, which provides uniform charge and smooth surface for subsequent deposition. And the number 2 indicates the number of immersions. (c) The sensor's surface with the basement membrane was dipped into the negatively charged PSS solution for 30 min, rinsed with ultrapure water, and dried with nitrogen. (d) Subsequently, the sensor's surface was dipped into the PAA-ran-PAAPBA solution for 30 min, rinsed with ultrapure water, and dried with nitrogen (Fig. 4). The PAA-ran-PAAPBA can be considered as an cationic macromolecule at pH 4.8 because of protonated amino groups at acidic pH. Steps (c) and (d) were repeated until 6 or 12 bilayers, i.e., $(PSS/PAA\text{-}ran\text{-}PAAPBA)_2$, were assembled on the gold film of the SPR sensor. The layer-by-layer assembly process of polymer was monitored by quartz crystal microbalance (QCM). For 6 bilayer film, the frequency shift was 447 Hz, and the thickness of polymer film was ca. 6.1 nm calculated by Sauerbrey's Eq. (1). Used the same method, the frequency shift was 621 Hz, and the thickness was ca. 8.5 nm for 12 bilayer film. After assembly, as shown in Fig. 5, we can see the different morphology of the surfaces using the atomic force microscopy (AFM). In addition, the surface roughness (Rq) for the bare sensor and assembled with 12 layers polymers are 5.19 nm and 4.10 nm, respectively.

3. Measurement system for glucose concentration

3.1. SPR sensor

An SPR sensor in the Krestchman structure (ICx Technologies) (Fig. 6) was used in this study, and the exterior and interior structures included a LED, an aperture and polarizing film, a gold film, a reflecting mirror, a photodiode array, and a memory chip. When polarized light of a given wavelength is reflected from the sensing surface over a range of incident angles, surface plasmon resonance occurs at the interface of the prism and gold film at the SPR angle. The intensity of the reflected light decreases markedly, which can be detected by a photodiode array. The software can convert the SPR angle to a refractive index to obtain the concentration of the analyte [19,20].

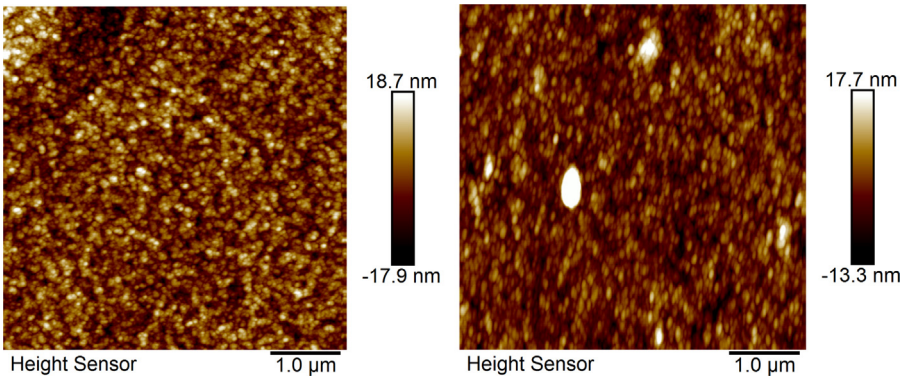

Fig. 5. The different morphology of the surfaces of the SPR sensors. (a) The surface morphology of the SPR sensor without polymer. (b) The surface morphology of the SPR sensor with 12 layers of polymer.

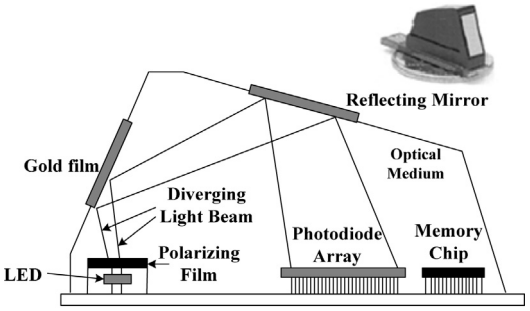

Fig. 6. Structure of the SPR sensor.

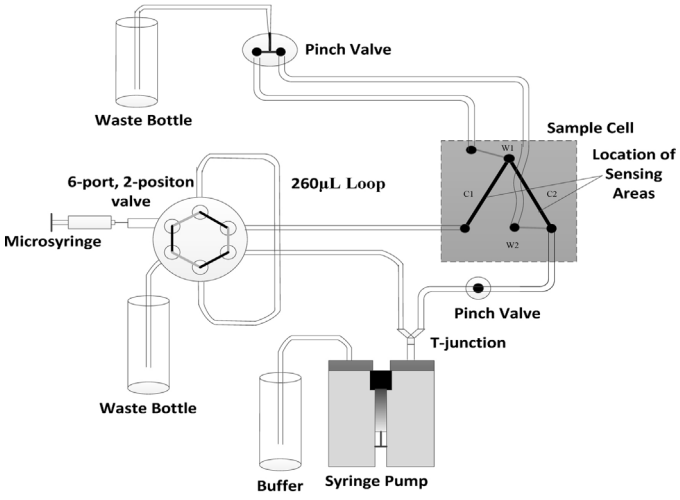

Fig. 7. Fluidic system used for the SPR measurement.

3.2. Fluidic system

As shown in Fig. 7, the SPR glucose measurement system includes a sample cell, a six-port two-position valve, a sample loop, a syringe pump, and a waste bottle. The microsyringe injects the sample into the 260-μL sample loop through the injection port and six-port, two-position valve. The valve then changes to the injecting position, and the syringe pump pushes buffer into the valve such that the sample is pushed into the sample cell. When pinch valve 1 and W1 are opened and W2 is closed, the buffer is stored in the C2 sensing area, and the sample can only flow through the C1 area and flow out from W1. If pinch valve 1 is closed, W1 is closed, and W2 is opened, the sample will pass through C1, C2, and W2 and then flow into the waste bottle [19,20].

4. Experiments and discussion

4.1. Reagent preparation

4.1.1. PBS (phosphate buffered saline)

$Na_2HPO_4{\cdot}12H_2O$ (3.5814 g; M_w = 358.14, Jiang Tian Chemical Technology Co., Ltd., China) was dissolved in 1000 mL of deionized water (Jiang Tian Chemical Technology Co., Ltd., China) to obtain 0.01 mol/L PBS (pH 9.0). 8 g of NaCl, 0.2 g of KCl (Jiang Tian Chemical Technology Co., Ltd., China), 3.63 g of $Na_2HPO_4{\cdot}12H_2O$, and 0.24 g of KH_2PO_4 (Jiang Tian Chemical Technology Co., Ltd., China) were dissolved in 1000 mL of deionized water to obtain PBS (pH 7.4).

4.1.2. Glucose solution

Because of the p*Ka* of PBA (8.8), borate polymer ions exist at alkaline solution, which form a stable complex with glucose. Almost all of the studies are performed under specific alkaline conditions (usually close to or higher than the p*Ka* of PBA). However, for the future application *in vivo*, we further investigated glucose detection at physiological conditions. The reproducibility experiments were repeated 5 times, and the other experiments were repeated 3 times.

The glucose solutions in group A featured PBS (pH 9.0) as the solvent, and the glucose solution (pH 9.0) with a concentration of 1000 mg/dL was serially diluted. In this experiment, the glucose solutions were divided into three ranges: 1–10 mg/dL (Δ = 1 mg/dL), 10–100 mg/dL (Δ = 10 mg/dL), and 100–1000 mg/dL (Δ = 100 mg/dL).

The glucose solutions of group B featured PBS (pH 7.4) as the solvent, and the glucose solution (pH 9.0) with a concentration of 1000 mg/dL was diluted to obtain the same concentrations as those in group A.

4.2. Effect of the number of layers

4.2.1. Measurement using the SPR sensors without polymer

PBS (pH 9.0) was used as the buffer, and the glucose solutions of group A were sequentially injected from low to high concentration through the surface of the SPR sensor without polymer. The ΔRUs between the different glucose solutions and the buffer solution were obtained. The experimental data were fitted by a quadratic curve, and the *R*-squared value was used to evaluate the curve fit. The results are shown in Fig. 8(a)–(c). The R-squared value of the glucose solution concentration-ΔRU fitted curve was 0.28664 in the concentration range of 1–10 mg/dL, which indicates a lack of linearity at low concentrations. The *R*-squared values of the

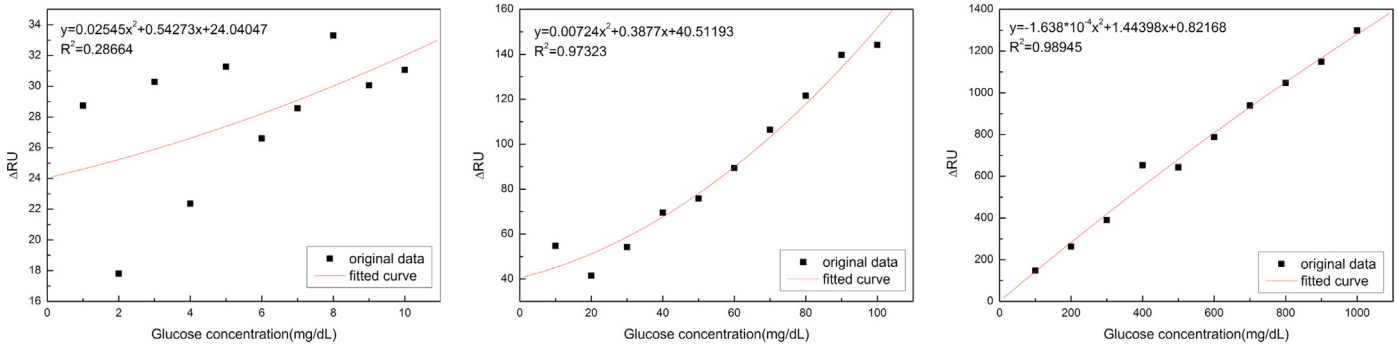

Fig. 8. Fitted curve of the ΔRU-glucose concentration without polymer. (a) 1–10 mg/dL, (b) 10–100 mg/dL, (c) 100–1000 mg/dL.

glucose solution concentration-ΔRU fitted curve for the concentration ranges of 10–100 mg/dL and 100–1000 mg/dL were 0.97323 and 0.98945, respectively.

4.2.2. Measurement using the SPR sensor with 6 polymer layers

PBS (pH 9.0) was used as the buffer, and the glucose solutions of group A were sequentially injected from low to high concentration through the surface of the SPR sensor with six immobilized layers of the borate polymer. The results are shown in Fig. 9(a)–(c). In the concentration range from 1 to 10 mg/dL the *R*-squared value was 0.64253, which is better than that obtained with the sensor without polymer. In addition, the *R*-squared values were 0.99024 in the concentration range of 10–100 mg/dL and 0.99348 in the concentration range of 100–1000 mg/dL.

4.2.3. Measurement using the SPR sensor with 12 polymer layers

For the SPR sensor with 12 polymer layers, PBS (pH 9.0) was used as the buffer, and the glucose solutions of group A were sequentially injected from low to high concentration. The results are shown in Fig. 10(a)–(c). In the concentration range from 1 to 10 mg/dL, the R-squared value was 0.78823, which is better than that obtained using the sensor with six polymer layers. The *R*-square values in the concentration ranges from 10 to 100 mg/dL and 100 to 1000 mg/dL were 0.99525 and 0.99780, respectively.

The glucose concentration of human interstitial fluid is always in the range of 30–600 mg/dL. After extracting the interstitial fluid through transdermal extraction, the interstitial fluid was diluted ten-fold, which means that the performance of the SPR sensor in the ranges of 1–10 mg/dL and 10–100 mg/dL are significant. The results of the above-described experiments are shown in Table 1. In the ranges of 1–10 mg/dL and 10–100 mg/dL, the performance of the sensors bound to the borate polymer was better than that of the sensor without the polymer. At pH 9.0, the sensitivity of the sensor with 12 polymer layers was better than that of the sensor with 6 polymer layers. In the low-concentration range, the measurement accuracy was improved with an increase in the number of polymer layers. From Fig. 10(a), the glucose concentration of 1–10 mg/dL (Δ = 1 mg/dL) in the buffer of PBS were subsequently injected to the surface of the SPR sensor bound to twelve polymer layers in a pH 9.0 environment, and the measurement resolution of glucose solution was 1 mg/dL. In this paper, the buffer was PBS, which has many components, such as sodium, potassium ions, thus the SPR sensor with borate polymer PAA-ran-PAAPBA has good specificity and sensitivity.

4.3. Regenerability

As shown in Fig. 3, the reaction between glucose and the SPR sensor with the borate polymer is reversible, which means that the measurement of the glucose concentration using this sensor, as opposed to that afforded by the enzyme electrode sensor, does not require the consumption of glucose. The regenerability is used to evaluate the affinity of the sensor for glucose. When the concentration of glucose increases, a condensation reaction occurs; in contrast, when the concentration decreases, dissociation occurs. To test the regenerability of the sensor with the borate polymer, the glucose solutions of group A were sequentially injected from high to low concentration through the surface of the SPR sensor bound to 12 polymer layers. The experimental data were fit to a quadratic curve, and the results are shown in Fig. 11(a)–(c). In the range of 1000–100 mg/dL, the *R*-squared value of the fitted curve was 0.98825, and *R*-squared values of 0.98837 and 0.52453 were obtained for the concentration ranges of 100–10 mg/dL and 10–1 mg/dL, respectively. The result shows good regenerability of the SPR sensor.

Compared with the results shown in Fig. 10(a)–(c), the *R*-squared values of the three datasets were all high, and there was no obvious difference between them. Thus, there is no relationship between the measurement sequence and the experimental result.

4.4. Reproducibility

In this paper, the SPR sensor without borate polymer and the sensor bound to six or twelve layers of polymer were used to discuss the effects of the number of layers and the pH environment. The experiment results in this paper are based on abundant experiments, and the data shows good reproducibility, thus only one set of experimental result was selected in each part of the paper. Fig. 12 is the standard deviation diagram of the sensor bound to twelve polymer layers in pH 9.0, and all the experiments were repeated five times respectively under the same condition. The result shows good reproducibility of the SPR sensor.

4.5. Stability and response speed

To test its stability characteristics, the SPR sensor with 12 polymer layers was preserved for three months at room temperature. The glucose solutions from group A were injected from low to high concentration, and the results are shown in Fig. 13. In the range of 1–10 mg/dL, the *R*-squared value of the glucose solution concentration-ΔRU fitted curve was 0.85468, whereas the *R*-squared values for the concentration ranges of 10–100 mg/dL and 100–1000 mg/dL were 0.99561 and 0.99963, respectively.

The SPR glucose measurement system and the data processing software used in the experiment was SensiQ (SensiQ Technologies, Inc.), and the SPR spectrum is handled into the real time kinetic curve through the software. Use the maximum RU value substrates the baseline RU value, and then the response of different glucose concentration is got. To compare the data of different sensors, only the value of ΔRU were provided though the real time kinetic curve

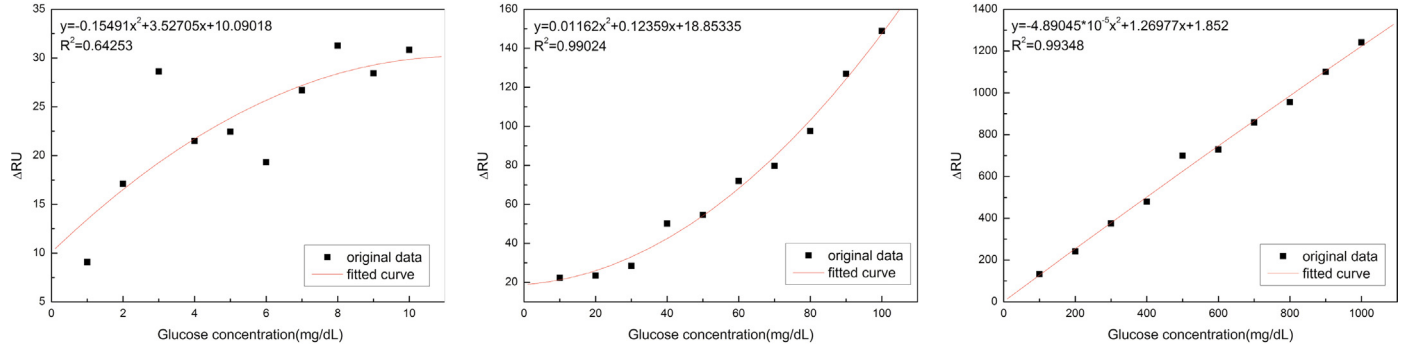

Fig. 9. Fitted curve of ΔRU-glucose concentration with 6 layers of polymer. (a) 1–10 mg/dL, (b) 10–100 mg/dL, (c) 100–1000 mg/dL.

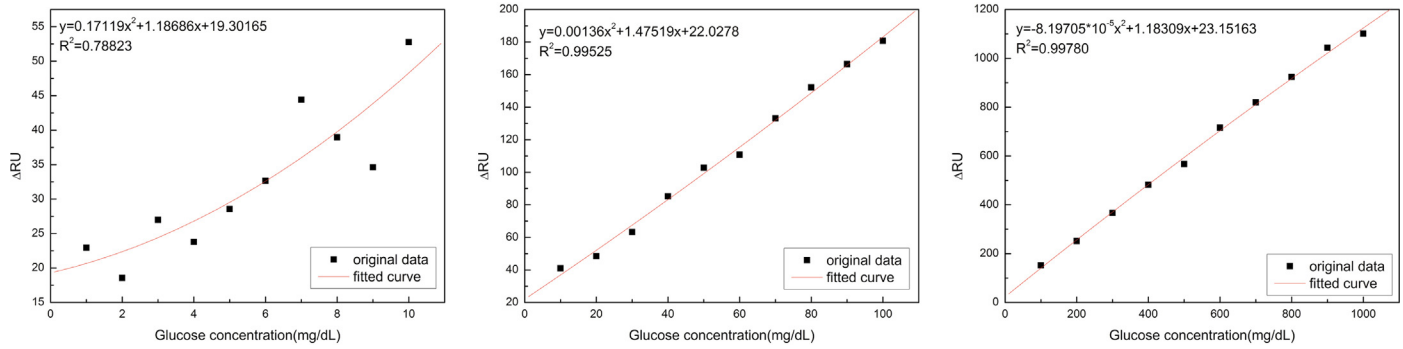

Fig. 10. Fitted curve of ΔRU-glucose concentration with 12 polymer layers. (a) 1–10 mg/dL (b) 10–100 mg/dL (c) 100–1000 mg/dL.

Table 1
R^2 values obtained with different numbers of polymer layers.

R^2	1–10 mg/dL	10–100 mg/dL	100–1000 mg/dL
SPR sensor without polymer	0.28664	0.97323	0.98945
SPR sensor with 6 layers of polymer	0.64253	0.99024	0.99348
SPR sensor with 12 layers of polymer	0.78823	0.99525	0.99780

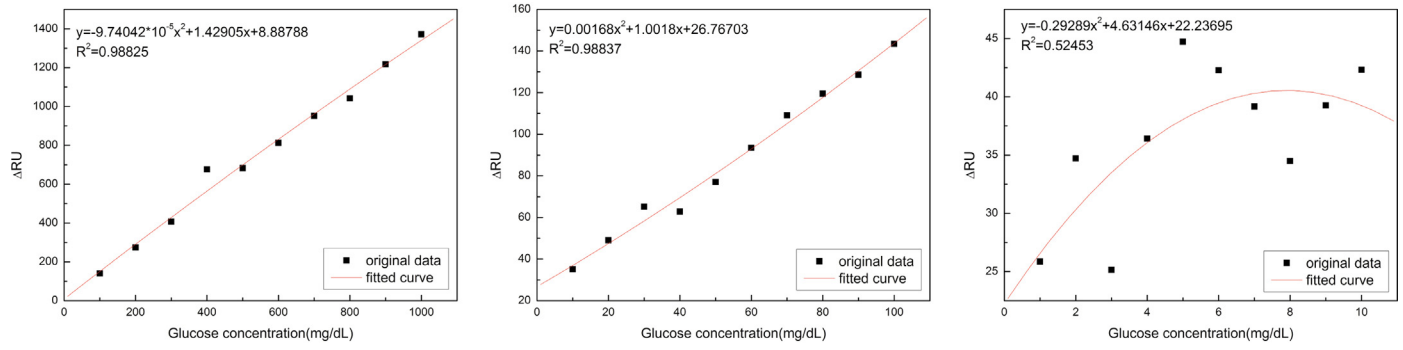

Fig. 11. The glucose solution was injected from high to low concentration through the surface of the SPR sensor bound to 12 polymer layers. (a) 100–1000 mg/dL, (b) 10–100 mg/dL, (c) 1–10 mg/dL.

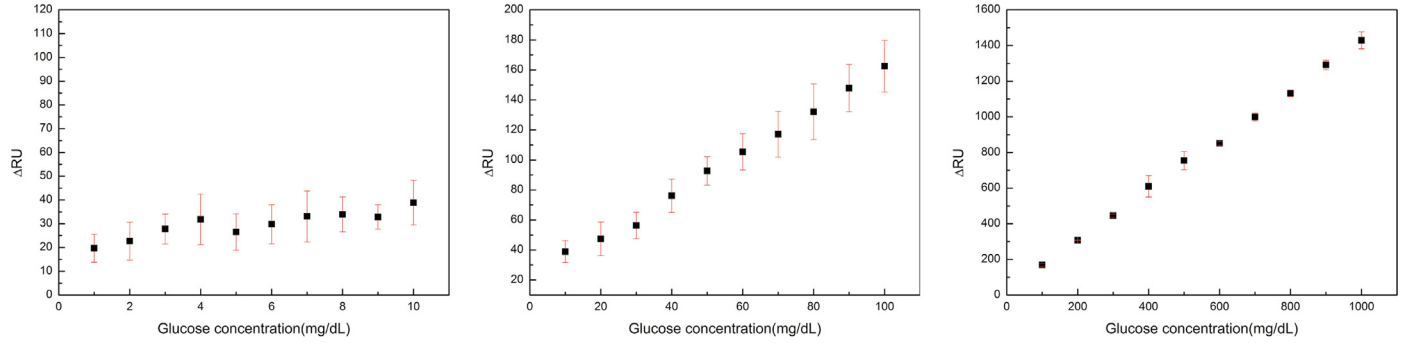

Fig. 12. The reproducibility of the SPR sensor bound to twelve polymer layers in pH 9.0. (a) 1–10 mg/dL, (b) 10–100 mg/dL, (c) 100–1000 mg/dL.

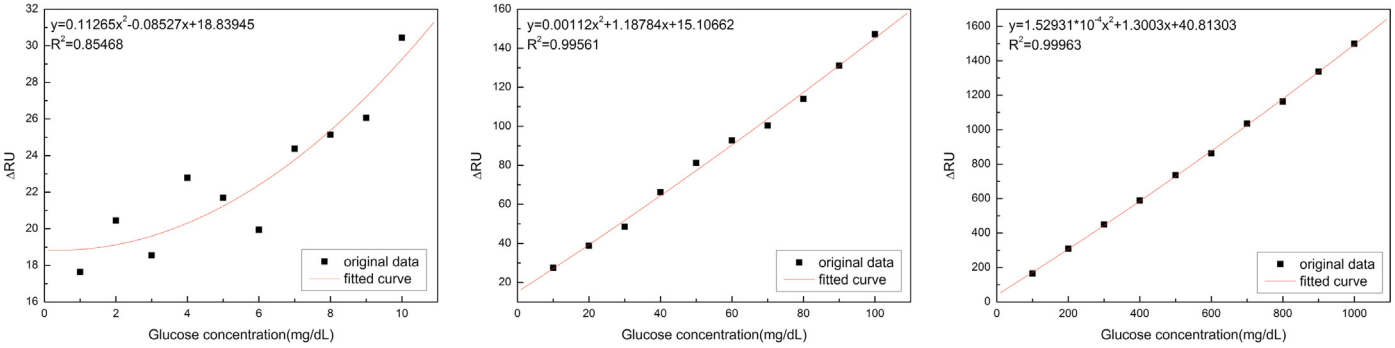

Fig. 13. Stability characteristics of the SPR sensor with 12 polymer layers. (a) 1–10 mg/dL, (b) 10–100 mg/dL, (c) 100–1000 mg/dL.

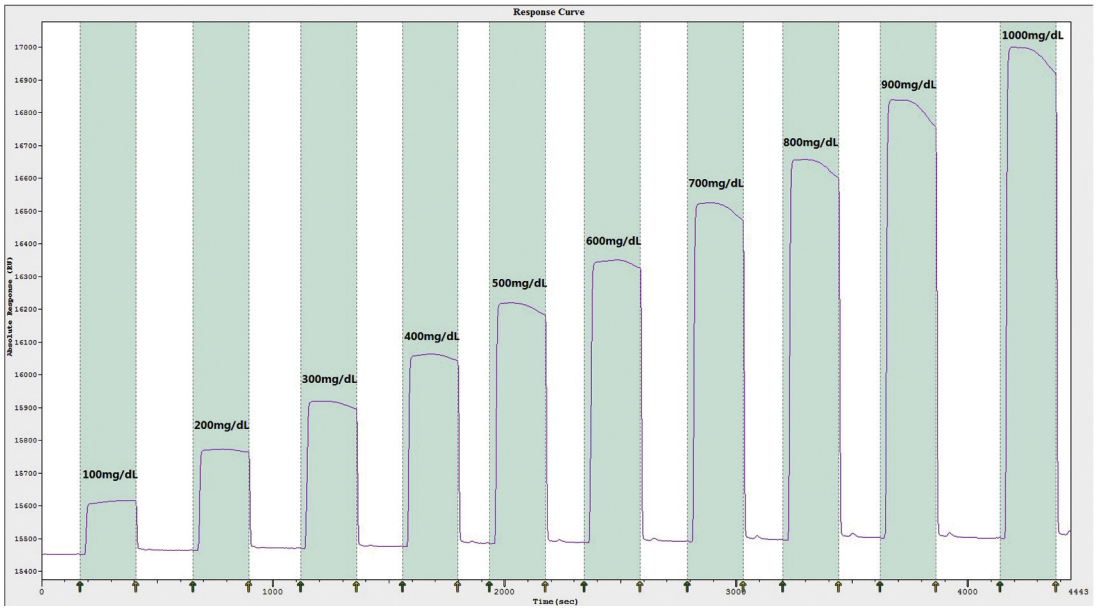

Fig. 14. The sensorgram of the stability experiment at the range of 100 mg/dL–1000 mg/dL.

shows the surface plasmon resonance response intuitively. Fig. 14 is the real time kinetic curve of the stability experiment when the glucose concentration ranges from 100 mg/dL to 1000 mg/dL.

The experimental results show that the sensor with 12 polymer layers was still in good condition after three months of preservation at room temperature, and its stability was better than that of the sensor bound to GGBP.

To discuss the response speed of the sensor, the response time was calculated from the real time kinetic curve of the stability characteristics experiment of the SPR sensor bound to twelve polymer layers in pH 9.0, and the average response time was 17.6 s, which indicates that the response time of the SPR sensor is fast.

4.6. Glucose detection at physiological conditions

Almost all of the studies are performed under specific alkaline conditions (usually close to or higher than the p*Ka* of PBA). However, for the future application in vivo, we further investigated glucose detection at physiological conditions, the pH of which is 7.4. Using the SPR sensor bound to 12 polymer layers and the

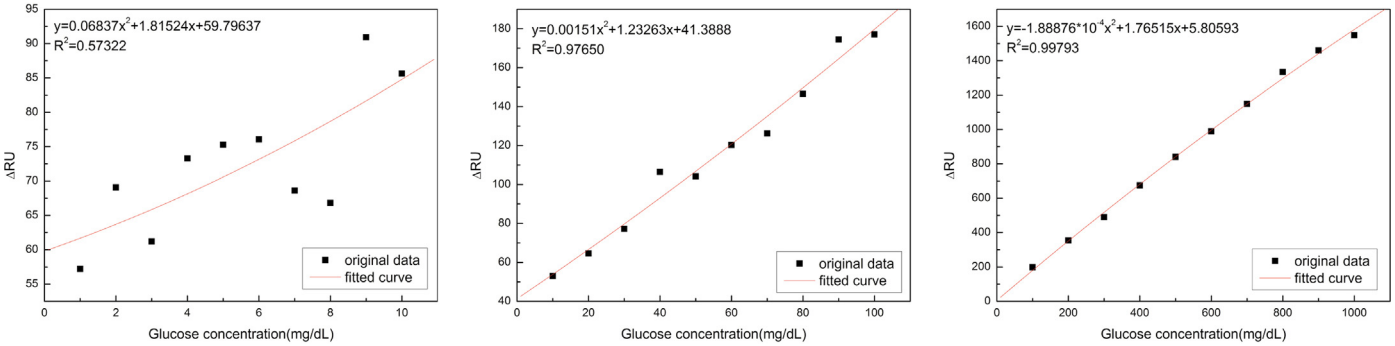

Fig. 15. Fitted curve of ΔRU-glucose concentration with 12 polymer layers at physiological conditions. (a) 1–10 mg/dL, (b) 10–100 mg/dL, (c) 100–1000 mg/dL.

PBS (pH 7.4) as the buffer, and the glucose solutions of group B were sequentially injected from low to high concentration. The ΔRUs between the different glucose solutions and buffer solutions were obtained. The experimental data were fit to a quadratic curve, and the results are shown in Fig. 15(a)–(c). In the range of 1–10 mg/dL, the *R*-squared value of the fitted curve was 0.57322, which is worse than that obtained with PBS at pH 9.0. Because of the p*Ka* of PBA (8.8), borate polymer ions exist at alkaline solution. The *R*-squared values in the concentration ranges of 10–100 mg/dL and 100–1000 mg/dL were 0.97650 and 0.99793, respectively. From the experiment results, we can draw the conclusion that the SPR sensor bound to 12 layers of polymer works well at physiological conditions.

5. Conclusions

The borate polymer PAA-ran-PAAPBA was introduced into a SPR sensor to detect glucose concentration. The layer-by-layer self-assembly method was used to bind the borate polymer to the surface of the SPR sensor and to control the number of layers such that the measurement range and resolution of the SPR sensor could be adjusted. In this study, 6 and 12 layers of polymer were immobilized on the surface of the SPR sensor, and the experimental results indicated that the linearity of the SPR sensor was highly improved with the use of the borate polymer. The *R*-squared values of the glucose concentration-ΔRU fitted curves of the SPR sensor with 12 polymer layers in the concentration ranges of 1–10 mg/dL and 10–100 mg/dL were as high as 0.78823 and 0.99525, respectively, and those of the sensor with 6 layers polymer were 0.64523 and 0.99024, respectively, which means that the measurement accuracy improved with an increase in the number of polymer layers. The sensor works well at physiological conditions and exhibited good regenerability, reproducibility and stability, which lay the foundation for the development of an implantable device for continuous glucose measurement.

Our group focuses on the research of the continuous measurement of glucose concentration in vivo for many years. The colleague in our group is now developing a fiber optic SPR (FO-SPR) sensor for glucose monitoring. The miniaturized size of the FO-SPR sensor renders it particularly appropriate for implantable measurement of the glucose concentration in the interstitial fluid by detecting the refractive index to replace the enzyme electrode sensor. In the continuous glucose measurement system of FO-SPR, the fiber optic SPR sensor is the only part implanted under the skin, which means only the optical signal passes through the subcutaneous tissue and the other parts such as the light source, the spectrograph, the electrical circuit and the PC will be out of human body. As a result, compared to the enzyme electrode sensor, the effects of bioelectricity could be eliminated during the measurement. In the near future, the methods and the results in vitro testing in this paper will be used to modify the surface of the FO-SPR sensor to realize the glucose affinity measurement in vivo testing.

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References

[1] W. Hou, S.B. Cronin, A review of surface plasmon resonance-enhanced photocatalysis, Adv. Funct. Mater. 23 (13) (2013) 1612–1619.
[2] C. Boss, et al., Novel chemico-mechanical approach towards long-term implantable glucose sensing, Proc. Chem. 1 (1) (2009) 313–316.
[3] Y. Yonemori, et al., Biosensor system for continuous glucose monitoring in fish, Anal. Chim. Acta 633 (1) (2009) 90–96.
[4] P. Valdastri, et al., Wireless implantable electronic platform for blood glucose level monitoring, Proc. Chem. 1 (1) (2009) 1255–1258.
[5] F. Pillet, et al., Dendrimer functionalization of gold surface improves the measurement of protein–DNA interactions by surface plasmon resonance imaging, Biosens. Bioelectron. 43 (2013) 148–154.
[6] X. Huang, et al., A MEMS affinity glucose sensor using a biocompatible glucose-responsive polymer, Sens. Actuators B: Chem. 140 (2) (2009) 603–609.
[7] K.-C. Liao, et al., Percutaneous fiber-optic sensor for chronic glucose monitoring in vivo, Biosens. Bioelectron. 23 (10) (2008) 1458–1465.
[8] Y. Xie, et al., Metallic-like stoichiometric copper sulfide nanocrystals: phase- and shape-selective synthesis, near-infrared surface plasmon resonance properties, and their modeling, ACS Nano 7 (8) (2013) 7352–7369.
[9] S. Singh, M. McShane, Enhancing the longevity of microparticle-based glucose sensors towards 1 month continuous operation, Biosens. Bioelectron. 25 (5) (2010) 1075–1081.
[10] S. Li, et al., Development of boronic acid grafted random copolymer sensing fluid for continuous glucose monitoring, Biomacromolecules 10 (1) (2008) 113–118.
[11] A. Pasic, et al., Miniaturized fiber-optic hybrid sensor for continuous glucose monitoring in subcutaneous tissue, Sens. Actuators B: Chem. 122 (1) (2007) 60–68.
[12] M.J. Polking, et al., Controlling localized surface plasmon resonances in GeTe nanoparticles using an amorphous-to-crystalline phase transition, Phys. Rev. Lett. 111 (3) (2013) 037401.
[13] M. Tomassetti, et al., Comparison of three immunosensor methods (surface plasmon resonance, screen-printed and classical amperometric immunosensors) for immunoglobulin G determination in human serum and animal or powdered milks, J. Pharm. Biomed. Anal. 73 (2013) 90–98.
[14] M. Ermini, et al., Coupling nanotechnology to optical affinity sensing: the case of surface plasmon resonance imaging for DNA detection, in: Sensors, Springer, 2014, pp. 103–106.
[15] L.L. Kegel, D. Boyne, K.S. Booksh, Sensing with prism based near infrared surface plasmon resonance spectroscopy on nanohole array platforms, Anal. Chem. 86 (7) (2014) 3355–3364.
[16] F.-X. Huang, et al., Measurement of glucose concentration by surface plasmon resonance with D-galactose/D-glucose binding protein, Nami Jishu yu Jingmi Gongcheng 8 (2) (2010) 132–136.
[17] R. Méjard, et al., Tuneable and robust long range surface plasmon resonance for biosensing applications, Opt. Mater. 35 (12) (2013) 2507–2513.
[18] A. Giorgini, et al., Surface plasmon resonance optical cavity enhanced refractive index sensing, Opt. Lett. 38 (11) (2013) 1951–1953.
[19] P. Bhatia, P. Yadav, B.D. Gupta, Surface plasmon resonance based fiber optic hydrogen peroxide sensor using polymer embedded nanoparticles, Sens. Actuators B: Chem. 182 (2013) 330–335.
[20] M. Baltzinger, et al., Dissecting the oligonucleotide binding properties of a disordered chaperone protein using surface plasmon resonance, Nucleic Acids Res. 41 (22) (2013) 10414–10425.
[21] G. Marchesini, et al., Spreeta-based biosensor assays for endocrine disruptors, Biosens. Bioelectron. 22 (9–10) (2007) 1908–1915.
[22] G.S. Jun Yan, S. Deeter, Binghe Wang, The relationship among pK a, pH, and binding constants in the interactions between boronic acids and diols – is not as simple as it appears, Tetrahedron 60 (2004) 11205–11209.
[23] X. Wang, et al., Doxorubicin delivery to 3D multicellular spheroids and tumors based on boronic acid-rich chitosan nanoparticles, Biomaterials 34 (19) (2013) 4667–4679.
[24] X. Zhang, et al., Highly stable and degradable multifunctional microgel for self-regulated insulin delivery under physiological conditions, Nanoscale 5 (14) (2013) 6498–6506.
[25] C. Zheng, et al., Amphiphilic glycopolymer nanoparticles as vehicles for nasal delivery of peptides and proteins, Eur. J. Pharm. Sci. 49 (4) (2013) 474–482.

Biographies

Dachao Li received the Ph.D. degree in Precision Instrument and Mechanics from Tianjin University, Tianjin, China, in 2001. From 2004 to 2006, he did post-doctoral research at Institute of Microelectronics, Peking University, Beijing, China. From 2006 to 2008, he was a research associate at Department of Electrical Engineering and Computer Science, Case Western Reserve University, Cleveland, USA. Presently, he is an associate professor in College of Precision Instrument and Optoelectronics Engineering, Tianjin University. His research specializations focus on micro sensor and optofluidics.

Di Yang received her B.E. degree in Precision Instrument from Tianjin University, Tianjin, China, in 2012. At present, she is a master student at the Precision Instrument and Mechanics from Tianjin University, Tianjin, China. Her current research interests involve the measurement techniques of surface plasmon resonance and localized surface plasmon resonance.

Jia Yang received her B.E. degree in Precision Instrument from Hebei university of Technology, Tianjin, China, in 2010 and the M.E. degree in Precision Instrument and Mechanics from Tianjin University in 2013. Her research interests involve the measurement techniques of surface plasmon resonance bound to the GGBP and the borate polymer.

Yuan Lin received her B.S. and Ph.D. degree in materials science and engineering from Jilin University, Changchun, China, in 2003 and 2008. From 2006 to 2007, she studied in the Department of Food Science, Rutgers University, USA as a joint Ph.D. student. From 2008 to 2010, she did post-doctoral research at Department of Chemistry and Biochemistry, University of South Carolina, USA. Presently, she is an associate professor in State Key Lab of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Changchun, China. Her research interest includes the design functional biomaterials and the interaction between cell and substrate.

Yingjuan Sun received her Bachelor degree in Polymer Material and Engineering from Anhui University, Hefei, China, in 2012. From 2012 to now, she studied in the State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Changchun, China, as a Ph.D. candidate in Qian Wang's group. Her research focus is mainly on studying the self-assembling conductive polymer patterns with ordered topography and electrical stimulation for the directional growth of neural cells.

Haixia Yu received the Ph.D. degree in precision instrument and optoelectronics engineering from Tianjin University, Tianjin, China, in 2007. From 2008 to 2010, she studied in the Department of Electrical Engineering & Computer Science, Case Western Reserve University, USA as a joint Ph. D. student. From 2011 to 2013, she did post-doctoral research at Tianjin University, Tianjin, China. Presently, she is an associate professor in College of Precision Instrument and Optoelectronics Engineering, Tianjin University. Her research specialization is on the biomedical microfluidics and the micro biomedical sensors.

Kexin Xu received the Ph.D. degree in precision instrument engineering from Tianjin University, Tianjin, China, in 1988. From 1992 to 1999, he was a senior research fellow and team leader for non-invasive glucose measurement techniques in the Laboratory of Basic Technologies, KDK Corporation, Kyoto, Japan. He obtained his professorship from Tianjin University in 2000. Since 2002, he has been the Dean of the College of Precision Instrument and Opto-Electronic Engineering, Tianjin University. His research interest includes the design theory of acousto-optic tunable filter and spectroscopic technology, rapid detection of milk ingredients, monitoring of air and flue gas composition, non-invasive glucose monitoring, etc.