Ultra-sensitive poly(boric acid) hydrogel-coated QCM sensor by using UV pressing-assisted polymerization for saliva glucose monitoring

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ABSTRACT: Quartz crystal microbalance (QCM) has attracted extensive attention in the field of biological analysis and detection because of its high sensitivity, fast response, real-time measurement, good operability, and low-cost production. However, the detection of trace amounts of small molecules, such as the detection of low concentrations of salivary glucose under physiological conditions, is still a major challenge. Herein, the surface of a QCM chip was coated with a poly(boronic acid)-basedhydrogel usingUV pressing-assisted polymerization to obtain a simpledevice for glucose detection. The designed QCM sensor shows record-low detection limit of glucose (3 mg/L at pH 7.5) among reported boric acid polymer-based QCM sensors, which is about 30 times lower than that of sensors fabricated by conventional surface initiation-spin coating. The outperformance of the poly(boric acid) hydrogel-coatedQCM sensor is probably owing to the uniform and compactmicrostructure, and the presence of sufficient glucose binding sites resulting from thehydrogel coating generated by UV pressing-assisted polymerization. This method provides an important solution for the detection of trace amounts ofsmall organicmolecules or ions, and has the potential to push forward the practical applications of QCM sensors.

1. Introduction

Quartz Crystal Microbalance (QCM) is a very sensitive quality detector, which can detect sub-nanometer mass changes in biosensors.[1–3] Owing to its high sensitivity, fast response, low-cost production, ability of real-time measurement and simple integration compared with other electronic components, QCM isconsidered an important microbiosensor in the field of biological analysis and detection.[4–9] Current research on QCM focuses on the exploration of mechanism and kinetics of binding processes of large biomolecules[10,11]as well as the detection of DNA,[12,13] proteins,[14,15] bacteria,[16] etc.In addition, recent reports have examined the application ofQCMsin reproductive medicine, e.g.,thein-situ assessment of the postejaculatory dynamics of human seminal fluids (viscosity, volume, and sperm concentration).[17,18] Furthermore, relatively few reports focus on the detection of organic molecules and ions with QCM in liquids.[19]

In 2019, approximately 463 millionpeoplehave been diagnosed with diabetes worldwide (aged 20–79), and the number of diabetics will reach 578.4 million by 2030. Diabetes and its complications impose significant economic consequences for individuals, families, health systems and countries. American diabetes association (ADA) has conclusively shown that self-monitoring of blood glucose (SMBG) is an important part of diabetes management and individualized treatment. SMBG results are helpful to evaluate the degree of glucose metabolismdisorderin patients with diabetes, develop a reasonable hypoglycemic program, and reflect the effect of hypoglycemic treatment,which allows appropriate adjustment of the hypoglycemic program.[20,21]However, the inconvenience, expense, pain and complexity involved in SMBG leads to underutilization.[22,23] Therefore, an accurate, painless and easy-to-use

device is urgently needed to achieve glucose monitoring.Reports have shown that glucose concentration in saliva is highly correlated withblood sugar levels.[24–26]Therefore, saliva has become an ideal marker for non-invasive blood glucose monitoring due to its safety, non-invasiveness, convenience and realtime moitoring.[27,28] However, the concentration of glucose in saliva is extremely low, accounting for approximately 1/100–1/50 of the blood glucose concentration. In addition,glucose is a small molecule monosaccharide (MW=180.16 g/mol). Therefore, changes of the glucose concentration in saliva cause only a small signal, which is difficult to be detected by QCM. This presents a critical factor that limits applications of glucose-QCM sensors.[29,30]

To achieve low-concentration glucose detection under physiological conditions, a glucose-sensitive, film-coated QCM electrode with high specific adsorption is desirable. Compared with conventional glucose-responsive materials–enzymes and lectins, boronic acid polymers benefit from their stability, durability, low cost, and high glucose sensitivity and are widely used in glucose identification materials.[31–33] QCM electrodes are coated with polymers via chemical methods,where the initiator is fixed on the surface of the QCM electrode, and the polymer is subsequently grown in situ on its surface via free radical polymerization.[34,35]However, certainglucose sensors based on QCM are sensitive enough tosteadily detect low glucose concentrationsin saliva (20–200 μmol/L; after conversion: 3.6–36 mg/L)[36] under physiological conditions (Table S2 in the Supporting Information).

Herein, a new method of coating poly(boric acid) hydrogel onto the surface of a QCM electrode by UV pressing-assisted polymerization was demonstrated (denoted as UVpressing). The functional poly(boric acid) hydrogel coating obtained by this method has a dense structure, sufficient glucose binding sites, excellent stability, uniformity, and is easy to operate. Inert gas atmosphere is not required during the UV-induced reaction, which simplifies the experimental process and saves resources. In combination with our lab-built QCM platform, glucose can be successfully detected in PBS and artificial saliva under physiological conditions and a glucose detection limit of 3 mg/L was obtained at pH 7.5. This work provides a new method for detecting low concentrations of small moleculesbasedon QCM.

2. Results and discussion

2.1. Synthesis of the hydrogel

Apolymerizable double bond was grafted onto the upperside of QCM electrode (Figure 1-i, Figure S1 in the Supporting Information). The synthesis of thesurface-grafted hydrogel on the electrode can be roughly divided into three steps (Figure 1). First, a clean quartz plate was prepared, and a small amountof pre-polymerization solution was dropped onto the quartz plate. Then, the treated QCM electrode was placed face down on the pre-polymerization solution, and the bottom side was pressed with uniform force. After exposure to ultraviolet light for a short time, the hydrogel formed on the electrode surface. Finally, the hydrogel-coated QCM (Figure 1-ii) was separated from the working solid substrate-crystal plateby immersion in distilled water.

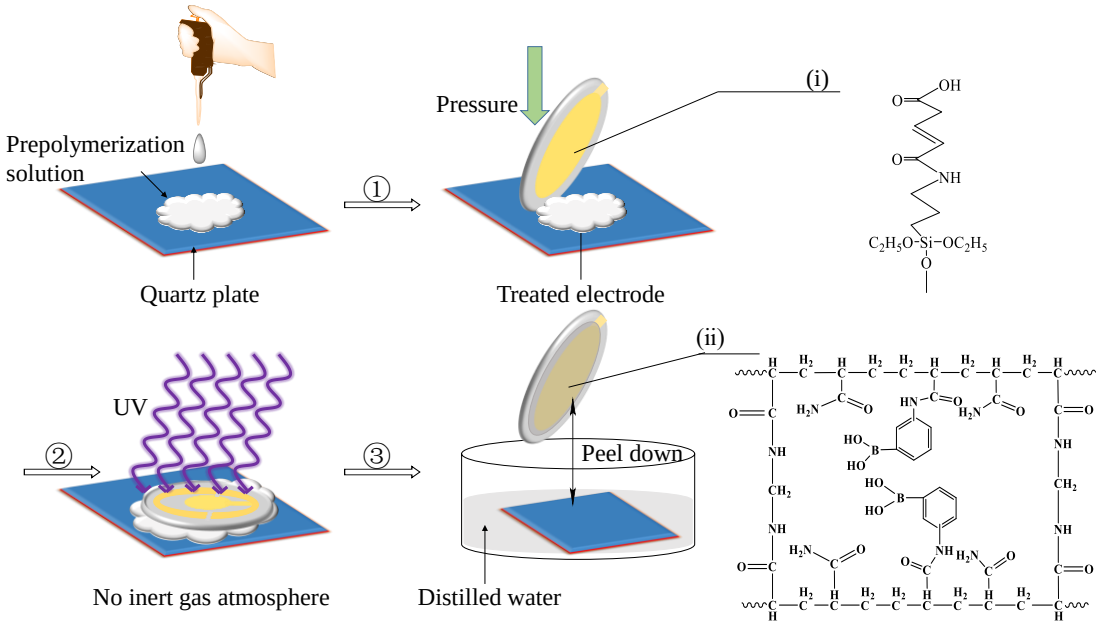

Figure 1. Synthesis of hydrogel-coated QCM electrode byUVpressing. ① Dropping of the prepolymerized solution onto the electrode surface and application of external pressure. ② UV polymerization. ③ Peeling offof thehydrogel-coated QCM sensor from the working solid substrate-crystal plate.

Films synthesized by two different synthesis methods (UV pressing and surface initiation-spin coating) were analyzed by SEM,aspresented in Figure 2. Figure 2a (top view) shows that the surface of the hydrogel synthesized by UV pressing was complete and relatively flat. In contrast,Figure 2b (top view) reveals that the film synthesized by spin coatingis not complete and exhibits many spots (SEM image of doubled bond–QCM chip is shown in Figure S3 in the Supporting Information). Figure 2a,b (cross section)shows that the hydrogel synthesized by UV pressing is more compact, which will increase the amount of boric acid groups in the hydrogel. The thickness of the prepared films by either method is about 420 nm.

In order to prove the superiority of UV pressing,the detection limits of hydrogel-coated

QCM sensors preparedby both methods were studied by lab-built QCM platform (Figure S10 in the Supporting Information).In this work, the detection limit refers to the minimum glucose concentration that can be detected by boric acid hydrogel-coated QCM sensor (Figure S6 in the Supporting Information).As described above, Figure 2c,dshows that the detection limit of the hydrogel-coated QCM sensor prepared by UV pressingis 3 mg/L,which is significantly lower than the detection limit of100 mg/L determined for the sensor prepared by spin coating.This indicates that the film prepared by UV pressing has abetter glucose response.

The main reason for the excellent glucose binding performance of the hydrogel prepared by UV pressing is the external pressure applied during the fabrication process. Because the pre-polymerized solution is confinedbetween the electrode and quartz plate, the hydrogel formed by polymerization possesses uniformity and compact microstructures.In addition, the polymerization does not require inert gas protection, as the air in the pre-polymerization liquid is eliminated by the applied pressure. Other advantages include thefacilesynthesis process and the possibility to control the thickness ofthehydrogel by tuning the applied pressure (see Figure S9 in the Supporting Information).

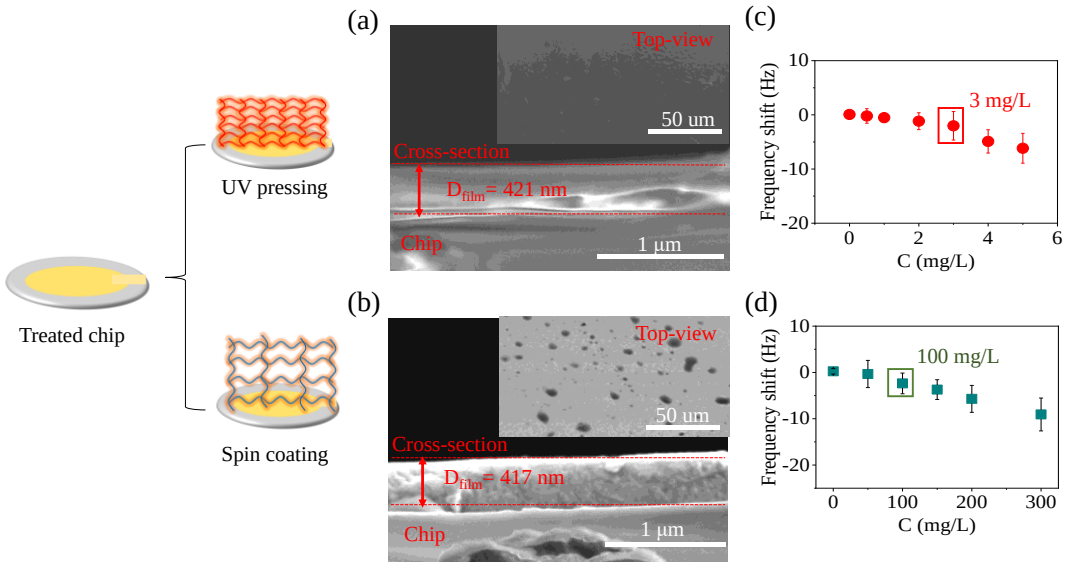

Figure 2. Comparison of hydrogel-coated QCM electrodes prepared by different methods. (a) Hydrogel coating fabricated by UV pressing exhibited a uniform and dense structure. (b) Hydrogel coating generated by spin coating showed a heterogenous structure with obvious spots. (c)The thickness of hydrogel films synthesized by the two methods was about 420 nm. (d) The detection limit of the hydrogel-coated QCM sensorsynthesized by UV pressing is 3 mg/L, while that of the other sensorsynthesized by spin coating is 100 mg/L.

2.2 Characterization

The hydrogel-coated electrode was characterized by FTIR. As shown in Figure 3a,the peak at ~3195 $cm^{-1}$is attributed to theN–H stretchingvibrationof the NH_2group,and the peak at ~1658 $cm^{-1}$is attributed to the C=O stretching vibration.[37] Characteristic absorption peaks of C_6H_6 appear at ~1423 cm^{-1},[38]and the absorption peak at ~1345 $cm^{-1}$is assigned to B–O.[39]These results confirm theformation of the boric acid hydrogel.

The surface roughness of the hydrogel-coated electrode was analyzed by Atomic Force

Microscopy (AFM) using a near-field microscope. The AFM results show that the surface ofthehydrogel is homogeneous (peak-valley roughness ~35 nm,Figure 3b).SEM and AFM analysis of hydrogel-coated chip in dry state and glucose solution are shown in Figure S4a-d in the Supporting Information**.**

Figure 3c shows XPS survey scan of the hydrogel-coated electrode, where the peaks of C1s, O1s,and N1s are attributed to various reactants (3-acrylamidophenylboronic acid, acrylamide, and N,N'-methylenebisacrylamide). The B1s signal at 191 eV indicates the presence of boronic acid groups (Figure 3d).[40,41]The element distribution of B1s, C1s,and O1s is 6.6±1.7%, 43.5±2.0%, and 24.8±1.4%, respectively.

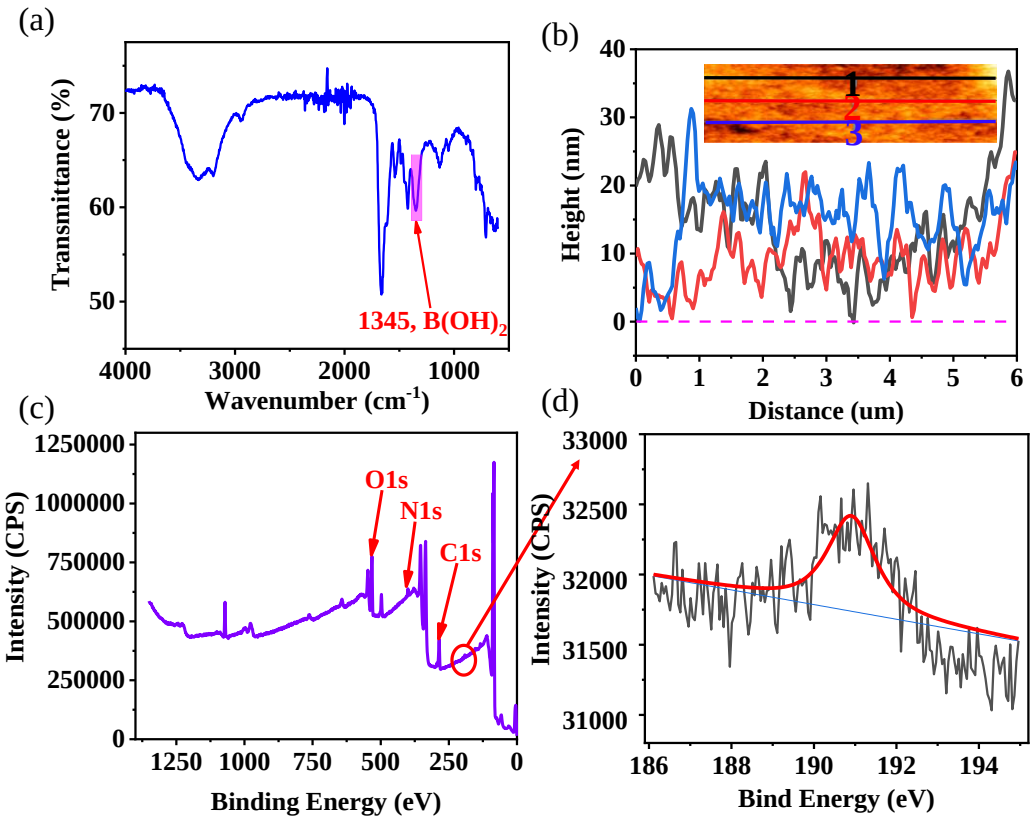

Figure 3. (a) FTIR spectrum of hydrogel-coated electrode. (b) Homogenoushydrogel surface (peak-valley roughness ~35 nm).(c) XPS survey scan of hydrogel-coated chip.

(d) B1ssignal at 191 eV is attributed to the boronic acid groups (black line: actual measurement; red line:Gaussian fit through the XPS data; blue line: software-generated baseline of Gaussian fit).

2.3 Hydrogel properties

The performance of the hydrogel was studied in PBS, and the resonant frequency shift (△F= F(glucose sample) – F (blank sample)) was measured to evaluate the glucose sensor performance. First, the stability of the sensor has been tested, which presents a very important aspect of the performance. The hydrogel-coated QCM electrode was placed in a flow cell, and PBS was injected by a peristaltic pump at room temperature. The hydrogel-coated QCM sensor shows a tiny fluctuation of ~13 Hz in PBS for about 20 h (Figure 4a).

Boronic acids can covalently bind glucose and reversibly form a boronic acid diester (Figure S2a in the Supporting Information).[42,43]Hydrogel-coated QCM sensor can recognize glucose molecules (Figure S2b in the Supporting Information). For a control experiment, the hydrogel was synthesizedunder the same conditions as the boric acid hydrogel but in the absence of 3-(acrylamido) phenylboronic acid (3-APB) in the prepolymer solution. As shown in Figure 4c,as the glucose concentration changes, the △F of thecontrolhydrogel did not show any obvious changes. In contrast, the boric acid hydrogel shows△F response to glucose in the concentrationrange of 0–160 mg/L, which encompasses the concentration range of glucose in saliva of both healthy people and diabetics.[44] When the glucose concentrationgradually increases from 0 to 160 mg/L,

the absolute value of △F increases rapidly, suggesting the rapid binding between boronic acid groups of the hydrogel and glucose molecules. With increasing reaction time, ΔF gradually stabilizes. As theglucose concentration gradually decreases from 160 to 0 mg/L, the absolute value of △F gradually decreases, indicating that glucose molecules are released from the hydrogel (detection under different pH is shown in Figure S8 in the Supporting Information). These results indicate that 3-APB inthehydrogel is the active molecule that binds glucose in solution.The response time is defined as the time when 90% of the total frequency shift is achieved.[45,46]Magnification of the data(Figure 4c, green box), shows a fast response of the hydrogel to glucose of about 52 s (Figure 4b).

In addition, the pH value of the detection medium plays an important role for the binding ability of the hydrogel. Figure 4d shows the sensor response to glucose in the concentration range of 0–160 mg/L at different pH values, revealing a favorable linear relationship between glucose concentration and △F at pH values of 6.8–7.5 (physiological pH range of human saliva). With increasing pH, the absolute value of △F increases, due to higher pH promotingionization of boric acid and increasing the number of glucose molecules that bind to boric acids. Glucose concentration and △F are linearly correlatedwith linear correlation coefficients of 0.9532,0.9393, and 0.9858 at pH 6.8, 7.2, and 7.5, respectively.

Furthermore, the influence of the hydrogel thickness on △F was studied. Upon increasing film thicknessfrom 160 to 420 nm at a constant glucose concentration, the

absolute value of △F increases (Figure 4e). In contrast, when film thickness increases to 600 nm, the absolute value of △F decreases (The data of △F, △R and △M are shown in Figure S7a-c in the Supporting Information).

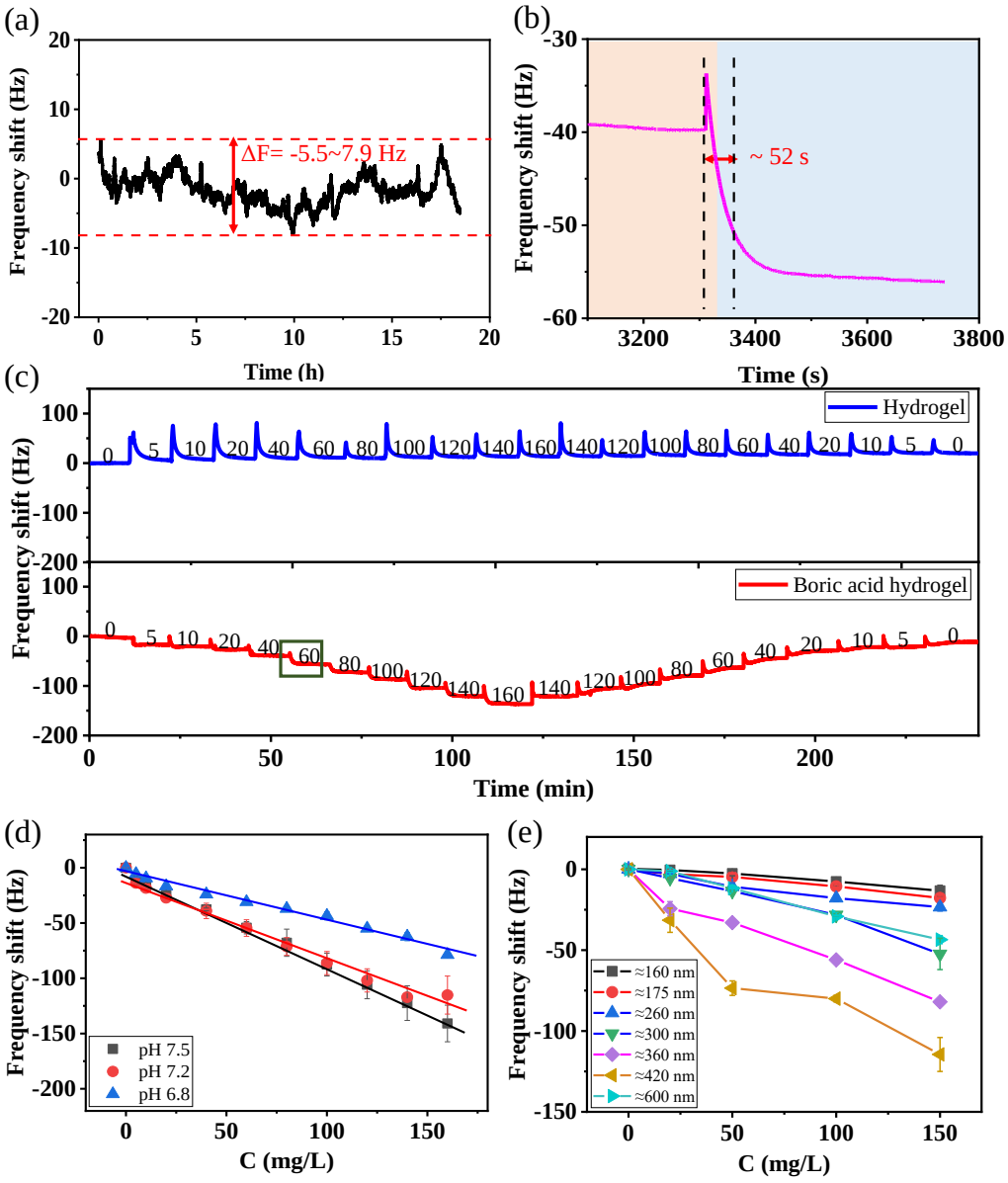

Figure 4. (a) The hydrogel-coated QCM sensor shows a tiny fluctuation of ~13 Hz in PBS (pH 7.5) for at least 20 h. (b) The response time of thesensor in the detection of glucose was 52 s in PBS at pH 7.5. (c) Thecontrolhydrogel (in the absence of3-APB) did not show any frequency change, while the hydrogel prepared with 3-APB showed

good response to different concentrations of glucose in PBS at pH 7.5 (glucose concentrationincreased from 0 to 160 mg/L and then decreased from 160 to 0 mg/L). (d)Favorable linear relationship between frequency shifts and different glucose concentrations (from 0 to 160mg/L) in PBS from pH 6.8 to pH 7.5. (e) Impact of hydrogel thickness on glucose detection, revealing an optimal hydrogel thickness of about 420 nm.

2.4 Artificial saliva test

The prepared glucose sensor was then used to detect glucose in artificial saliva to further evaluate the performance of the hydrogel. As shown in Figure 5a, the absolute value of △F increaseswith increasing glucose concentration. Conversely, the absolute value of △F gradually decreases with decreasing glucose concentration. Furthermore, the hydrogel recovered well, which is comparable to the results obtained in PBS buffer solution.

As presented in Figure 5a, the absolute value of △Fis relatively smallbetween pH 6.8–7.5. As pH value increases, the absolute value of △F gradually becomes lager (Figure 4d). Therefore, the pH is adjusted to 7.9 to increase the signal value (At pH ⩾ 8.0, the baseline fluctuation of the sensor was unstable.Fluctuations of △F were almost twice as high as at pH 7.5 (Supplementary Information Figure S5)). No significant changes are observed when each glucose sample is tested ten times, as shown in Figure 5b (R^2=0.9979). The RSD (relative standard deviation) of each glucose concentration is less than 10% (Table S1 in the Supporting Information). This experiment proves the

high stability and accuracy of the hydrogel.

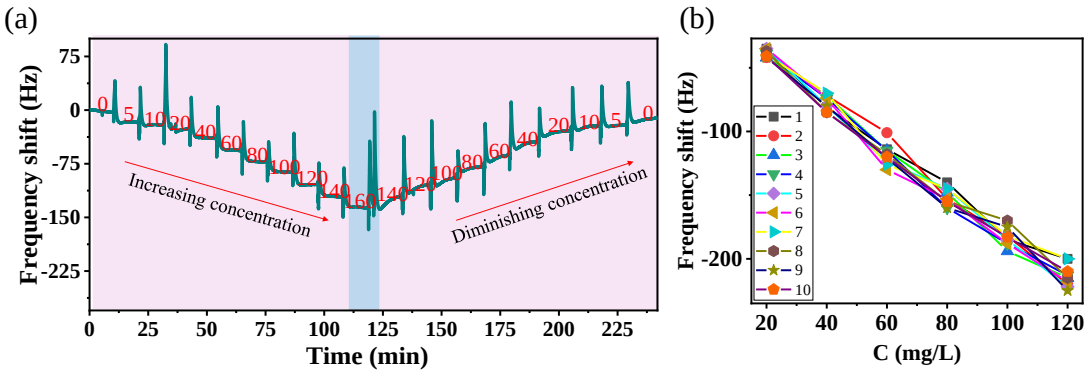

Figure 5. (a) The sensor shows good response to different concentrations of glucose in artificial saliva at pH 7.5 (glucose concentration increases from 0 to 160 mg/L and then decreases from 160 to 0 mg/L). (b) The frequency shifts remain largely stable during repeated testing (10 measurements)ofartificial saliva containing different glucose concentrations with the hydrogel-coated sensor at pH 7.9.

3. Conclusions

Herein, the fabrication of an ultra-sensitive poly(boric acid) hydrogel-coated QCM electrode by UV pressing and its application as sensor for the detection of glucose are reported. The hydrogel coating has a dense structure, sufficient glucose binding sites, excellent stability and uniformity. These unique properties endow the QCM sensor withrecord-low glucose detection limit under physiological conditions, making it suitable for saliva glucose monitoring. QCM measurements revealed that the glucose detection limit is as low as 3 mg/L and the detection range of glucose is 0–160 mg/Lwith good linearity in the range of pH 6.8–7.5. Repeated glucose tests of artificial saliva showed negligible deviations between measurements, indicating good reproducibility

(RSD<10%, n=10, pH=7.9). In follow-up studies, the hydrogel-coated QCM sensor will be gradually optimized for glucose detection in real saliva.

4. Experimental design and procedure

4.1 Instruments and reagents

Instruments. The QCM chip was purchased inthe SiYi coating material business departmentof Danyang development zone. The fundamental frequency of the QCM chip is 5 MHz, and has agold electrode surface. Glucose tests were performed usingQCM 200 (SRS) andlab-built QCM platform. Surface morphologies and material thicknesses of the hydrogels were investigated by scanning electron microscopy (S–4800 and SU8220). Thehydrogelsurface morphologies in glucose solution were investigated by environmental scanning electron microscopy (HD–Q–200) and atomic force microscopy (M8-HR). The functional groups of the hydrogel werecharacterized by micro-infrared spectrometry (SP–200i), and the transmission was measured by attenuated total reflection. The chemical composition of the hydrogel wasanalyzedby X-ray photoelectron spectrometry (ESCALAB250Xi). The surface roughness of the hydrogel was analyzed by atomic force microscopy using Neaspec s-SNOM. During the preparation of the cross-linked polymer films, pressure wasapplied using atailor made pressure film machine. Ultraviolet polymerizationat 365 nmwas inducedusingaUV lamp.

Reagents. Acrylamide (AM, 98.5%) was purchased from Xilong Chemical Co., Ltd., 2,2-dimethoxy-1,2-diphenyl-ethanone (DMPA, >98%) was purchased from TCI

Development Co., Ltd. (Shanghai), 3-aminopropyltriethoxysilane (APTES, 98%) was purchased from Alfa Aesar Chemicals Co., Ltd. (China), 3-(acrylamido) phenylboronic acid (3-APB, 98%) was purchased from Frontier Scientific, N,N'-methylenebisacrylamide (BIS, 98%) was purchased from Sinopharm Chemical Reagent Ltd., andglucose (99.8%) was purchased from National Institutes for Food and Drug Control.Dimethyl sulfoxide (DMSO), glucose, sodium phosphate dibasic dodecahydrate, potassium dihydrogen phosphate, and maleic anhydride were all purchased as pure analytical reagents.

Preparation of PBS. First,35.82 g $Na_2HPO_4 \cdot 12H_2O$ was dissolved in 1L distilled water, and 7.8 g $KH_2PO_4 \cdot 2H_2O$ was dissolved in 500 mL distilled water. Then, 770 mL Na_2HPO_4 solution and 230 mL KH_2PO_4 solution were mixed to obtain the required PBS solution.The pH was adjusted usingNaOH and HCl solution.

Preparationof artificial salivaaccording to ISO 10271–2011.[47]The specific compositionof artificial salivais NaCl 0.4 g/L, $CaCl_2 \cdot H_2O$ 0.795 g/L, KCl 0.4 g/L, $Na_2S \cdot 2H_2O$ 0.005 g/L, $Na_2HPO_4 \cdot 12H_2O$ 0.78 g/L, urea 1 g/L,andPBS 1L. NaOH and HCl were added to adjust the pH. The obtained artificial saliva was filtered throughapolyethersulfone membrane withapore size of 0.22 μm.

4.2 Hydrogel synthesis

Surface modification of QCM electrode. A QCM electrode was sonicated for 10 min in piranha solution (mixture of H_2SO_4(96%, w/w) and H_2O_2 (30%, w/w) ina volume ratio of 7:3). The processed electrode was washed with redistilled water and dried with

N_2 gas. Then, the electrode was immersed in a mixed solution of 3-aminopropyltriethoxysilane (100 μL) and ethanol (50 mL) at room temperature. After 12 h, the electrode was rinsed with ethanol and subsequently dried with N_2 gas.The dried electrode was immersed in a mixed solution of maleic anhydride (1 g) and N, N´-dimethylformamide (50 mL) for 12 h. Finally, the treated electrode was rinsed with ethanol and dried with N_2 gas.[48,49]

Hydrogel film synthesis by UV pressing. First, 5 mol/L pre-polymer solution was prepared, which consisted of 18% wt.3-APB, 2% wt. BIS, 78% wt. AM, and 2% wt.DMPA in dimethyl sulfoxide. Second, 25 μL pre-polymerization solution was dropped onto a quartz plate (10 x 10 cm). The electrode was placed face down on the pre-polymerization solution and pressed with appropriate force. The crystal plate was irradiated with UV lamp (λ = 365 nm) for 30 min. Then, the crystal plate was placed in distilled water for about 1h to automatically detach the hydrogel-coated QCM sensor from the working solid substrate-crystal plate. Finally, the film-coated electrode was rinsed with redistilled water. For comparison, a control hydrogel was synthesized according to the same procedure without addition of3-APB.

Hydrogel synthesis by spin coating. Electrodepretreatmentand formulation of the pre-polymerized solution were the same as described above for the hydrogel synthesis by UVpressing. Then, 50 μL pre-polymer solution was deposited onto the electrodeand spin-coated for 1 min at 3000 rpm. The electrodecoated with the prepolymer was placed in a customized glass container, which was repeatedly evacuated to remove O_2. For

polymerization, the electrode was irradiated with UV light (λ= 365 nm) underN_2atmosphere for 30 min. Finally, the obtained film-coated QCM electrode was repeatedly rinsed with redistilled water.

Glucose detection with hydrogel-coated QCM sensor. A QCM chip coated with a glucose-sensitive hydrogel was dried with nitrogen and installed into the reaction cell of the QCM. PBS was pumped into the reaction cell using a peristaltic pump, and the frequency of the QCM chip was monitored in real time using the QCM data acquisition software. After △F was stabilized, the glucose detection capacity of the hydrogel was evaluated. During the test, the glucose solution (2 mL) was injected with a flowof 200 μL/s. When the glucose solution was fully injected into the reaction cell, the peristaltic pump was closed. After △F stabilized, the next sample was injected.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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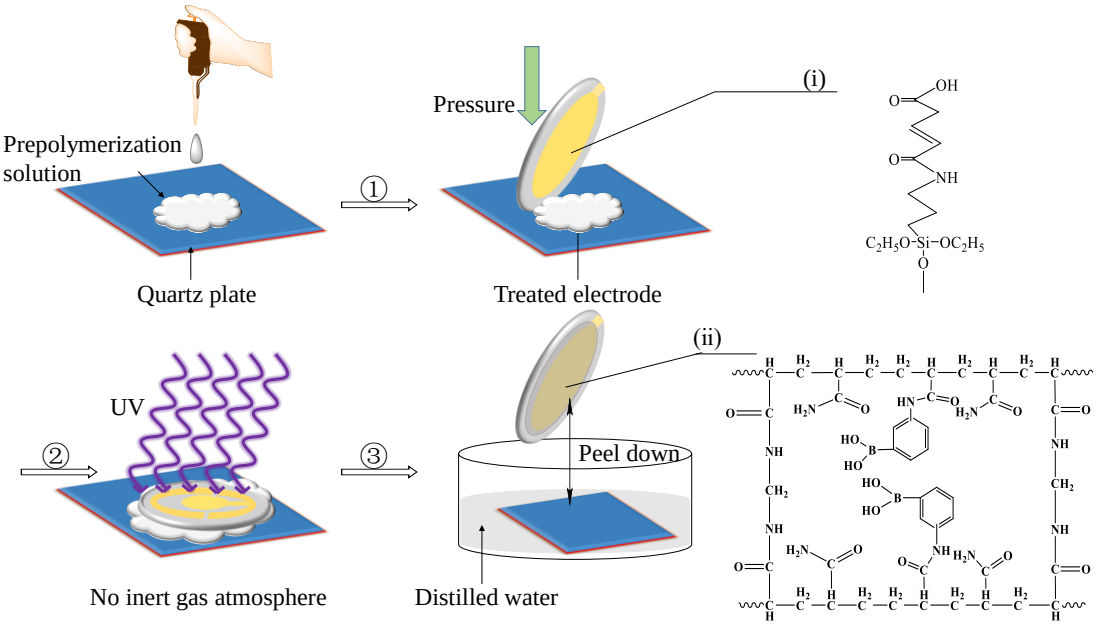
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