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Noninvasive glucose monitoring using saliva nano-biosensor

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ABSTRACT

Millions of people worldwide live with diabetes and several millions die from it each year. A noninvasive, painless method of glucose testing would highly improve compliance and glucose control while reducing complications and overall disease management costs. To provide accurate, low cost, and continuous glucose monitoring, we have developed a unique, disposable saliva nano-biosensor. More than eight clinical trials on real-time noninvasive salivary glucose monitoring were carried out on two healthy individuals (a 2–3 h-period for each trial, including both regular food and standard glucose beverage intake with more than 35 saliva samples obtained). Excellent clinical accuracy was revealed as compared to the UV Spectrophotometer. By measuring subjects' salivary glucose and blood glucose in parallel, we found the two generated profiles share the same fluctuation trend but the correlation between them is individual dependent. There is a time lag between the peak glucose values from blood and from saliva. However, the correlation between the two glucose values at fasting is constant for each person enabling noninvasive diagnosis of diabetes through saliva instead of blood. Furthermore, a good correlation of glucose levels in saliva and in blood before and 2 h after glucose intake was observed. Glucose monitoring before and 2 h after meals is usually prescribed by doctors for diabetic patients. Thus, this disposable biosensor will be an alternative for real-time salivary glucose tracking at any time.

1. Introduction

The International Diabetes Federation estimates 382 million people worldwide had diabetes in 2013, and the number is forecasted to reach 592 million by 2035 (a 55% increase) [1]. There were 5.1 million diabetes-related deaths globally in 2013, equaling to one death every 6 s, an 11% increase over 2011 [2]. Early diagnosis, on-time treatment and continuous management are vital to patients' life quality and to avoid complications such as circulatory problems, kidney failure, heart disease, stroke, and blindness [3,4]. Current practices for diabetes management rely on monitoring blood glucose levels. Blood glucose measurements are required to determine insulin dosage and to detect abnormal glucose levels indicating illnesses, dietary changes, or adverse medication responses. These intrusive tests are generally disliked because of the pain and inconvenience caused by finger pricking, resulting in fewer tests and inadequate blood glucose control. Poor blood glucose control results in more complications and even higher management costs. Particularly, repeated painful finger sticks are a major problem for young children and result in similar negative consequences for disease management.

Glucose sensing started in 1841 when it was performed in urine, but unfortunately the correlation between urine and plasma glucose was inconsistent [5]. Monitoring of blood glucose levels is currently the only recognized and widely used method for the diagnosis and control of diabetes. There are many different types of blood glucose meters on the market; however, they all require users to prick their fingers multiple times a day to obtain blood samples. Some minimally invasive or noninvasive techniques for blood glucose monitoring were studied, including infrared (IR) spectroscopy, fluorescence spectroscopy, Raman spectroscopy, and surface plasmon resonance. However, the results still have to be correlated with direct blood glucose measurements, and the sensitivity and reliability are limited by spectral signal-to-noise level and skin thickness. For example, in 2002, Cygnus Inc. introduced a wearable GlucWatch device measuring the glucose electroosmotically extracted across skin [6]. Nevertheless, the difficulty of use due to the sweat collection process and the low level of accuracy resulted in its removal from the market. Another product, the OrSense NBM device provided by OrSense Ltd. [7], which detects blood glucose concentration via an optical method called "occlusion spectroscopy" [8], has not achieved any significant success. Although optical technologies for glucose determination are available, most of them are for laboratory use due to the size, cost, and complexity of operation. Thus, a noninvasive, convenient,

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accurate, easy-to-use, portable, and low-cost diagnostic tool for diabetes is highly demanded.

As summarized by Lei et al. [9], there are three necessary prerequisites for most clinical applications: (i) a simple and inexpensive method for collecting biological samples with minimal discomfort, (ii) specific biomarkers associated with health or disease, and (iii) an accurate, portable and easy-to-use technology for disease diagnosis and health screening. Saliva, commonly considered as the 'mirror of the body', is very attractive as a biomedium for clinical diagnostics. Its unique properties, such as noninvasive accessibility and the presence of plentiful disease biomarkers, make it particularly attractive for disease diagnosis and monitoring [10,11]. Saliva can be easily collected by individuals with modest instruction and it dramatically reduces the discomfort of the tests. Changes in saliva are believed to indicate the wellness of an individual. There are a large number of diagnostic analytes present in saliva, including glucose [12,13], steroid hormones [14], and the HIV antibody [15]. Saliva was first demonstrated to have diagnostic power comparable to that of blood in differentiating smokers from non-smokers through thiocyanate ions levels [16]. Results from blood, saliva, and urine as biomedia were compared and saliva was recognized as the most sensitive one. Saliva is also revealed to be more accurate than blood in detecting oral cancer [17,18]. Furthermore, the concentration of some other disease biomarkers in saliva was found to exceed that in blood, illustrating a further advantage of using saliva for clinical diagnostics [19,20].

Regarding to the technologies for determining salivary glucose levels, optical measuring systems such as Liquid Chromatography–Mass Spectrometry (LC–MS) and UV–VIS Spectrophotometry were reported [21,22]. However, the measurements can only be done in a laboratory as they require significant processing time, expensive reagents, sophisticated instrument, and highly trained professionals. Consequently, these methods cannot be used for individual glucose monitoring at home or in daily activities. Until now, there is not a suitable product for home care measurement of glucose using saliva. Technologies, including microchips and microfluidic devices, show great potential in developing a robust, cost-effective, accurate, portable, and easy-to-use diagnostic tool for saliva analysis [11,23]. Miniaturized saliva-based diagnostic technologies will enable the use of trace amount of biofluids to provide quick and reliable results for clinical decision-making and treatment outcomes-predicting.

A positive correlation between blood glucose and salivary glucose is revealed by many studies [21,24–27]. Other than salivary glucose, no other parameters in saliva were found to be markedly affected in diabetes mellitus [13]. Therefore, salivary glucose can be utilized as an alternative diagnostic method for diabetes and as a general screen for prediabetes and undiagnosed diabetes.

Here we proposed an on-chip disposable nano-biosensor providing a painless test methodology with sufficient sensitivity. It is disposable and thus eliminates extensive cleaning or electrode pretreatment between measurements. The working electrode is functionalized with single-walled carbon nanotubes (SWNT) and multilayers of chitosan (CS), gold nanoparticles (GNp) and glucose oxidase (GOx), using a layer-by-layer (LBL) assembly technique [28]. The biosensor can detect glucose down to 0.1 mg/dL and provide noninvasive, reliable (high resolution), highly reproducible, convenient, fast, and continuous salivary glucose monitoring for personal and point-of-care use.

2. Experimental design and procedure

The chemicals and facilities used are listed here, and more importantly, we introduced the sensor configuration and fabrication procedures and preparation for clinical trials.

2.1. Reagents and apparatus

Glucose oxidase (GOx, 17, 300 units/G solid) from Aspergillus niger, gold nanoparticles (GNp, 20 nm diameter), chitosan (CS), poly(allylamine) (PAA, 20 wt% solution in water), acetate buffer solution (pH 4.65), D-(+)-glucose, phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma Aldrich. COOH functionalized single-walled carbon nanotube suspension (SWNT, diameter: 1–2 nm; length: 2–5 μm, 4000 mg/L in distilled (DI) water with ~5–7 wt% COOH groups at the end) was purchased from Brewer Science Company. Dulbecco's phosphate-buffered saline (DPBS, no calcium, no magnesium) was purchased from Life Technologies. Glucose Assay kit (100 assays) was purchased from BioVision company. UV-cuvette, ultra-micro, 15 mm was purchased from BrandTech Scientific Inc. Westran S, 0.2 μm PVDF blotting membranes were purchased from Sigma–Aldrich. 3 mL syringes with Luer-Lok™ tip were purchased from Becton Dickinson Company. Aluminum 50 mesh was purchased from TWP Inc. and Crosstex dental cotton was purchased from SAFCO Dental Supply Co.

Silicon wafers (diameter 3″, boron doping, ⟨100⟩ orientation, resistivity 0–100 Ω, thickness 406–480 μm, one-side polished) were purchased from University Wafer; and Platinum Pellets (1/8″ diameter × 1/8″ length, per gram, 99.99% pure) were purchased from Kurt J. Lesker Company.

Facilities used in Gorge J. Kostas Nanoscale Technology and Manufacturing Research Center include wet bench wafer cleaning system, bruce furnace 7355B (oxidation), nanospec thickness measurement machine, brewer/laurell spinner, quintel 4000 mask aligner, unaxis ICP etch (Plasma Therm 790), E-beam deposition system, micro automation 1006 dicing saw, and supra 25 SEM. Facilities used in Environmental lab include UV-mini 1240 Spectrophotometer from Shimadzu and uVISC™ Portable Viscometer Control Advanced System from Cole-Parmer.

2.2. Device fabrication

Fabrication of the disposable nano-biosensor is described in this section. It includes the micro-fabrication of the sensor chip (Fig. 1a) and LBL assembly of sensor electrode modification (Fig. 1b).

The on-chip electrochemical sensing device contains at least one working electrode, a counter electrode and a reference electrode and one possible electrode geometry is shown in Fig. 1a. The small rectangle (purple) marks out the reactive area while the larger one (blue) indicates where sample drops on. Device – S2D2 is of size $20 \times 10\,mm^2$ with the reactive area $32\,mm^2$. Microelectrodes were fabricated by photolithography followed by electron-beam evaporation of Cr/Pt (20 nm/200 nm) layer onto a silicon oxide substrate.

Electrode modification was done through a LBL assembly of SWNT and multilayer films composed of CS–GNp–GOx (Fig. 1b) [28]. The CS–GNp–GOx unit was repeated several times to form a multilayered coating. The number of layers can be adjusted to achieve the best sensing performance.

2.3. Saliva sampling procedures for test subjects

Two healthy volunteers of age-group 20–30 years were enrolled in this study, following the Northeastern University's Institutional Review Board (IRB), wherein the individuals singed consent form and received a $12.00 gift card at the completion of each session with their identities unrevealed.

The following protocol was introduced to all subjects and executed in all preclinical tests:

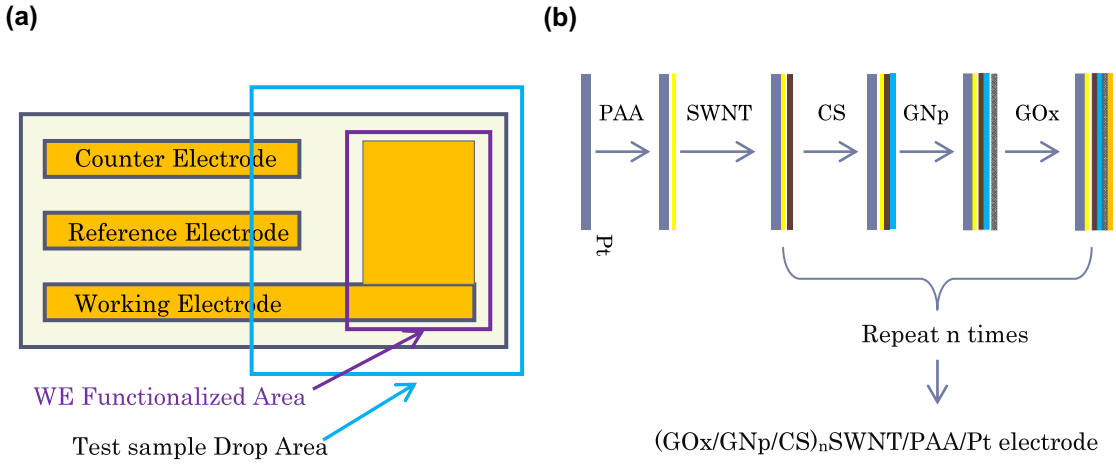

Fig. 1. Schematic diagram of the (a) biosensor; (b) modification procedure of the sensor electrode.

(1) Wait for 2 min after rinsing mouth with water.
(2) Minimize swallowing and hold saliva in mouth (typically <1 min).
(3) Place dental sterilized cotton sponge in mouth and chew until it is soaked with saliva (typically <1 min).
(4) Deposit sponge into syringe directly from the mouth without touching it to avoid contamination.
(5) Insert plunger into syringe.
(6) Squeeze saliva through preinstalled membrane (PVDF membrane) in the bottom of syringe into sterilized tubes gently (approx. 100 μL, per sensor per measurement needed).
(7) Preserve sample tubes in 4 °C chill box while producing samples.
(8) Take pipette to drop saliva (100 μL) onto glucose sensor.
(9) Perform amperometric measurement (30 s) and display.
(10) Dispose sensors after washing out the residue salivary specimen on it.

2.4. Electrochemical measurements and characterization

Cyclic Voltammetry (CV) and Amperometric measurements were performed in PBS (0.1 M, pH 7.4) at room temperature (~23 °C) using a mini potentiostat. Amperometric measurements for determining the salivary glucose levels were performed of filtered unstimulated saliva samples at room temperature (~23 °C) using a mini potentiostat. A Supra 25 Scanning Electron Microscope (SEM) was employed for the surface morphological characterization of the SWNT, GNp, and GOx on the sensor electrode reactive region.

2.5. UV Spectrophotometer measurements

Part of the filtered saliva samples were boiled at 100 °C for 30–40 min and then cooled down to room temperature. After centrifuging at 12,000*g* for 5 min to remove any particulate materials, each supernatant was transferred to new micro-centrifuging tubes and reacted with the glucose assay kit. The glucose content of each saliva sample was determined by the absorbance at 570 nm using UV-mini 1240 Spectrophotometer.

2.6. Viscosity measurements

400 μL of each sample was mounted into uVISC™ Portable Viscometer Control Advanced System and viscosity value was obtained within 1 min. We kept track of the viscosity change of the raw saliva and the PVDF membrane filtered saliva of one healthy subject with one Trutol® 75 Glucose Tolerance Beverage intake. Approximately 1 mL of each saliva samples was collected before (−30 min) and 60, 120, and 180 min after the glucose beverage intake and measured three times using this uVISC™ Portable Viscometer Control Advanced System.

3. Results and discussion

3.1. Sensor characterizations

3.1.1. On-chip disposable enzyme-based nano-biosensor

The uniformity of assembled SWNT, GNp, and GOx layers was inspected by SEM (Fig. 2). SWNT and GNp were well distributed (Fig. 2a and b). Three layers of GOx matrix covered the whole reactive surface on the working electrode (Fig. 2d) while one layer could only cover partially the reactive surface (Fig. 2c). SWNT, represented by the short white line (about several hundred-nm long), were successfully assembled onto the functional area (Fig. 2a). Besides some white dots, which were believed to be aggregated SWNTs or small contaminants, the overall coating uniformity was very good. GNp can be assembled on chitosan through electrostatic interactions [29]. The SEM image (Fig. 2b) also proved that GNp were uniformly distributed with a minor amount of aggregation. SWNT could be clearly observed underneath the GNp layer. The dark flocculent areas presented potential chitosan molecules connecting SWNT with GNp. Finally, GOx was adsorbed onto GNp through $-NH_2$ groups. Fig. 2c shows one layer of GOx assembled on the sensor electrode. The scattered molecular clusters were very likely due to the insufficient GNp sites or the space repulsive force between GOx molecules. After assembling two more GOx matrix, the accumulated glucose oxidase successfully covered the whole reactive surface and its pattern was guided by the underlying SWNT (Fig. 2d).

We compared the detection results of glucose in buffer solution using sensors with one layer of GOx and three layers of GOx coating (Fig. 2e and f) and it confirmed sensor functionalized with PAA/SWNT/$(CS/GNp/GOx)_3$ film had much better sensitivity and repeatability. Furthermore, multi-layered GOx coating enhanced the linearity of the sensor response.

Our on-chip glucose sensor determines salivary glucose levels by keeping track of the electrons passed through the glucose oxidase enzyme coated on the working electrode. The charge transfer complex formed by the functional layers of SWNT–CS–GNp between glucose oxidase and the electrode surface has permitted direct electron transfer between the active center of glucose oxidase and the electrode allowing direct oxidation of the enzyme.

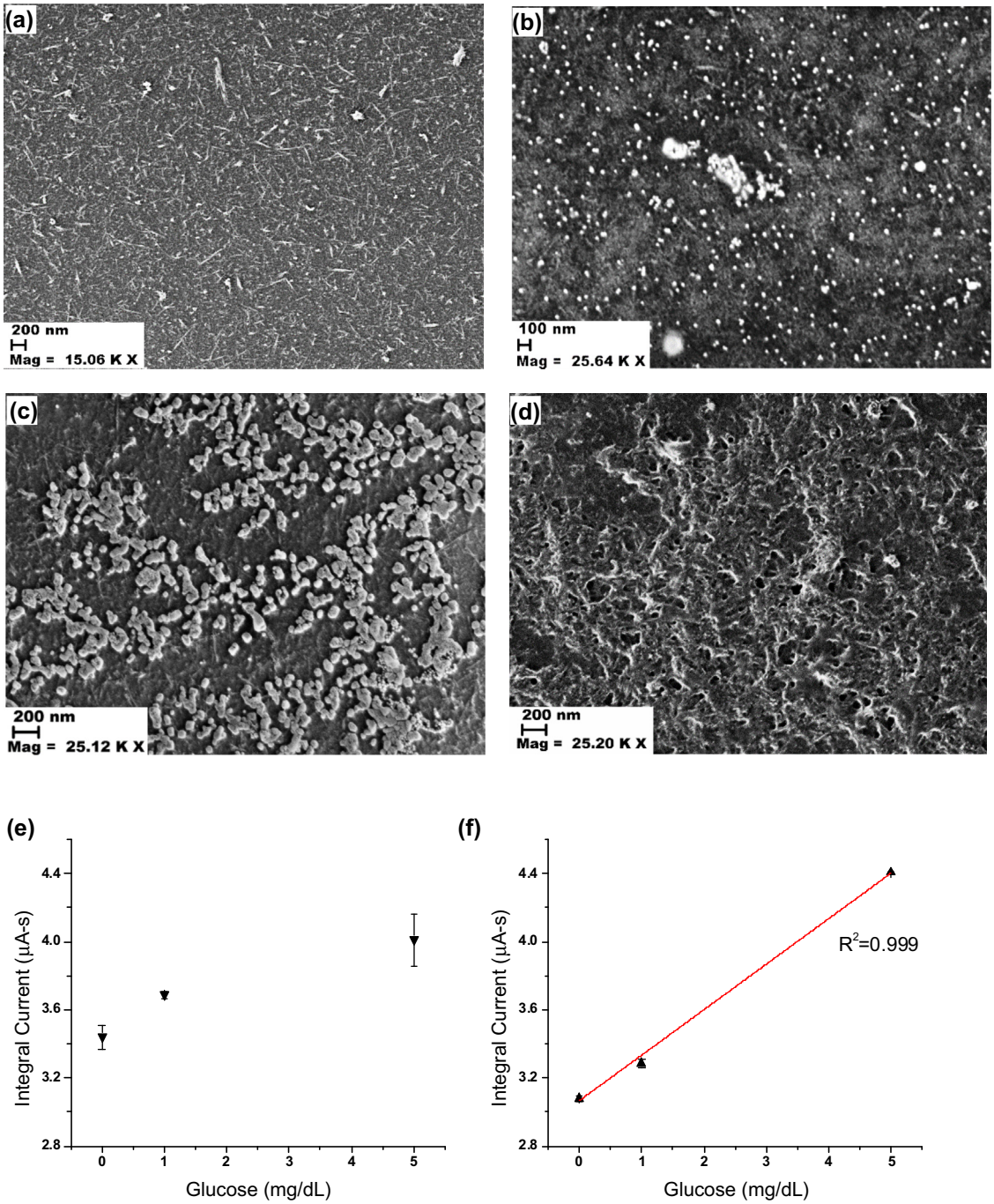

Fig. 2. (a–d) SEM images of functional layers of the glucose sensor: (a) one layer of SWNT; (b) one layer of SWNT/GNp; (c) PAA/SWNT/CS/GNp/GOx film on the sensor electrode surface; (d) PAA/SWNT/$(CS/GNp/GOx)_3$ films on the sensor electrode surface; (e and f) amperometric measurements of glucose buffer solutions at applied potential 0.2 V using sensors functionalized with (e) PAA/SWNT/CS/GNp/GOx film; and (f) PAA/SWNT/$(CS/GNp/GOx)_3$ film. Error bars = ±standard deviation and $n = 3$.

It eliminates the inherent limitations of redox mediators in bio-electrocatalytic applications [30]. The mediator-free glucose sensing mechanism provides effective electrical communication between enzyme molecules and the electrode surface.

3.1.2. Sensing properties of the on-chip disposable nano-biosensor

Cyclic Voltammetry (CV) measurements were conducted to detect glucose of different concentrations in PBS. Current response increased with the elevation of glucose concentration, and tended to reach a saturation value where all active sites of GOx were used up. It conformed with the characteristic of Michaelis–Menten kinetics. Each concentration was measured three times with three individual sensors and a high reproducibility was revealed. The sensor demonstrated a reliable linear detection range over 0.1–20 mg/dL (0.017–1.11 mM) with sensitivity = 26.6 μA/mM-cm^2 and correlation coefficient of 0.995 [31].

We also observed the amperometric behavior of the modified electrode of glucose solutions at different concentrations at applied potential of 0.2 V. Each test lasted 30 s providing enough data for analysis. A data processing method was developed including data filtering and data integration steps. First, after obtaining raw output current data, the natural static noise was eliminated by using a filtering function. Then an integration of the current response in 18–21 s was recorded. This data integration method

demonstrated a much better accuracy than using a single data point. Furthermore, the low applied potential −0.2 V, can significantly reduce the possible interference from other electroactive species whose oxidization potentials are close to glucose [32–34]. Thus, we used 0.2 V as the applied potential and current integration as our output.

3.2. Salivary glucose monitoring

Saliva sampling procedure listed in Section 2 was developed based on our research objectives and referred from some literature on saliva analysis [26,35–36]. Saliva samples we collected from each participant were all unstimulated which revealed the participants' real metabolic conditions. It would increase the test accuracy.

Experimental saliva collection and sampling devices were shown in Fig. 3a. A dental cotton roll was used to soak enough saliva, then a PVDF (polyvinylidene fluoride) membrane was applied to filter out big molecules, and finally the iron wire gauze was applied to fix and stabilize the membrane at the bottom of the syringe. Filtered saliva samples were collected in self-standing screw cap tubes. The samples were then split to two parts. One part was used to immediately determine its glucose content by using our glucose sensors. The other part was boiled, centrifuged and measured by UV Spectrophotometer to assess the accuracy of our sensors.

The particular PVDF membrane we selected has a protein binding capacity over 200 μg/cm^2 and a pore size of 0.2 μm. It has maximum immobilization of proteins during sample transfers and minimized sample loss. The small pore size also eliminates 'blow-through' and increases protein binding over a wide range of molecular weights. Thus, it can filter out most of the proteins in saliva rendering the glucose determination more accurate. Another perspective for selecting the membrane is to decrease the viscosity of saliva samples. Viscosity of human saliva varies between individuals and is easily affected by the biological environments [37,38]. We kept track of the viscosity of raw saliva and the membrane filtered saliva of one healthy subject with one Trutol® 75 Glucose Tolerance Beverage intake (Fig. 3b). The subject was asked not to eat or drink anything from 10 pm the night before the test. Approximately 1 mL of each saliva samples was collected before (−30 min) and 60, 120, and 180 min after the glucose beverage intake and measured three times. As demonstrated in the viscosity monitoring results, we managed to successfully reduce the viscosity of saliva via this membrane. The filtered saliva samples had a viscosity of 1.07–1.13 mPa-s which was believed not to affect the glucose detection.

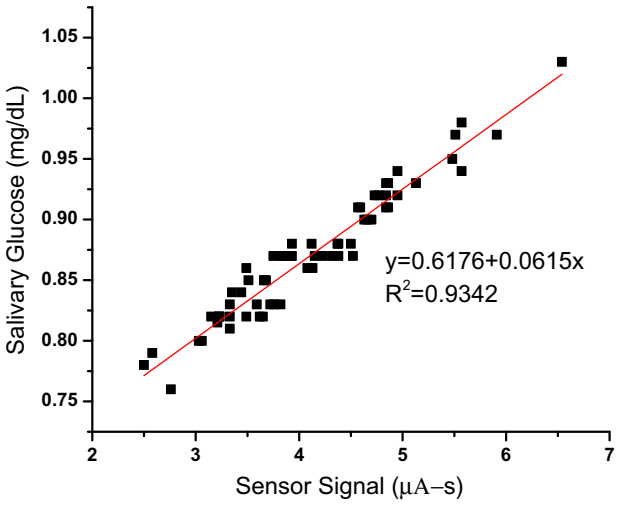

Fig. 4. Sensor calibration with UV Spectrophotometer.

We did sensor calibration with the UV Spectrophotometer of 75 fasting saliva samples from two healthy individuals on different days and obtained a linear correlation between them (Fig. 4).

Based on this, we compared subject A's blood glucose measured by blood glucose meter and salivary glucose measured by our sensors at fasting on different days (Fig. 5).

There is clearly a constant correlation between blood glucose and salivary glucose at fasting state on different days. It permits saliva analysis to be an alternative noninvasive diagnostic method for diabetes and our sensor as a general screen tool for prediabetes and undiagnosed diabetes.

Furthermore, we measured the glucose levels in both fasting and after-meal saliva samples using our sensors and the UV Spectrophotometer and did error grid analysis to quantify the clinical accuracy of salivary glucose estimates generated by sensors as compared to UV Spectrophotometry method (Fig. 6). The subjects were asked to fast overnight and to have a regular meal after taking fasting saliva and blood samples. Several more sets

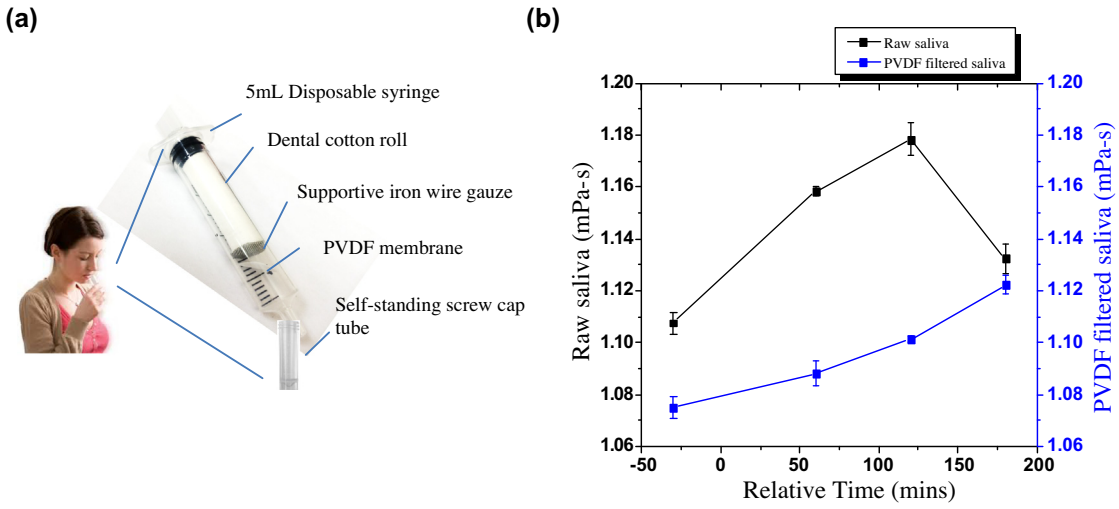

Fig. 3. (a) Schematic of saliva sampling procedures; (b) viscosity changes with 75 g glucose tolerance beverage intake of raw saliva (black); PVDF filtered saliva (blue).

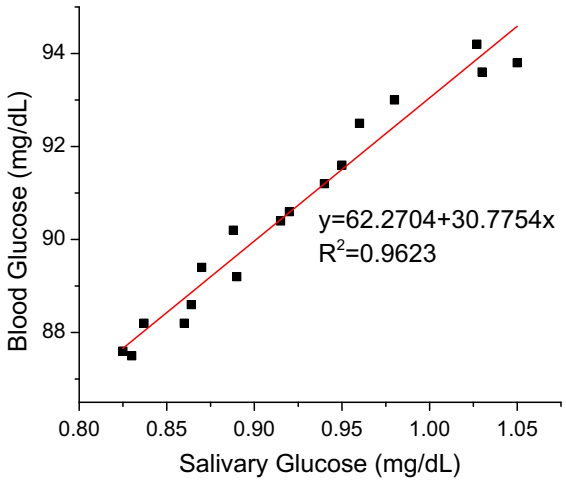

Fig. 5. Correlation between blood glucose and salivary glucose at fasting state.

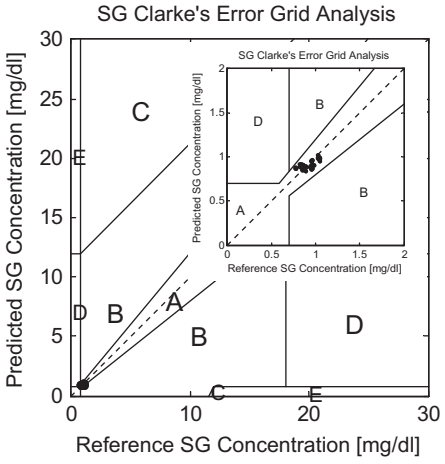

Fig. 6. Clinical accuracy evaluation of salivary glucose estimated by sensors compared to values obtained from UV Spectrophotometer with the zoom-in image as an inset.

of saliva and blood samples from them were measured after meal. There were 68 saliva samples obtained from two healthy young adults, and the glucose concentrations were between 0.6 and 1.2 mg/dL, in agreement with results from many other clinical studies [25,27,39]. Region A are those values within 20% of the reference method, while Region B to E contain points that are outside of 20% would not/would lead to inappropriate treatments in a severity-level ascending order. With all data falling in Region A, it well demonstrates the salivary glucose values measured by our sensors are within 20% of the reference UV Spectrophotometry method, thus, it has a very high clinical accuracy.

Here are some other findings in our glucose monitoring clinical trials for healthy young adults where saliva and blood glucose were measured simultaneously before and after glucose-content food/beverage intake and each sample was measured at least three times:

(1) Salivary glucose value reaches its peak value approximately 15–40 min after food intake while the blood glucose increases to its highest value at around 30–60 min after. The time difference in reaching peak values is regarded as a normal physical mechanism [21,25,39]. After peak, both salivary glucose and blood glucose begin to decrease until both drop to normal ranges within 3 h.

(2) Intense physical exercise after food intake can cause both salivary glucose and blood glucose concentrations to be very low (e.g. 70 mg/dL) even after 3 h but still in healthy range.

(3) The physiological responses after food intake vary from individuals, and their normal salivary glucose and blood glucose ranges can also be different.

(4) Different individuals have different carbohydrate metabolism after food intake, and the fluctuations in salivary glucose and blood glucose levels also vary for one subject on different days. However, the rise and fall of glucose levels in saliva and blood were observed and the fluctuation in salivary glucose measured by our sensors was in compliance with that in blood glucose. Moreover, the little variance between the salivary glucose measured by our sensors and by the UV Spectrophotometer also proved the great accuracy and reliability of our sensing system.

(5) Changes of glucose levels both in blood and in saliva are different whether the subjects take regular meals or 75 g glucose tolerance beverage. Regular food contains not only sugar (which can be directly digested to glucose) but also protein and fat, and some of the protein or fat will not be turned into glucose unless necessary. Glucose beverage, on the other hand, is just 75 g glucose dissolved in water which can be directly transited into blood and into saliva. The difference in body metabolism upon the intake of different types of carbohydrates was also revealed in other studies [12,21,25,39–41]. The mechanism of human carbohydrate metabolism is beyond our knowledge but we will carry out more research to understand these dynamic shifts in both saliva and blood glucose, and develop a better way to interpret salivary glucose levels in real time.

(6) In our study, the fasting salivary glucose levels of two healthy young individuals were found to be between 0.6 and 1.2 mg/dL and it was consistent with the results reported by other groups using different salivary glucose sensing methods [14,25,39]. The peak salivary glucose concentrations after subjects taking regular meals or 75 g glucose tolerance beverage could reach 1.1 mg/dL and 2.8 mg/dL respectively. However, even higher levels of fasting salivary glucose of non-diabetic subjects (ranging 3–12 mg/dL) were also reported [42–44]. The studies were done in Iran and India with different age groups being compared. The reason of their much higher fasting salivary glucose levels is most likely due to their carbohydrate-rich dietary pattern of the Iran and Indian population. Thus, we believe subjects' age, living regions, and life styles including their dietary patterns play important roles in their glucose tolerance levels.

(7) A high correlation of glucose levels in saliva as measured by our sensors and blood glucose levels as measured by the standard finger stick technique in healthy volunteers before and 2 h after an intake of a 75 g glucose containing beverage was observed. Two-hour is a common interval prescribed by doctors for diabetic patients to check blood glucose levels before and after meals. Thus, the high feasibility to use saliva analysis as an alternative, noninvasive, and convenient diagnosis method for diabetes and for glucose monitoring in diabetes patients is well established. Our sensor will allow a convenient and painless determination of equivalent blood glucose levels through salivary glucose monitoring.

4. Conclusions

We have developed an innovative, simple, and low cost on-chip electrochemical glucose sensing system. It can provide noninvasive, reliable, convenient, fast, and continuous salivary glucose monitoring for personal and point-of-care use. It also demonstrates

excellent clinical accuracy as compared to salivary glucose obtained by UV Spectrophotometer. The constant correlation between blood glucose and salivary glucose for each person enables noninvasive diagnosis of diabetes through saliva. Although the correlation between blood glucose and salivary glucose profiles is highly individual dependent, there is a good correlation between glucose levels in saliva and in blood before and 2 h after glucose intake. Thus, this disposable glucose sensor is very powerful for real-time convenient tracking of glucose levels in saliva at any time, and can further be used for both diagnosis of diabetes and glucose monitoring.

The sensor can also be miniaturized and optimized in shape or re-configured for other applications. The sensitivity can be optimized by adjusting the coating procedure for the working electrode and by precise automatic production. The sensor can serve as a stand-alone device, or be incorporated into another device. Furthermore, it provides a good platform for on-chip electrochemical sensing of various other chemicals and biomolecules.

Limitation of this study is that only healthy individuals were included so that there were no extreme high/low glucose values detected. The study of the relationship between blood glucose and salivary glucose based on age, gender, medical, and health conditions is highly needed. These outcomes would improve statistical confidence by increasing the study population. The sensor is designed to measure as low as 0.1 mg/dL and as high as 20 mg/dL glucose in saliva, which is sufficient to be medically applicable for diabetic diagnosis and health surveillance.

Conflict of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript. Wenjun Zhang; Yunqing Du; Ming L. Wang.

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