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Noninvasive glucose monitoring using saliva nano-biosensor

Wenjun Zhang [a,*], Yunqing Du [a], Ming L. Wang [a,b]

[a] Interdisciplinary Engineering Program, College of Engineering, 400 SN, Northeastern University, 360 Huntington Ave, Boston, MA 02115, USA
[b] Bioengineering Department, 429 SN, Northeastern University, 360 Huntington Ave, Boston, MA 02115, USA

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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.

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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,

* Corresponding author. Tel.: +1 617 373 3010.
E-mail address: zhang.wenj@husky.neu.edu (W. Zhang).

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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:

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