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智能手机驱动的电化学狗，用于血液β-酮的护理点监测

Smartphone-Powered Electrochemical Dongle for Point-of-Care Monitoring of Blood β-Ketone

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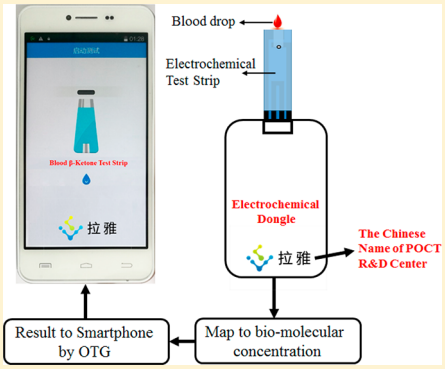
ABSTRACT: A smartphone-powered medical dongle as a miniaturized electrochemical analyzer associated with an enzymatic β-hydroxybutyrate test strip for accurate characterization of blood ketone in peripheral whole blood at the point-of-care, which is capable of providing critical guidance for the evaluation and treatment of diabetic ketoacidosis (DK) and diabetic ketosis acid (DKA), is reported. The measured results of blood ketone by the medical dongle were compared with the clinical results from a bulky biochemical analyzer, and the analysis showed good agreement. The proposed medical smartphone-powered dongle was demonstrated to be a very promising platform as a miniaturized electrochemical analyzer for point-of-care monitoring of the critical biochemical parameters such as blood ketone and a good solution for mobile health management.



Under regular circumstances, there is a small amount of ketone, with the concentration ranging from 0.03 to 0.5 mmol/L, in blood, which is the normal phenomena when fat is oxidized to provide energy for the body. The amount of ketone bodies utilized in the extrahepatic tissues is proportional to the concentration of ketone bodies in arterial blood.[1−4] When the concentration of ketone in the blood rises to 7 mmol/L, the utilization capacity of extrahepatic tissues is saturated. The threshold of renal ketosis is diagnosed with ketone bodies above 70 mg/dL. When the concentration of ketone bodies in the blood rises over this threshold, the capacity of filtration of ketone bodies over the reabsorption by glomerular tubule leads to ketonuria. In this situation, ketoplasia synthesis capacity is more paramount than the utilization by extrahepatic tissues. It causes blood ketone bodies to increase, which leads to ketoacidosis. The excess ketoplasia is excreted along with urine. This clinical symptom is called ketonuria.[5−7]

Diabetic ketoacidosis (DK) and diabetic ketosis acid (DKA) diabetes are common acute complications and also the most common pathological state of ketosis. Delayed diagnosis and treatment may lead to death. In the early stage of DK/DKA, acetoacetate was converted into betaβ-hydroxybutyric acid, so that the blood β-hydroxybutyric acid/acetoacetate significantly increases from a normal level of 2−3:1 to a level of 16:1.[8,9] The blood ketone level is the critical marker for the diagnosis of DK and DKA. Blood ketone concentration also plays a very important role in the whole process of DK/DKA detection, diagnosis, treatment, and prevention.[10,11] Both the blood ketone and urine ketone can directly and indirectly reflect the in vivo levels of ketone bodies in blood; however, the specificity of traditional urine ketone detection is poor, which reflects the indirect condition, and the result is obviously delayed. Therefore, blood ketone was utilized as a clinical marker to diagnose DK/DKA due to the higher specificity compared to the urine ketone. Moreover, the conventional detection of blood ketone with a bulky biochemical analyzer is time-consuming and tedious, which is not able to meet the clinical requirements at the point-of-care scale.[12−15]

Recently, the market of mobile health-based chronic disease management is undergoing a rapid growth and forecasted to be extremely large in the future global aging society. Good medical service is urgently needed. In addition, the hardware of miniaturized biomedical device also plays a critical role in the whole industry chain. Therefore, biomedical microdevices related to the smartphone have drawn the attention of many research groups due to the ubiquitous availability of the smartphone as well as its capability as a biomedical information transfer station. The smartphone with its powerful accessibility allows people to down/upload their personal biomedical information and interact with their family doctor. The biomedical device associated with the smartphone is capable

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of providing medical, biochemical, and biophysical information, which can be sent to a personal health center for a remote doctor. Guo demonstrated a research work which implemented blood uric acid monitoring with the utilization of a smartphone as a miniaturized electrochemical analyzer.[16] Guo and Ma also reported a meaningful electrochemical test strip with a dual electrochemical reaction channel.[17] The dual structure allowed the simultaneous detection and characterization of uric acid and glucose. A smartphone-based immunosensor was successfully demonstrated for the diagnosis of HIV with enzyme-linked immunosorbent assay (ELISA).[18] A smartphone-based fluorescence immunoassay was presented to perform the detection of prostate specific antigen (PSA).[19] In addition, smartphone-based molecular diagnosis for pathogens at the point-of-care was also reported.[20] The above-mentioned works demonstrated that the smartphone-based biomedical analytical platform is powerful and meaningful for biochemical, immune, and molecular level analytics in clinical application. The electrochemical biosensor plays a critically important role in point-of-care diagnosis due to its portability, cost-effectiveness, ease of use, fast analysis, and compactness. Therefore, it is a very promising solution to utilize a smartphone with the electrochemical dongle for point-of-care monitoring of blood ketone for DK/DKA evaluation.

In this Letter, a medical smartphone-powered dongle as a miniaturized electrochemical analyzer incorporated with a disposable test strip for accurate evaluation of blood ketone in finger whole blood at the point-of-care was demonstrated. As Figure 1a shows, a one-shot electrochemical blood ketone strip was connected to the medical electrochemical dongle which is powered by the smartphone through an OTG (On-The-Go: a kind of device communication standard). There was no battery within the medical dongle; the power source was obtained from the smartphone through OTG. The concentration of blood ketone was linearly proportional to the electrochemical reaction current, which was mapped into the measured concentration of blood ketone and displayed onto the smartphone screen. Simultaneously, the personalized medical data was synchronized to the person's corresponding personal health center. Figure 1b illustrates the electronic structure of the proposed medical dongle, consisting of a microprocessor, a power source managing module, an OTG module, etc. The advantages of the proposed platform were summarized such as low-cost, compactness, rapidity, and higher sensitivity and specificity. The whole cost of the proposed platform except the smartphone was determined to be less than $10, under the condition of massive production. The real finger stick blood samples were applied on the electrochemical one-shot test strip for blood ketone characterization. The measured results of blood ketone were compared to the results of the bulky biochemical analyzer, and the analysis showed good agreement. According to the signal-to-noise level, the limit of detection by the proposed system can reach 0.001 mmol/L. The electrochemical current is linearly proportional to the blood ketone concentration with the range between 0.001 and 6.100 mmol/L. The specificity of the ketone strip was determined by the selectivity of the β-hydroxybutyrate dehydrogenase. The effect of the other substances in the whole blood can be suppressed by adding some antijamming reagents to the enzyme solution. Therefore, the specificity was adjustable by minor modification of the immobilized reagents.

However, there always exists some difference in measured results between the proposed system and the bulky biochemical analyzer. The hematocrit (HCT) of blood had a negative effect on the measured result by the medical dongle. It is still a difficult issue for electrochemical detection of biochemical molecules since the effect of HCT on the measurement was hard to describe accurately. By doing the HCT compensation in the algorithm, the accuracy of the measurement can meet the standard clinical criterion. It was demonstrated that the proposed medical smartphone powering dongle was very meaningful as a miniaturized electrochemical analyzer for point-of-care monitoring of the critical biochemical parameters such as blood ketone under variable circumstances, which is believed to be useful in fulfilling the needs of health mobility.

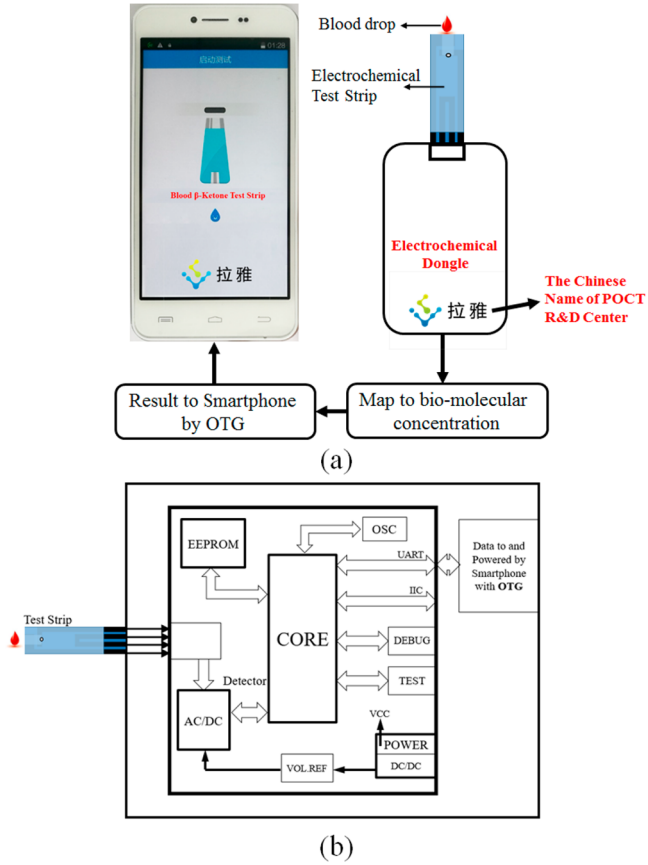

Figure 1. (a) Practical photograph of the proposed medical dongle with a blood ketone test strip; the medical dongle was powered and communicated with a smartphone. (b) Schematic illustration of the medical dongle: microprocessor, power source managing module, OTG module.

THEORY AND METHODS

Figure 2a shows a practical photo of the proposed medical electrochemical dongle with connection to the smartphone through an OTG wire. An electrochemical ketone test strip works as a biosensor, which converted the concentration of ketone to electronic current, which was then analyzed and read by the dongle. The working principle of the ketone test trip is based upon the β-hydroxybutyrate dehydrogenase method. The blood ketone can be accurately evaluated by the direct detection of blood β-hydroxybutyrate levels since the concentration of β-hydroxybutyrate is highly linearly correlated with the blood ketone level. Figure 2b indicates the working principle of the blood ketone test strip. At the beginning of the enzyme-catalyzed process, β-hydroxybutyrate was catalyzed to

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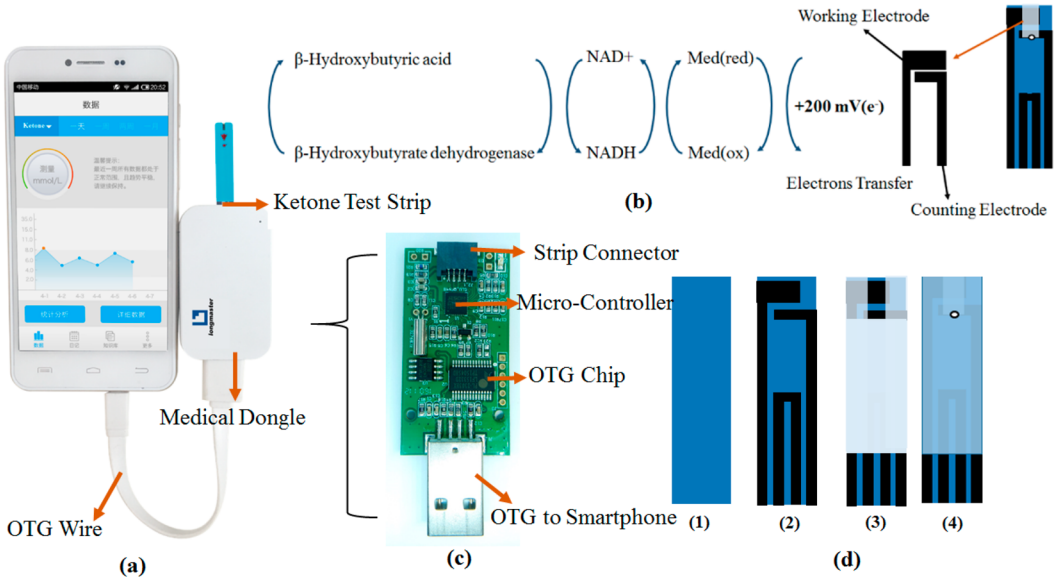

Figure 2. (a) Photograph of the medical dongle with OTG connecting to the smartphone; (b) the working mechanism of the blood ketone test strip; (c) the PCB and electronic elements in the medical dongle; (d) the fabrication procedure of the blood ketone test strip.

acetylacetic acid under the help of *β*-hydroxybutyrate dehydrogenase. At the same time, nicotinamide adenine dinucleotide (NADH) was oxidized into NAD^+ with the reduction of Fe(III) to Fe(II). By applying the 200 mV direct current (DC) between the working and counter electrode, the electrochemical current was generated. The medical dongle can directly read the current signal by the electronic contact with the electrodes printed on the test strip. The electrochemical current was mapped into the measured blood *β*-hydroxybutyrate concentration which was directly displayed and saved in the smartphone. Figure 2c shows the circuit board of the medical dongle, which consists of a test trip connector, a microcontroller, an OTG controlling chip, and peripheral functional circuits. Figure 2d displays the schematic fabrication process of the *β*-hydroxybutyrate test strip. The one-shot electrochemical test strip was manufactured by a screen-printer. First, the carbon ink was printed on the polyethylene terephthalate (PET) (as Figure 2d.1). In the following step, PET with carbon electrodes was baked within the specified drybox at 65 °C for 40 min (as Figure 2d.2). After the drying process, an insulating stick double layer was pasted on the PET leaving the working area (as Figure 2d.3). Paramount *β*-hydroxybutyrate dehydrogenase was immobilized within the working area. Subsequently, the test strip with enzyme was baked in a tunnel oven at 35−45 °C for 20−30 min. The hydrophilic layer and upper cover plate were pasted on the PET to form the final strip (as Figure 2d.3).

RESULTS AND DISCUSSION

The medical dongle works as an electrochemical analyzer to provide the chronoamperometric (CA) characterization of the proposed ketone test strip since this typical method is precise, sensitive, and effective. The chronoamperometric curves as an electrochemical catalyzed result of the existence of *β*-hydroxybutyrate with the applied electrical potential at 200 mV on the working electrode are depicted in Figure 3. The two chronoamperometric curves correspond to the two blood droplets taken from one person. During the early stage of the electrochemical reaction, the numbers of electrons are

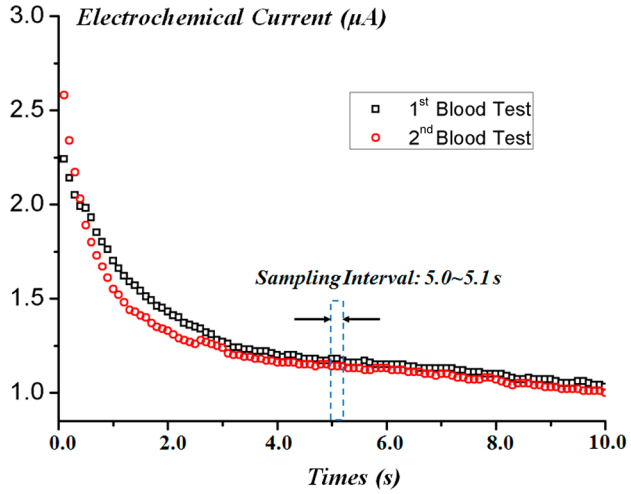

Figure 3. Electrochemical characterization of the proposed ketone test strips: the typical chronoamperometric curves for real blood sample from the normal objective with blood ketone: 0.36 mmol/L; the two droplets of blood were from the same person.

accumulated and transferred on the surface of the working electrode under the enzyme-catalyzed reaction, which stage experienced a very short time. As a subsequent process, the electrochemical current decays exponentially as Figure 3 indicated due to the exhaustion of the *β*-hydroxybutyrate molecules above the surface of the working electrode. The exponential decay lasted around 2 s. Then, the electrochemical current arrived at a relatively steady level since the mass transfer of *β*-hydroxybutyrate molecules from bulky solution to the surface of the electrode dominated the electrochemical reaction. In the relatively steady stage, the electrochemical current was linearly proportional to the concentration of *β*-hydroxybutyrate and was able to be described by the Cottrell equation:

$$I = \frac{nFACD^{1/2}}{\pi^{1/2}t^{1/2}} \quad (1)$$

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where n is the number of electrons, F is the Faraday constant, A is the electrode area, C is the concentration of β-hydroxybutyrate, D is the diffuse coefficient, and t is the time. The electrochemical current was acquired by the ADC (Analog to Digital Converter) in the medical dongle. The current acquisition time interval was set between 5.0 and 5.1 s, and the sampling interval was set at 5 ms. The characterized current was determined by averaging the sampled current value of 20 sampling points. After 10 s, the blood ketone concentration was displayed in the smartphone by resolving the electrochemical current.

$$I_c = \frac{1}{20}\sum_{i=0}^{i=19} I_i \quad (2)$$

The monitoring of blood ketone plays an indispensable role in the diagnosis, treatment, and prevention of DK/DKA (as shown in Table 1). In the diagnosis of ketosis, the patients were diagnosed with DKA on the basis of three factors: (1) blood β-hydroxybutyrate >3 mmol/L; (2) blood glucose >13.9 mmol/L; (3) blood HCO_3 <15−18 mmol/L or artery blood pH <7.3. When the patient was diagnosed with DKA, clinical therapy with the utilization of insulin was required. The efficacy of the formatted therapy was assessed by the evaluation of blood ketone level, as Table 1 indicated. Continuously monitoring blood glucose and ketone can prevent the DK/DKA. Therefore, blood ketone is the critical marker for chronic disease management.

Table 1. Detection of Blood Ketone Bodies Runs through the Whole Cycle of Diagnosis, Treatment, and Prevention of DKA

diagnosis of ketosis	DKA diagnosis standard: β-hydroxybutyrate >3 mmol/L glucose >13.9 mmol/L blood HCO_3 <15−18 mmol/L or artery blood pH <7.3
treatment test	if β-hydroxybutyrate decreases with the rate >0.5 mmol/L/hour, keep monitoring for 2 days if β-hydroxybutyrate decreases with the rate <0.5 mmol/L/hour, increase insulin dosage (1 U/h) until blood ketone stays at a normal level
assessment of efficacy	β-hydroxybutyrate <3 mmol/L, venous blood pH >7.3, patients can eat food, recommend subcutaneous insulin therapy
health prevention and care	keep monitoring blood glucose and ketone to prevent the DK/DKA

In order to assess the accuracy of the measured results of the proposed medical dongle, the results obtained from the medical dongle and the bulky clinical biochemical analyzer were compared by investigating the linear correlation between the measured data of the medical dongle (finger whole blood from one patient) and the data of the bulky biochemical analyzer (human serum from the same patient) of those patients who were diagnosed with DKA. The volunteer recruiting and sample collection were all conducted under the framework of governing law and hospital regulation. Both fingertip peripheral blood and venous blood taken from the same patient were utilized for the comparison test. There were 109 blood samples taken from 109 patients of clinical trials participating in the clinical experiment. The patients were required to sit down to have a rest for 5 min before the blood samples were taken. One chosen finger was professionally pricked with a one-shot medical needle. The second finger blood droplet was collected and applied on the ketone test strip for the medical dongle reader. Subsequently, the venous blood sample was taken and collected with a medical 3 mL vacuum tube (with EDTA anticoagulant) by puncturing the venous vein. In the following step, the blood sample in the tube undergoes the process of centrifugation at 4 °C, with a rotation speed of 3000 rpm for half hour. The blood serum of the corresponding patient was collected by obtaining the supernatant part in the tube, and the obtained sample was examined in the bulky biochemical analyzer. The associated curve between the bulky biochemical analyzer and the medical dongle acquired from 109 patients is plotted in Figure 4, with a slope of 1.01028 (intercept, −0.04194, the linear regression coefficient, 0.987) ranging from 0 to 4 mmol/L. It was demonstrated that the preciseness and stability of the measured data by the medical dongle as compared to the bulky biochemical analyzer are highly reliable and applicable in clinical use. Blood serum ketone determined by the bulky biochemical analyzer is the gold standard in clinical use. There are some differences between the two measured results in Figure 4. One main reason is the utilization of different blood samples (medical dongle: finger pricked whole blood; bulky biochemical analyzer: venous blood). Besides, some other critical factors exist to make an obvious effect on the accuracy for the electrochemical biosensor. In the amperometric method, the existence of blood cells with a specific concentration is capable of having a destructive effect on the measured result through influencing the targeting molecules transfer from bulky samples to the reaction surface above the working electrode. The mechanical influence principle has been investigated by previous research. The large amount of erythrocytes in the finger pricked blood can mechanically hinder the blood plasma's diffusion into the working electrode. The hematocrit of blood obviously influences the viscosity of blood, which leads to slower blood capillary flow within the electrochemical reaction channel. It was reported that other blood species such as protein sediment, hemolysis, fibrin aggregation, and blood platelet can influence the measured results with the electrochemical method. Therefore, an allowable deviation existed in the national standard (such as glucose monitoring: a difference of less than 15% was allowed between the point-of-care test device and the bulky biochemical analyzer).

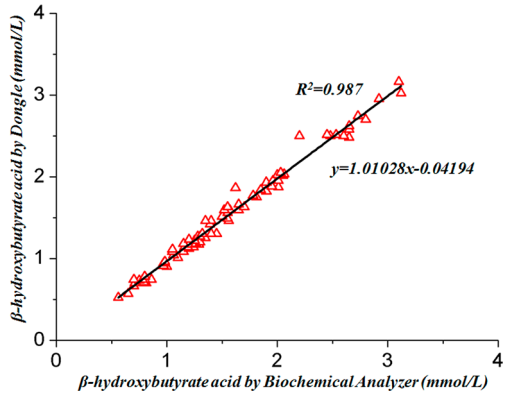

Figure 4. Measured concentration of blood β-hydroxybutyrate comparison between the medical dongle reader and the bulky biochemical analyzer.

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Low, middle, and high concentrations of blood ketone from DKA patients were used to calculate the CV% (coefficient of variation) with respect to the proposed electrochemical ketone test strip, respectively. Real blood samples from three DKA patients, A (with low level of blood β-hydroxybutyrate), B (with middle level of blood β-hydroxybutyrate), and C (with high level of blood β-hydroxybutyrate), were applied to the ketone test strips with the medical dongle, and each test was continuously repeated 5 times for each blood sample. Table 2 summarized the detailed record of the test with respect to the low, middle, and high range of blood ketone and calculated CV %, which indicated the test strip had good reproducibility and reliability.

Table 2. Three Patients with Blood Ketone Concentrations at Low, Middle, and High Levels Were Tested with the Proposed System[a]

blood sample ID	measured blood β-hydroxybutyrate (mmol/L)						
	1st	2nd	3rd	4th	5th	averaged value	CV (%)
A	0.63	0.59	0.65	0.62	0.59	0.616	3.79
B	1.62	1.57	1.64	1.59	1.62	1.608	1.54
C	3.94	3.73	3.85	3.90	3.79	3.842	1.96

[a]Every test for each patient was continuously repeated 5 times, producing 5 measured results for one patient. Coefficient of variation was evaluated, respectively.

CONCLUSIONS

A medical dongle powered by a smartphone was demonstrated to successfully work as a miniaturized electrochemical analyzer for point-of-care monitoring of blood ketone by incorporation with an electrochemical ketone test strip. The measured result from the medical dongle was compared with the test result from a bulky biochemical analyzer, and this analysis showed satisfactory agreement. The medical dongle with the connection to the smartphone allows the test results to be uploaded and stored in the patient's personalized health center easily, which is capable of providing critical important guidance and a record for both patients and their family doctors. Therefore, the medical dongle is believed to have a great applicable potential in mobile health applications.

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Notes

The author declares no competing financial interest.

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