NFC-ENABLING SMARTPHONE-BASED PORTABLE PHOTOTHERMAL SENSING INTEGRATED WITH PAPER-BASED MICROFLUIDIC DEVICES FOR ENZYME-FREE GLUCOSE DETECTION

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ABSTRACT
In this work, we present the first development of a photothermal-based microfluidic paper analytical device (PT-µPADs) integrated with Near-Field Communication (NFC) technology and smartphone readout for enzyme-free glucose quantification in human samples. With the properties of gold nanoparticles (AuNPs) serving as both nanozyme and photothermal substrate, there is no need for costly reagents such as enzymes or instrumentation for the selective and sensitive detection of glucose. Herein, the temperature signal caused by the plasmonic resonance of the AuNPs is easily measured and read out using an NFC tag and smartphone application, demonstrating the potential for real-world applications. By integrating the benefits of microfluidics, nanomaterials, and NFC technology for wireless readout, our sensor shows great promise as an accessible, affordable, and shelf-stable device for glucose quantification.

KEYWORDS
Photothermal, microfluidic paper-based analytical devices (µPADs), gold nanomaterial, Near-Field Communication (NFC) technology, glucose, point-of-care (POC) testing.

INTRODUCTION
Diabetes mellitus is a severe chronic metabolic disease caused by the pancreas's inability to produce sufficient insulin (Type I diabetes) and its failure to activate glucose uptake (Type II diabetes). Normal glucose levels range from 3.9 to 5.6 mmol L^{-1} in blood and 0.80 mmol L^{-1} in urine samples. Elevated glucose levels can indicate signs of diabetes and may lead to potentially lethal consequences if not managed early [1]. Recently, numerous commercial devices are available for glucose monitoring, including glucometers and glucose test strips [2]. While these convenient devices allow unskilled users to test at home, they suffer from low stability and short lifespan, which may lead to detection errors, since they rely on enzyme substrates to improve assay selectivity. Additionally, some methods exhibit no enough sensitivity for lower glucose level detection, which cannot rapidly diagnose some related diseases. To address these drawbacks, the development of glucose sensor without the use of enzyme substrates is highly reasonable.

Gold nanomaterials (AuNPs) have garnered considerable attention as a promising material for various analytical applications [3]. With their distinctive properties as nanozymes, numerous researchers utilize them as glucose oxidase-like and/or peroxidase-like materials for glucose and hydrogen peroxide (H_2O_2) detection, resulting in increased assay stability [4]. Also, AuNPs serve as outstanding photothermal substrates capable of converting light energy to heat owing to their plasmonic characteristics [5]. Importantly, there is evidence that the photothermal method is more sensitive than the colorimetric method, thereby enhancing assay detectability [6]. With these features of AuNPs, the sensitive and selective quantification of glucose level accordingly is highly feasible.

Near Field Communication (NFC) holds considerable promise for analytical sensing applications by reducing analysis costs associated with sophisticated instruments, while also enhancing portability and accessibility for users [7]. Presently, NFC technology has been developed and integrated into electrochemical applications [8]. However, it is noteworthy that there is currently no literature on its implementation for temperature measurement. Therefore, integrating this technology with photothermal detection presents an excellent solution to overcome previous limitations in photothermal analysis, which required bulky laser equipment and thermometers.

In this study, we introduce the fabrication of a photothermal-based microfluidic paper-based analytical device (PT-µPAD) integrated with NFC technology for enzyme-free quantification of glucose. By leveraging the unique characteristics of AuNPs, this technique reduces reagent consumption and analysis costs while enhancing assay selectivity and sensitivity. Furthermore, the temperature signal can be easily read using NFC technology, providing increased accessibility and portability. Upon adding the sample solution to the sample zone of the PT-µPAD, the solution immediately flows to the detection area where AuNPs gradually disappear due to the catalytic system with glucose. Subsequently, the PT-µPAD is inserted into the fabricated portable NFC platform, and a 535 nm green LED light is activated to irradiate the AuNPs. The resulting photothermal heat from the detection zone is measured by an embedded temperature-sensing NFC tag and communicated to a smartphone for near-field contactless readout. This proposed technique represents the first demonstration of NFC readout with PT-µPADs for glucose monitoring and holds potential for further applications in the analytical field.

FABRICATION OF THE PORTABLE NFC PLATFORM
The portable NFC platform was designed using SolidWorks (Dassault Systems SolidWorks Corporation, Waltham, MA). Afterwards, the designed platform was fabricated through 3D printing using matte black PLA filament on an Ultimaker S5 fused deposition modelling printer. (Utrecht, Netherlands). **Figure 1** shows the component of the proposed platform including the customized battery charging system, the battery, the 535 nm LED light, and the SmartTag 1 NFC system.

FABRICATION OF PT-µPADS AND GENERAL PROCESS
The establishment procedure of the PT-µPADs is illustrated in **Figure 2**. Concisely, the patterned paper was designed using Adobe Illustrator software program with a sample channel (diameter 8.0 mm), a straight channel of 11.0 mm x 2.50 mm (length x width), and a detection channel (diameter 6.0 mm). The total size of our designed paper was 23.0 mm x 23.0 mm (length x width). Subsequently, the paper was printed on Whatman No. 1 filter paper using a wax printer (Xerox ColorQube 8870, Japan). After that, the printed paper was baked at 120 °C for 2.0 min and then cooled to room temperature. Following that, the back side of the paper was taped with adhesive tape to prevent the solution leakage from the device. Finally, 7.5 µL of AuNP solution was coated onto the detection channel and allow it to dry at room temperature.

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The general procedure initiates through introduction of 30.0 µL of glucose and sample solutions into the sample zone of the device and then let it to dry at room temperature for 15 min. Subsequently, the paper was inserted into the portable NFC platform and the 532 nm LED light was turned on for 5 min. Next, the temperature signal was recorded using a temperature sensing NFC tag, such as the ST Microelectronics SmartTag 1 and read out by its accompanying NFC smartphone application by bringing the smartphone close to the PT-µPAD. In this experiment, the difference temperature signal (ΔT) between initial temperature signal (T_0) and produced temperature signal (T_p) was determined as an analytical signal. Ultimately, images of the PT-µPADs were taken using a smartphone camera (iPhone 11).

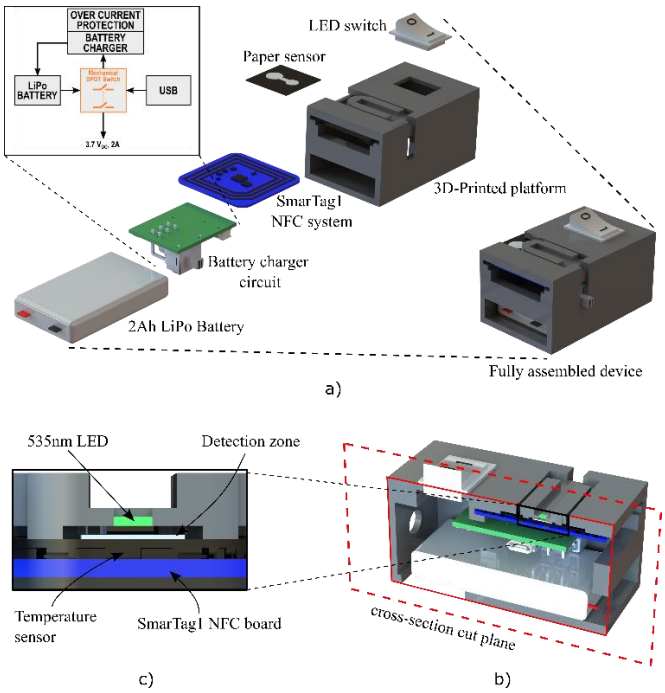

Figure 1 *Schematic design and fabrication of the portable NFC platform and its charging circuit for our technique.*

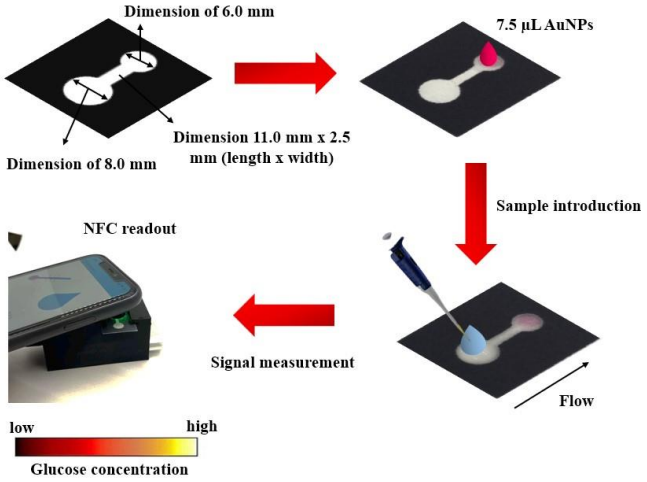

Figure 2 *Schematic representation of (a) the PT-µPADs preparation and (b) NFC-enabled PT-µPADs for monitoring glucose levels in the proposed method.*

WORKING PRINCIPLE OF THE PROPOSED METHOD

The major principle underlying the development of our developed sensor for glucose detection is related to the oxidation of glucose by AuNPs in the present of oxygen (O_2), resulting in the generation of H_2O_2 and gluconic acid [9]. The produced H_2O_2 can subsequently be oxidized by the AuNPs in the presence of O_2 to form hydroxyl radical (˙OH). The AuNPs can be then etched by ˙OH which leads to change in their size and color owing to the located surface plasmon resonance (LSPR) characteristics [10]. We investigated this assay by measuring the absorbance spectrum of the AuNPs mixed with both phosphate buffer solution (PBS), as a blank, and glucose solution at 10.0 µmol L^{-1}. The absorbance peak at 520.7 nm indicated significant difference between blank and glucose solution mixed with AuNPs. Apparently, it can also be noticed that the red color of the AuNPs turned to pale-pink. Similarly, the size of the AuNPs reduced from 10.0 ± 1.0 nm to 7.0 ± 2.0 nm upon exposed to glucose content (**Figure 3(A)**).

We further tested the photothermal analysis on µPAD sensor for glucose determination by measuring temperature signals of blank and glucose at 10.0 µmol L^{-1}. As shown in **Figure 3(B)**, there is a dramatic increase in the ΔT signal for glucose detection, which can be distinguished from the blank signal. As a result, we can confirm that the utilization of the AuNPs properties as both GOx-like and peroxidase-like, and photothermal substrate is highly capable of enzyme-free detecting glucose in order to enhance assay sensitivity and stability and reduce analysis costs.

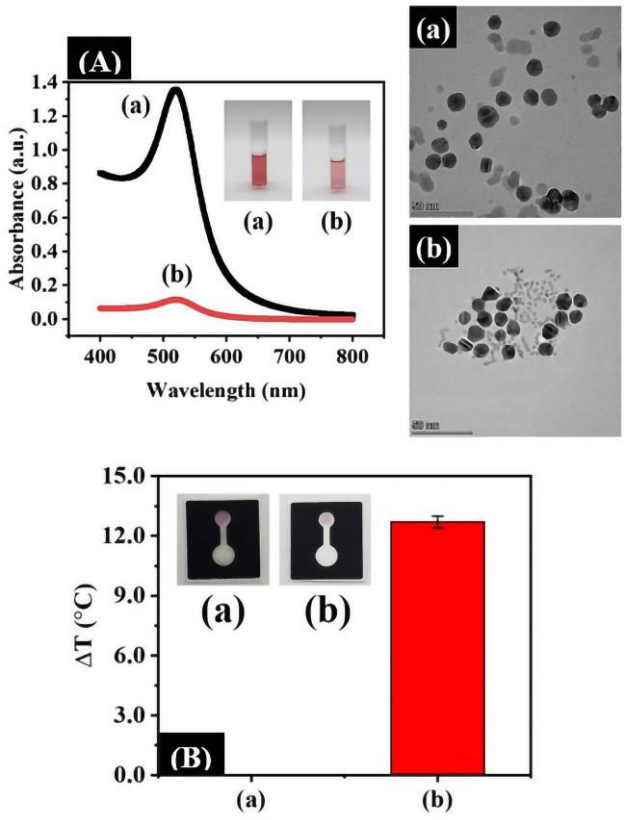

Figure 3 *(A) the absorbance spectrum and TEM image of AuNPs solution with (a) PBS solution and (b) glucose solution at 10.0 µmol L^{-1} and (B) the displays of µPADs and the temperature signals of (a) blank and (b) glucose concentration at 10.0 µmol L-1 using our developed method (n = 3).*

RESULTS AND DISCUSSIONS

The analytical efficiency of our sensor was evaluated through detection of a blank solution and glucose solutions at various concentrations. The ΔT signal of the blank solutions was 0.0 °C while it was 4.0 and 8.3 °C when measuring glucose concentrations at 5.0 and 7.5 µmol L^{-1}, respectively, demonstrating a direct change in temperature signal as glucose concentrations. In **Figure 4**, the linear range between 5.0 and 20.0 µmol L^{-1} with R^2 value of 0.9967 was obtained. Furthermore, the LOD was found to be 25.0 nmol L^{-1} by calculating from 3S.D./m. where m is a slope. We also measured the ΔT signal of this glucose level compared to the blank signal for ten times (n = 10). The resultant ΔT signals were 0.2 and 1.0 °C for blank and glucose at 25.0 nmol L^{-1}, respectively, confirming that this glucose level is the LOD of the proposed method. Additionally, this assay shows remarkable precision for glucose monitoring with the highest standard deviation (%RSD) of 3.90%. Moreover, we found that our method can be stored for at least ten weeks, which is advantageous to the practical use (**Figure 5**).

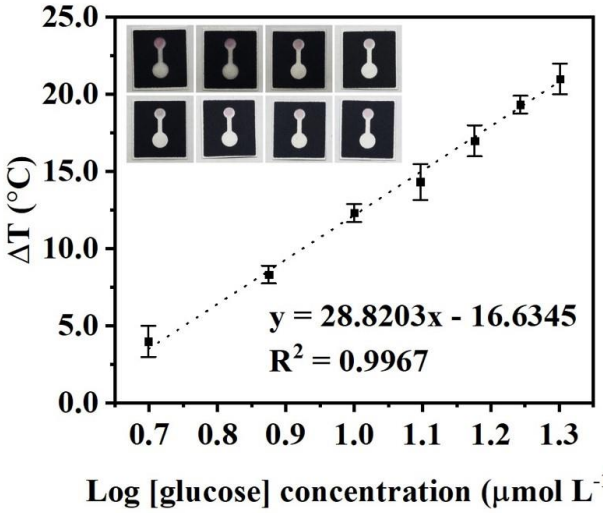

Figure 4 *Working linear range of glucose detection using our NFC-enabled photothermal µPADs (n = 3).*

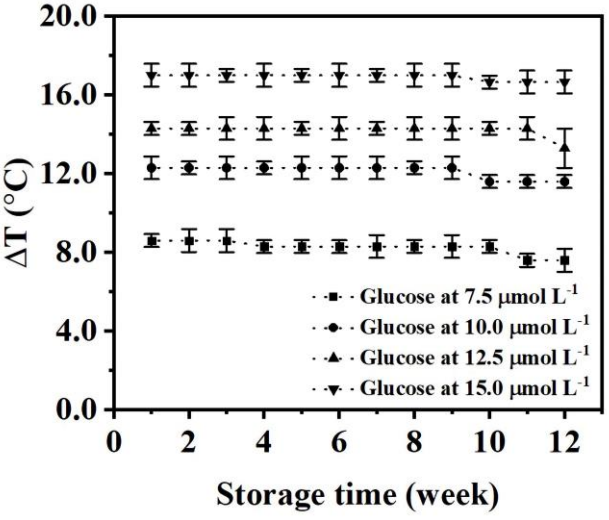

Figure 5 *Demonstrate the temperature signals of storage time of the developed PT-µPADs sensor for glucose detection (n = 3).*

We investigated the selectivity of the developed sensor for glucose monitoring by detecting the ΔT signals from mixing glucose with the interfering substrates that may be present in real samples. In **Figure 6**, none of these substrates significantly affected the ΔT signal for glucose detection, as there was no dramatic change in temperature over 5.0% compared with the signal obtained from measuring only glucose. Consequently, this method is highly selective towards glucose, even though it does not require enzymes.

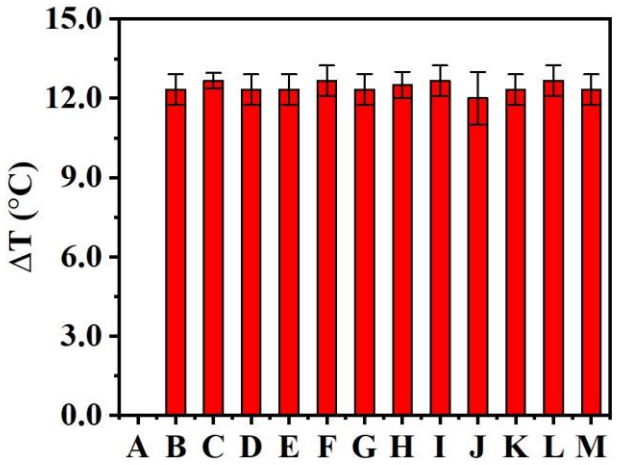

Figure 6 *Demonstrate the temperature signals obtained for (A) a blank solution, (B) glucose (10.0 µmol L^{-1}), and (C-M) mixtures of glucose (10.0 µmol L^{-1}) and with potentially interfering substances, substances including (C) BSA (100.0 mmol L^{-1}), (D) CRP (12.0 mmol L^{-1}), (E) creatinine (1.0 mmol L^{-1}), (F) cortisol (50.0 mmol L^{-1}), (G) dopamine (50.0 nmol L^{-1}), (H) fructose (10.0 mmol L^{-1}), (I) IL-6 (120.0 nmol L^{-1}), (J) lactic acid (50.0 µmol L^{-1}), (K) TNF-α (10.0 pmol L^{-1}), (L) urea (50.0 mmol L^{-1}), and (M) uric acid (50.0 µmol L^{-1}) (n = 3).*

To validate the developed method for biomedical applications, we spiked a standard glucose solution at different concentrations into human control serum samples. Prior to spiking, the glucose level in the human control serum was determined to be 5.29 mmol L^{-1}, consistent with the results from the attached material certificate. In **Table 1**, the percentages of recovery ranged between 99.73% and 101.03% with the highest relative standard deviation (%RSD) of 3.33%. This shows that our sensor can accurately and precisely monitor glucose levels.

Table 1 Result of the recovery studies for glucose detection in human control serum samples using our proposed method (n = 3).

Glucose standard added (mmol L^{-1})	Total found (mmol L^{-1})	%Recovery
0.0	5.29	99.73
2.5	7.83	103.38
5.0	10.41	101.03
7.5	12.92	100.97
10.0	15.94	100.91

CONCLUSIONS AND FUTURE WORK

We successfully developed the PT-µPADs integrated with the temperature signal NFC technology for enzyme-free glucose determination in human biological samples. The design and fabrication of both PT-µPADs and the portable NFC platform are straightforward and affordable. Furthermore, by employing the feature of the AuNPs, the stability of the device is better than other previous commercially available glucose sensor relied on the enzyme reaction. Results indicates that our proposed method provided the acceptable accuracy and precision, and also selectivity for glucose detection, while there is no need for expensive substrate. So, this developed approach can be an alternative analytical and diagnostic device for sensitively and selectively monitoring glucose level without requiring any instrument and has potential to point-of-care (POC) testing.

Future research will focus on the utilization of this developed approach to detect glucose level in real samples of individuals and/or patients. Correspondingly, further research will extend this photothermal analysis using the NFC readout in the analytical application for detecting other biomolecules.

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