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IoT PCR for pandemic disease detection and its spread monitoring

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A B S T R A C T

During infectious disease outbreaks, the centers for disease control need to monitor particular areas. Considerable effort has been invested in the development of portable, user-friendly, and cost-effective systems for point-of-care (POC) diagnostics, which could also create an Internet of Things (IoT) for healthcare via a global network. However, at present IoT based on a functional POC instrument is not available. Here we show a fast, user-friendly, and affordable IoT system based on a miniaturized polymerase chain reaction device. We demonstrated the system’s capability by amplification of complementary deoxyribonucleic acid (cDNA) of the dengue fever virus. The resulting data were then automatically uploaded via a Bluetooth interface to an Android-based smartphone and then wirelessly sent to a global network, instantly making the test results available anywhere in the world. The IoT system presented here could become an essential tool for healthcare centers to tackle infectious disease outbreaks identified either by DNA or ribonucleic acid.

1. Introduction

Dengue fever, ranked as the most important mosquito-borne viral disease with pandemic potential, emerged as a serious public health concern. The World Health Organization (WHO) reported that in the past fifty years, the number of cases has increased 30 times, causing huge human and economic cost which has yet to be tackled [1]. According to studies [2,3], there were an estimated 390 million annual cases of dengue fever and an estimated 3.9 billion people in 128 countries are at risk of being infected by dengue fever virus (DENV) with different serotypes. The DENV is affecting numerous areas in Eastern and Southeast Asia, such as China [4]; the lowlands of Nepal [5]; the slums in Delhi, India [6]; Hanoi, Vietnam [7]; and Iran [8] as well as other parts of the world, such as the Middle East, North Africa [9], and South America [10]. Due to the lack of effective treatment of severe dengue fever [11], accurate and early detection methods to identify the DENV serotype [5,12,13] are required in order to provide careful patient monitoring and to prevent the disease’s progression to a more severe stage. Mobile laboratories suitable for tackling highly contagious diseases such as Ebola, have been developed [14], but they are too bulky and expensive for non-contagious DENV, malaria or similar diseases. An easy-to-use, affordable and reliable point of care (POC) device to perform rapid and economical DENV diagnostic tests in high-risk areas is badly needed. Moreover, it is of the utmost importance to immediately report the results of the POC, including its time and location, to authorities in a centralized location to take required measures.

These days, a vast amount of the human population has possession of a cell phone with an embedded global positioning system (GPS) connected to mobile networks via base stations using different generations of cellular mobile communications, such as second (global system for mobile communications) (2G), third (universal mobile...
telecommunications system) or fourth (long term evolution (LTE)/ worldwide interoperability for microwave access). Connecting the easy to use and cost-effective POC devices providing the DENV diagnoses via a mobile network would create an Internet of Things (IoT) [15] for healthcare [16,17], an essential tool to tackle any infectious disease outbreak. The IoT would speed up the information transfer from POC devices to a centralized location and then based on models using big data analysis [12,18,19], suggest targeting specific sites. The IoT would be able to communicate with the POC systems, inform its operator where to go next to perform testing, remotely change the test protocols or provide other important information to the POC device user. This year, the fifth generation of cellular mobile communications will be released with a massively increased data rate compared to those of previous generations. Nevertheless, the IoT for POC applications such as DENV testing can rely on the oldest generation of 2G, as the POC communication with the centralized location requires a low data rate, an important feature for cash strapped countries of the developing world.

DENV is often detected by a paper-based immunochromatographic test either alone [20] or in combination with malaria [21], enzyme-linked immunosorbent assay (ELISA) [22], and/or reverse transcription polymerase chain reaction (RT-PCR) [23] (allowing identification of the DENV serotype) [12]. The paper-based lateral flow immunochromatographic test is fast and easy to use therefore is often used in remote areas. However, due to its lower sensitivity as well as specificity, a confirmation using a second detection method (ELISA and/or RT-PCR) is often required. The ELISA method relies on antibody production by the human immune system, the method being more suitable for advanced stages of the disease. The RT-PCR is the preferred method as, in principle, it can detect a single copy of specific ribonucleic acid (RNA) [24,25] having both sensitivity and specificity required for early disease detection (lowering the cost of treatment). Unfortunately, RT-PCR tests are typically only carried out in hospitals or certified diagnostic laboratories after the onset of DENV symptoms. The RT-PCR method is highly specific and reliable; the problem is that not every infected patient makes it to the hospital to be positively diagnosed. As an outcome, the diagnostic results are not available as quickly and comprehensively as required for disease outbreak control.

Recently, a lot of effort was invested in the development of a portable system for POC diagnostics [26–29]. Numerous methods were developed using a smartphone for data communications as well as fully integrated systems [30] such as a smartphone-based optical system for high throughput [19]. We previously reported a hand-held polymerase chain reaction (PCR) system capable of four simultaneous reactions, demonstrating its performance by amplification of complementary deoxyribonucleic acid (cDNA) of avian influenza A (H7N9) RNA, proving its principle of operation [31]. Later on, a reverse transcription step was added and RNA of an Ebola virus was detected with RT-PCR while using human transcript glyceraldehyde-3-phosphate dehydrogenase RNA as a positive control [31].

Here, we report an IoT PCR device that can be used for pandemic disease detection and its spread monitoring. It is a portable system with a weight of ≈ 170 g and physical dimensions of (≈ 60 × 100 × 34) mm² equipped with Bluetooth (BT) communication between the PCR and the Android wireless tool, such as a smartphone or a tablet. We utilized it to test cDNA of a DENV, which could also be used for RNA detection. Once the DENV was detected, the unit sent this information, including the GPS coordinates to a centralized location via the LTE network, thus helping the authorities to map the disease spread (as suggested in Fig. 1).

2. Material and methods/PCR device

2.1. PCR chip: fabrication and assembly

The core of the system consists of four virtual reaction chambers (VRC) on a hydrophobically coated glass (Fig. 2C) which were successfully tested earlier for ultrafast real-time PCR with external optics [32], and a fully integrated real-time PCR [33]. The glass was placed on a silicon chip made by micro-electro-mechanical system (MEMS) technology developed earlier [32]. We improved the layout of the MEMS chip containing heaters and sensors using a Nanolithography toolbox [34] by adding an electrically grounded guard ring between the thin film sensor and the heater. We also increased the chip size to (≈ 18 × 18 mm²) to assure easier handling. The chip was placed on its own printed circuit board (PCB) and was connected to the motherboard of the PCR unit by a connector for simple replacement and calibration (Fig. 2B and D). The electronics as well as the optics (Fig. 2A) was also improved but it is not a subject of this contribution. The PCR systems were then assembled and calibration were ready for deployment.

2.2. DNA templates and primers

We tested the PCR device performance using cDNA of DENV prepared by synthesis. Real field testing of the device would have to start with sample collection and preparation followed by reverse transcription before the PCR can be conducted [25,35]. Also, reagents for single step qRT-PCR are needed. More details are in the discussion section.

We selected an amplicon with a total length of 177 base pairs (bp) from 9937 bp to 10113 bp, as follows:

\[
\text{ACAAGTGAACAACACTGTCATCACAAGCGAAAACATGAGTGTGACA}
\]

\[
\text{AACAACGGAAGACATGTCTGAGCTGTGAAACAGGTTGTGATTACAGA}
\]

\[
\text{AAAACCCATGGATGGAAGAAAAATCCCTCAGTGAATCATGGGAGGAAA}
\]

\[
\text{TCCCATATTTGGGAAAGAGAGACAAATGTGTCGGCCG using forward}
\]

\[
\text{and reverse primers with sequences ACAAGTGAACAACCTGGTCCAT}
\]

\[
\text{and GCCGCACATTGTCCTCCTC, respectively.}
\]

2.3. PCR master mix and protocol

We prepared a solution to conduct the PCR by mixing ≈ 0.3 μL of a commercial polymerase with contents of 5 units μL⁻¹, ≈ 1 μL of a PCR buffer consisting of 100 mM tris (hydroxymethyl) aminomethane-HCl (pH ≈ 8.3), 500 mM KCl, ≈ 15 mM MgCl₂, ≈ 0.8 μL of a deoxyribonucleotide triphosphate mixture containing of adenine, thymine, cytosine, and guanine each with a concentration of ≈ 2.5 mM, ≈ 0.4 μL of forward as well as reverse primer, ≈ 0.5 μL of Eva Green intercalating dye with original concentration 20× (diluted as per manufacturer instruction), ≈ 1 μL of bovine serum albumin with contents of ≈ 5 mgmL⁻¹, and ≈ 1 μL of cDNA template with varied contents depending on the experiment. Furthermore, the total volume was adjusted by adding ≈ 4.6 μL of sterilized water to a total volume of ≈ 10 μL.

Prior to testing on an IoT PCR device, we verified the master mix performance and its values of critical threshold (Ct) and the melting temperature (Tm) using a commercial real-time PCR system (Supplementary Section A) beginning with a hot start at 95°C for 30 s followed by 40 cycles of PCR amplification consisting of DNA denaturation at 95°C for 8 s, primer annealing at 60°C for 30 s, and DNA sequence elongation at 72°C for 10 s, then followed by melting curve analysis (MCA) from 72°C to 95°C. Once the master mix performance was verified, we ran the same protocol on the IoT PCR system.

2.4. Bluetooth module design

Wireless communication between mobile platforms, such as a mobile phone or tablet, and the PCR system was provided via a BT module, which comprised a commercial unit supporting BT version 2.1. This unit has its own dedicated 5 V power supply with a universal serial bus (USB) connector, including AC-DC converter and voltage stabilizer, making it compatible with a standard USB power supply or power bank. The independent power supply for the BT module eliminates its influence on PCR system stability. Data transfer between the BT unit and
PCR system is conducted bi-directionally via a universal asynchronous receiver-transmitter interface. Based on a request from a mobile system, such as a smartphone or tablet, the last PCR measurement data are sent to the mobile system.

2.5. Cell phone/PC APP

We developed an application (APP) for Android devices that allows them to receive data via the BT communication module from the PCR system, save them into a text file, and represent them in graphical format. The smartphone display is split into two parts: the top is used to show the PCR amplification curve and the bottom for MCA. The system has the option of sending the data to a dedicated place with GPS coordinates to inform authorities about the presence of the infectious disease. After pairing the Android device with the BT unit of the PCR system, data from the PCR system are downloaded into the device and plotted on its display. They can also be automatically sent via LTE network to a dedicated place monitoring the disease outbreak. For convenience, we also created a personal computer (PC) APP for PCR system programming (Fig. 3A) either via USB or BT interface.

3. Results

We performed two basic real-time PCR experiments. The first test was conducted with four samples having a master mix with identical contents of the cDNA of DENV template to verify real-time PCR system uniformity and repeatability as well as temperature settings by performing MCA. The second test mimicked the actual field test having three samples and a no template control (NTC) (Supplementary Section B). The captured analog-to-digital converter output values of PCR system, data from the PCR system are downloaded into the device and plotted on its display. They can also be automatically sent via LTE network to a dedicated place monitoring the disease outbreak. For convenience, we also created a personal computer (PC) APP for PCR system programming (Fig. 3A) either via USB or BT interface.
amplification data, as well as MCAs, were displayed on the integrated thin film transistor (TFT) screen of the PCR unit. Then they were transferred to the smartphone via BT to be displayed by the mobile system and also further processed by extracting the background noise and normalization. The data were then averaged from four consecutive runs and plotted in a linear (Fig. 3B) and logarithmic (Fig. 3C) scale. We then calculated the \( C_\text{T} \) as the value of the cycle number with 10% of relative fluorescence amplitude as \((20.07 \pm 0.17)\) cycle (mean \pm standard deviation from four measurements), showing an excellent point to spot result uniformity. We also extracted the MCA (Fig. 3D) and performed the curve fitting using a modified Boltzmann curve obtaining a \( T_M \) value of \((81.98 \pm 0.16)\)°C (mean \pm standard deviation from four measurements). We subsequently performed a numerical differentiation of the fitted curves for better visualization (Fig. 3E) by showing the values of \( T_M \) as peaks of the \(-dF/dT\) curve. Concurrently, we performed identical testing using a commercial real-time PCR system getting a \( T_M \) of \((81.97 \pm 0.16)\)°C (mean \pm standard deviation from four measurements). (D) MCA of samples after PCR with curve fitting and (E), the first derivatives of the fitted curves. The peaks corresponded to the value of \( T_M \) as \((81.98 \pm 0.16)\)°C (mean \pm standard deviation from four measurements).

4. Discussion

We developed an IoT PCR system equipped with BT communication and a supporting Android APP for smartphones as well as a PC APP for convenient PCR programming with a projected manufacturing cost between 100–200 USD.

This real-time PCR is a robust device with no moving parts and uses four standard light emitting diodes (LEDs) with a diameter of 5 mm as a light source with a lifetime of more than 50,000 h. The most fragile part is the micromachined silicon chip, here mounted on its own replaceable PCB.

We performed a set of experiments using a cDNA of DENV with 40 cycles of a three-step PCR protocol followed by an MCA taking \( \approx 34 \) min in total.

The testing time can be shortened either by system redesign [36] or using probe-based PCR and performing a single PCR cycle as fast as 10 s [32], thus having a total required time for the 40 cycle PCR protocol as short as \( \approx 6 \) min. The system can be further expanded using multiplexing just by reprogramming and effectively doubling the throughput [36], reprogramming the PCR readout, or using multicolor LEDs with a dual bandpass filter.

Once the PCR was completed, the data were transferred via BT to a portable Android platform and then they could be sent via LTE network together with the PCR unit number, time of test completion and GPS coordinates of its location to a centralized location. The positive result could indicate the spread of the infectious disease on a map in a similar form as the hypothetical results from Xi’an, Shaanxi Province, P. R. China (Fig. 4). We performed the first test in our laboratory capturing PCR data as well as the GPS location, and then we emulated the tests performed through the city by capturing the locations only (Supplementary section C). This PCR with BT and Android-based wireless communication could be a key part of an IoT system for healthcare ready for deployment to tackle infectious disease outbreaks.

An actual approach to tackle infectious disease spreading would require obtaining samples from patients, processing them depending on the body fluid type, then mixing them with the reagents and pre-concentrating them into volume compatible with the miniaturized PCR, which is 0.5 μL or less. Samples collected from cheek swabs or from saliva have very low contents of DNA, thus hindering the PCR analysis. There are readily available single-step kits for real-time PCR systems capable of processing samples as they are collected [37,38]. The only requirement is mixing the sample with these reagents. After the mixing step, the cells are enzymatically lysed, so that DNA/RNA from cells is released, followed by heating to stop the lysis enzymes [39] and activation of the polymerase, which is then followed by PCR thermal cycling. The last two steps would be performed on the hand-held PCR.

Samples that originate from blood contain albumin and other PCR inhibitors; these have to be first removed by a method compatible with hand-held PCR as shown before [25,35] using functionalized para-magnetic beads. This sample preparation also includes sample pre-concentration to process small sample volumes by hand-held PCR.

Any part of the system getting into contact with the actual sample has to be disposable due to extremely high contagion of sample to be tested. Both systems, potential sample preparation as well as the PCR reaction should be conducted on a disposable microscope cover slip glass coated with suitable fluorosilanes or Teflon making their surface hydrophobic [40]. All other parts of the system can be non-disposable.
since they do not come into contact with either the reagents or the sample. Once the test is completed, the used glass objects are disposed of and are then replaced with fresh ones. The device is now ready making the entire process of diagnoses economical.

Here we tested a DENV as a typical representative of an infectious disease spread by mosquitos. The same system can also be applied to monitor the spreading of other diseases, such as malaria. In principle, its deployment could also be possible for highly contagious diseases such as Ebola, severe acute respiratory syndrome (SARS), and other viruses, but in such circumstances, the system would have to be handled by highly trained professionals due to the highly infectious nature of the viruses. Also, for practical reasons, the hand-held PCR system would have to be redesigned for autoclaving to prevent any contamination of the operator.

5. Conclusions

We tested an IoT real-time PCR system and demonstrated that the device sensitivity and reproducibility were improved. The PCR device performance is comparable with that of a commercial system. The protocol consisting of 40 cycles to detect the cDNA of a DENV required \( \approx 34 \) min. The system was connected via BT communication with an Android-based smartphone and then with the whole world via LTE. This could become a basic block of the IoT, thus helping to tackle infectious disease outbreaks.

The device can be used for other diseases such as any type of influenza, malaria, and human immunodeficiency virus. It can be used as a “first-defender” tool to perform rapid early diagnosis and, more importantly, the data collected can be used to prevent pandemic outbreaks.

Authors’ contributions

Pavel Neuzil, Ciprian Iliescu and Hanliang Zhu wrote the manuscript, Hanliang Zhu performed the experiments and data analyses, and Pavel Podesva improved and assembled the PCR systems.

Xiaocheng Liu and Haoqing Zhang fabricated the PCR chip, Tomas Teply programmed the internal PCR software as well as the Android APP; Honglong Chang participated in the organizing and writing of the manuscript; Ying Xu conceived the PCR as an IoT device and participated in writing the manuscript; Airong Qian, Yingfeng Lei, and Yu Li chose the cDNA sequence and designed the primers; Andreea Niculescu wrote the IoT part of the manuscript; and Pavel Neuzil designed the portable PCR concept as well as the micromachined chip.

Declaration of competing interest

Authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi: https://doi.org/10.1016/j.snbb.2019.127098.

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