Thesis for the Degree of Master of Engineering

# A Carbon-black-poly(dimethylsiloxane) Patterned Paper Device using Photothermal-based Nucleic Acid Amplification for Energy-efficient Point-of-care Testing

by

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Pukyong National University

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# 에너지 효율이 좋은 현장 진단을 위해 광열 기반의 핵산 증폭을 이용한 카본-블랙-폴리디메틸실록산이 패터닝된 종이 소자

Advisor: Prof. Joong Ho Shin

by

Ye Lin Kim

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A dissertation

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# ABBREVIATIONS

## Abbreviations

| | |
|---|---|
| Nucleic acid amplification test | NAAT |
| Polymerase chain reaction | PCR |
| double-stranded DNA | dsDNA |
| single-stranded DNA | ssDNA |
| Deoxynucleotide triphosphate | dNTP |
| Helicase-dependent amplification | HDA |
| Strand-displacement amplification | SDA |
| Transcription-mediated amplification | TMA |
| Loop-mediated isothermal amplification | LAMP |
| Forward inner primer | FIP |
| Backward inner primer | BIP |
| Forward outer primer | F3 |
| Backward outer primer | B3 |
| Forward loop primer | LF |
| Backward loop primer | LB |
| Point-of-care testing | POCT |
| World Health Organization | WHO |
| Poly(dimethylsiloxane) | PDMS |
| Poly(methyl methacrylate) | PMMA |
| Positive temperature coefficient | PTC |

| | |
|---|---|
| Infrared | IR |
| *Escherichia coli* | *E. coli* |
| *Staphylococcus aureus* | *S. aureus* |
| *Salmonella typhimurium* | *S. typhimurium* |
| weight/weight | w/w |
| Polyethlene terephthalate | PET |
| Lysogeny-broth | LB |
| Deionized water | DI water |
| Limit of detection | LOD |
| Non template control | NTC |
| Green intensity | G value |
| Red intensity | R value |
| Near infrared | NIR |
| Light-emitting diode | LED |
| Platinum | Pt |

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# 에너지 효율이 좋은 현장 진단을 위해 광열 기반의 핵산 증폭을 이용한 카본-블랙-폴리디메틸실록산이 패터닝된 종이 소자

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## 요 약

핵산 증폭법은 분자 진단에서의 중요한 기술 중 하나로 병원체, 바이러스성 질병, 유전병 진단, 포렌식 등 다양한 분야에서 사용되고 있다. 이 중 가장 보편화된 기술은 중합효소연쇄반응 (PCR)으로, 각기 온도가 다른 세 가지의 단계를 한 사이클로 하여 30–40 회의 반복을 통해 핵산을 수백만개로 증폭시킨다. 단계마다의 다른 온도로 인하여 정밀하고 빠른 온도 변화가 중요하며 이에 따라 온도 전환 기기를 필수적으로 필요로 한다. 하지만 이는 별도의 검사실이 아닌 환자가 있는 장소에서 진단을 수행할 수 있는 현장 진단에 적합하지 않다. 이에 따라 일정한 온도 아래에서 핵산 증폭을 수행할 수 있어 PCR 에 비해 훨씬 간단한 등온 증폭법이 개발되었다. 많은 종류의 등온 증폭법 중 민감도와 특이도가 높은 loop-mediated isothermal amplification (LAMP)가 많이 사용되고 있다. LAMP 의 경우, 65 ℃에서 핵산 증폭이 수행된다. 하지만 이 경우에도 상온보다 높은 온도에서 반응이 일어나기 때문에 오븐, 핫 플레이트와 같이 많은 전력을 사용하는 외부 장치가 필수적이다. 따라서, 핵산 증폭법을 현장 진단에 적용하기 위해서는 작고 가벼우며, 휴대성이 높고, 전력 사용과 외부 장치로의 의존성을 최소화하는 것이 중요하다.

이에, 본 연구에서는 카본-블랙-폴리디메틸실록산 (PDMS)이 패터닝된 종이 기반 소자를 핵산 증폭을 위한 가열 플랫폼으로써 제작하였다. 카본 블랙은 특정 빛을 흡수하여 열로 전환시킬 수 있는 광열 물질로, 808 nm 의 레이저 빛 아래에서 열을 발생시키기 위하여 사용되었다. 이 광열효과를 이용하여, 단 시간 내에 타겟 유전자 서열을 증폭할 수 있는 loop-mediated isothermal amplification (LAMP)를 수행하여 식인성 병원체인 *Escherichia coli* (*E. coli*) O157:H7 DNA 의 *rfbE* 유전자를 증폭하였다. 종이 기반 소자에 패터닝되는

카본 블랙의 농도와 PDMS 의 혼합 비율을 변경하여 타겟 온도를 최적화하였으며, LAMP 를 수행하는 데 필요한 최소한의 레이저 출력 세기 또한 최적화하였다. 본 연구에서 제안하는 종이 기반 소자를 이용하여 LAMP 수행 시 15 분 이내에 결과 확인이 가능하며 종이 기반 소자의 색 변화를 통하여 타겟 DNA 의 존재 유무를 육안으로 확인할 수 있다. 종이 기반 소자를 활용하여 타겟인 *E. coli* O157:H7 DNA 의 *rfbE* 유전자를 $10^2$ CFU $mL^{-1}$ 의 검출 한계를 가지고 특이적으로 검출하였다. 종이 기반 소자를 현장 진단에 적용할 수 있는 가능성을 높이기 위해, 808 nm 레이저 다이오드를 이용한 장치 또한 개발하였다. 레이저 다이오드 장치는 전력 소비량을 최소화할 수 있도록 휴대용 배터리를 통하여 구동되었으며, 2 W h 의 매우 적은 전력 소비량을 가지고 있다. 본 연구에서 제안하는 종이 기반 소자는 저렴하고 일회성이며, 손쉽게 제작이 가능하다. 뿐만 아니라, 레이저 다이오드 장치는 작고 가벼워 휴대성이 높으며, 적은 전력 소비량을 보인다. 이를 통해, 종이 기반 소자와 레이저 다이오드 장치는 용이한 현장 진단을 위한 도구로써 활용할 수 있을 것이라 사료된다.

# CHAPTER 1: INTRODUCTION

## 1.1 Research Background

Nucleic acids are small biomolecules that are essential for all known life including genetic materials, DNA and RNA. Nucleic acids can store and transmit genetic information of cells. However, due to the low quantities of nucleic acids, the technique which can detect small amounts of nucleic acid was required. Nucleic acid amplification tests (NAATs) are the most common technology which can detect target genetic materials by amplifying target nucleic acid sequences. NAATs are widely used to detect viral or bacterial disease such as SARS-CoV-2 [1], tuberculosis [2], bacterial foodborne infections [3], and human immunodeficiency virus [4] due to their high specificity and sensitivity.

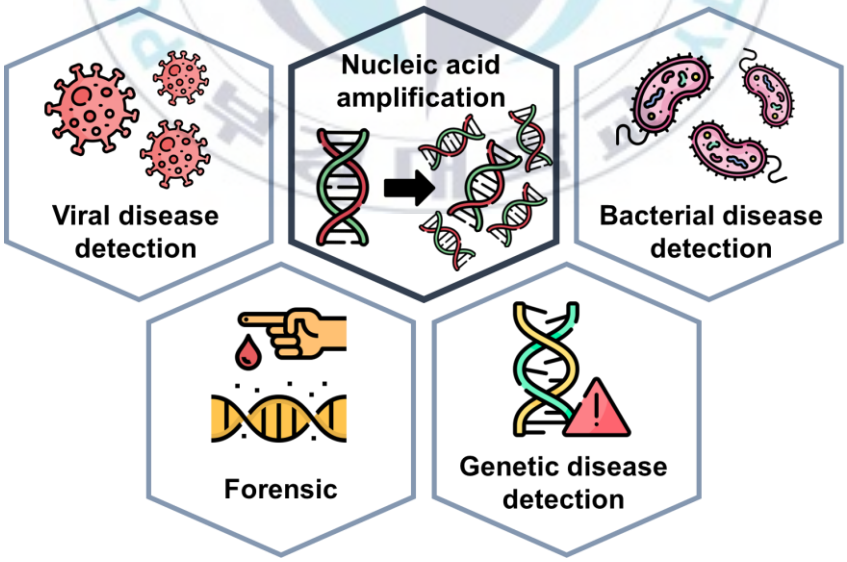



**Figure 1.** Applications of nucleic acid amplification tests.

Polymerase chain reaction (PCR) is one of the most widely used type of NAATs. PCR can achieve large amount (millions to billions) of amplified products from a very small number of DNAs by thermal cycling. PCR is based on the enzyme reaction which has three steps, denaturation, annealing, and extension, to amplify target DNA (Figure 2). The denaturation step requires heating at 95 ℃ to separate double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA). After denaturation, lowering the temperature (50 to 65 ℃) enables the primers to attach to the complementary site. Finally, change the temperature to 72 ℃ to activate DNA polymerase and synthesize new complementary DNA strand using deoxynucleotide triphosphates (dNTPs) from attached primers. These three steps are cyclical and repeated about 30 to 40 times (Figure 3). Through this thermal cycling, exponential amplification can be achieved in 1–2 hours. However, precise thermal cycling processes require thermal cycler, which can be bulky, expensive and require a lot of power consumption. Therefore, isothermal amplification methods have been reported as alternative amplification methods to eliminate these limitations.

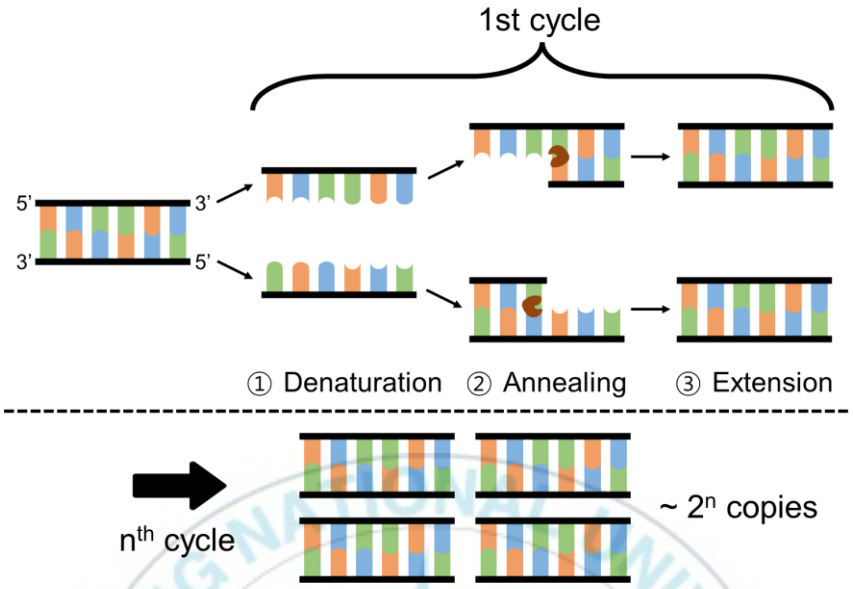



**Figure 2.** Amplification process of PCR.

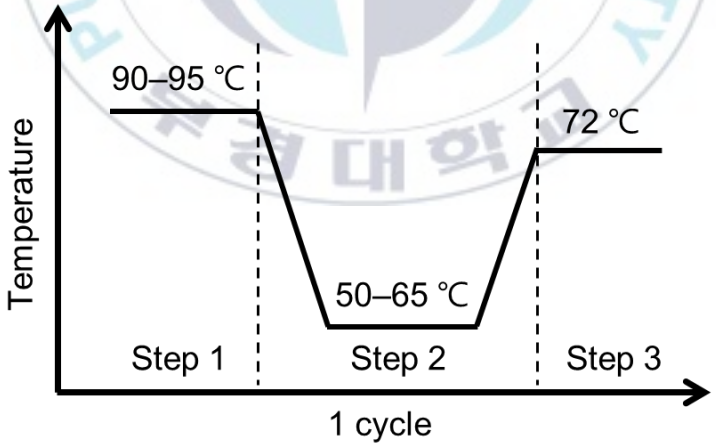



**Figure 3.** The temperature cycle of PCR.

Step 1: Denaturation at 90–95 ℃. Step 2: Annealing at 55–65 ℃.

Step 3: Extension at 72 ℃.

Isothermal amplification methods of nucleic acids such as helicase-dependent amplification (HDA), strand-displacement amplification (SDA), transcription-mediated amplification (TMA), and loop-mediated amplification (LAMP) have been developed. Isothermal amplification can be performed under the constant temperature condition without a thermal cycler. Since there is no need for temperature change for each stage which can eliminate the time for temperature conversion, isothermal amplification is more simple and rapid compared to conventional PCR.

Among the various isothermal amplification methods, LAMP is one of the widely used method. LAMP was first developed by Notomi et al. in 2000 [5]. LAMP can recognize 6–8 distinct regions of target nucleic acid specifically using 4–6 primers, including forward inner primer (FIP), backward inner primer (BIP), forward outer primer (F3), backward outer primer (B3), and optional two loop primers (LF and LB) which can accelerate amplification. At 60–65 ℃, the reaction is initiated by invasion of FIP and extension by a strand displacing DNA polymerase. Then, ssDNA strand is released by F3 primer, and it acts like the template for BIP and B3. And extension using BIP and B3 with strand displacing DNA polymerase occurred. Through this reaction, products with dumbbell structures are formed which can eliminate the denaturation step (Figure 4). Due to the 6–8 target sequences, LAMP can achieve high specificity and sensitivity results. Also, it has simple detection methods such as turbidity, fluorescence, and colorimetric assay. Therefore, performing LAMP for detecting pathogens has been reported [6-8].

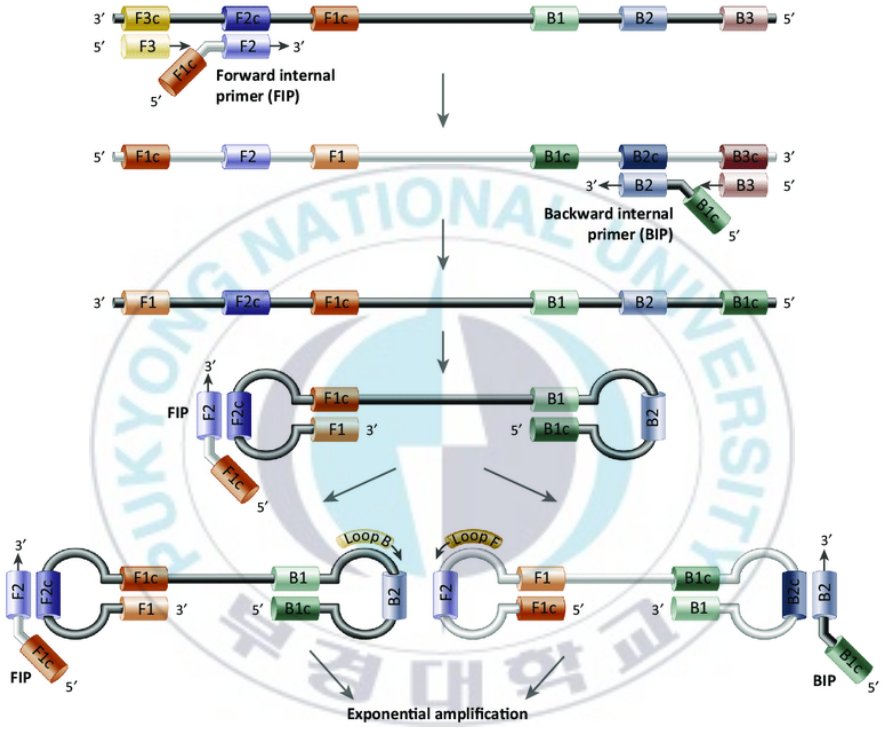



**Figure 4.** Amplification process of LAMP.

**Table 1.** Comparison of PCR and LAMP

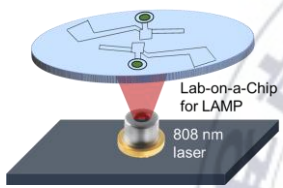
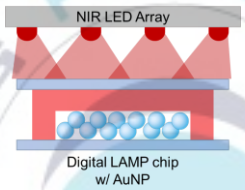
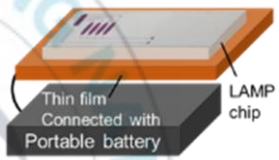
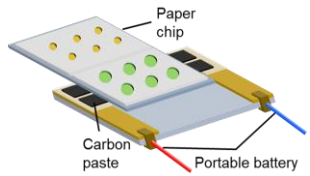
| | **PCR** | **LAMP** |
|---|---|---|
| Temperature | Cycling<br>95 ℃ (Denaturation)<br>→ 60 ℃ (Annealing)<br>→ 72 ℃ (Extension) | Constant<br>60–65 ℃ |
| Time | 1–2 hours | 0.5–1 hour |
| Primer | 2 | 4–6 |
| Detection methods | Gel electrophoresis, Fluorescence | Gel electrophoresis, Fluorescence, Turbidity, Colorimetric assay |

## 1.2 Related Research Trend

To minimize using of bulky and expensive external equipment such as thermocycler, isothermal amplification which do not require thermal programming and controlling is developed. By using the isothermal amplification to detect various diseases, the results can be easily obtained in a short time. However, most of the isothermal amplification is occurred at a higher temperature than room temperature which means that an external heating equipment is also required such as water bath, hot plate, or oven [8,9]. These are having high power consumption and are difficult to apply for point-of-care testing (POCT). POCT is an on-site diagnostic test that is performed at or near to the patient without conducting the test in a laboratory. For the POCT application, according to the World Health Organization (WHO), "ASSURED" criteria: Affordable, Sensitive, Specific, User-friendly, Robust and Rapid, Equipment-free, Deliverable to end-users have been guided [10]. Thus, many researches about detecting pathogens using nucleic acid amplification for POCT are reported [11,12].

To perform NAATs in miniaturized system for POCT, research using a microfluidic chip or system have been investigated. Microfluidic chips such as lab-on-a-disc [13,14], poly(dimethylsiloxane) (PDMS) chip [15,16] and poly(methyl methacrylate) (PMMA) chip [17,18] are usually used (Table 2). However, these microfluidic chips have complex fabrication step and require expensive fabrication equipment. Also, additional external devices such as centrifuge and syringe pump are required.

**Table 2.** Microfluidic devices for NAATs

| **Ref. #** | **[13]** | **[15]** | **[17]** | **[19]** |
|---|---|---|---|---|
| Schematics |  |  |  |  |
| Amplification platform | Polycarbonate disc | PDMS chip | PMMA chip | Polycarbonate film |
| Amplification method | LAMP | Digital LAMP | LAMP | LAMP |
| Integrated heater | Laser diode | NIR LED arrays | Thin-film heater | Thin-film heater |
| Time | 40 min | 45 min | 60 min | 30 min |
| Power consumption | 2.5 Wh | 9 Wh | 10 Wh | 3.75 Wh |

Therefore, paper-based devices are widely used as a platform using NAATs for POCT to take the advantages of their cheap, easy-to-fabricate and handle, and disposable properties [20-22]. Furthermore, in case of paper-based device using LAMP takes 15 min of running time which can leads to quicker analysis compared to the LAMP performed in tubes that takes more than 30–60 min [23,24].

Especially, due to the essential heating step in NAATs, integrated heating systems for amplification, and detection are extensively reported [25,26]. To apply heater integrated microfluidic chips or paper-based device platforms to POCT, it should be portable and requires minimum power consumption. Generally, resistive heating system using thin film is used as an integrated heater platform for nucleic acid amplification due to its easy control of temperature using current change and high thermal conductivity [27]. Thin film such as copper [28], and positive temperature coefficient (PTC) heater [29,30] can be integrated directly with microfluidic chip. However, heat is generated by applying the electricity which needs power supply, and it can reduce the portability and energy efficiency. For example, thin-film heaters which can control the temperature for a portable PCR device requires 6.5 W of power [31]. The heating system using aluminum heat blocks for each PCR step need 10 W of power [32]. An infrared (IR) tungsten lamp for heating microfluidic chip which is used for PCR requires 50 W [33].

Also, heaters can be integrated with the disposable paper-based or microfluidic devices. For example, screen printed resistive microheater was used as a PCR cycling heater performed on microfluidic chip [34]. An inkjet-printed resistive microheater integrated with paper-based device was also reported [35]. Moreover, silver paint was injected directly into the PDMS-based amplification device to integrate heating system for POCT [36]. Lastly, thin-film resistive heater with carbon-paste was integrated with paper-based device [19].

Additionally, photothermal-based heating devices for nucleic acid amplification have been recently reported due to the advantages such as rapid heating, easy mounting in system, and compactness [37,38]. The photothermal effect is a phenomenon in which a photothermal material absorbs light in a specific wavelength and converts it into thermal energy. Because of this property, photothermal material such as gold and silver nanoparticles were used as a heating platform for nucleic acid amplification [15,39,40]. To cause the photothermal effect, the device which can emit the specific wavelength of the light is essential such as laser device that can irradiate the light continuously. However, these devices are expensive, and require high power consumption which is not ideal for POCT application.

## 1.3 Research Concept and Purpose

In this study, we developed a paper-based device patterned with carbon-black-PDMS mixture which is used for DNA detection using loop-mediated isothermal amplification through photothermal effect and a laser-diode device to test the applicability for POCT as a detection platform. Carbon-black is an inexpensive material which can absorbs specific light and convert it to heat, and the heat caused by the photothermal effect between carbon-black and 808-nm laser was applied to LAMP that can amplify target sequence isothermally. The carbon-black concentration and the mixing ratio of PDMS base and curing agent were optimized for the required LAMP temperature. Also, the power of the laser was considered to minimize the power consumption for laser irradiation.

Here, the *rfbE* gene of *E. coli* O157:H7 DNA was amplified using a paper-based device patterned with carbon-black-PDMS mixture (Figure 5). The results were analyzed by the color change of the paper-based device. The detection limit of *E. coli* O157:H7 in paper-based device was confirmed and the specificity of paper-based device was also confirmed by comparing the results of *E. coli* O157:H7, *Staphylococcus aureus* (*S. aureus*), and *Salmonella typhimurium* (*S. typhimurium*). Additionally, LAMP was performed using a paper-based device and lab-made laser-diode device to demonstrate the applicability for POCT. The lab-made laser-diode device was operated by using the portable battery which can make the device portable. The carbon-black-PDMS patterned paper-based device is cheap, disposable, and easy-to-fabricate. Also, the lab-made laser-diode device has minimum power consumption, light weight and is portable. We expect that the suggested carbon-black-PDMS patterned PDMS with portable laser-diode device will have the potential to be applied in POCT.

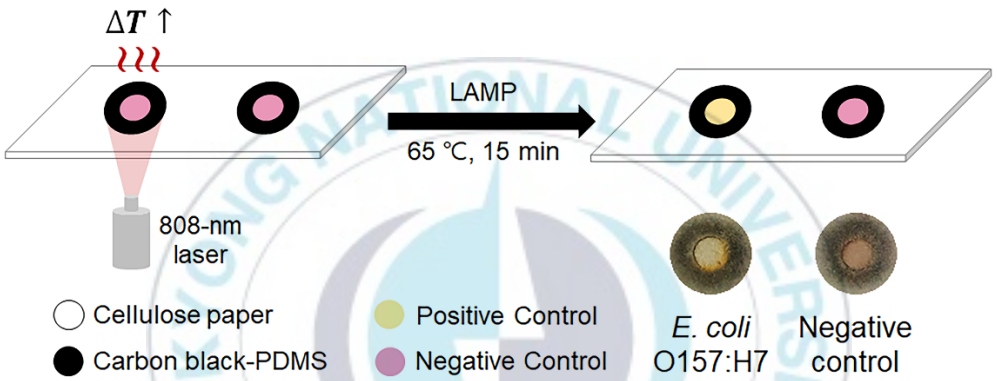



**Figure 5.** Overview of carbon-black-PDMS patterned paper-based device. Using the heat generated by a photothermal effect of carbon-black and 808-nm laser, LAMP was conducted to amplify *rfbE* gene of a foodborne pathogen *E. coli* O157:H7

# CHAPTER 2: MATERIAL AND EXPERIMENTAL

## 2.1 Materials and reagents

Poly(dimethylsiloxane) (PDMS, SYLGARD 184) was obtained from Sewang Hitech Co., Ltd. (Gyeonggi-do, Korea). Activated carbon black powder (Carbon black) was purchased from Sigma-Aldrich (St. Lou-is, MO, USA). Cellulose paper was purchased from Ahlstrom-Munksjö (Helsinki, Finland). A portable battery was obtained from Xiaomi (Beijing, China). Tryptone was obtained from Duksan Pure Chemical (Gyeonggi-do, Korea). Yeast extract and sodium chloride were obtained from Daejug Chemical & Metals Co. (Gyeonggi-do, Korea). WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) was obtained from New England BioLabs Inc. (Ipswich, MA, USA). An 808 nm laser was purchased from Changchun New Industries (Changchun, China).

## 2.2 Fabrication of the paper-based device

A PDMS base and curing agent were mixed in 10:1 ratio. Then, 1% weight/weight (w/w) of carbon-black was put into PDMS mixture and mixed well. 20 μL of a Carbon-black-PDMS mixture was dispensed on the cellulose paper and absorbed into cellulose paper for 10 min at room temperature. An adhesive polyethylene terephthalate (PET) film was bonded to backside of a cellulose paper, and it was cured in a drying oven at 85 ℃ for 45 min. After curing, center of the carbon-black-PDMS patterned cellulose paper was punched by 3 mm of biopsy punch.

Then, a new cellulose disc with a 3 mm diameter was put into this hole. Here, the cellulose disc refers to the region where the sample is loaded (Figure 6).

For the rectangular-area paper-based device, the center of the cellulose paper was cut into rectangular shape by the laser cutter in 8 mm × 1 mm (width × depth). Then, the carbon-black-PDMS mixture was loaded to paper and cured. After curing carbon-black-PDMS mixture, a new rectangular shaped cellulose paper was inserted into the cut hole. Here, the paper-based device or the circular-area paper-based device refers to the paper-based device having a circular sample loading area and rectangular-area paper-based device means the paper-based device having a rectangular shaped cellulose paper for sample loading area.

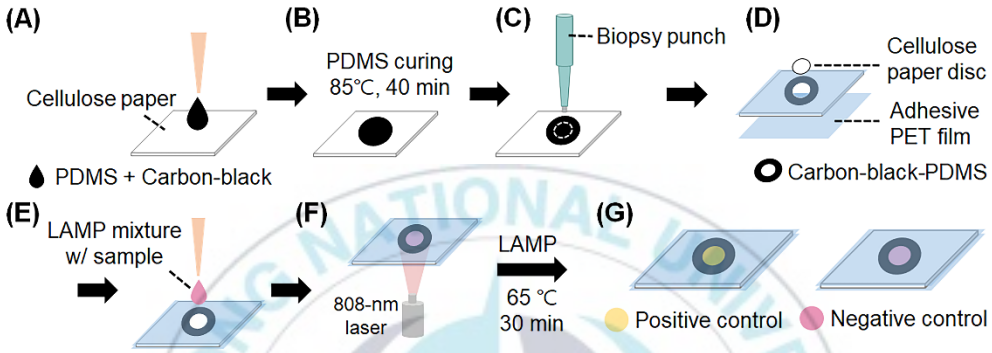



**Figure 6.** Fabrication method of paper-based device patterned with carbon-black-PDMS mixture. (A) Carbon-black-PDMS mixture was dropped on the cellulose paper. (B) After the absorption of carbon-black-PDMS mixture into the cellulose paper, it was cured in an oven. (C) The middle part of the carbon-black-PDMS spot was punched. (D) Adhesive PET film was bonded at the bottom of the cellulose paper and the new cellulose disc was put. (E) LAMP mixture with sample was loaded and (F) 808-nm laser was used for LAMP. (G) The results can be confirmed by naked eye through the color change of the cellulose disc.

## 2.3 Design and fabrication of the laser-diode device

SolidWorks software (Dassault Systèmes SolidWorks Co., France) was used to design the housing of laser-diode device and was printed using 3D printer (Sindoh, Seoul, Korea). A portable battery and an Arduino UNO board were used to provide the power necessary to operate 808 nm laser diodes. The laser-diode device was made of three floors containing operation floor (first floor), irradiation floor (second floor), and LAMP floor (third floor). The circuit with LM317 driver, Arduino UNO, and portable battery are located on the first floor. Two fans and two laser diodes located on the second floor are connected with the laser diode operating circuit from the first floor. These can be turned on or off by the connection of a portable battery. On the third floor, the evaporation-prevention case containing the paper-based device was located.

## 2.4 Bacteria culture

Three different bacteria: *E. coli* O157:H7 (ATCC 35150), *S. aureus* (ATCC 12600), and *S. typhimurium* (ATCC 14028) were streaked on Lysogeny-broth (LB) agar plates first. LB agar was mixed with sodium chloride, tryptone, yeast, and agar in 1 L of deionized water (DI water) in a 10:10:5:15 (grams) ratio. Then a colony from LB agar plates of each bacterium was cultured for 16 hours in 5 mL of LB media at 37 ℃ and 200 rpm. LB media consists of sodium chloride, tryptone, and yeast extract in 1 L of DI water at a ratio of 10:10:5 (grams).

## 2.5 DNA extraction

Cultured *E. coli* O157:H7 was diluted into $10^1$–$10^8$ CFU $mL^{-1}$ in 10-fold for limit of detection and specificity test. *S. aureus* and *S. typhimurium* were diluted into $10^4$ CFU $mL^{-1}$ for specificity test. After diluting the bacteria samples to each concentration, bacteria samples were accumulated by centrifugation for 1 min at 10,000 rpm and the supernatant was taken off to gather the pellet of the bacteria. Then, bacterial cell lysis, washing, and elution was performed by following the protocol of the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea).

## 2.6 LAMP in the paper-based device

In this study, the same primers as the previous study reported by Fei et al. [41] were used to perform LAMP (Table 3). A DNA sample (1 μL) was added to 24 μL of LAMP mixture. LAMP mixture is containing WarmStart® Colorimetric LAMP 2X Master Mix (12.5 μL), primers (1.6 mM of FIP and BIP, 0.2 mM of F3 and B3, 0.4 mM of LF and LB, total 2.5 μL), and of DI water (9 μL). The LAMP mixture (5 μL) containing sample (1 μL of DNA or DI water) was loaded onto the cellulose disc of the paper-based device. And the PET film which was used as an evaporation prevention lid was attached on the top side of the paper-based device. Then, 808-nm laser diode was irradiated to the bottom of the paper-based device for 15 min, and the results were confirmed by naked eye through color change. Also, the results were analyzed by ImageJ software (National Institute of Health, USA) using the value of the RGB.

**Table 3.** LAMP primer sets for amplifying the target gene of bacteria.

| Target bacteria | Target gene | Primer | Sequences (5'to 3') | Base pair (bp) |
|---|---|---|---|---|
| ***Escherichia coli* O157:H7 (ATCC 35150)** | *rfbE* | FIP | CTCTCTTTCCTCTGCG GTCCGATGTTTTTCAC ACTTATTGGAT | 43 |
| | | BIP | TAAGGAATCACCTTG CAGATAAACTAGTAC ATTGGCATCGTGT | 43 |
| | | F3 | AACAGTCTTGTACAA GTCCA | 20 |
| | | B3 | GGTGCTTTTGATATTT TTCCG | 21 |
| | | LF | CCAGAGTTAAGATTG AT | 17 |
| | | LB | CGAAACAAGGCCAGT TTTTTACC | 23 |

## 2.7 Experimental setup and operation

An 808-nm laser was used to irradiate the light which can cause the photothermal effect of the paper-based device patterned with carbon-black-PDMS having the circular disc to optimize the carbon-black concentration, laser power, mixing ratio of PDMS, stability of paper-based device, limit of detection, and specificity of the paper-based device. To irradiate the 808 nm laser light to the bottom of the paper-based device, acrylic housing which has a hole in the center to pass the light without loss was used. The spacing between the paper-based device containing sample and the 808-nm laser was fixed at 2 cm to allow the light of the laser to cover the cellulose disc and the patterned carbon-black-PDMS of the paper-based device. By irradiating 0.5 W power of 808-nm laser, the temperature of the paper-based device was increased through the photothermal effect. Using this heat, LAMP was performed on the paper-based device for 15 min. The temperature of the paper-based device was measured every minute for 15 minutes by the thermal imaging camera (Teledyne FLIR LLC, Wilsonville, OR, USA). To increase the accuracy of the temperature measurement, thermal imaging camera was perpendicular to the paper-based device. Also, to confirm the accuracy of the temperature, K thermocouple was also used to compare the temperature of the cellulose disc of the paper-based device.

Also, the laser-diode device was used to test the sensitivity of the rectangular-area paper-based device. In this sensitivity test, two rectangular-area paper-based devices in an anti-evaporation case, containing DI water and target DNA sample, respectively, were mounted on the acrylic plate placed between the second floor and the third floor of the laser diode device. By connecting the portable battery to Arduino UNO on the first floor, 808-nm laser was irradiated to the carbon-black-PDMS spot on the bottom of the paper-based device. Using the heat generated through the photothermal effect, LAMP was conducted for 15 min at 65 ℃. The temperature was measured with the thermal imaging camera and with the K

thermocouple to compare the temperature with the temperature of the thermal imaging camera. After 15 min, results were checked through the color difference by naked eye. Also, G/R value was analyzed using the ImageJ software.

# CHAPTER 3: RESULT AND DISCUSSION

## 3.1 Optimization of concentration of carbon-black

In the nucleic acid amplification tests, the temperature is one of the significant points. Thus, the temperature was measured using the paper-based device having several concentrations. Each concentration of carbon-black powder (0, 0.5, 1, 1.5% (w/w)) were mixed with the PDMS mixture. PDMS is a silicone, and it is used by mixing a PDMS base and curing agent. Here, PDMS base and curing agent were mixed in 10 : 1 (Base : Curing agent) ratio. PDMS mixture is an organic material that can maintain a liquid form at room temperature. This allows carbon-black, an organic material, to be uniformly dispersed in PDMS and this carbon-black-PDMS mixture can be absorbed onto paper substrate. In addition, when heat is applied to the paper-based device with absorbed carbon-black-PDMS mixture, PDMS can be cured in an absorbed state and is hydrophobic. Therefore, the cured carbon-black-PDMS mixture can act as a hydrophobic wall that makes the hydrophilic liquid to be positioned where desired. To make the environment similar with the amplification environment, DI water was loaded on the cellulose disc (amplification zone) of the paper-based device and PET film was attached to prevent evaporation. Then, 0.5 W of 808 nm laser was irradiated, and the temperature was measured for 10 min. In all carbon black concentration (0, 0.5, 1, 1.5% (w/w)), the maximum temperature of the paper-based device was reached within 1 min. In this experiment, the average temperature for 10 min was analyzed.

To obtain optimal conditions of concentration of carbon-black for LAMP, the temperature according to the carbon black concentration was confirmed. As shown in Figure 7, the temperature of the paper-based device increased, as the concentration of the carbon-black concentration increased.

However, 1.5% carbon-black concentration showed a temperature similar to that of 1% carbon-black concentration. This phenomenon can be explained as follows. According to Wang et al., when high concentration of the photothermal materials are present, most of the light energy is absorbed by the top section of photothermal material [42]. Since the remaining photothermal material beneath cannot generate much heat, the paper-based device did not show an increase in temperature at a specific threshold of carbon black concentration.

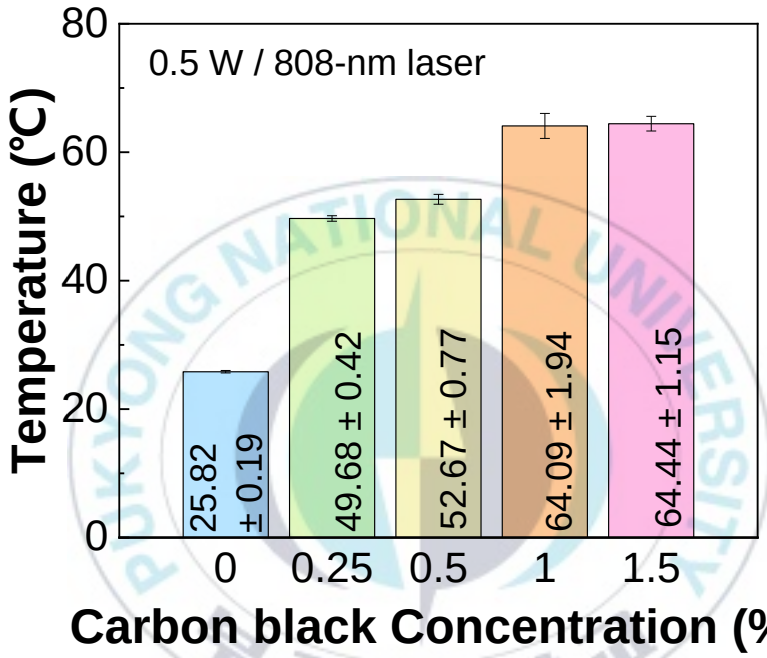



**Figure 7.** Temperature according to the concentration of carbon black. As the concentration of carbon-black increase, the temperature of the paper-based device reached to higher temperature. However, when the carbon-black concentration was above 1%, the temperature was maintained.

## 3.2 Optimization of output power of 808-nm laser

Theoretically, when high concentration of photothermal material present, more heat can be produced. So, the laser power capable of maintaining the temperature of LAMP (65 °C) was confirmed at each carbon-black concentration to minimize the power of 808-nm laser needed for LAMP. Here, the power of the 808 nm laser was confirmed by using a laser power meter and the laser power which can maintain the paper-based device at 65 °C for 10 min with minimal fluctuation was set as the required laser power.

As hypothesized, the power of an 808-nm laser was decreased when the concentration of carbon-black increased. On the other hand, to generate more heat, higher power was needed at lower carbon-black concentration. Furthermore, there was no increase of the carbon-black concentration over 1% which result in maintaining the laser power for heating paper-based device up to 65 °C (Figure 8). Through these results, optimized carbon black concentration and laser power is 1% and 0.5 W, respectively.

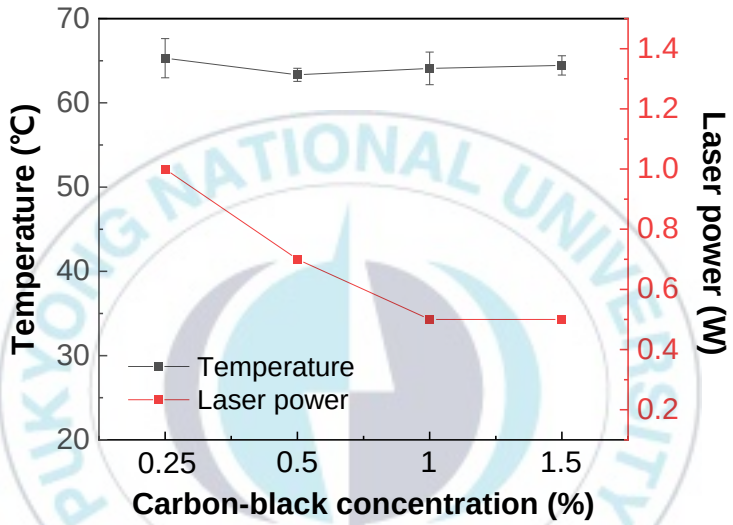



**Figure 8.** Laser power according to the carbon-black concentration which can maintain 65 °C for LAMP. Lower power was needed to reach 65 °C in higher concentration.

## 3.3 Optimization of PDMS mixing ratio

As mentioned earlier, PDMS is composed of base and curing agent. The commonly used mixing ratio of PDMS is 1 : 10 (Curing agent : Base). By increasing the amount of curing agent, the amount of cross-linking between the monomers increases. As a result, the flexibility of PDMS is decreased and stiffness is increased [43]. Using this property, temperature of the paper-based device was monitored by applying different ratio of PDMS with fixed carbon black concentration to paper-based device. Here, 1 : 5, 1 : 10, 1 : 15, and 1 : 20 (Curing agent : Base) of mixing ratio and 1% of carbon black powder was used to fabricate the paper-based device. 0.5 W of 808-nm laser was irradiated for 30 min continuously, and the temperature produced by photothermal effect was observed.

Although the laser power and carbon-black concentration were fixed, the paper-based device reached to a higher temperature when the amount of curing agent was increased (Figure 9). There was no significant difference between the paper-based device made with 1 : 10 and 1 : 15 ratios. However, the paper-based device made with 1 : 5 ratio showed around 10 °C higher temperature compared to the paper-based device made with 1 : 20 ratio. This phenomenon can be explicated by the cross-linking network of the curing agent. If the curing agent ratio in the PDMS mixture increases, the amount of cross-linking using covalent bond between the polymers increased. And heat transport through this covalent bonded cross-linking polymer also increased [44,45]. For this reason, heat transfer efficiency can be increased regardless of same carbon black concentration and output power of 808-nm laser. Therefore, target temperature can be controlled easily by changing the mixing ration of PDMS mixture. From this, the paper-based device made with various PDMS mixing ratios can be adapted to many different nucleic acid amplification methods, not just LAMP.

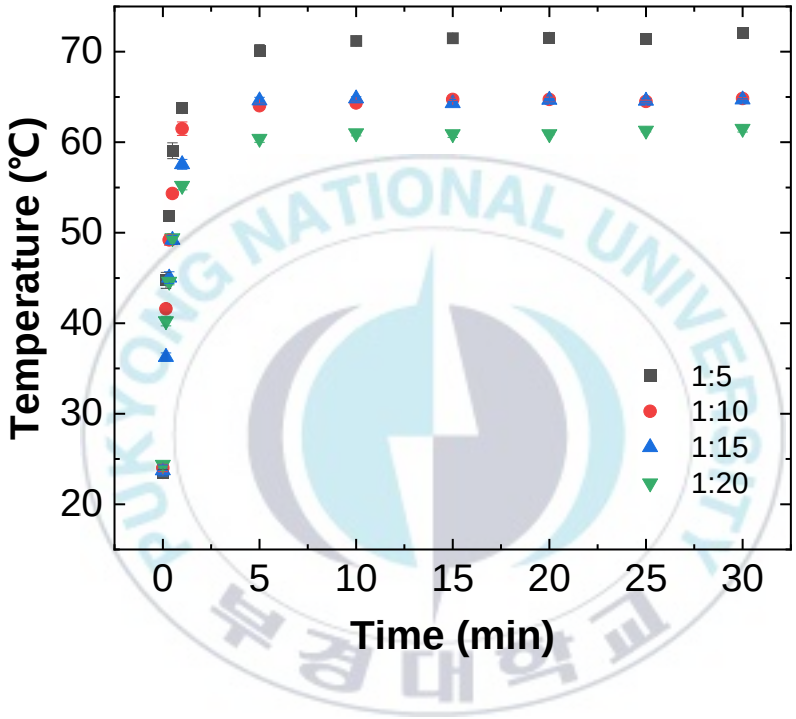



**Figure 9.** The temperature of the carbon-black-PDMS patterned paper-based device according to the PDMS mixing ratio.

### 3.4 Stability of the paper-based device

Using the optimized condition (1% carbon-black concentration mixed with 1 : 10 (Base : Curing agent) PDMS mixture and 0.5 W of 808-nm laser), the stability of the paper-based device was confirmed. The temperature of the paper-based device was measured using a thermal imaging camera every 10 min (Figure 10). When the laser was irradiated for 1 h, the temperature of the paper-based device reached between 60 °C and 65 °C within 1 min. After 1 min, there was no significant fluctuation in the temperature for 1 h (Figure 11). Also, in IR images, the cellulose disc of the paper-based device showed around 60 °C at 1 min and maintained around 65 °C for 1 hour. This means that the paper-based device can reliably produce heat under laser irradiation for a long time without any effect. Therefore, all of the following experiments were performed by irradiation 0.5 W 808-nm laser on the paper-based device patterned with 1 : 10 (Base : Curing agent) PDMS mixture mixed with 1% carbon black.

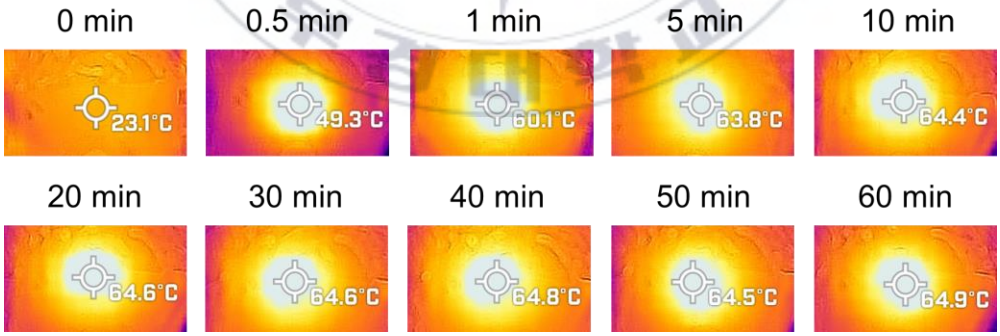



**Figure 10.** Infrared image of the paper-based device patterned with the carbon-black-PDMS. The sample loading disc located in the center of the paper-based device showed stable temperatures for 1 hour.

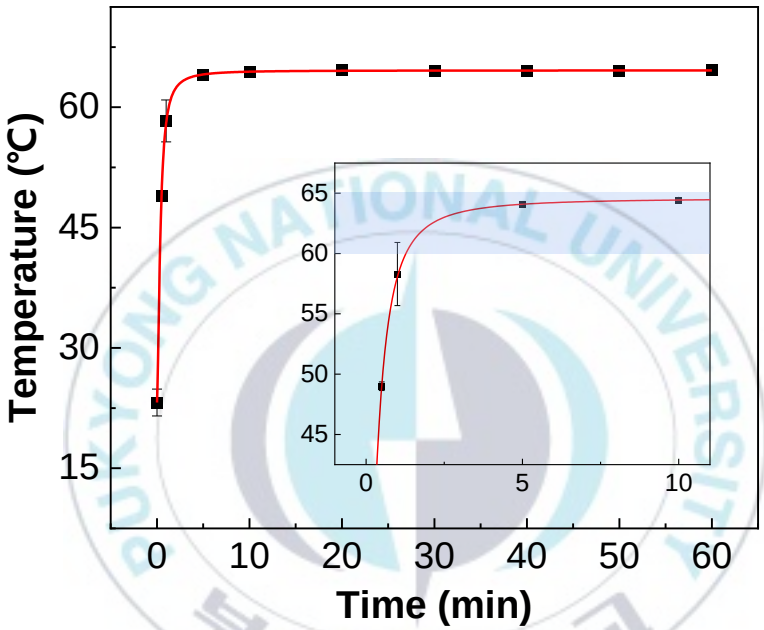



**Figure 11.** Stability of the paper-based device. The temperature of the paper-based device was maintained around 65 °C which is required for LAMP performance without significant fluctuation.

## 3.5 Comparison of heating methods

To confirm whether target DNA could be amplified using the paper-based device through the photothermal effect. In addition, in order to confirm the difference between the heating methods, the results using hot plate which is common external heater used for LAMP and 808-nm laser to use the photothermal effect were compared. In the LAMP master mix, phenol red, pH indicator, is contained. In this reaction, phenol red was used as a visual indicator of the LAMP reaction. Phenol red is red color in alkaline environment and shows yellow color in acidic environment. In a positive reaction (when the target DNA is contained in the sample), DNA polymerase incorporates dNTPs into nascent DNA and by-products such as pyrophosphate and hydrogen ions are released and accumulated. And these protons drop the pH (alkaline to acidic), turning phenol red from pink to yellow [46].

Despite the difference of heating platform, NTC showed pink color after 15 min in all cases, and both platforms showed yellow color in *E. coli* O157:H7 DNA sample after 15 min (Figure 12A, 12B). Also, the results were analyzed through RGB analysis. Due to the color change from pink to yellow after 15 min when the target is successfully amplified, the significant increase of green intensity was confirmed. Accordingly, the green intensity (G value) was used as a marker which able to confirm the negative or positive results. For standardization, G value was divided into the red intensity (R value). In the case of NTC, there was no significant change in both heating platforms, and in the case of *E. coli* O157:H7 samples, it was confirmed that the G/R value significantly increased to 0.91 after 15 min in both hot plate and 808-nm laser (Figure 12C, 12D).

In both heating platforms, the LAMP was conducted within 15 min. Typically, LAMP takes 30–60 min to get the results when performed in a tube. However, the LAMP using the paper-based device proposed in this study took a

shorter time than LAMP performed in the tube regardless of the heating method. These can be explained by surface-to-volume ratio. High surface-to-volume ratio can increase heat transfer efficiency. Comparing the surface-to-volume ratio of the 0.2 mL PCR tube (20 μL) and paper (3 mm of diameter), the paper (4.7 $mm^2$ $\mu L^{-1}$) is about 3 times higher than the PCR tube (1.5 $mm^2$ $\mu L^{-1}$). Therefore, it was possible to conduct LAMP faster and confirm the results when using the paper-based device.

To compare the results between the results using hot plate and the results using 808-nm laser statistically, the p-value was confirmed. As a result, both NTC and *E. coli* O157:H7 sample showed a p-value higher than 0.05 (P = 0.11 and P = 0.33, respectively), indicating that there was no significant difference between the G/R values, that is, no difference depending on the heating method.

Therefore, it was confirmed that there was no affect according to the heating platform. Additionally, both in the color change and G/R value, there was no significant difference which means 808-nm laser can be used as a photothermal heating platform for LAMP.

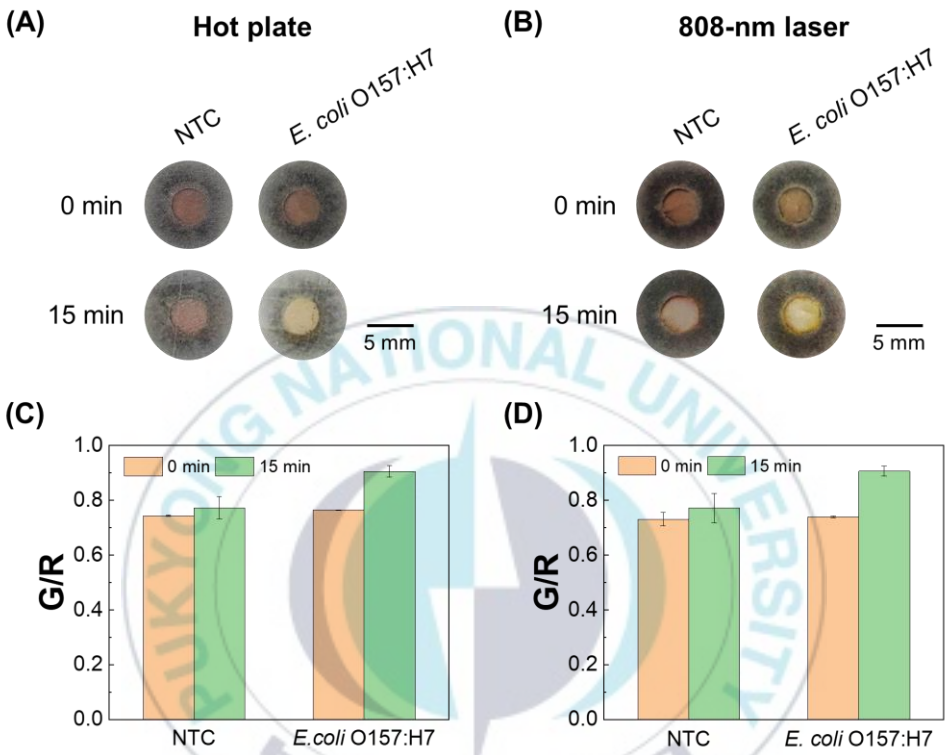



**Figure 12.** Comparison of the results between the heating devices. (A) Colorimetric results of hot plate which is generally used. (B) Colorimetric results of the 808-nm laser for photothermal heating. (C) G/R value of the NTC and *E. coli* O157:H7 sample in 0 and 15 min using hot plate. (D) G/R value of the NTC and *E. coli* O157:H7 sample in 0 and 15 min using 808 nm laser. There was no significant difference between the hot plate and 808-nm laser in both color and G/R value.

## 3.6 Detection limit of the paper-based device

To confirm the limit of detection (LOD) of the paper-based device, *rfbE* gene of *E. coli* O157:H7 bacteria was used as a target sample. *rfbE* gene of *E. coli* O157:H7 is specific gene that can produce the O157 antigens which is usually used as a target gene to detect *E. coli* O157:H7. From the various concentration of *E. coli* O157:H7 bacteria ($10^1$ to $10^8$ CFU $mL^{-1}$), DNA was extracted. Then each concentration of DNA or DI water was mixed with the LAMP mixture and was dropped onto the cellulose disc in the middle of the paper-based device. After bonding the PET film on the top surface, 808-nm laser diode was irradiated in 0.5 W power. By the heat produced from the carbon-black, LAMP was conducted. Through the color change, results were confirmed by naked eye after 15 min of laser irradiation.

After 15 min of laser irradiation, non-template control (NTC) and $10^1$ CFU $mL^{-1}$ showed pink color which means absence of target DNA, and the concentration above $10^2$ CFU $mL^{-1}$ showed yellow color which means presence of target DNA (Figure 13A). As well as color change, RGB value was also analyzed by ImageJ software. In NTC and $10^1$ CFU $mL^{-1}$, G/R value was about 0.75 and from $10^2$ CFU $mL^{-1}$, G/R value was about 0.9 after 15 min (Figure 13B). Compared to the G/R value at 0 min, in samples with concentrations more than $10^2$ CFU $mL^{-1}$, it was increased about 0.15. Otherwise, NTC and $10^1$ CFU $mL^{-1}$showed increase in G/R value about 0.05. As a result, LOD of paper-based device was confirmed to be $10^2$ CFU $mL^{-1}$. This LOD is comparable with the LOD achieved from the previous studies which was detected same target DNA [47,48].

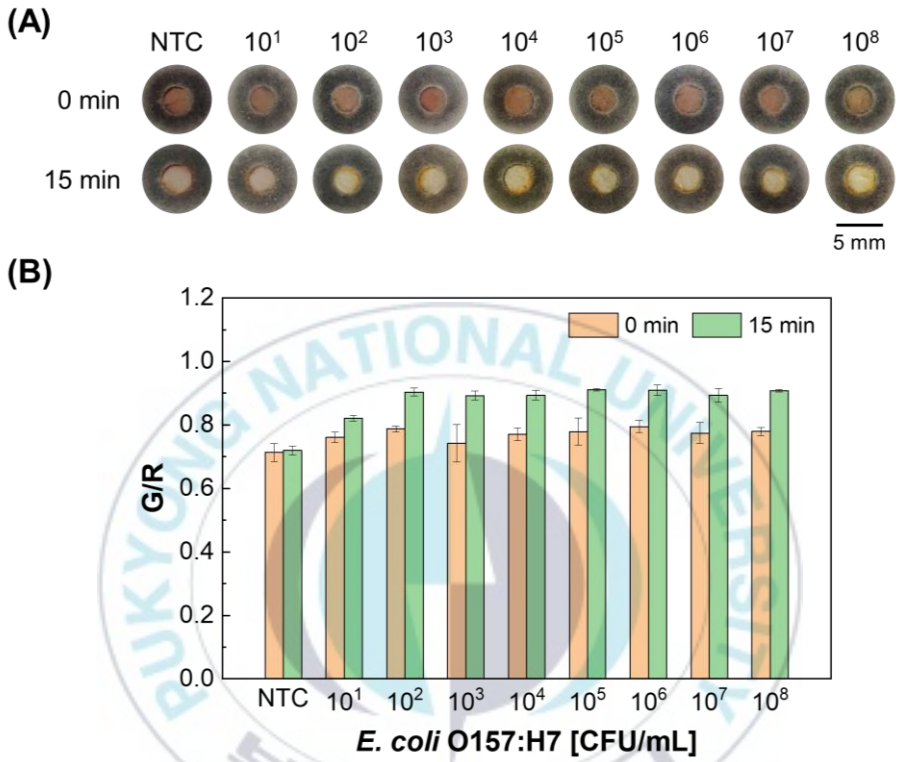



**Figure 13.** Limit of detection of the paper-based device. (A) Photo of the paper-based device with various concentrations at 0 min and 15 min. The color of the paper-based device changed into yellow after 15 min from the $10^2$ CFU $mL^{-1}$. (B) G/R value according to the concentration of the sample. G/R value was significantly increased from $10^2$ CFU $mL^{-1}$ compared to the that of NTC and $10^1$ CFU $mL^{-1}$.

## 3.7 Specificity of the paper-based device

To confirm the specificity, ability to detect only the target to be detected, of the paper-based device, we compared the results after amplifying the $10^4$ CFU $mL^{-1}$ of *E. coli* O157:H7, *S. aureus*, and *S. typhimurium* DNA, respectively. The target was the *E. coli* O157:H7 DNA. Each of the DNA from different bacteria was mixed with the LAMP mixture and was dropped on the amplification disc of the paper-based device. After the adhesive PET film was bonded, the 808-nm laser was irradiated in 0.5 W power. The results were observed by the naked eye through the color change and analyzed by ImageJ program.

After 15 min of laser irradiation, the paper-based device with the *E. coli* O157:H7 sample showed yellow color which means successful amplification. And the paper-based device with the NTC, *S. aureus* and *S. typhimurium* remained pink color which means no target DNA was present (Figure 14A). G/R value analysis showed same results. *E. coli* O157:H7 sample showed increased G/R value after 15 min and NTC, *S. aureus*, and *S. typhimurium* sample showed similar G/R value with the 0 min after 15 min (Figure 14B). Therefore, the paper-based device showed high specificity which can only detect the target DNA even though there is non-target DNA.

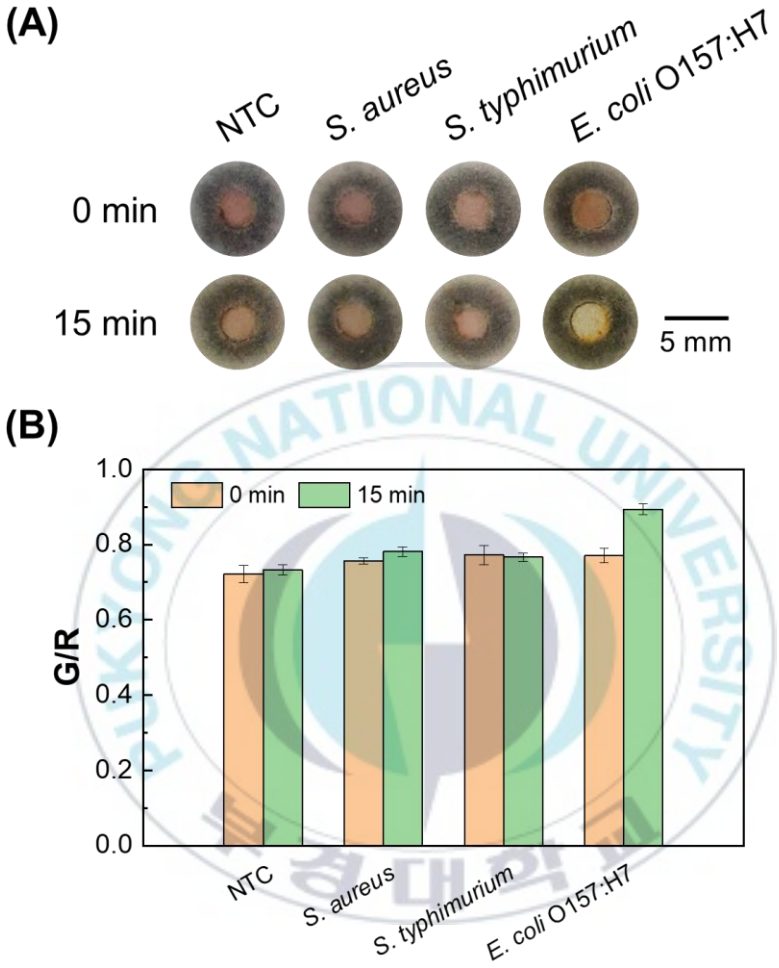



**Figure 14.** Specificity of the paper-based device. (A) Photo of the paper-based device containing different bacteria samples. (B) G/R value according to the bacteria sample. Only the *E. coli* O157:H7 sample showed difference in the color and G/R value after 15 min.

## 3.8 The laser-diode device

To cause the photothermal effect which can generate the heat for LAMP performance, an optical device is required. In this study, carbon-black powder was used as a material that can generate the heat through the photothermal effect. Carbon-black can absorbs various wavelength (500 nm–1000 nm) [49]. In this study, portable laser device to apply the paper-based device in POCT was suggested. Using the characteristics of carbon-black and photothermal effect, the laser diodes which have 808 nm of wavelength and maximum power 1 W was used as a light source. The laser diode is cheap and can be easily purchased.

The laser-diode device consists of three floors. First, the circuit with laser diode driver LM317 for laser diode device connected with Arduino UNO board, and a portable battery were located on the first floor. To control the power of the laser diode, resistance in the circuit was adjusted. And two laser-diodes and two fans are located in the second floor which are connected to the laser diode circuit on the first floor. When the portable battery was connected with Arduino UNO board, two laser diodes and two fans were turned on at the same time. Two laser diodes were used as a light source that can emit 808 nm wavelength of light in 0.5 W of power. Two fans were used to cool the heat generated by self-heating of laser diode to protect the overheating which can shorten the lifespan of the laser diodes. Between the second floor and the third floor, an acrylic plate was positioned. An acrylic plate was used to minimize the effect of the fans located on the second floor for cooling the laser diode while allowing the laser light to pass through without loss. On the third floor, the paper-based devices are located (Figure 15). To minimize the evaporation of the sample from the paper-based device, two paper-based devices were put in an anti-evaporation case to enclose the carbon-black-PDMS spot tightly. Additionally, to align the paper-based devices with the location that light of the laser diodes irradiated,

anti-evaporation case with the paper-based devices were slid into a sliding holder and it was placed on the third floor (Figure 16).

In order to conduct two tests, one to validate the presence or absence of a target DNA and the other to serve as an NTC, two laser diodes were used. The dimension of the lab-made laser-diode device was 150 mm × 115 mm × 95 mm, and its weight is 542 g (without the portable battery) and 818 g (with the portable battery). Also, the laser-diode device is affordable, can be operated by using a portable battery and require minimum power which can makes it more applicable to POCT. The power is 4 W and the power consumption is 2 W h which is required for conducting LAMP for 15 min when using the two laser diodes and two fans at the same time. These power and power consumption are quite lower than those of the heating platforms reported previously (Table 4). The carbon-black-PDMS patterned paper-based device suggested in this study have highest energy efficiency compared to the heating platforms using the LAMP. However, there are some heating platforms that showed lower power using the PCR compared to the laser-diode device suggested in this study. Photothermal heating platform using 450 nm LED array for PCR used gold film as a photothermal material. But the device suggested in this study is not a portable device and they need additional step to use the gold film as a photothermal material. And the heating platform using Platinum (Pt) resistors showed better energy efficiency, but it required complex fabrication which needs expensive additional equipment. The advantage of the paper-based device suggested in this study is that a photothermal heating platform with easy temperature control can be fabricated more easier without complex steps. Also, a 10,000-mA h portable battery which is used to operate the paper-based device can run the tests 14 times, which can be applied POCT usefully.

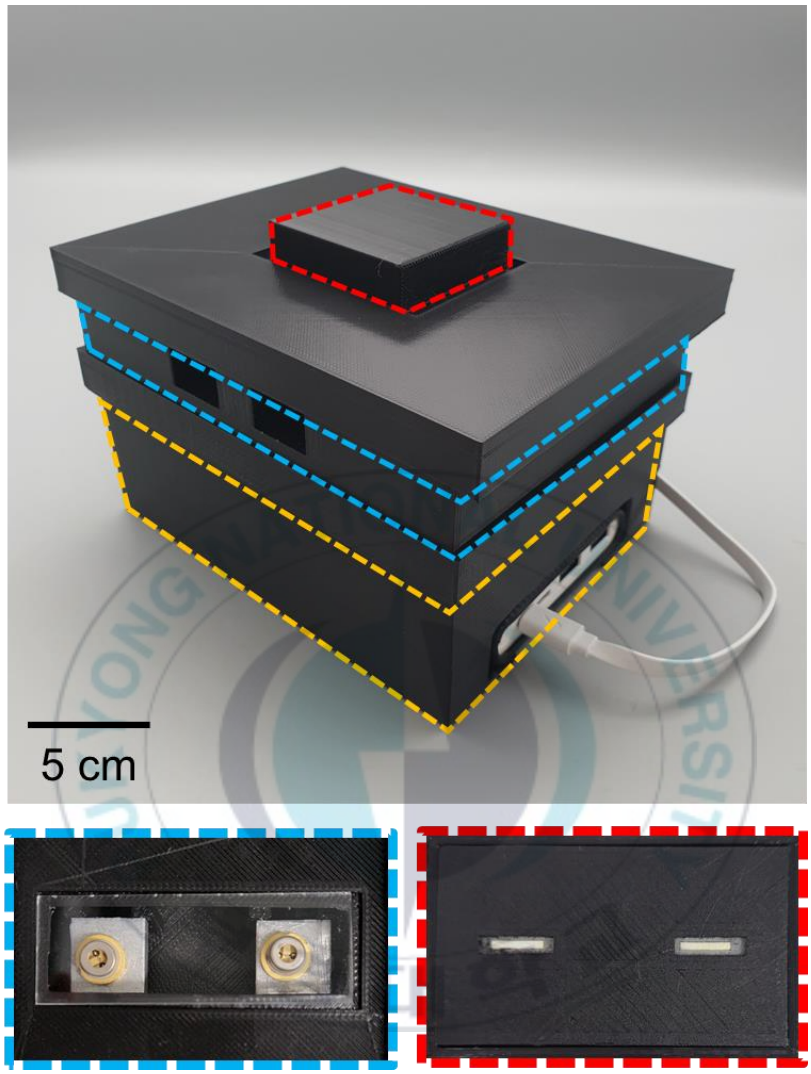



**Figure 15.** Photo of the laser-diode device. Laser-diode device consists of three floors. The first floor (yellow line) contains laser-diode circuit, Arduino UNO, and portable battery. The second floor (blue line) contains two laser diodes and two fans. Third floor (red line) contains the paper-based device with the anti-evaporation case.

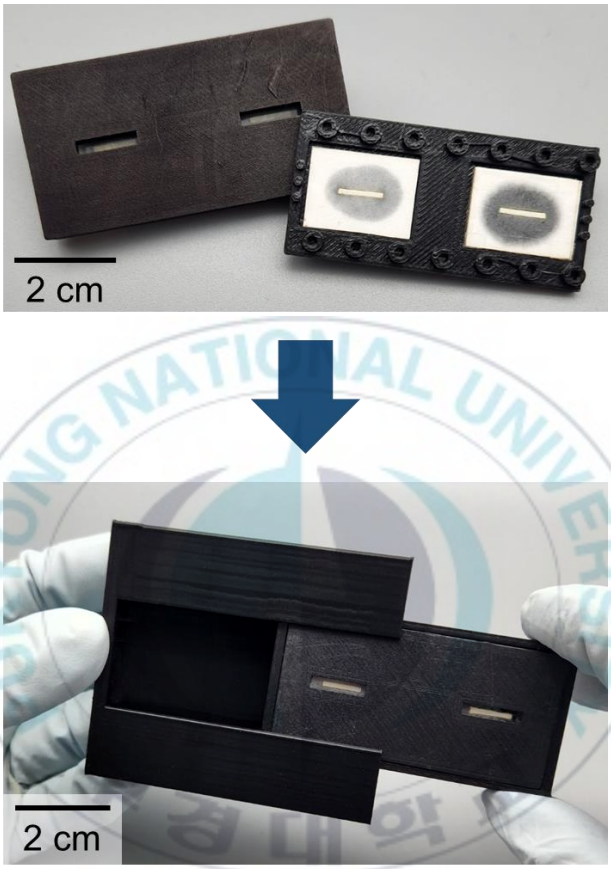



**Figure 16.** Photo of the mounting process of the paper-based device and evaporation prevention case. Paper-based device was inserted to the anti-evaporation case, and it was inserted to second case to place the reaction spot of the paper-based device in the exact position where the light comes out from the laser-diode.

**Table 4.** Comparison of the maximum power and power consumption of heating platforms for nucleic acid amplification.

| Ref. # | Heating platform | Maximum power [W] | Power consumption [W h] | Time required to perform assay |
|---|---|---|---|---|
| **LAMP using photothermal heating** | | | | |
| This work | Near infrared (NIR) laser diodes | 4 | 2 | 15 min |
| [15] | NIR light-emitting diode (LED) array | 12 | 9 | 45 min |
| [13] | NIR laser diode | 3.75 | 2.5 | 40 min |
| **LAMP using resistive heating** | | | | |
| [19] | Thin-film heater | 7.5 | 3.75 | 30 min |
| [17] | Thin-film heater | 10 | 10 | 60 min |
| **PCR using photothermal heating and resistive heating** | | | | |
| [32] | Aluminium heat blocks | 10 | 5 | None |
| [50] | 450 nm LED array | 3.5 | 0.3 | 5 min |
| [51] | Platinum (Pt) resistors | 4.11 | 3.2 | None |
| [52] | Pt resistors | 1.18 | 0.295 | 15 min |

## 3.9 Specificity of the paper-based device using the laser-diode device

We confirmed the specificity of the paper-based device using a lab-made laser-diode device to evaluate the applicability for POCT of the platform suggested in this study. Due to the characteristics of the laser diode that irradiates light in a rectangular shape with a thickness of 1.5 mm, the paper-based device with a circular shape was not enough to generate the heat as when using a commercialized laser device. To reach the temperature for conducting the LAMP by increasing the area that can be in contact with the light of the laser diode, the shape of the paper-based device was changed from circular shape to rectangular shape. The fabrication process of the rectangular-area paper-based device is same as that of the circular-area paper-based device, but the sample loading area was fabricated using a laser cutter instead of a biopsy punch. The sample was loaded on the rectangular loading area and the light was irradiated from 808-nm laser diode in the laser-diode device for 15 min to perform the LAMP.

With no significant difference from the results of the specificity test using circular-area paper-based device, the paper-based device containing *E. coli* O157:H7 DNA as a sample was only changed the color from the pink to yellow. And other samples (NTC, *S. aureus*, and *S. typhimurium*) showed no color change (Figure 17A). In addition, only the devices containing *E. coli* O157:H7 DNA as a sample showed significant increase in the G/R value, and in the case of the *S. aureus* and *S. typhimurium*, the G/R value showed a similar trend to NTC (Figure 17B).

The shape of the paper-based device had to be changed due to the shape of the laser-diode. The rectangular shape of the light from the laser-diode can be converted into a circular shape by using a suitable optical setup such as an aspherical lens or an off-axis mirror [53,54]. This setting was not applied in this study given the scope of the study. Nonetheless, the specificity of the suggested paper-based device was confirmed and demonstrated its potential as a platform for nucleic-acid amplification which can be used in POCT settings.

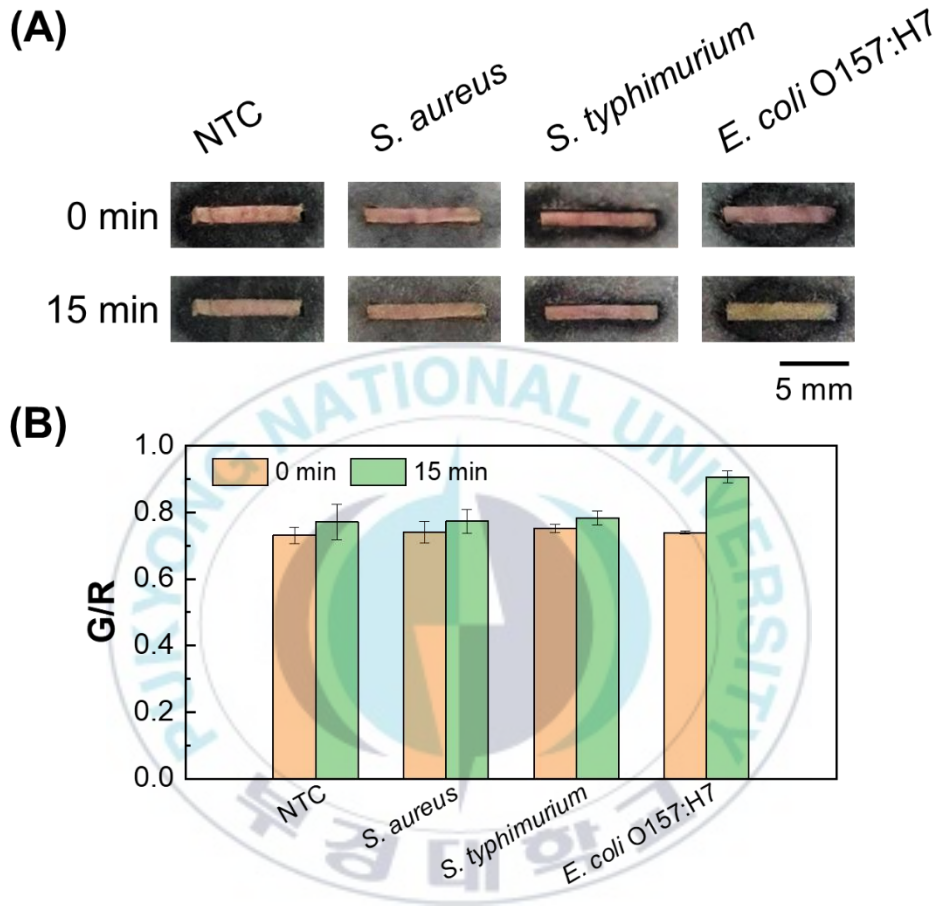



**Figure 17.** Specificity of the paper-based device using laser-diode device. (A) Photo of the paper-based device amplified using laser-diode device. (B) G/R value of the paper-based device according to the bacteria sample. Compared to the results of paper-based device using commercial laser, no significant difference was confirmed.

# CHAPTER 4: CONCLUSION

In summary, a foodborne pathogen *E. coli* O157:H7 was successfully amplified using the paper-based device patterned with the carbon-black-PDMS and the lab-made portable laser-diode device. Through the photothermal effect between the carbon black and 808-nm laser, the heat required for LAMP was generated. The heat generated from the paper-based device can be easily controlled by changing the carbon black concentration, output power of the laser, and PDMS mixing ratio. These properties make the paper-based device and the laser-diode device applicable to various nucleic acid amplification methods. When performing LAMP using the paper-based device proposed in this study, the result can be confirmed within 15 min, and the presence or absence of the target DNA can be visually detected in naked eye through the color change of the paper-based device. Using the carbon-black-PDMS patterned paper-based device and laser-diode device, *rfbE* gene of *E. coli* O157:H7 was specifically amplified and showed detection limit of $10^2$ CFU $mL^{-1}$. This indicates that the paper-based device and the laser-diode device was applicable for detecting foodborne pathogen. The paper-based device is cheap, disposable, and have simple fabrication step and the laser-diode device is small, portable, and requires low power which indicates suitability for POCT. Through these results, the paper-based device and the laser-diode device can be used as a NAATs and POCT platform positively.

Furthermore, additional conditions such as the size of the paper-based device, thickness of the paper-based device, and dimension of the patterned carbon-black-PDMS mixture spot can be optimized for temperature control for different amplification methods. Also, it is expected that the possibility of the contamination when loading the sample and packing to prevent the evaporation can be minimized

through the foldable paper-based device which can minimizes contact with the sample loading surface and sliding the folded paper-based device into the heating device directly. In addition, additional processes such as fully integrated device including DNA extraction using paper-based heat lysis, multiplexed detection, and pre-stored reagents on paper-based device are expected to further increase the possibility of application of the paper-based device to POCT.

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마지막으로, 언제나 저를 응원해주고 믿어주고, 힘들 때마다 큰 버팀목이 되어준 사랑하는 부모님 그리고 내 동생 예진이, 승민이에게 감사하다는 말을 전하고 싶습니다.

**February 2023**

**Ye Lin Kim**

# PUBLICATIONS

**International journal**

**1.** Ye Lin Kim, Donghyeok Kim, Jihoon Park, Minseok Kwak, and Joong Ho Shin, *"Carbon-black-embedded poly (dimethylsiloxane)-paper hybrid device for energy-efficient nucleic-acid amplification in point-of-care testing",* Analytical Methods, 14(26), 2569–2577, 2022.

(Selected as an inside front cover)

**International conference**

**1.** Ye Lin Kim, Joong Ho Shin, "Amplification of nucleic acids in microwell using photothermal effect", The 25th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2021), Palm Springs Convention Center and virtual, USA, (October 10-14, 2021).

**2.** Ye Lin Kim, Joong Ho Shin, "Carbon black-PDMS embedded paper-based device for pathogen detection using photothermal effect", The 26th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2022), Hangzhou International Expo Center and virtual, China, (October 23-27, 2022).

**Domestic conference**

**1.** Ye Lin Kim, Joong Ho Shin, “Polymerase chain reaction in microarray using photothermal effect”, 한국바이오칩학회 2021 춘계학술발표회, 홍천 (2021.06.16-18).

**2.** Ye Lin Kim, Joong Ho Shin, “Loop-mediated isothermal amplification in a paper-based device using photothermal effect”, 한국바이오칩학회 2021 추계학술발표회, 제주도 (2021.11.17-19).

**Domestic patent**

**1.** Joong Ho Shin, Ye Lin Kim, Min Seok Kwak, "Detection kit, detection apparatus and method of manufacturing the detection device," 10-2022-0014893 (Filling date: 2022.02.04)