Integrated pumpless microfluidic chip for the detection of foodborne pathogens by polymerase chain reaction and electrochemical analysis

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ABSTRACT

As food poisoning has increased interest in public hygiene, the study for a novel monitoring system for pathogen evaluation has been significantly explored. In this study, we established a simple single-chip pathogen analysis system by integrating the film-based PCR module, electrode module and the polydimethylsiloxane (PDMS)-based finger-actuated microfluidic modules. The film-based PCR module and electrode module were employed for gene amplification and electrochemical analysis, and the PDMS-based finger-actuated microfluidic modules were utilized for sample migration and mixing, which can replace external pumping systems. The integrated pumpless microfluidic chip contains the three parallel microfluidic channels for the simultaneous analysis of pathogens from multiple samples that channels are operated simultaneously by pressing the button without external pumping systems. Using the integrated pumpless microfluidic chip, the target gene of foodborne pathogens was amplified by a thermal cycling polymerase chain reaction (PCR) method in the film chamber, and the amplified gene was electrochemically quantified by using the square wave voltammetry. \textit{Escherichia coli} O157:H7 was selected as a target pathogen and the pathogen was sensitively evaluated with the limit of detection (LOD) of 10\textsuperscript{2} colony-forming units (CFU). In regard to the significant featuring of the integrated microfluidic chip, the sensing platform developed could be used for pathogen screening as a promising tool in the field of point-of-care testing.

1. Introduction

Foodborne illness is one of the world’s health-threatening diseases that causes diarrhea, fever, chills, vomiting, and even death\textsuperscript{[1,2]}. It is important to screen foodborne pathogens in an early stage before contaminated foods are delivered to end-users. Conventionally, culture-based methods have been used to detect foodborne pathogens by counting the number of bacteria colonies on an agar plate\textsuperscript{[3,4]}. However, it takes several days to get analysis results, so it is unsuitable for rapid on-site detection as the pathogens should be enriched in an incubator and plated and cultured on an agar plate for colony formation. As an alternative, the polymerase chain reaction (PCR) has been widely applied for the rapid detection of foodborne pathogens that amplifies the specific gene of the pathogens under thermal cycling, and amplified genes are analyzed\textsuperscript{[5,6]}. The signal analysis of amplified genes, gel electrophoresis is the most commonly used method for analyzing the amplified genes, but it is difficult to quantitatively assess the amplified genes. Therefore, various methods have been used to quantitatively evaluate the amplified genes based on colorimetric\textsuperscript{[7,8]}, electrical\textsuperscript{[9,10]}, and fluorescent signals\textsuperscript{[11,12]}. Additionally, compared to gel electrophoresis that requires bulky and complicated equipment, the assessment of the amplified genes is enabled by using a compact signal analyzer\textsuperscript{[13,14]}. Although foodborne pathogens are detectable by PCR with high sensitivity and specificity, it is still challenging to realize a PCR-based detection system as an on-site detection platform that includes various functions on a compact single device.

On the other hand, microfluidics and lab-on-a-chip technologies have been fascinating for the miniaturization and integration of conventional laborious equipment\textsuperscript{[15]}. Various functions can be realized in small-sized devices and they can be integrated into a single device via microfluidic channels. In this manner, many efforts have been carried
out for miniaturization and integration of various functions for the detection of foodborne pathogens by PCR [16–19]. Although overall systems have been miniaturized and various functions are integrated into the single device, external pumping systems are required to operate the microfluidic devices in addition to the existing equipment for PCR and signal analysis, which can make an overall system more bulky and complicated. Therefore, the miniaturization of conventional benchtop systems for the operation of microfluidic devices should be miniaturized for the rapid on-site detection of foodborne pathogens in an early stage.

In this regard, various user-friendly microfluidic devices have been reported that can simply apply pressure into a microchannel without an external pumping system [20]. Among various technologies, finger-actuated microfluidic devices can control the flow in a microchannel on-demand by simply pushing and releasing the buttons without any external equipment. Finger-actuated microfluidic devices have facilitated various functions of conventional microfluidic devices, including droplet generation [21], immunoassay [22,23], anti-microbial resistance assessment [24], and PCR mixture preparation [25]. Recently, an indirect pressurization method has been developed to decrease user-dependent variation in finger-actuated microfluidic devices that can control a set amount of reagents regardless of various end-users [26–28].

By applying the finger-actuated microfluidic devices as functional modules, here, we report an integrated pumpless microfluidic chip for the detection of foodborne pathogens by PCR and electrochemical analysis. Amplification of genomic DNA and detection of amplified genes by electrochemical signals were performed in an integrated microfluidic chip and the finger-actuated microfluidic modules were applied to mix and transfer the reagents instead of conventional external pumping systems. After characterizing the transferrable volume and the mixing ratio of the finger-actuated microfluidic modules and the component of electrolyte, the target analyte, the genomic DNA of *Escherichia coli* (E. coli) O157:H7, was amplified by PCR and amplified genes were detected by electrochemical analysis.

2. Materials and methods

2.1. Apparatus and materials

GoTaq® DNA polymerase (M3005) was obtained from Promega. *E. coli* O157:H7 (ATCC 43894) was obtained from ATCC. **(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5-di-1H-benzimidazole trihydrochloride hydrate, bisBenzimidize (Hoechst 33258),** (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), and erioglaucine were obtained from Sigma-Aldrich (St. Louis, MO, USA). A polydimethylsiloxane (PDMS) precursor and thermos-initiator (Sylgard 184) was purchased from Dow Corning (Midland, MI, USA). SU-8 2100 was purchased from MicroChem Corp (Westborough, MA, USA). A spectrometer (Spectra Max 250) was purchased from Molecular Devices (Sunnyvale, CA, USA). A PXI (PXIe-1060Q) system for instrumentation was purchased from National Instruments (Austin, TX, USA). An ultraviolet (UV) visualizer (LIAS Slite 140) was purchased from Avegene Life Sciences (Taipei, Taiwan). The DNA lysis solution (Qiamp DNA mini kit, 51304) was purchased from QIAGEN (Hilden, Germany). The electrochemical analysis was performed with an electrochemical analyzer 630B (CH Instruments, Austin, TX, USA). A plotting cutter (FC4600C-50 PRO) was obtained from GRAPHTEC (Yokohama, Japan). An E-beam evaporator (EBS400) was purchased from EVA TEC (Trübbach, Switzerland).

2.2. Pathogen preparation in broth and real sample

*E. coli* O157:H7 was cultured in 10 mL Luria-Bertani (LB) medium for 16 h at 37°C, including 1 g sodium chloride, 0.5 g yeast extract, and 1 g tryptone in 100 mL autoclaved deionized (DI) water. To investigate the colony-forming units (CFU), the colonies were counted using the colony counting principle. The suspected *E. coli* O157:H7 samples were inoculated into the broth. To prepare a target pathogen concentration from 10^3 to 10^6 CFU per 1 mL broth, the cells were serially diluted. To prepare the real sample-based pathogen samples, the 1.0 × 10^5 CFU of *E. coli* O157:H7, *S. enteritidis*, and *B. cereus* were spiked into the 10-times diluted milk. The samples were stored at 4°C until use. Prior to testing the prepared sample, the cells were harvested by centrifugation at 13,000 rpm for 10 min.

2.3. gDNA amplification of pathogens

Based on the GenBank, the genomic sequences of eaeA gene of *E. coli* O157:H7 were selected as target genes. The forward primer was 5'-GACCGGGCAACAAGCATAGC-3' and the reverse primer was 5'-CCACCTGCAGCACAAGAGG-3'. The amplicon sizes of the target gene were 384 bp for *E. coli* O157:H7. The *Salmonella enteritidis* (S. enteritidis) and *Bacillus cereus* (B. cereus) were also selected. The forward primer was 5'-CAAACGACGTGGTTCCAAGCG-3' and the 5'-GCTGTTGCGAACCAATGT-3', and the 5'-TGGTGACGGTAGGTGCCG-3' and the 5'-TCCGCTTCTACA- CATCTTACAG-3' were used as reverse primer. The amplicon sizes were 498 bp and 443 bp for *S. enteritidis* and *B. cereus*, respectively. The pathogen gDNA was reacted with a PCR mixture containing dNTP (dATP, dGTP, dCTP, and dTTP), MgSO_4, primers, and polymerase. The solution was manually loaded into the inlet hole, and the solutions in the integrated microfluidic chip were migrated by operating the finger-push button. The integrated microfluidic chip was then applied to the NI device which is a customized heater for PCR thermal cycling. The gene amplification was implemented under the following thermal cycling conditions: 300 s for pre-denaturation at 95°C, 30 s for denaturation at 95°C, 30 s for annealing at 60°C, 30 s for DNA synthesis at 72°C, and 300 s for final elongation at 72°C. The 30 times of heating cycles were repeated.

2.4. Fabrication of the integrated microfluidic chip

The integrated microfluidic chip consists of four components containing finger-actuated microfluidic modules for injection and mixing module, film-based PCR module, and electrode module (Fig. 1). PDMS-based finger-actuated microfluidic modules consist of four layers, including three PDMS layers (thickness = 3 mm) and one PDMS membrane (thickness = 30 μm) (Fig. 2A and C). Cover layer, and fluidic, pneumatic channels layers were fabricated by soft lithography using the mixture of PDMS precursors and thermos-initiator (mixing ratio = 10:1) over the bare silicon wafer, and 100 μm height of micro-mold which is fabricated by photolithography using SU-8 2100. PDMS membrane was obtained by spin-coating a 7:1 PDMS to thermos-initiator mix on bare silicon wafer at 1500 rpm for 60 s. To fabricate the finger-controlled microfluidic devices, each layer is bonded with each other after the oxygen plasma treatment for 60 s. To prevent the weir structure of Valve 1 and 2 being bonded to the PDMS membrane, the weir structures were passivated by using permanent marker before oxygen plasma treatment. The film-based PCR module was assembled by using the various type of film (Fig. 2B). Cover film consisted of polytetrafluoroethylene (PTFE) and polyethylene (PET) film. The PET was also employed for middle film and bottom film. The polyimide (PI) film was used for PCR chamber and microfluidic channels. To assemble the film-based PCR module, the double-side adhesive PI and PET were cut by a floating cutter, and the films were stacked alternately [29]. The electrode module was fabricated by assembling the gold electrode on the glass with double-side adhesive PI and PET films (Fig. 2D). Then, using the adhesive PVC film, the electrode module was directly conjugated with finger-actuated mixing module chip. The thermal oxidation-treated glass substrate was patterned by using the photolithography. The Ti (20 nm) and Au (200 nm) layers were sequentially deposited on the glass substrate using E-beam evaporation. The electrodes, including working, counter, and reference electrodes (1 μm × 6 μm), were patterned. The three
individual electrode were conjugated with each three chambers for simultaneous analysis on a single chip. All electrodes are made of gold in regard to the previous reports [30–32]. The fabricated chip was used for single-use to prevent the contamination between different samples which may results in false negative or false positive signals.

2.5. Assessment of mixing ratio and dispensed volume

To evaluate the mixing ratio, 1 mM erioglaucine solution was mixed with distilled water and the concentration of the mixture was measured. To measure the concentration of the mixture, a calibration curve was first obtained by measuring the absorbance of the mixture between 10 μL of 0.1 to 1 mM erioglaucine solution and 200 μL of distilled water at 406 nm using the spectrometer. Based on the calibration curve, the concentration of the mixture was calculated by measuring the absorbance of the mixture between 10 μL of sample solution and 200 μL of distilled water at 406 nm.

2.6. Electrochemical behavior of gold electrodes

The electrochemical characteristic of the gold electrode was evaluated by cyclic voltammetry (CV) scanning method in the voltage range from 0 to 0.6 V (versus Au) with a scan rate from 10 to 500 mv/s in the 5 mM ferrocyanide. The stability test of electrode was performed by scanning the 30-times CV cycling at the 10 and 50 mV/s in the 5 mM ferrocyanide.

Fig. 1. Demonstration of the integrated pumpless microfluidic chip for the detection of foodborne pathogen. (A) A schematic of the proposed device. (B) Working principle of the finger-actuated pumping unit. (C) Working principle of the electrochemical detection of amplified genes. (D) An image of the integrated pumpless microfluidic chip.

Fig. 2. Configuration of each module of the integrated pumpless microfluidic chip. (A) Finger-actuated microfluidic module for injection of reagents. (B) Film-based PCR module for PCR. (C) Finger-actuated microfluidic module for mixing electrolyte and amplified genes. (D) Gold electrode module for the electrochemical detection, including film-based chambers.
2.7. Quantitative analysis of amplified pathogenic genes

The electrochemical analysis was performed by analyzing the current signal of Hoechst. The Hoechst mixture solution on the gold electrode was evaluated by using the square wave voltammetry (SWV) with an electrochemical analyzer. The potential from 0.1 to 0.6 V (versus Au) was scanned with an amplitude of 0.025 V and a frequency of 25 Hz at 50 mV/s. The analysis time for a single assay was approximately 25 s.

3. Results and discussion

3.1. Working principle of the integrated microfluidic chip

As shown in Fig. 1A, the integrated microfluidic chip has the three buttons on the single chip. The solutions in the chip could be precisely transferred at the same volume by operating the buttons. The structure and working principle of the finger-actuated pumping unit are presented in Fig. 1B. Each pumping unit consists of two pneumatic valves and one actuation chamber, in which Valve 1 and actuation chamber are operated depending on the pressure change of the pneumatic channel, while Valve 2 is operated depending on that of the fluidic channel. When the button is pushed, increased pressure in the pneumatic channel closes Valve 1 and compresses the actuation chamber, which discharges fluid in the actuation chamber by opening Valve 2. When the button is released, Valve 1 is opened and the actuation chamber is decompressed that causes a decrease in the pressure of the fluidic channel. Therefore, Valve 2 is passively closed and the fluid is charged into the actuation chamber. By repeating the push and release of the button, fluid can be dispensed into a single direction without backflow. The whole valve on the chip was made by PDMS, which has high softness and elasticity, enabling the original structure to be maintained for repeated operation. Thus, the designed finger-actuated modules translocate the solution continuously and stably during the test. Each button on the chip controls three parallel channels, so the three samples could be analyzed simultaneously on a single chip. The pathogen samples were firstly injected into the inlet and the samples were migrated into the PCR chamber by operating Button 1. The chip was then interfaced with the PXI system which provides heating for the PCR. The amplified genes were moved away from the PCR chamber using Button 1 and mixed with Hoechst 33258 as an electrochemical signaling molecule by operating Button 2. To analyze the signals, the blended solution was migrated into the electrode chamber by pushing Button 3. The Hoechst 33258 is specifically intercalated with double-strand DNA, reducing the electrochemical signals on the surface of electrodes. In the case of a positive test, the low signal was generated owing to the low concentration of free Hoechst 33258, while the high signal could be obtained from a negative test, the low signal was generated owing to the low concentration of free Hoechst 33258 (Fig. 1C). Based on the designed operation principle, the integrated microfluidic chip was fabricated as shown in Fig. 1D.

To successfully assemble each functional component, including injection, PCR, mixing, and electrode modules on a single chip, adhesion films were employed to assemble modules on a polycarbonate (PC) plate. As shown in Fig. 2A, the PDMS was mainly employed for the finger-actuated injection module to effectively pump the sample solution. The injection module consists of four layers including cover layer, fluidic channels layer, PDMS membrane, and pneumatic channel layers, which are all made by PDMS. The channels in the injection module were directly conjugated with the film-based PCR module and the sample solutions were moved by pressing the button. The PCR module was made of various types of films such as PET and PI with three individual reaction chambers for DNA amplification (Fig. 2B). After the PCR, the sample solutions were then moved into the mixing module through the connected fluidic channels. The layer component of the mixing module is similar to the injection module, while the mixing module contains the two push buttons (Fig. 2C). The first button is for mixing the amplified DNA and electrolyte and for the movement into the mixing chamber, and the second button is for the movement of the mixture from the mixing chamber to the electrode module. The electrode module contains three individual sets of gold electrodes under the three individual chambers (Fig. 2D). The patterned gold electrodes were fabricated on the glass by using photolithography. The currents resulting from the concentration of free Hoechst on the electrode surface are reversely proportional to the concentration of the amplified gene. By using the integrated pumpless microfluidic chip, the gDNA of target pathogen could be simply analyzed by button pushes.

3.2. Characterization of the finger-actuated microfluidic modules

For the characterization of the finger-actuated microfluidic devices, the controlled volume by each button was assessed. As the device was designed to control a set amount of volume without backflow, the controllable volume by a single button push was determined by the compressed volume of the actuation chamber (ΔV) (Fig. 3A) [26–28]. Therefore, the geometry of the actuation chamber should be designed according to the amount of desired controllable volume. Buttons 1 and 3 are designed to control 4 μL volume in each row, while Button 2 is designed to control the total volume of 4.5 μL (1.5 μL for the amplified genes + 3 μL for the electrolyte). Therefore, regardless of initially loaded volume in the inlet chambers, the amount of reagent moved to target region is determined by the number of button pushes. To confirm whether the designed volume was controlled by each button and each row can control the same amount of volume, the volume dispensed was analyzed as shown in Fig. 3B. Although there was no significant difference in the dispensed volume of each row, there was an approximately 10% decrease in the measured volume compared to the designed dispensible volume which was also represented in previous work [26]. Furthermore, it was analyzed that pumping units operated by Button 2 can mix electrolyte and amplified genes with a mixing ratio of 2:1. To visualize the test, 1 mM erioglaucine solution was injected into the inlet for amplified nucleic acids and distilled water was injected into the inlet for the electrolyte. The concentration of the mixture from the mixing chamber was about 0.33 mM in every row and it can be noted that 1 mM erioglaucine solution was mixed with distilled water at a ratio of 1:2. Although the dispensed volume decreased slightly compared to the designed value, there was no significant difference in the mixing ratio.

3.3. Electrochemical behavior of the electrode

The integrated microfluidic chip contains the electrode to electrochemically detect the amplified genes of the pathogen. To confirm the property of the electrode, the electrochemical behavior was determined. First, the ferrocyanide-based CV was carried out with various scan rates. As shown in Fig. 4A, the oxidation and reduction voltammogram was clearly detected with regular responses of the electrochemical signal throughout the detection range, and each peak current was altered in accordance with the applied scan rate from 10 to 500 mV/s. Based on the result obtained, the linearity was verified by using each redox peak current from the graph (Fig. 4A), and the obtained redox peak was linearly changed in accordance with the scan rate (Fig. 4B). Using the represented signals, the linearity of R² from each oxidation and reduction peak was calculated as to be 0.96, and 0.95, respectively. The result reveals the remarkable electrochemical characteristics of the redox response toward the fabricated electrode.

To investigate the reproducibility, the CV method was repeated with a scan rate of 10 and 50 mV/s. From Fig. 4C, the results for 5, 10, 15, 20, 25, and 30 cycles for both scan rates, and the uniform signals were observed in the whole applied condition. To precisely verify reproducibility, the narrow voltage range from 0.2 to 0.35 V of the oxidation peak was verified as presented in the inset of Fig. 4C. The highly reproducible signals were obtained with a coefficient of variation (COV) of 2% in the whole applied condition. Based on the findings, the test result reveals the high stability and reproducibility of the
3.4. Optimization of electrochemical signaling

To electrochemically detect the pathogen by using the developed integrated microfluidic chip, the non-specific adsorption of materials in the cocktail solution to the channel surface should be verified and optimized. In our previous study, Hoechst was successfully employed in the film-based biosensing chip as a signaling molecule [32]. However, PDMS should non-specifically absorb the various materials based on its hydrophobicity and stickiness. Thus, to accurately use the Hoechst in the PDMS chamber, the composition of the electrolyte was optimized. Concerning the test condition of our previous research [32], 50 and 100 mM of Hoechst were employed with 0.1% Triton X-100 to the both of tube- and chip-based tests. To precisely optimize the test condition, the tests were performed at least three times under the same condition and the electrochemical behavior was measured by SWV.

In the tube-based test result with 50 mM Hoechst, approximately 1.2 μA was obtained (Fig. 5A), while approximately 0.3 μA was measured in the PDMS chip-based test (Fig. 5B). Considering the use of the same volume of Hoechst and the same type of electrode in both tests, Hoechst should be non-specifically adsorbed on the surface of the PDMS, causing the decrease of the electrolyte concentration and the electrochemical signal. Thus, the prevention of the PDMS surface is required for precise analysis, so the Triton X-100 was added as a surfactant to the Hoechst solution. As a result of 50 mM Hoechst with 0.1% Triton X-100 in the tube-based test, 0.35 μA was obtained. Compared with the result from 50 mM Hoechst, approximately 0.85 μA was changed, indicating that the surfactant on the electrode interfered with the electrochemical signal. However, in the PDMS chip-based test, the signal was slightly increased compared to the result from 50 mM Hoechst. The phenomenon was that the surfactant was deposited on the surface of PDMS. Thus, the relatively high concentration of Hoechst could be effectively migrated on the electrode. Based on the findings, we confirmed that the surfactant is proper to apply for the PDMS chip as a surface blocking agent. Although the significant result is obtained, the signal variation is nearly similar. The large signal change is required to precisely and quantitatively analyze the various concentrations of the pathogens, and thus, a 100 mM Hoechst test was performed. In the tube-based test result, about 1.3 μA was acquired, while 1 μA was acquired in 50 mM Hoechst test result. However, in the PDMS chip-based test, 0.7 μA was obtained, and the signal was twice higher than the test result from 50 mM Hoechst. Then, the surfactant test was subsequently implemented. In the tube-based test, the signal was nearly halved compared with the Hoechst without surfactant. However, the PDMS chip test result demonstrated the highest signal in the whole applied condition. Moreover, the electrochemical signal was similar in both tube- and PDMS-based tests. Therefore, the test condition of 100 mM Hoechst with 0.1% Triton X-100 was selected as the optimized condition and the solution was employed to the pathogen analysis.

3.5. E. coli O157:H7 detection using the integrated microfluidic chip

To detect the pathogens using the fabricated integrated microfluidic chip, E. coli O157:H7 was employed as a target analyte. The concentration ranges from 10⁵ to 10⁶ CFU/mL were prepared and the three samples were simultaneously measured in a single chip. Briefly, the prepared E. coli O157:H7 samples were firstly mixed with a lysis buffer and the mixture solution was injected into the injection module. The template gene was amplified by film-based PCR methods as previously described [29]. The amplified gene was blended with Hoechst and the cocktail solution was moved into the electrode. The pathogen was finally measured by SWV methods. Every step of the solution migration in the integrated microfluidic chip was operated by only finger-push. The test results were demonstrated in Fig. 6A. In the whole detection range, the obtained electrochemical signals were changed in accordance with the concentration of E. coli O157:H7. The result indicated that the gDNA from the pathogen was clearly amplified and quantified using the integrated microfluidic chip. To precisely analyze the linearity from the voltammogram obtained, the calibration curve was presented using the peak signal in current at 0.4 V. As shown in Fig. 6B, the signals from the calibration curve was inversely proportional to the target E. coli O157:H7 concentration. The electrochemical signals from 10⁵ to 10⁶ CFU/mL of E. coli O157:H7 exhibited a linear correlation response and the current was saturated at 10⁷ CFU/mL. Although the results were similar for both 10⁵ and 10⁶ CFU/mL, these signals were clearly distinguished from the negative control test, demonstrating that the developed chip was able to precisely investigate the low concentration of the target gene.

Considering the saturation concentration, we assumed that the limit of detection (LOD) of the integrated microfluidic chip for the evaluation of E. coli O157:H7 was the 10² CFU/mL. To confirm the reproducibility of the target gene, the results were repeated at least three times under the same conditions, including temperature, pH, and reaction time. The calculated COV in the whole applied concentration was approximately 8%. The correlation coefficient in the linear range from Fig. 6B was also calculated and the 0.94 of R² was obtained. The results reveal high reproducibility and reliability. The amplified gene in the chip was analyzed within about 5 min, including electrolyte mixing, solution migration, and electrochemical analysis. Considering the analysis time of conventional methods such as electrophoresis for 30 min, we successfully reduced the detection time. Additionally, in comparison with the large scale instrument with a complex detection system in the medical and commercial field, the developed chip was significantly simplified the detection principle and scale by integrating the various functional device into a single chip. Therefore, the simple continuous measurement from the sample-in to result-out is made possible on the basis of the finger-actuated microfluidic modules.

To confirm the practical usability of the developed integrated chip, the pathogens spiked into the real samples was tested. The 1.0 × 10⁵ CFU of E. coli O157:H7, S. enteritidis, and B. cereus were prepared based on the milk, and then each pathogen was applied to the integrated chip under the same condition with previous methods. The Fig. 7 shows the
obtained electrochemical result. The peak current from *E. coli O157:H7*, *S. enteritidis*, and *B. cereus* were registered as 1.7, 1.5, and 1.7 $\mu$A, while the 2.9 and 3.7 $\mu$A were obtained from milk and DI water, respectively. The result indicates that the applied gDNA in the milk samples were successfully amplified by using the integrated chip, and the Hoechst-based electrochemical signal was effectively conducted. The observed result from all positive samples were clearly distinguishable with the negative test, allowing the accurate analysis of various types of pathogen on a single integrated chip. In the negative control test, the result from milk was about 0.8 $\mu$A lower than that of DI water due to the signal interference in milk such as albumin, casein, and enzymes. In regard with the milk containing various ingredients, the developed biosensing chip demonstrated practical usability to the real sample-based pathogen analysis.

Although the benchtop electrochemical analyzer and thermal cycler were used for the proposed method, it is meaningful noting that the need for external equipment for reagent transfer is eliminated. The use of thermal cycler can also be eliminated by using isothermal amplification methods including LAMP or RPA [33], and the portable electrochemical detector can be used instead of benchtop electrochemical analyzer [34]. Additionally, the four main steps are required for pathogen analysis as follows: (i) the solution migration for 2 min, (ii) heating for 30 min, (iii) solution mixing for 3 min, and (iv) electrochemical analysis for 5 min. Thus, approximately 40 min is required for completion of pathogen analysis. The total assay time is similar with conventional methods, while the developed sensing system is significantly minimized in comparison with commercial PCR principle using the lab-scale instrument.

In terms of cost of the chip, a single chip could be made under a dollar. The PMDS was mainly employed to fabricate the integration chip, and the about 0.3$ was costed for a single chip. The Au chip used in the integration chip was made in large quantities based on 8-inch lithography principle, and price for single chip was approximately 0.5 $. By using the prepared materials, each integration chip was manually assembled, and a single integration chips could be fabricated in 5 min. With regard to these findings, we have successfully established the usability of molecular diagnosis, which reduces the device scale, the
sample volume, and the analysis time, enabling point-of-care testing (POCT) analysis.

4. Conclusion

This study successfully established a novel biosensing platform by hybridizing PDMS-based, finger-actuated microfluidic modules with film-based PCR module and electrode module. The simple integration and assembly of hetero-materials enable various functionalities, including PCR, transfer and mixing of reagents, and electrochemical analysis on a single chip. Additionally, owing to the use of inexpensive materials with the simple operation system, the assay cost and analysis time would be significantly reduced without the use of a sophisticated instrument or system, while the proposed biosensing platform demonstrated the high reproducibility with 10% COV and high sensitivity with LOD of $10^2$ CFU/mL for E. coli O157:H7. The designed biosensing platform provides a practical use in the field based on simple finger actuation along with advanced functionalities for sample migration, mixing, and rapid analysis, enabling high applicability in the general public for pathogen evaluation as a POCT device.

Credit author statement

Yoo Min Park: Original draft; Investigation; Data curation.
Juhwan Park: Original draft; Methodology; Conceptualization.
Sun Young Lim: Validation; Investigation; Analysis.
Yeji Kwon: Chip fabrication; Investigation; Validation.
Nam Ho Bae: Chip fabrication; Validation.
Je-Kyun Park: Funding acquisition; Supervision; Review & editing.
Seok Jae Lee: Funding acquisition; Supervision; Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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