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An easy-to-use nucleic acid-based fluidic platform for the profiling of oral microbiome from swab and mouthwash that consists of functionalized microbeads and a multi-chamber aliquoting structure

Hyejin Lee [a,1], Yun Guang Li [a,1], Jiatong Kang [a], Seung Won Shin [b], Changyoon Baek [a], Junhong Min [a,*]

[a] School of Integrative Engineering, Chung-Ang University, Heukseok-dong, Dongjak-gu, Seoul 06974, South Korea
[b] Department of Bioengineering, College of Engineering, California Institute for Quantitative Biosciences, QB3, University of California Berkeley, Berkeley, CA 94720, USA

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ABSTRACT

The oral microbiome is an important indicator of health status; however, it has the limitation of varying significantly over time and under different life conditions. Therefore, rapid and convenient analysis through automation is required. In this study, we developed an automated oral microbiome analysis platform for the rapid and convenient diagnosis of health using mouthwash as the sample. Functionalized microbeads coated with graphene oxide (GO) were employed to concentrate 5 mL of mouthwash sample into 300 μL within 3 min, achieving an eight-fold concentration. Effective adsorption of bacteria onto the functionalized microbeads was confirmed using scanning electron microscopy. Furthermore, nucleic acids were extracted from bacteria adsorbed on the functionalized microbeads via bead beating (3000 rpm) for 3 min, achieving approximately 100 % yield compared with conventional methods. The extracted nucleic acid solution was then automatically aliquoted into PCR 8-tube strips (2 μL each) with high accuracy (CV% = 2.63 %) using a simple fluidic aliquoting structure that incorporated an access channel and retention area. Oral microbiome profiling at the phylum level was compared between the developed fluidic cartridge method and commercial approaches, including next-generation sequencing (NGS) analysis. The automated platform, which integrates the developed sample pre-treatment and aliquoting processes into a fluidic cartridge, successfully analyzed the oral health status of both humans and companion animals within 3 h, demonstrating analytical accuracy equivalent to that of NGS.

1. Introduction

The oral microbiome refers to the community of microorganisms, such as bacteria, viruses, and fungi, that reside within the oral cavity, including the mouth, teeth, gums, tongue, and salivary glands [1]. This microbiome is composed of thousands of diverse species of bacteria, viruses, fungi, and archaea, among others, which coexist and interact to form the oral ecosystem [2]. The microorganisms within the oral cavity perform various functions, with the majority playing beneficial roles in maintaining oral health [2,3]. Some microorganisms help prevent oral diseases by breaking down food remnants [4] and preventing tooth decay [5]. Additionally, certain bacteria regulate the human immune system, enhancing resistance to oral infections [6].

The oral microbiome has been associated with various systemic diseases. Some studies have shown an association between oral health status and cardiovascular diseases, such as heart disease and stroke. Bacteria associated with oral inflammation can travel through the bloodstream to the cardiovascular system, causing inflammation and damaging blood vessel walls [7–9]. Furthermore, some studies suggest that changes in the oral microbiome are closely linked with insulin resistance and the risk of developing diabetes [10,11]. Moreover, alterations in the oral microbiome have been associated with inflammatory bowel disease, respiratory infections, Alzheimer's disease, and pregnancy complications [12–16].

To analyze the oral microbiome, it is necessary to collect samples first. Typically, various methods such as the use of swabs, mouthwash,

* Corresponding author.
E-mail address: junmin@cau.ac.kr (J. Min).
[1] These authors contributed equally to this work.

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dental floss, and mouth rinse are employed for sample collection [17–19]. However, it is crucial to select the appropriate method based on the purpose and requirements of the study. Among these, mouthwash has several advantages compared with other sampling tools, including 1) wide sampling coverage, 2) non-invasiveness and ease of use, 3) standardization through the use of a specific mouthwash solution, and 4) preservation of microbial viability by adding preservatives. However, it also has a severe drawback in that the sample is diluted [20].

The second step of microbiome research is to uniformly extract the genomic DNA (gDNA) of all bacteria present in the sample. There are various methods for extracting gDNA and RNA from the cells. Many studies have already been conducted using chemical methods involving chaotropic salts and high-concentration detergents, or biological methods involving the use of proteinase [21–23]. Commonly available kits for gDNA extraction are also widely used in this regard. However, chemical methods often encounter issues with differential DNA extraction efficiency between Gram-positive and Gram-negative bacteria [24,25]. Additionally, the use of proteinase can lead to inconveniences regarding kit storage and usage. In recent years, we have reported that a physical method using bead beating can achieve constant efficiency in DNA extraction, as demonstrated in previous studies [26–28].

Next-generation sequencing (NGS) is the commonly used method for analyzing the human microbiome, but requires relatively complex equipment and consumes significant time and resources [29,30]. Therefore, NGS is unsuitable for studying the oral microbiome, which requires continuous monitoring due to its compositional fluctuations in response to time and environmental factors.

This study demonstrated a quantitative PCR (qPCR)-based, fully automated oral microbiome analysis cartridge and system using graphene oxide (GO)-coated functionalized microbeads and channels for uniform sample distribution. Most prior studies [31,32], including our own earlier work [27,28] employing GO-coated beads for bacterial enrichment and nucleic acid extraction, have evaluated only a single step (either bacterial concentration or DNA extraction) or have performed sample pretreatment and downstream analysis separately. For platform integration, we used a PCR-based analytical approach while aiming to achieve performance similar to conventional one pot NGS systems. This required a more uniform process for sample preparation and dispensing. To improve this uniformity, we concluded that combining and automating these steps would be the most efficient strategy. Therefore, we integrated the sample preparation and dispensing processes into a single, streamlined workflow. By using this platform, all bacteria present in the mouthwash samples were adsorbed and concentrated on functionalized microbeads. In contrast, mouthwash samples contain various proteins and impurities that may affect bacterial adsorption efficiency when using GO microbeads. However, previous studies conducted by our research group have demonstrated successful concentration of target pathogens—including adenovirus, *S. aureus*, and *E. coli* [27], *C. difficile* [28], Cyanobacteria [33], Norovirus [34], SARS-CoV-2 [35]—from a wide range of complex samples rich in proteins and organic matter, such as river water, fecal samples, and surface samples. These results indicate that GO microbeads can reliably adsorb target pathogens even in samples containing substantial amounts of proteins, organic materials, and contaminants. Therefore, the process presented in this study can be considered robust and applicable across diverse sample conditions. Thus, the effectively adsorbed bacteria on the GO microbeads allowed uniform extraction of 16S ribosomal DNA (16S rDNA) using a simple bead-beating method, without requiring complex procedures, high-concentration chemical treatments, or proteinase. The extracted 16S rDNA sample was divided into eight separate 2 μL aliquots and transferred into PCR 8-tube strips containing PCR master mixtures (eight kinds) using a simple aliquoting fluidic method. The efficiency of the simple 2 μL aliquoting fluidic method was compared with manual pipetting in terms of PCR efficiency. Microbiome analysis of mouthwash samples at the phylum level was performed using a qPCR amplification assay and compared with NGS methods. Finally, the developed automatic system was directly applied to canine and feline oral healthcare, showing excellent correlation with clinical diagnostic results.

2. Materials and methods

2.1. Materials

Sodium acetate and Triton X-100, both from Sigma-Aldrich (MO, USA), and acetic acid from Daejung Chemicals & Metals (South Korea) were used to prepare the binding buffer (sodium acetate, 50 mM, pH 5; 0.5 % Triton X-100). To prepare the lysis buffer (Tris-HCl, 100 mM, pH 9), Trizma base and hydrochloric acid (HCl) were obtained from Sigma-Aldrich and Samchun Chemicals (South Korea), respectively.

Glass microbeads (40–70 μm; Daihan Scientific, South Korea), graphite powder, potassium permanganate ($KMnO_4$), triethoxysilane (Sigma-Aldrich), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2; Daejung Chemicals & Metals), and ethanol (Merck Millipore, Germany) were prepared for the fabrication of the functionalized microbeads.

2.2. Performance analysis based on bacteria counting by qPCR

The total amount of bacteria was quantified using qPCR (LightCycler® 96 Instrument, Roche, Switzerland) with a commercial nucleic acid extraction and purification kit (QIAamp DNA Mini Kit, Qiagen, Germany) and TB Green Premix Ex Taq II Master Mix (Takara Bio, Japan). In particular, the bacterial nucleic acid extraction method using the QIAamp DNA Mini Kit was used as a standard protocol for bacterial quantification. To quantify total bacteria, the PCR primers targeted the universal region of the 16S rDNA [36].

Before employing real clinical swab and mouthwash samples, an artificial sample consisting of *Staphylococcus aureus* (*S. aureus,* ATCC 6538P) and *Salmonella typhimurium* (*S. typhimurium*, ATCC 14028) at a concentration of 10^6 CFU/mL was utilized to optimize the operational conditions.

Binding efficiency (%) was indirectly determined by comparing the bacterial amount present in the solution entering the sample treatment chamber with that in the solution exiting the chamber: 100 × [(bacteria amount in the entering solution – bacteria amount in the exiting solution) / bacteria amount in the entering solution].

To observe the adsorption of bacteria in mouthwash samples on the functionalized microbeads, a scanning electron microscope (Emcrafts, South Korea) was used.

Lysis efficiency (%) was determined based on a vigorous agitation method using a vortex mixer (Scientific Industries, NY, USA), as described in our previous research [27], which demonstrated comparable or higher efficiency compared to the commercial lysis method, the Qiagen method. Lysis efficiency (%) was defined as the ratio of the amount of 16S rDNA extracted from bacteria adsorbed on the surface of functionalized microbeads using the lysis module to the amount extracted using a vortex mixer (50 Hz). Lysis efficiency (%) can be expressed as (extracted amount of 16S rDNA via bead beating using the lysis module) / (extracted amount of 16S rDNA via bead beating with vortex under the same conditions) × 100 (%). The extracted amount was quantified using calibration of the Ct values obtained through qPCR.

Relative concentration efficiency (%) was defined based on the performance of the commercial QIAamp DNA Mini Kit with mouthwash samples, and calculated as follows: 100 × [total 16S rDNA amount eluted after concentration using fluidic cartridge / total 16S rDNA amount eluted using commercial kit].

2.3. Mouthwash and swab samples preparation

Mouthwash samples were obtained from eight healthy volunteers. Each mouthwash sample was collected after at least 30 min of fasting from food and drink. Volunteers washed their mouths with 5 mL of

distilled water by gargling 10 times, and the mouthwash sample was collected into a 50 mL conical tube (SPL Life Sciences, South Korea) containing 1 mL of binding buffer. The mouthwash samples were left standing for 1 min to allow sedimentation, and the supernatant was used for the subsequent experiment. All experiments on mouthwash samples were approved by the IRB of Chung-Ang University Gwangmyeong Hospital (approval number CAUGH 2503–266–041).

Oral samples from canines and felines were collected by Gyeyang Sky Animal Medical Center using sterile swabs by rubbing the gingival and canine tooth margins as well as the oral cavity until the swab was sufficiently soaked. Specifically, back-and-forth movements between the gums and teeth were performed at least five times, and circular movements within the oral cavity were performed at least five times. The saliva-containing swabs were then placed into tubes containing 5 mL of transport medium and stored at −20 °C until further processing. Only the resulting solution was used for subsequent experiments.

2.4. GO-coated functionalized microbeads for bacteria concentration

GO was synthesized using our previous method [34]. Briefly, graphite powder was dispersed in concentrated H_2SO_4 and stirred for 12 h. The mixture was allowed to react with $KMnO_4$ for 75 min. To terminate the oxidation reaction, distilled water and H_2O_2 were added. The resulting GO was collected and subsequently washed several times with HCl and distilled water.

To fabricate the GO-coated functionalized microbeads, the glass microbeads were pre-cleaned with piranha solution and then functionalized by incubation with triethoxysilane in 95 % ethanol. Finally, the silanized glass microbeads were dispersed in a GO–ethanol solution and subjected to 150 W ultrasonic agitation using a probe-type sonicator (VCX 750, Sonics & Materials, CT, USA) to achieve uniform coating of the microbeads.

2.5. Design of integrated fluidic cartridge and operator

The integrated fluidic cartridge consisted of a sample treatment chamber containing functionalized microbeads, a sample chamber, a fluidic cartridge, and commercial PCR 8-tube strips (Roche). The dimensions of the fluidic cartridge were 110 mm × 60 mm × 90 mm (L × W × H). All components except the PCR 8-tube strips were made of polycarbonate. The sample chamber had a maximum capacity of 13 mL (H = 25 mm; ø = 27 mm). It was directly connected to the sample treatment chamber (H = 44 mm; ø = 10 mm), which contained 0.6 g of functionalized microbeads and a single neodymium magnet (L = 6 mm; ø = 3 mm).

The fluidic cartridge consisted of three layers. The 1st layer contained four valves and functioned as a sample treatment chamber holder. The multi-chamber aliquoting structure was in the 2nd layer. This multi-chamber aliquoting structure consisted of an access channel (L = 82 mm; W_A = 2 mm; H_A = 1 mm) and a semicircular retention area (R_R = 1.6 mm; H_R = 0.5 mm) with a 0.4 mm ($ø_R$) hole in the bottom to trap the solution. The solution could be released into the PCR 8-tube strips through this hole. The 3rd layer was composed of two types of waste chambers: waste chamber 1 (mouthwash) for mouthwash samples from the sample chamber that moved toward valve 1, and waste chamber 2 (aliquoting) for lysis buffer that passed through the multi-chamber aliquoting structure toward valve 4.

The operator (Fig. 1, bottom left) capable of driving the fluidic cartridge was 220 mm × 245 mm × 155 mm (L × W × H). The lid, which had a connecting tube that could supply air while fixing the fluidic cartridge to the operator, was manufactured to measure 135 mm × 245 mm × 155 mm (L × W × H). The operator consisted of one syringe pump, two solenoid valves, and one lysis module, with the syringe pump connected in parallel to the two solenoid valves. When the fluidic cartridge was combined with the lid and mounted on the operator, the program connected to the operator, the syringe pump, the solenoid valve, and the lysis module, thereby moving the solution inside the fluidic cartridge.

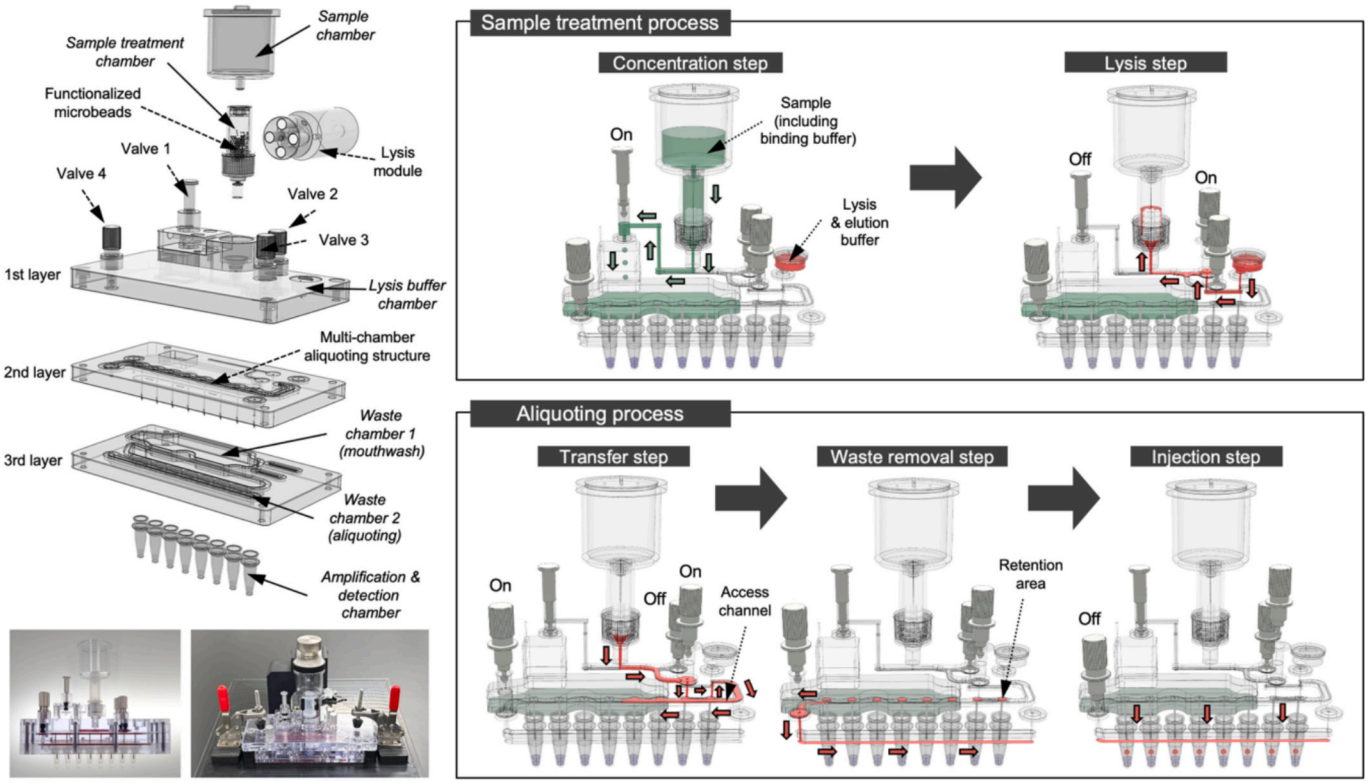


Fig. 1. Overview of the integrated fluidic cartridge and the microbiome analysis process for the mouthwash sample.

2.6. Phylum level oral microbiome analysis using qPCR and NGS

The microbiome profiling results of the fabricated fluidic platform were compared with the results of a well-established commercial method at the phylum level. For phylum level analysis of the oral microbiome, five pairs of primers targeting Actinomycetota, Bacillota, Bacteroidota, Fusobacteria, and Proteobacteria were used (Table S1, Supplementary Material) [37–40]. As a well-established commercial method developed for nucleic acid extraction, a QIAamp DNA Mini Kit was used according to the manufacturer's instructions, and the NGS results were provided by Macrogen (South Korea). Relative abundance (%) was calculated as the ratio of phylum level to the amount of 16S rDNA extracted from the same sample using a fluidic platform and a well-established commercial method.

To verify the nucleic acid purity of the samples for analytical comparison, the eluted samples were spiked with adenovirus DNA (not originally present in the sample), and qPCR efficiency was compared to that obtained when the same DNA was spiked into pure distilled water. Human adenovirus type 5 was obtained by Professor Dai-Wu Seol (College of Pharmacy, Chung-Ang University, South Korea), and adenovirus DNA was extracted using the QIAamp MinElute Virus Spin Kit (Qiagen, Germany). qPCR analysis was performed following the protocol described in our previous study [34].

2.7. Oral bacteria profiling using qPCR for the detection of periodontitis in companion animals

In addition to the phylum level analysis, oral samples from canines and felines were collected using swabs to analyze seven bacterial species—*Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Eubacterium nodatum* (En), *Fusobacterium nucleatum* (Fn), and *Campylobacter rectus* (Cr)—known to be periodontal disease/halitosis factors (Table S1, Supplementary Material) [41,42]. Species abundance (%) was calculated as the ratio of the amount of each bacterial species to the amount of 16S rDNA extracted from the fluidic platform.

3. Results and discussion

3.1. The whole process for microbiome analysis using mouthwash samples with an integrated fluidic platform

Oral microbiome analysis is largely divided into three steps. The first step is the sample treatment process, which concentrates bacteria from mouthwash samples and extracts 16S rDNA. The second step is dispensing a solution containing the extracted 16S rDNA and finally performing qPCR. Utilizing the integrated fluidic platform designed in this study, the collected mouthwash sample/binding buffer is loaded into the sample chamber, the lid is closed, and when the syringe pump connected to the lid is operated with positive pressure, the mouthwash sample/binding buffer mixture is passed through the sample treatment chamber and moves toward valve 1 (valve 1 on, valves 2, 3, 4 off) to waste chamber 1. At this time, bacteria are adsorbed on the surface of functionalized microbeads packed in the sample treatment chamber (concentration step in the sample treatment process). Afterward, the solenoid valve in the operator is opened to the lysis buffer chamber side, and the syringe pump is operated to inject air in the positive direction, so that 0.5 mL of lysis buffer contained in the lysis buffer chamber moves toward valve 2 (valve 2 on, valves 1, 3, 4 off) and enters the sample treatment chamber. By utilizing the rotation of the external lysis module, the neodymium magnet inside the sample treatment chamber is moved, and the functionalized microbeads are disturbed to perform bead beating, thereby extracting the 16S rDNA from the bacteria (lysis step in the sample treatment process). The movement of the mouthwash sample/binding buffer mixture and the lysis buffer is indicated with arrows in Fig. 1. When the solenoid valve in the operator is opened again to the sample chamber side and the syringe pump is operated in the positive direction, the extracted 16S rDNA moves toward valve 3 (valves 3, 4 on, valves 1, 2 off) and flows into the multi-chamber aliquoting structure (transfer step in the aliquoting process), and moves along the access channel to the waste chamber 2 (waste removal step in the aliquoting process). When 2 μL of solution is filled in the retention area, valve 4 is turned off and air is injected into the cartridge using the syringe pump. Next, the 16S rDNA, aliquoted in 2 μL, is introduced into the amplification & detection chamber containing the PCR mixture (injection step in the aliquoting process) and then placed in a PCR device to perform nucleic acid amplification. The movement of the 16S rDNA extracted during the aliquoting process is also indicated with arrows in Fig. 1.

3.2. Bacteria concentration and lysis in the sample treatment chamber consisted of functionalized microbeads

Fig. 2a illustrates the optimization of the rate for adsorbing bacteria to functionalized microbeads, which concentrates and isolates bacteria present in the sample. An artificial sample (10 mL) containing *S. aureus* and *S. typhimurium* is used, and the binding efficiency of the bacteria is found to be over 80 % at a sample loading rate of up to 9 mL/min, and over 95 % when adsorbed at 5 mL/min. When the sample loading rate increases from 1 to 5 mL/min, the binding efficiency decreases by about 5 %, while the binding time decreases significantly from 10 to 2 min. When the sample loading rate increases from 5 to 9 mL/min, the binding time decreases from 2 to 1 min, but the binding efficiency decreases by 8 %. Therefore, the optimal sample loading rate is determined to be 5 mL/min, which drastically reduced the binding time for adsorption without decreasing the binding efficiency.

To confirm that bacteria are adsorbed on the functionalized microbeads coated with GO, the bead surface is observed using scanning electron microscopy (SEM), and it is found that bacteria are adsorbed on the surface either singly or in aggregates (Fig. 2b). With this result, it is confirmed that bacteria present in mouthwash samples could be concentrated by effectively adsorbing them to functionalized microbeads.

As shown in Fig. 2c, because an external lysis module is present and the functionalized microbeads are disturbed by a neodymium magnet in the sample treatment chamber, the lysis efficiency of the functionalized microbeads is compared according to the rotational speed of the external lysis module. When the rotational speed of the lysis module is 3000 rpm, it is confirmed that the lysis efficiency of the adsorbed bacteria is equivalent to or higher than that obtained using a vortex mixer (50 Hz). It is found to be consistent with the 3 min bead beating condition of 50 Hz, which was suggested in our previous study [26] for effective nucleic acid extraction.

Fig. 2d shows the results of verifying the efficiency (relative concentration efficiency) of the entire sample treatment process using an actual mouthwash sample at the optimized sample loading rate, specifically, the rotational speed of the lysis module. In addition, to optimize the mouthwash sample volume, the DNA extraction amount (300 μL of the solution volume recovered after the sample treatment process) and absorbance at a wavelength 600 nm is measured according to the mouthwash volume (3, 5, and 7 mL), and compared with the method using a commercial nucleic acid extraction and purification kit (mouthwash sample volume 500 and 300 μL of the solution volume recovered after the sample treatment process). As expected, the relative concentration efficiency increases as the mouthwash sample volume increases, but although the 5 mL mouthwash sample showed a relative concentration efficiency that was more than eight times higher than the result obtained with the commercial kit using 500 μL, the relative concentration efficiency did not increase any further when a 7 mL sample was used. This suggests that as the mouthwash sample volume increases, not only the total amount of bacteria in the sample but also impurities, such as proteins, increase, so the concentration of bacteria by adsorption

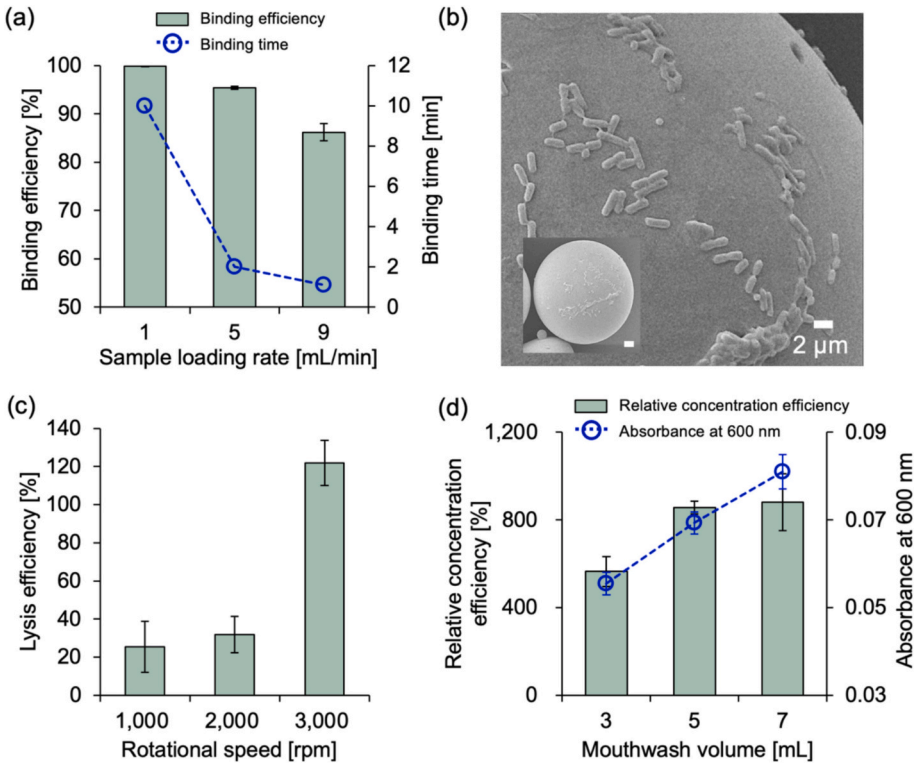


Fig. 2. Optimization of sample preparation process condition; (a) the effect of the mouthwash sample loading rate on the binding efficiency, (b) scanning electron microscopy images of the bacteria adsorbed on the functionalized microbeads surface, (c) bacteria lysis efficiency by rotational speed of lysis module, (d) relative concentration efficiency of mouthwash sample volume.

on the surface of functionalized microbeads could be inhibited. The increase in impurity concentration is predicted by measuring the solution recovered after the final sample treatment process at a wavelength of 600 nm. As the mouthwash sample volume increases, impurities also become concentrated and are included in the final product (the solution recovered after the sample treatment process). Based on the experimental results, the optimal mouthwash sample volume in this study was determined to be 5 mL.

However, in this study, the system was designed to minimize the influence of proteins on bacterial adsorption by ensuring sufficient bead surface area proportional to the volume of the mouthwash sample. The amount of functionalized microbeads used in the experiments was selected based on conditions under which the adsorption efficiency of both proteins and bacteria remains stable within the tested mouthwash volume. Consequently, in this process, bacterial adsorption efficiency is governed primarily by the ratio between sample volume and available bead surface area, rather than by the presence of impurities. Furthermore, considering our previous studies [27,28,33–35], which demonstrated that GO microbeads can reliably adsorb and concentrate target pathogens from various complex samples rich in proteins and organic matter, it can be inferred that the process presented in this work is applicable for bacterial adsorption regardless of protein content or other sample impurities.

3.3. *Two-microliter aliquoting performance result using a fluidic cartridge*

To automatically dispense extracted nucleic acid solutions without the use of a micropipette, an aliquoting structure is fabricated within a fluidic cartridge. Its performance was verified. Fig. 3a shows the design and results of a fast and simple equal distribution fluidic channel based on the principles of surface tension and pressure drop. The aliquoting structure consists of an access channel (W_A = 2 mm; H_A = 1 mm) into which the solution is injected and a retention area (R_R = 1.6 mm; H_R = 0.5 mm; inner volume = 2 μL). At the bottom of the retention area, which has a H difference of 0.5 mm from the bottom of the access channel, there is a hole ($ø_R$ = 0.4 mm) through which the solution can move to the PCR 8-tube strips. These structural parameters were designed through an optimization process, as shown in Fig. S1.

When the nucleic acid solution extracted from the sample treatment chamber is pushed by injecting air (Fig. 3a ①), the solution fills not only the 2 mm wide access channel but also the retention area, which has a hole on the bottom through which air can vent. To further explain, the air present in the access channel and retention area is expelled through the vent connected to the access channel output and the retention area output hole. As this air is released, the nucleic acid solution flows in and fills the retention area, as shown in Step 1. However, the size of the hole is very small compared to the size of the access channel, so the solution is not discharged through the hole in the retention area (Fig. 3a ②). To explain this in more detail, because the hydrophobic channel connecting the output hole of the retention area to the amplification & detection chamber functions as a hydrophobic valve, the nucleic acid solution filling the retention area is prevented from exiting through the output hole [43,44]. If the air is continuously pushed, the air layer removes the solution existing in the access channel, but the solution in the retention area does not drain (Fig. 3a ③ and ④). At this stage, all remaining solution in the access channel is discharged and directed into waste chamber 2 (aliquoting). The reason the solution existing in the retention area is not removed together with the solution filling the access channel is that the area of the solution existing in the retention area in contact with the surface of the retention area is approximately 13 mm^2, which is more than eight times the area of the solution existing in the access channel in contact with the surface (1.6 mm^2), thus it is considered that the solution is easily separated due to the height difference. This can be explained by the capillary effect, which is expressed in Step 2. Finally, with the vent connected to the output of the access channel closed off, if the air is pushed with a short but strong pressure, the solution in the retention area moves to the PCR 8-tube strips (amplification & detection chamber) located below through the 0.4 mm ($ø_R$) hole located in the

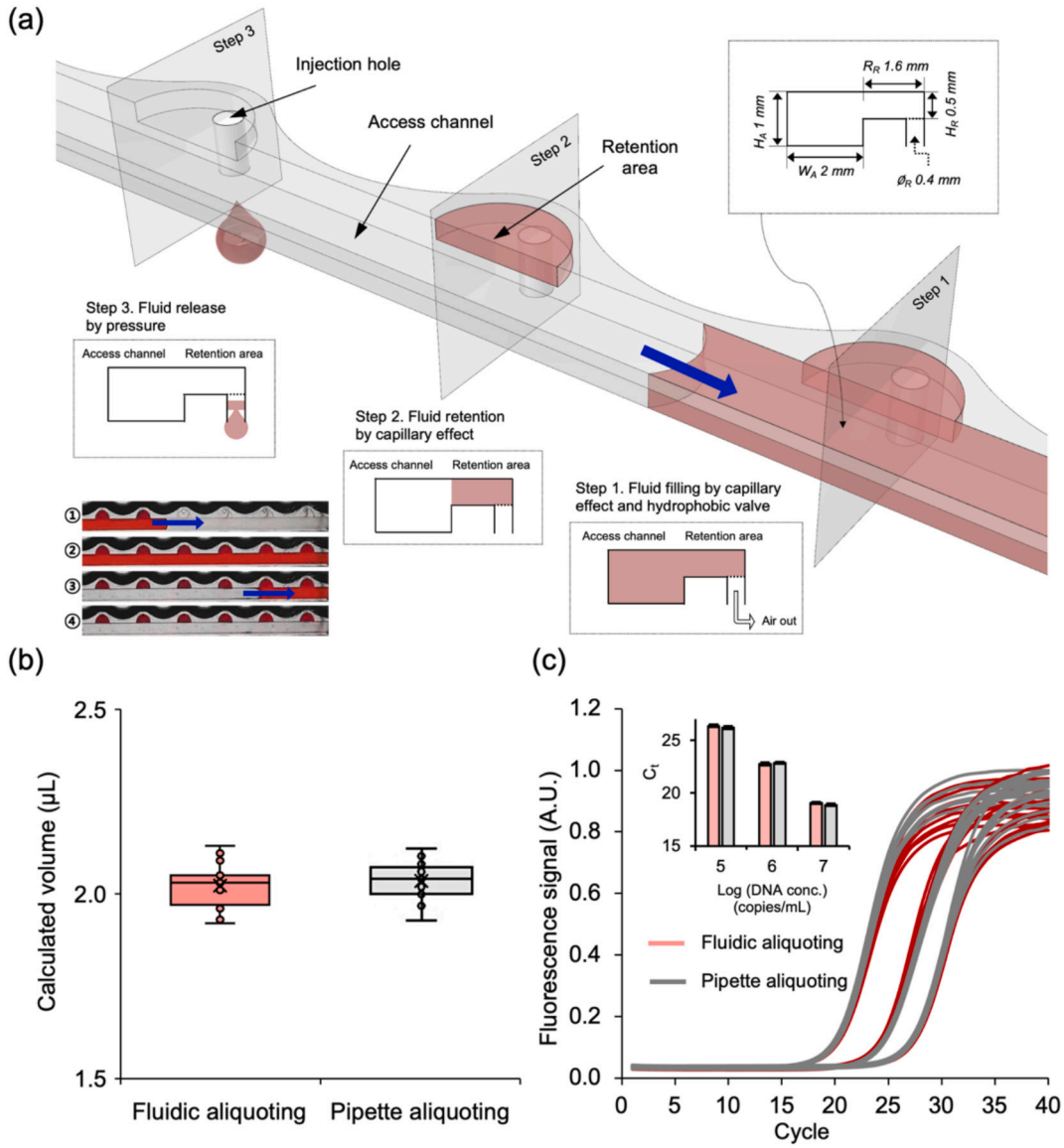


Fig. 3. Two-microliter aliquoting performance result of the integrated fluidic cartridge; (a) images of an operation for aliquoting in each chamber, (b) comparison of fluidic and pipette aliquoting in the same chamber, (c) fluorescence signal result by comparisons of fluidic and pipette aliquoting using qPCR (Inset: cycle threshold value).

center of the bottom of the retention area (Step 3).

Fig. 3b shows the comparative results obtained by repeating the manual method using a micropipette and the aliquoting structure method 30 times with a 2 μL dispensing amount. Manual pipette aliquoting and fluidic aliquoting showed statistically equivalent performances, with average volumes of 2.03 ± 0.05 and 2.02 ± 0.05 μL, respectively (*t*-test, *p*-value >0.05). The coefficient of variance (CV%) of each method was 2.22 % for micropipettes and 2.63 % for fluidics, demonstrating high reproducibility comparable to that of micropipettes.

To quantitatively verify the performance of the aliquoting structure, 2 μL was dispensed using pipette aliquoting and compared to fluidic aliquoting. The DNA at different concentrations is verified using qPCR in the next step (Fig. 3c). At 10^7 copies/mL of DNA, the average cycle threshold (C_t) value is 18.89 ± 0.11 for fluidic aliquoting and 19.05 ± 0.10 for pipette aliquoting, a difference of 0.16. At 10^6 copies/mL, the average C_t value is 22.88 ± 0.10 for fluidic aliquoting and 22.75 ± 0.16 for pipette aliquoting, a difference of 0.13. At 10^5 copies/mL of DNA, the average C_t value is 26.20 ± 0.13 for fluidic aliquoting and 26.36 ± 0.12 for pipette aliquoting, a difference of 0.16. In this way, a difference of 0.15 in the average C_t value is shown at various concentrations, corresponding to a difference of 0.15 cycles, and a quantitative difference of $2^{0.15}$ is approximately 11 %. This can be attributed to pipetting and equipment noise [45]. As a result, fluidic aliquoting using the fabricated fluidic cartridge demonstrated similar dispensing performance to pipette aliquoting, demonstrating that simple and accurate dispensing can be achieved using the integrated fluidic cartridge fabricated in this study.

3.4. Comparison with a commercial method for phylum-level microbiome analysis of mouthwash samples

To verify whether the analytical performance of the qPCR method in this study is comparable to that of commercial NGS method, it is first necessary to compare the pretreatment efficiency of the developed process and the commercial process in terms of the characteristics of the samples used, as pretreatment constitutes the initial step of the analysis. All samples analyzed consisted of purified DNA solutions, and verification of nucleic acid purity was considered essential to ensure robust

performance under realistic operating conditions. Accordingly, Fig. 4a presents the results demonstrating that samples extracted through the preprocessing process developed in this study exhibited nucleic acid purity similar to that obtained using a commercial nucleic acid extraction and purification kit (QIAamp DNA Mini Kit). No statistically significant difference in PCR efficiency was observed between the two methods (*t*-test, $p > 0.05$), confirming that the developed process achieves nucleic acid purity comparable to that of the commercial kit.

Based on this verification, to confirm the feasibility of PCR for microbiome analysis, the results of qPCR and NGS performed for microbiome analysis at the phylum level are compared and shown in Fig. 4b and Fig. 4c. Bacterial 16S rDNA is extracted from mouthwash samples collected from eight healthy volunteers through a sample treatment process, and qPCR and NGS were performed on the extracted samples. The primers used in qPCR are shown in Table S1 (Supplementary Material). Raw data using these primer sets, which are important for assessing reproducibility, are shown in Table S2. The relative abundance (%) of five major phyla (Actinomycetota, Bacillota, Bacteroidota, Fusobacteria, and Proteobacteria) in the oral microbiome of eight healthy individuals is compared, and it is found that the qPCR method is sufficient for phylum level microbiome analysis. Taken together, these results indicate that both the preprocessing and analytical workflows perform at levels equivalent to the commercial standard, supporting the feasibility of this method as a viable alternative.

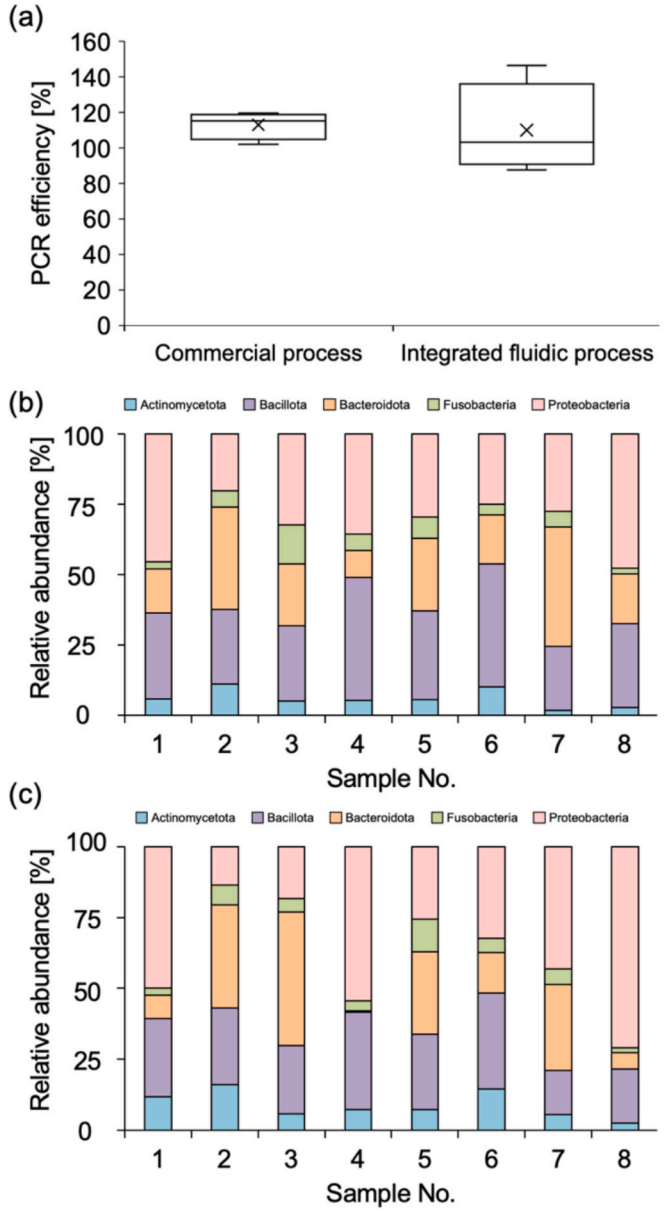


Fig. 4. Comparison of (a) PCR efficiency between commercial process and integrated fluidic process, the relative abundance of five major microbiomes at the phylum level using (b) NGS and (c) qPCR method.

3.5. Comparison result of the developed integrated fluidic platform with the commercial method for microbiome analysis of mouthwash sample at the phylum level

It is known that 96 % of the total bacteria present in the oral cavity of a healthy person are composed of *Bacillota*, *Bacteroidota*, *Fusobacteria*, and *Proteobacteria* [46,47]. In this study, we compared the characteristics of the oral microbial community between the integrated fluidic process—where DNA extracted using the integrated fluidic platform developed in this study was analyzed by qPCR—and the commercial process, in which DNA extracted with the QIAamp DNA Mini Kit, a commercial nucleic acid extraction and purification kit, was analyzed by NGS. The primers used for qPCR and the corresponding raw data are shown in Table S1 and S3. As shown in Fig. 5, we confirm that there is no difference between the two analysis methods at all phylum levels (*t*-test, *p*-value >0.05). In addition, in both methods, *Proteobacteria* appeared similarly as the phylum corresponding to the greatest proportion in most samples. Likewise, *Fusobacteria* made up the smallest proportion, confirming that the bacterial phylum corresponding to the lowest proportion is the same in both methods. Furthermore, both *Bacillota*, representing gram-positive bacteria, and *Bacteroidota*, representing gram-negative bacteria, show similar relative abundances between the commercial process and the integrated fluidic process developed in this study. Additionally, to perform a more detailed analysis based on microbial type, the communities analyzed in Fig. 5 were divided into gram-positive and gram-negative bacteria, and the results of the comparative analysis between processes are presented in Fig. S2. The relative abundance (%) of gram-negative bacteria was newly defined as the sum of the relative abundances (%) of Bacteroidota, Fusobacteria, and Proteobacteria, excluding Bacillota, which belongs to gram-positive bacteria. The relative abundance (%) for gram-positive bacteria was maintained as the relative abundance (%) of Bacillota. Based on this, comparison of the integrated fluidic platform process developed in this study with the commercial process (NGS) showed that the analytical patterns were highly similar between the processes, regardless of classification as gram-positive or gram-negative. This suggests that the developed platform does not exhibit performance variations based on microbial type. Accordingly, it demonstrates the potential to reliably process and analyze diverse microbiota regardless of their characteristics. These results indicate that the integrated fluidic platform developed in this study successfully analyzed the oral health status within 3 h and has a reliable level of reproducibility comparable to well established commercial methods, suggesting that it has the potential to replace or complement commercial methods in oral microbiome analysis.

3.6. Oral bacteria analysis of swab sample for canines and felines

To confirm the applicability of samples collected from oral swabs as well as mouthwash samples, oral samples from canines and felines were used. The oral conditions of 10 canines and 10 felines are evaluated by dividing them into periodontal disease and halitosis. Regarding periodontal disease, the veterinarian visually observed the disease and indicated the severity with a number (0 to 3). Halitosis was quantified (0 to 3) by the same veterinarian using measurement results obtained with a halitosis meter. These numbers are compiled to display a clinical score. The subjects are listed in order of severity of oral disease, and the results of analysis using qPCR are shown in Fig. 6. Bacteria related to periodontal disease are Aa, Pg, Tf, Td, En, and Cr, and bacteria related to

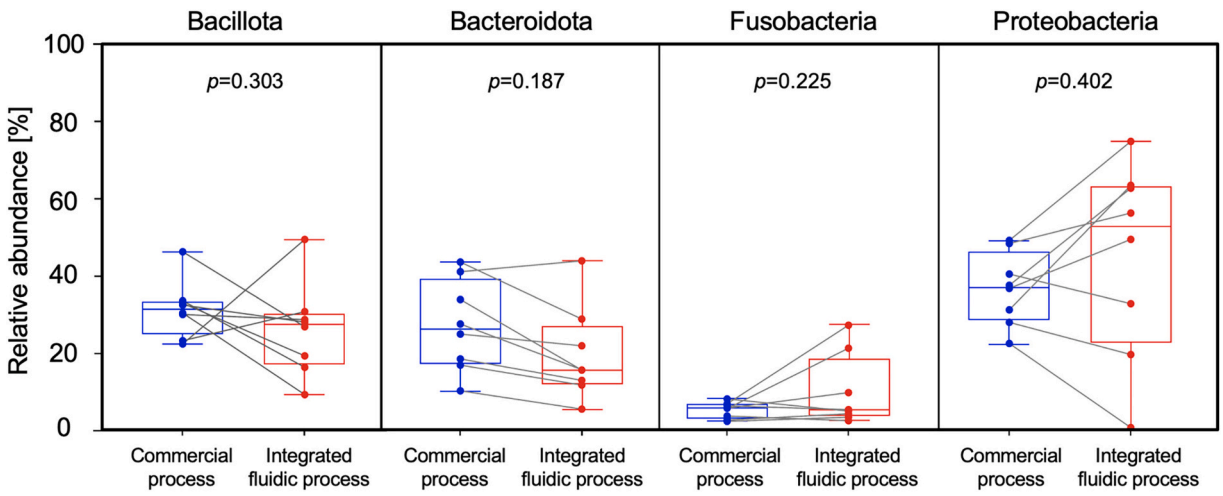


Fig. 5. Comparison of the relative abundance of four major microbiomes at the phylum level using the integrated fluidic process (DNA extracted using the integrated fluidic platform developed in this study and analyzed by qPCR) and the commercial process (DNA extracted using the QIAamp DNA Mini Kit, a commercial nucleic acid extraction and purification kit, and analyzed by NGS).

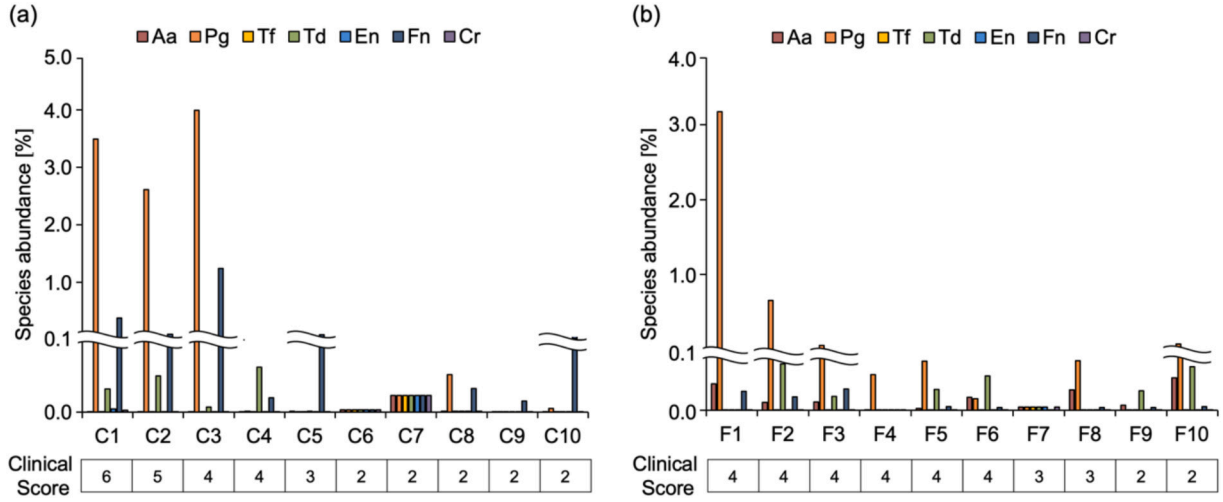


Fig. 6. Oral bacteria profiling using the integrated fluidic platform for the detection of periodontitis in companion animals; (a) canines and (b) felines.

halitosis are Pg, Tf, Td, and Fn [48,49]. The qPCR primer sets and their raw data is presented in Table S1, S4, and S5. Among the canines, individuals found to have both periodontal disease and halitosis (clinical score 4 or higher) have high Pg and Fn ratios. In the case of felines, the Pg ratio is measured to be relatively high. These results are similar to those obtained from oral condition diagnosis by veterinarians, suggesting that various types of oral samples can be applied to this fluidic platform. As the veterinarian's diagnostic method is expressed only as a score, it is not possible to immediately determine whether a specific bacterial species is present. However, the fluidic platform developed in this study can also confirm the diversity of bacterial species, allowing for the establishment of a customized treatment strategy accordingly.

4. Conclusion

Most microbiome research and the commercialization of that research, including the gut microbiome, are based on traditional sample treatment methods, results derived from NGS, and interpretation through group samples. However, when conducting longitudinal studies, such as personalized microbiome analysis or daily health monitoring, automated equipment that is fast and easy to use is essential.

To address this issue, we developed a sample treatment process (concentration and purification) and an automated aliquoting process, both of which are essential for automated analysis, using GO-coated beads. Through this integrated approach, we overcame the limitations of conventional GO-coated bead–based sample preparation methods and achieved performance comparable to that of one-pot NGS systems. In this study, mouthwash is used as a sample, and 2 μL of the solution is dispensed using fluidic technology to fit PCR 8-tube strips. However, this technology can be applied to various complex samples, such as feces and food, and the dispensing can be expanded to accommodate the type of marker to be measured. Furthermore, when using multiplex qPCR, the number of markers that can be analyzed simultaneously is likely to increase in diversity. Consequently, it is expected that complex nucleic acid-based analysis processes will be performed automatically, and fluidic based dispensing technology, rather than micropipette-type technology, will have a high possibility of expansion in the future.

However, comparison of the relative abundances obtained from the commercial process and the integrated fluidic process showed that many microbial taxa produced consistent results, while others exhibited different patterns. These outcomes suggest that sample-specific characteristics may influence the observed profiles, indicating that the current findings may have limited generalizability. In addition, we acknowledge

that the clinical sample sizes used in this study ($n = 8$ humans, $n = 10$ canines, and n = 10 felines), while acceptable for technical proof-of-concept, are limited for biological validation. In future work, we aim to increase the number of clinical samples and conduct large-scale validation to demonstrate the robustness of the proposed method. It is expected that this will further increase the usability of the integrated sample preparation and PCR analysis platform of this study.

CRediT authorship contribution statement

Hyejin Lee: Writing – original draft, Investigation. **Yun Guang Li:** Writing – original draft, Investigation. **Jiatong Kang:** Validation, Conceptualization. **Seung Won Shin:** Conceptualization. **Changyoon Baek:** Conceptualization. **Junhong Min:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbsr.2025.100931.

Data availability

No data was used for the research described in the article.

References

[1] J.J. Rajasekaran, H.K. Krishnamurthy, J. Bosco, V. Jayaraman, K. Krishna, T. Wang, K. Bei, Oral microbiome: a review of its impact on oral and systemic health, Microorganisms 12 (2024) 1797, https://doi.org/10.3390/microorganisms1209179.

[2] M. Kilian, I. Chapple, M. Hannig, P.D. Marsh, V. Meuric, A.M. Pedersen, M. S. Tonetti, W.G. Wade, E. Zaura, The oral microbiome – an update for oral healthcare professionals, Br. Dent. J. 221 (2016) 657–666, https://doi.org/10.1038/sj.bdj.2016.865.

[3] Y. Zhang, X. Wang, H. Li, C. Ni, Z. Du, F. Yan, Human oral microbiota and its modulation for oral health, Biomed. Pharmacother. 99 (2018) 883–893, https://doi.org/10.1016/j.biopha.2018.01.146.

[4] M. Lu, S. Xuan, Z. Wang, Oral microbiota: a new view of body health, Food Sci. Hum. Wellness 8 (2019) 8–15, https://doi.org/10.1016/j.fshw.2018.12.001.

[5] J.L. Baker, A. Edlund, Exploiting the oral microbiome to prevent tooth decay: has evolution already provided the best tools? Front. Microbiol. 9 (2018) 3323, https://doi.org/10.3389/fmicb.2018.03323.

[6] P.N. Deo, R. Deshmukh, Oral microbiome: unveiling the fundamentals, J. Oral Maxillofac. Pathol. 23 (2019) 122–128, https://doi.org/10.4103/jomfp.JOMFP_304_18.

[7] J.Y. Kim, K. Lee, M.G. Lee, S.J. Kim, Periodontitis and atherosclerotic cardiovascular disease, Mol. Cells 47 (2024) 100146, https://doi.org/10.1016/j.mocell.2024.100146.

[8] M.S. Zaman, S.M.G. Alam, M.S. Razzaque, Oral hygiene and cardiovascular health, Hygiene 5 (2025) 14, https://doi.org/10.3390/hygiene5020014.

[9] D.F. Gualtero, G.I. Lafaurie, D.M. Buitrago, Y. Castillo, P.K. Vargas-Sanchez, D. M. Castillo, Oral microbiome mediated inflammation, a potential inductor of vascular diseases: a comprehensive review, Front. Cardiovasc. Med. 10 (2023) 1250263, https://doi.org/10.3389/fcvm.2023.1250263.

[10] S. Yang, S. Zhang, Q. Cao, G. Zhu, J. Liu, G. Li, M. Zhu, Association between oral microbial diversity (only bacteria) and diabetes in U.S. adults: analysis of NHANES 2009–2012 data, BMC Oral Health 25 (2025) 837, https://doi.org/10.1186/s12903-025-06204-x.

[11] Y.R. Chang, W.C. Cheng, Y.C. Hsiao, G.W. Su, S.J. Lin, Y.S. Wei, H.C. Chou, H. P. Lin, G.Y. Lin, H.L. Chan, Links between oral microbiome and insulin resistance: involvement of MAP kinase signaling pathway, Biochimie 214 (2023) 134–144, https://doi.org/10.1016/j.biochi.2023.06.013.

[12] E. Read, M.A. Curtis, J.F. Neves, The role of oral bacteria in inflammatory bowel disease, Nat. Rev. Gastroenterol. Hepatol. 18 (2021) 731–742, https://doi.org/10.1038/s41575-021-00488-4.

[13] J.L. Pathak, Y. Yan, Q. Zhang, L. Wang, L. Ge, The role of oral microbiome in respiratory health and diseases, Respir. Med. 185 (2021) 106475, https://doi.org/10.1016/j.rmed.2021.106475.

[14] J. He, N. Mao, W. Lyu, S. Zhou, Y. Zhang, Z. Liu, Z. Xu, Association between oral microbiome and five types of respiratory infections: a two-sample Mendelian randomization study in East Asian population, Front. Microbiol. 15 (2024) 1392473, https://doi.org/10.3389/fmicb.2024.1392473.

[15] J. Wan, H. Fan, Oral microbiome and Alzheimer's disease, Microorganisms 11 (2023) 2550, https://doi.org/10.3390/microorganisms11102550.

[16] M. Saadaoui, P. Singh, S. Al Khodor, Oral microbiome and pregnancy: a bidirectional relationship, J. Reprod. Immunol. 145 (2021) 103293, https://doi.org/10.1016/j.jri.2021.103293.

[17] Z. Brookes, L. Teoh, F. Cieplik, P. Kumar, Mouthwash effects on the oral microbiome: Are they good, bad, or balanced? International Dental Journal 73 (Suppl. 2) (2023) S74–S81, https://doi.org/10.1016/j.identj.2023.08.010.

[18] H. Lu, P. Zou, Y. Zhang, Q. Zhang, Z. Chen, F. Chen, The sampling strategy of oral microbiome, iMeta 1 (2022) e23, https://doi.org/10.1002/imt2.23.

[19] X. Fan, B.A. Peters, D. Min, J. Ahn, R.B. Hayes, Comparison of the oral microbiome in mouthwash and whole saliva samples, PLoS One 13 (2018) e0194729, https://doi.org/10.1371/journal.pone.0194729.

[20] E. Zaura, V.Y. Pappalardo, M.J. Buijs, C.M.C. Volgenant, B.W. Brandt, Optimizing the quality of clinical studies on oral microbiome: a practical guide for planning, performing, and reporting, Periodontology 2000 (85) (2021) 210–236, https://doi.org/10.1111/prd.12359.

[21] G.L. Smith, C. Sansone, S.S. Socransky, Comparison of two methods for the small-scale extraction of DNA from subgingival microorganisms, Oral Microbiol. Immunol. 4 (1989) 135–140, https://doi.org/10.1111/j.1399-302x.1989.tb00240.x.

[22] J. Rosenbaum, M. Usyk, Z. Chen, et al., Evaluation of oral cavity DNA extraction methods on bacterial and fungal microbiota, Sci. Rep. 9 (2019) 1531, https://doi.org/10.1038/s41598-018-38049-6.

[23] F. Teng, S.S. Darveekaran Nair, P. Zhu, et al., Impact of DNA extraction method and targeted 16S-rRNA hypervariable region on oral microbiota profiling, Sci. Rep. 8 (2018) 16321, https://doi.org/10.1038/s41598-018-34294-x.

[24] F. Carbonero, G.M. Nava, A.C. Benefiel, E. Greenberg, H.R. Gaskins, Microbial DNA extraction from intestinal biopsies is improved by avoiding mechanical cell disruption, J. Microbiol. Methods 87 (2011) 125–127, https://doi.org/10.1016/j.mimet.2011.07.014.

[25] V. Wintzingerode, U.B. Göbel, E. Stackebrandt, Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, FEMS Microbiol. Rev. 21 (1997) 213–229, https://doi.org/10.1111/j.1574-6976.1997.tb00351.x.

[26] C. Baek, J. Min, Microvalve-assisted bead-beating system for selective nucleic acid preparation from bacteria and viruses, BioChip J. 9 (2015) 332–338, https://doi.org/10.1007/s13206-015-9409-7.

[27] W.-N. Lee, H.J. Yoo, H. Nguyen, C. Baek, J. Min, Semi-automatic instrumentation for nucleic acid extraction and purification to quantify pathogens on surfaces, Analyst 144 (2019) 6586–6594, https://doi.org/10.1039/C9AN00896A.

[28] C. Baek, Y.G. Li, H.J. Yoo, W.Y. Cui, J.T. Kang, J. Son, J. Min, Simple and portable on-site system for nucleic acid-based detection of Clostridium difficile in stool samples using two columns containing microbeads and loop-mediated isothermal amplification, Anal. Bioanal. Chem. 414 (2022) 613–621, https://doi.org/10.1007/s00216-021-03557-4.

[29] L.M. Petersen, I.W. Martin, W.E. Moschetti, C.M. Kershaw, G.J. Tsongalis, Third-generation sequencing in the clinical laboratory: exploring the advantages and challenges of nanopore sequencing, J. Clin. Microbiol. 58 (2019) e01315–e01319, https://doi.org/10.1128/JCM.01315-19.

[30] E. Caselli, C. Fabbri, M. D'Accolti, I. Soffritti, C. Bassi, S. Mazzacane, M. Franchi, Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture, BMC Microbiol. 20 (2020) 120, https://doi.org/10.1186/s12866-020-01801-y.

[31] D. Podbiel, F. Laermer, R. Zengerle, J. Hoffmann, Fusing MEMS technology with lab-on-chip: Nanoliter-scale silicon microcavity arrays for digital DNA quantification and multiplex testing, Microsystems & Nanoengineering 6 (2020) 82, https://doi.org/10.1038/s41378-020-00187-1.

[32] M. Keller, S. Wadle, N. Paust, L. Dreesen, C. Nuese, O. Strohmeier, R. Zengerle, F. von Stetten, Centrifugo-thermopneumatic fluid control for valving and aliquoting applied to multiplex real-time PCR on off-the-shelf centrifugal

thermocycler, RSC Advances 5 (2015) 89603–89611, https://doi.org/10.1039/C5RA16095B.
[33] G. Kim, Y.G. Li, Y. Seo, C. Baek, J.-H. Choi, H. Park, J. An, M. Lee, S. Noh, J. Min, T. Lee, Fabrication of graphene oxide-based pretreatment filter and electrochemical-CRISPR biosensor for the field-ready cyanobacteria monitoring system, Biosensors and Bioelectronics 237 (2023) 115474, https://doi.org/10.1016/j.bios.2023.115474.
[34] W.Y. Cui, H.J. Yoo, Y.G. Li, C. Baek, J. Min, Facile and foldable point-of-care biochip for nucleic acid based-colorimetric detection of murine norovirus in fecal samples using G-quadruplex and graphene oxide coated microbeads, Biosens. Bioelectron. 199 (2022) 113878, https://doi.org/10.1016/j.bios.2021.113878.
[35] H.J. Yoo, Y.G. Li, W.Y. Cui, W. Chung, Y.B. Shin, Y.S. Kim, C. Baek, J. Min, Discrimination and isolation of the virus from free RNA fragments for the highly sensitive measurement of SARS-CoV-2 abundance on surfaces using a graphene oxide nano surface, Nano Convergence 8 (1) (2021) 31, https://doi.org/10.1186/s40580-021-00281-8.
[36] N. Salzman, K. Hung, D. Haribhai, et al., Enteric defensins are essential regulators of intestinal microbial ecology, Nat. Immunol. 11 (2010) 76–82, https://doi.org/10.1038/ni.1825.
[37] C.B. Blackwood, A. Oaks, J.S. Buyer, Phylum- and class-specific PCR primers for general microbial community analysis, Appl. Environ. Microbiol. 71 (2005) 6193–6198, https://doi.org/10.1128/AEM.71.10.6193-6198.2005.
[38] M.A. Gorzelak, S.K. Gill, N. Tasnim, Z. Ahmadi-Vand, M. Jay, D.L. Gibson, Methods for improving human gut microbiome data by reducing variability through sample processing and storage of stool, PLoS One 10 (2015) e0134802, https://doi.org/10.1371/journal.pone.0134802.
[39] T. Iwase, M. Itano, Y. Yano, Primers for detecting Fusobacterium nucleatum by PCR methods and methods for detection, U.S. Patent Publication No. US 2004/0197849 A1, U.S. Patent and Trademark Office (2004).
[40] M. Mühling, J. Woolven-Allen, J. Murrell, et al., Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities, ISME J. 2 (2008) 379–392, https://doi.org/10.1038/ismej.2007.97.
[41] E.H. Kim, J.Y. Joo, Y.J. Lee, J.K. Koh, J.H. Choi, Y. Shin, J. Cho, E. Park, J. Kang, K. Lee, J. Bhak, B.C. Kim, J.Y. Lee, Grading system for periodontitis by analyzing levels of periodontal pathogens in saliva, PLoS One 13 (2018) e0200900, https://doi.org/10.1371/journal.pone.0200900.
[42] D. Kwon, K. Bae, H. Kim, S.H. Kim, D. Lee, J.H. Lee, Treponema denticola as a prognostic biomarker for periodontitis in dogs, PLoS One 17 (2022) e0262859, https://doi.org/10.1371/journal.pone.0262859.
[43] M. Hitzbleck, E. Delamarche, Advanced capillary soft valves for flow control in self-driven microfluidics, Micromachines 4 (1) (2013) 1–8, https://doi.org/10.3390/mi4010001.
[44] S. Wang, X. Zhang, C. Ma, S. Yan, D. Inglis, S. Feng, A review of capillary pressure control valves in microfluidics, Biosensors 11 (10) (2021) 405, https://doi.org/10.3390/bios11100405.
[45] A. Ruiz-Villalba, J.M. Ruijter, M.J.B. van den Hoff, Use and misuse of Cq in qPCR data analysis and reporting, Life 11 (2021) 496, https://doi.org/10.3390/life11060496.
[46] C. Bacali, R. Vulturar, S. Buduru, A. Cozma, A. Fodor, A. Chiș, O. Lucaciu, L. Damian, M.L. Moldovan, Oral microbiome: getting to know and befriend neighbors, a biological approach, Biomedicines 10 (2022) 671, https://doi.org/10.3390/biomedicines10030671.
[47] J. Yang, P. He, M. Zhou, S. Li, J. Zhang, X. Tao, A. Wang, X. Wu, Variations in oral microbiome and its predictive functions between tumorous and healthy individuals, J. Med. Microbiol. 71 (2022), https://doi.org/10.1099/jmm.0.001568.
[48] J.U. Choi, J.B. Lee, K.H. Kim, S. Kim, Y.J. Seol, Y.M. Lee, I.C. Rhyu, Comparison of periodontopathic bacterial profiles of different periodontal disease severity using multiplex real-time polymerase chain reaction, Diagnostics 10 (2020) 965, https://doi.org/10.3390/diagnostics10110965.
[49] K. Shinada, M. Ueno, C. Konishi, S. Takehara, S. Yokoyama, T. Zaitsu, M. Ohnuki, F.A. Wright, Y. Kawaguchi, Effects of a mouthwash with chlorine dioxide on oral malodor and salivary bacteria: a randomized placebo-controlled 7-day trial, Trials 11 (2010) 14, https://doi.org/10.1186/1745-6215-11-14.

Hyejin Lee received her bachelor's degree from Chung-Ang University (South Korea, 2024). She is currently studying for her master's degree in School of Integrative Engineering, Chung-Ang University.

Yun Guang Li received his master's degree from Chung-Ang University (South Korea, 2020). He is currently studying for his doctoral degree in School of Integrative Engineering, Chung-Ang University.

Jiatong Kang received his master's degree from Chung-Ang University (South Korea, 2022).

Seung Won Shin is currently an Assistant Project Scientist in the California Institute for Quantitative Biosciences at the University of California, Berkeley. He obtained his PhD from Sungkyunkwan University (SKKU, South Korea, 2017). He was a Postdoctoral Researcher at SKKU's Institute of Science and Technology (2017–2021) and a research professor in the School of Integrative Engineering at Chung-Ang University (2021−2023).

Changyoon Baek is currently Research Professor in the School of Integrative Engineering in Chung-Ang University (South Korea). She obtained her PhD from Chung-Ang University (South Korea, 2017).

Junhong Min is currently Professor in the School of Integrative Engineering in Chung-Ang University (South Korea). He obtained PhD from Sogang University (South Korea, 1998). He was a postdoctoral fellow in Cornell University (United States) (2000−2002). He worked for Innovative Biotechnologies in Grand Island, New York (United States) as a senior scientist (2002−2003) before being a project leader in Samsung Group (South Korea, 2003). He specialized in DNA/RNA purification/concentration, biosensor design, and nanomaterial application in advanced biotechnology and biochemical engineering.