

Lab on a Chip

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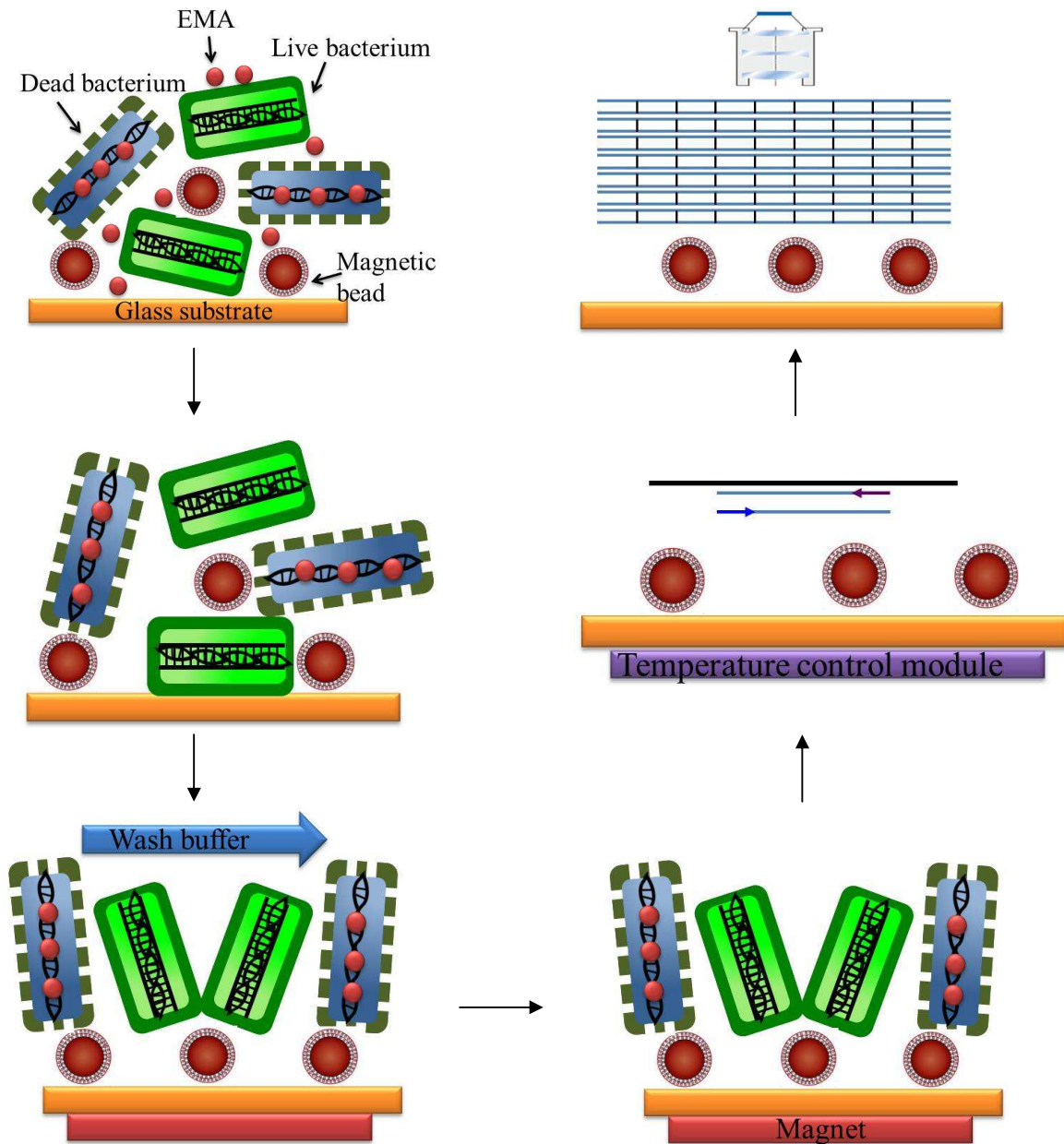
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An integrated microfluidic system capable of detecting live bacteria from clinical periprosthetic joint infection (PJI) samples within 55 minutes was developed in this study.



ARTICLE

Rapid isolation and diagnosis of live bacteria from human joint fluids by using an integrated microfluidic system

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Arthroplasty is a general approach for improving the life quality for patients with degenerative or injured joints. However, post-surgery complications including periprosthetic joint infection (PJI) poses a serious drawback to the procedure. Several methods are available for diagnosing PJI, but they are time-consuming or have poor sensitivity and specificity. Alternatively, reverse-transcription PCR can detect live bacteria and reduce false-positive results but cannot avoid the cumbersome RNA handling and human contamination issues. In response, an integrated microfluidic system capable of detecting live bacteria from clinical PJI samples within 55 minutes is developed in this study. This system employs an ethidium monoazide (EMA)-based assay and a PCR with universal bacterial primers and probes to isolate and detect only the live bacteria that commonly cause PJI. The experimental results indicated that the developed system can detect bacteria in human joint fluids with a detection limit of 10^4 colony formation unit/mL. Furthermore, nine clinical samples were analyzed using the microfluidic system. The results obtained from the microfluidic system were negative for all culture-negative cases, indicating that the proposed system can indeed reduce false-positive results. In addition, experimental results showed that the EMA sample pre-treatment process was crucial for successful detection of live bacteria. The culture-positive cases were diagnosed as positive by the proposed system only when the clinical samples were treated with EMA immediately after sampled from patients. Based on these promising results, the developed microfluidic system can be a useful tool to detect PJI and potentially be applied in other clinical situations.

Introduction

Arthroplasty has become an increasingly common approach for treating patients with degenerative or injured joints, rescuing them from immobility, and improving their quality of life. Due to an aging society, it is estimated that the number of patients requiring primary total knee arthroplasty may reach 3.48 million by the year of 2030¹. The number of periprosthetic joint infection (PJI), one of the most devastating complications of joint arthroplasty, is therefore forecasted to increase accordingly. Symptoms of PJI include joint pain, effusion, erythema, warmth at the implant site or draining sinus², and in severe cases, PJI may cause systemic sepsis. In addition to the discomforts and high reoccurrence rates, PJI is difficult to treat and may result in immense financial costs³.

To minimize the recurrence of PJI, hospitals worldwide commonly adopt a two-stage reimplantation process for performing arthroplasty, which consists of extensive debridement at the first stage followed by delayed reimplantation⁴. The timing of the second stage is crucial for the success of two-stage reimplantation. The delayed reimplantation can only be performed after confirming that there is no existence of live bacteria so that the prosthesis will not be implanted into an organ harboring live bacteria⁵. Therefore, the determination of infection and its eradication are critical before performing reimplantation procedure. There are several indicators used in clinical practices to detect the existence of live bacteria. Among those, culture of joint aspirates remains the gold standard for definitive diagnosis⁶. However, the culture assay has several drawbacks because it is time-consuming (3-7 days) and may be hampered by false-negative results if the infection is suppressed by antibiotics or the sampling is not representative⁷. Gram-staining, the erythrocyte sedimentation rates, C-reactive protein level, histopathological examination, white blood cell counts, radiographs and nuclear bone scans are commonly used to assess infection⁸⁻⁹. However, they have different intrinsic disadvantages such as poor specificity, poor sensitivity, or complicated, error-prone processes. Furthermore, these methods can only provide indirect information for the critical decision-making of the timing of the second-stage surgery. Therefore, a method with high sensitivity, direct detection of live bacteria, and time-saving is of great need for PJI treatment.

Recent advances in molecular diagnostics has improved the diagnosis of PJI. This is because the high sensitivity of molecular diagnostics can reduce false-positive detection obtained from conventional detection methods. One of the most popular methods for molecular diagnosis, polymerase chain reaction (PCR), has demonstrated the capability to detect infection in synovial fluid and intraoperative periprosthetic tissue samples¹⁰⁻¹⁵. However, these deoxyribonucleic acid (DNA)-based PCRs cannot distinguish live or dead bacteria because DNA remains intact after bacteria death. Patients carrying dead bacteria may therefore receive unnecessary antibiotic treatment due to the false-positive results. To overcome this problem, a 16S ribosome ribonucleic acid (rRNA)-based reverse transcription (RT)-PCR process to detect the existence of live bacteria was reported, as rRNA could be degraded shortly after bacteria death¹⁶. 16S rRNA-based RT-PCR indeed overcomes the problem of false-positive

65 result arising from dead bacteria. Unfortunately, handling and storage of RNA-bearing samples and RT-PCR are comparatively complicated and more susceptible to human-errors, which may cause biases to diagnostic results. Finally, all PCR-based detection methods require sample purified and nucleic acid extraction to minimize PCR inhibition from enzymes in joint fluids.

To solve the disadvantages from current PCR and RT-PCR-based detection methods, this work reports an integrated microfluidic system that incorporate an ethidium monoazide (EMA)-based assay, magnetic beads coated with vancomycin and a PCR with a primer set for the conserved gene region of bacteria to rapidly detect only live bacteria from clinical joint fluid samples for effective diagnosis of PJI. EMA, a DNA staining dye, was employed in this study because it can penetrate into the broken cell walls of dead bacteria and intercalate into DNA¹⁷. The irreversible intercalation prevents DNA from denaturation and stops it from serving as templates in subsequent PCR. Thus, only DNA of live bacteria will be amplified during the PCR process. Additionally, the cumbersome RNA handling issue can be alleviated. In this study, magnetic beads coated with vancomycin were used as a universal probe to capture bacteria from joint fluid because vancomycin can bind to cell wall of both Gram-positive and Gram-negative bacteria¹⁸⁻²⁰. The time and processes of nucleic acid extraction and purification therefore can be significantly reduced. Importantly, realizing these assay steps on an integrated microfluidic system – a technology that has made substantial impacts to the field of molecular diagnosis²¹ – can greatly reduce the risk of human error and contamination while speeding up the analysis and reducing sample and reagent consumption.

In this work, bacteria commonly found in PJI were first tested⁹. The limit of detection (LOD) was then determined by using bacteria spiked clinical joint fluid samples. Furthermore, nine clinical samples were diagnosed using the established experimental protocol to validate the feasibility of the proposed system. The experimental data demonstrated that the proposed system can detect live bacteria in clinical joint fluidic samples down to clinically relevant 10⁴ colony forming units (CFU)/mL, and hence diagnose PJI with minimal false positive and false negative, within 55 minutes, which is rapid enough for clinicians to avoid implanting a prosthesis into an infected joint. More importantly, this is the first time that an integrated microfluidic system has been used to detect live bacteria in joint-fluid clinical samples. The promising results indicate the proposed system could be a useful tool to assist clinicians for surgical decision-making in the near future.

Materials and methods

Experimental procedure

115 The experimental procedure of the proposed system is schematically shown in Figure 1. The left column of Figure 1 illustrates the experimental procedure and the right column demonstrates how the experimental procedure works on the integrated microfluidic chip. Ten 120 μL of vancomycin-coated magnetic beads, 50 μL of wash buffer and 20 μL of PCR reagents were first pre-loaded into their corresponding chambers before performing the live bacteria detection on the microfluidic chip. Then, 20 μL of samples incubated with EMA were loaded into 125 reaction chambers and exposed with visible light to trigger the EMA reaction (Figure 1(a)). Bacteria were

simultaneously captured by vancomycin-coated magnetic beads in this step (Figure 1(b)). After incubation for 10 minutes, a magnet was placed underneath the microfluidic chip to collect bacteria-bead complexes (Figure 1(c)). In the purification step, the wash buffer was pumped into the reaction chambers by the activation of transportation micro-pumps and normally-closed micro-valves to wash out PCR inhibitors (Figure 1(d)). Subsequently, the PCR reagents were pumped into the reaction chambers and the temperature of the microfluidic chip was regulated by a temperature control module of the integrated system to perform PCR (Figure 1(e)). The bacteria were then disrupted during a high temperature of 95°C during PCR and the DNA released from those live bacteria was amplified. After 25 PCR cycles, the fluorescence emitted from the PCR products were detected and analyzed (Figure 1(f)). Note that the whole process could be performed within 55 minutes automatically by using the integrated microfluidic system, which is faster when compared with the traditional process (115 min)¹⁶.

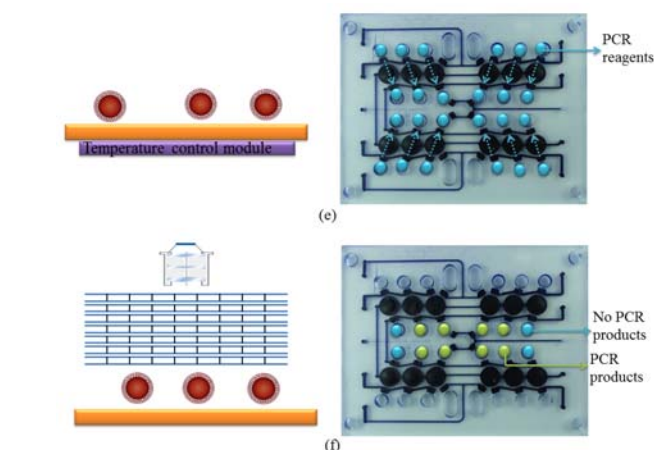
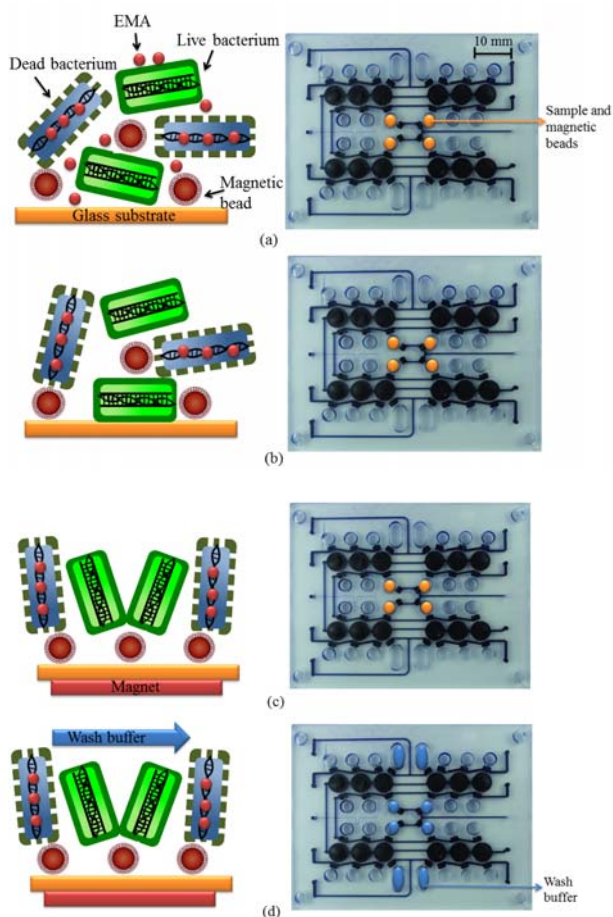


Figure 1. Experimental procedure performed on the integrated microfluidic system for live bacteria detection. (a) EMA treatment, (b) bacteria pre-concentration, (c) bacteria-magnetic beads complexes collection, (d) bacteria purification, (e) PCR, (f) fluorescent signal detection.

Chip design and fabrication

A microfluidic chip was developed to perform above-mentioned procedure, which is illustrated in Figure 2(a). Four identical modules that can be precisely and simultaneously controlled were incorporated in this microfluidic chip to perform four parallel reactions. Each module contains three reagent chambers, one wash buffer chamber, normally-closed valves, transportation units (micro-pumps), one reaction chamber and also one positive control chamber and one negative control chamber. Ten air via-holes were used to supply required air pressure to control the activation of valves and membranes. With these micro-components, the transportation of liquid is programmable and human intervention during the diagnosis process can be avoided. A video demonstrating the automatic transportation of liquid is shown in Electronic Supplementary Information (ESI).

In Figure 2(a), the blue line in the middle indicates the region for deploying the magnet and collecting magnetic beads in the reaction chambers. The red line in the middle indicates the region of the temperature control module of the integrated system. The chambers within this region are subject to temperature cycling so that PCR can be performed. The region is also where fluorescence signal is detected. The optical detection module of the integrated system will collect the fluorescent signals from these twelve chambers and analyze the results.

The photographs of the fabricated chip are shown in Figures 2(b) and 2(c). The microfluidic chip was composed of one bottom layer, one upper layer and one glass substrate, which were bonded by an oxygen plasma treatment²². The dimensions of the microfluidic chip were 74 x 58 mm (length x width).

The microfluidic chips used in this study were massively produced by Jabil Circuit Inc., Ltd., Taichung, Taiwan via a hot-embossing fabrication process. The microfluidic chip developed in this study is an improved version of our previous work²². In addition to the improvements of the micro-components configuration, the production method and the materials of the microfluidic chip were fine-tuned such that they could be mass-produced. Importantly, two micro-structures were re-designed in this work to ease the fabrication process and improve the device performance.

The first micro-structure is the roughened surface of the normally-closed valves, as shown in Figure 3(a). The results measured from a surface profilometer (VK-9700, KEYENCE Corp., Japan) indicated that the average surface roughness was 3.04 μm (Figure 3(b)). The operation of the normally-closed valves and the importance of the rough surface were schematically shown in Figure 3(d). First, the liquid was loaded in the left chamber. Then, the normally-closed valve and the thin membrane of the transportation unit were elevated by the suction force caused by vacuum. Next, another normally-closed valve was elevated. Therefore, a fixed volume of liquid was transported into the right chamber. Next, a compressed air was supplied to squeeze the thin membrane to push all liquid into the right chamber. Therefore, the operation of the normally-closed valve depends on flexible de-attachment of the structure. To prevent permanent bonding of the normally-closed valve to the glass substrate, a manual masking step was performed prior to the oxygen plasma bonding treatment²³. The rough surface of the normally-closed valve obviates the unreliable manual masking process and effectively prevents the valve from bonding together with the glass substrate during the oxygen plasma treatment.

Another new micro-structure proposed in this work is quantification pillars placed above the thin membrane of the transportation unit (Figure 3(c)), which served to improve the consistency of the transported volume among different chambers. The activation process with quantification pillars in the liquid transportation unit was illustrated in Figure 3(e). The amount of the transported liquid depends on the height of thin membrane elevation, which is difficult to control because the air pressure applied in each chamber sometimes differs. With the aid of the quantification pillars, thin membranes of the transportation unit will stop once it touches the quantification pillars so that the level of thin membrane elevation can be regulated and the consistence of the transported volume among different chambers could be then improved.

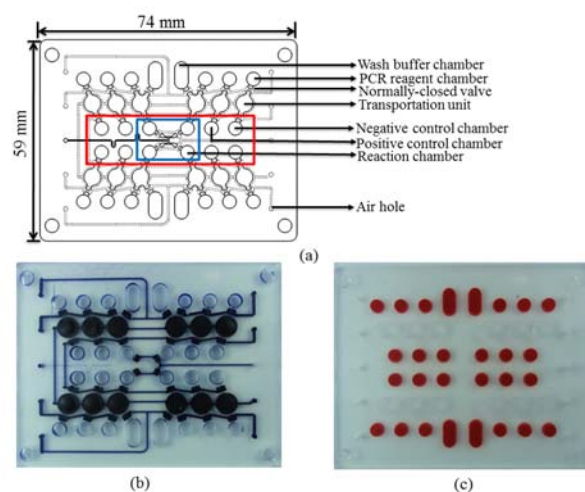


Figure 2. (a) The illustration, (b) the photographs of the bottom layer and (c) the upper layer of the microfluidic chip.

Preparation of EMA

EMA ($\text{C}_{21}\text{H}_{18}\text{BrN}_5$) purchased from Molecular Probes® (USA) was dissolved in ethanol to prepare 1 mg/ml stock and diluted by ethanol to create desired concentration before performing the diagnosis assay. Note that EMA may be harmful if swallowed or inhaled. There is no available report showing EMA has carcinogenic or mutagenic. However, it is one of the ethidium derivatives and therefore should be used with caution. Note that EMA should be stored at -20°C and kept from light exposure.

Preparation of vancomycin-conjugated magnetic beads

Magnetic-capture of bacteria during the purification step is achieved via vancomycin (Sigma-Aldrich Co. LLC., USA) coated magnetic beads (Dynabeads® MyOne™ Carboxylic Acid, Invitrogen Corp., USA). To prepare the beads, 30 μL of 100 nM vancomycin in phosphate buffered saline (PBS) and 950 μL of magnetic beads in PBS (4×10^6 beads/mL) were incubated with 20 μL of 120 mg/mL 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in distilled water, hydrochloride (Invitrogen Corp., USA) at 4°C overnight. After twice-washing with 1 mL of 0.02% Tween 20 in PBS and 1 mL of 0.1% sodium dodecyl sulphate in PBS for 3 minutes each, the magnetic surface was blocked with 0.1 M, ethanol amine in distilled water (Sigma-Aldrich Corp. LLC, USA). Then, 1 mL phosphate buffered saline (PBS) was used to wash out the residual ethanol amine and the magnetic beads were suspended in 1 mL PBS and stored at 4°C until use.

PCR primers and PCR reaction

The PCR primer set used in this study is a universal primer set that targets a conserved region of bacterial 16S rRNA gene (forward 5'- attagataacctgtagtccacgcc-3'; reverse 5'-cgctatccccacctcctcc- 3'), which was reported to detect many types of bacteria from PJI¹⁶. KAPA SYBR FAST kit (KAPABIOSYSTEM, USA) was used as PCR reagents. The 20- μL mixture for each reaction contained 10 μL of KAPA SYBR FAST PCR master mix, 0.4 μL 1

of 10 μM forward primer, 0.4 μL of 10 μM reverse primer, and 9.2 μL of distilled water. The thermal-cycling conditions were 95°C for 5 minutes, followed by 25 cycles of 95°C for 10 seconds, 62°C for 20 seconds and

5 72°C for 10 seconds. The PCR resulting DNA fragments in this study were approximately 400 base-pairs (bp) in length.

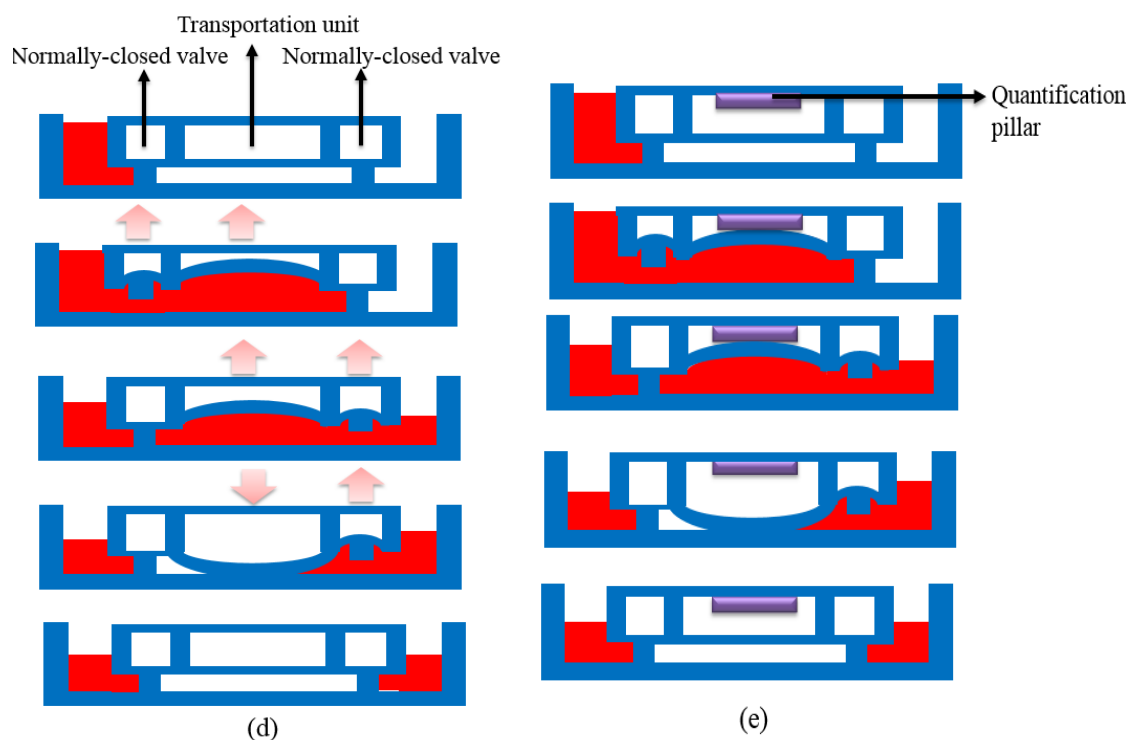
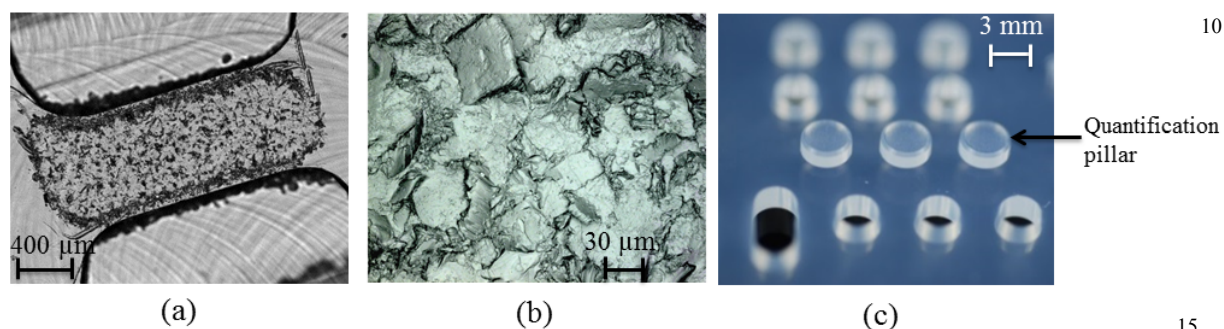


Figure 3. The micro-structures of the microfluidic chip; (a) The rough surface of the normally-closed micro-valve, (b) the surface roughness results from a surface profilometer, (c) the quantification pillars, (d) a side view of the original activation process for liquid transportation, (e) a side view of activation process for liquid transportation with quantification pillars.

Preparation of dead bacteria

Overnight bacterial culture heated at 95°C for 20 minutes was used as dead bacteria. Eighty μL of the heated bacteria culture was subject to the microfluidic assay and 40 μL of the heated bacteria culture was plated on the blood agar plate (BD, USA) to verify bacteria death. The plate without bacteria growth was confirmed as dead bacteria.

Positive control construction

PCR products amplified from *Escherichia coli* (*E. coli*) by the 16S rRNA primer set were used to create positive-control samples for reaction quality confirmation. The PCR product was cloned into pCR®2.1-TOPO® TA vector per manufacturer's instruction (TOPO TA Cloning®, Invitrogen Corp., USA). The cloned plasmid was confirmed by DNA sequencing. In addition, the cloned plasmids were successfully used as the target in subsequent PCR reactions using the 16S rRNA primer set. The results are shown in Supplemental Information (Figure S1), which

confirmed the utility of this plasmid as the positive control target.

Slab-gel electrophoresis

5 Slab-gel electrophoresis was used to confirm the SYBR Green-based fluorescent read-out for PCR reactions. Two grams of Low EEO agarose (FocusBio, Taiwan) were dissolved in 100 mL tris/borate/ethylenediamine-tetraacetic acid (TBE) buffer (Amresco, USA) and resulted in 2% agarose gel. 10 Five μL of the reaction mixtures containing DNA fragments were loaded into the agarose gel along with 1 μL DNA loading dye. Then, 100 V was applied for 30 minutes. Then, 100 V was applied for 30 minutes. 15 Ethidium bromide ($\text{C}_{21}\text{H}_{20}\text{BrN}_3$, 50 mg/mL, Sigma, USA) was used for gel staining in order to visualize DNA under ultraviolet exposure (302 nm, UVTM transilluminator, UVP, Canada). Note that ethidium bromide is a frameshift mutagen and should be used with extra caution. For measuring the size of the resulting DNA fragments, a 100-bp DNA ladder (DM003-R500, GeneDireX, USA) was used as a reference.

Clinical specimen

25 Clinical human joint fluid samples used in this study were obtained under the approval of Chang Gung Memorial Hospital Institutional Review Board (IRB number: 10134080A3 and 1031168C1).

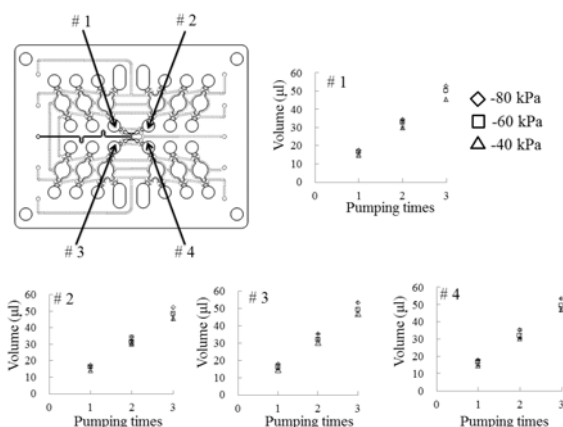
Statistical analysis

30 Two-tailed Student's *t*-test was used to perform all statistical analyses in this study and $p < 0.05$ was considered as statistically significant.

35 Results and discussion

The pumping rate of the transportation units

The pumping rate of the four transportation units on the microfluidic chip was first tested to verify if the new quantification pillars under the thin membrane of the transportation unit can improve the consistency of the amount of transported sample. The results were shown in Figure 4. As expected, the higher the negative force was provided, the more sample was transported from the reagent chamber into the reaction chamber. The maximum transported volume was measured to be 51 μL when a gauge pressure of -80 kPa was applied. The maximum volume needed to be transported on this microsystem is 50 μL of wash buffer. Therefore, three pulses under -80 kPa were sufficient to move the wash buffer. 45 Furthermore, two-tailed Student's *t*-test was used to analyze the transported volumes of chambers #1, #2, #3 and #4 and the statistical results showed no significant difference among each chamber ($p > 0.05$). These results demonstrated the consistency of the pumping rates of different transportation units, which were important in programming the automatic liquid transportation on the microfluidic chip and performing parallel diagnostic tests.



60 **Figure 4.** Consistency of pumping rates of different transportation units on the microfluidic chip.

Optimization of EMA pre-treatment of live bacteria diagnostic assay

65 The optimal condition for live bacteria detection from joint fluid samples was explored in this study. First, the EMA concentration required to prevent dead bacteria from producing PCR amplicons was determined. Twenty μL of dead *E. coli* at 10^6 colony formation units (CFU)/mL were spiked into an un-infected joint fluid sample and treated with 1 μL of EMA of different concentrations ranging from 1 mg/mL to 10 μg mL. After exposure under visible light (18 W, 1200 lm) for 30 minutes, the samples were PCR-amplified on the integrated microfluidic system. The results were analyzed by slab-gel electrophoresis, as shown in Figure 5(a). The results indicated that an EMA concentration of 1 mg/mL EMA effectively inhibited the PCR reaction. This concentration of EMA is comparable to that previously reported for detection of live *E. coli* in bacterial liquid culture (0.5 mg/mL)²⁴. Therefore, 1 mg/mL EMA was chosen as the experimental condition of the developed system.

85 In addition to the EMA optimal concentration, the optimal exposure time under visible light was also explored and the results were shown in Figure 5(b). Experimental data showed that 1-min light exposure was insufficient, as both live and dead *E. coli* could still function as PCR templates. However, after 5 minutes of visible light exposure, only the live *E. coli* could be amplified. The results indicated that the optimal exposure time was 5 minutes in the proposed system. Furthermore, in Figure 5(c), five bacteria genera commonly observed in PJI were tested in the microfluidic system and the electrophoretic results demonstrated all bacteria could be detected successfully. Of note, some groups reported using 650-W halogen light as the light source to trigger the cross-link reaction of EMA and DNA²⁴⁻²⁶, which could be completed in 1-2 minutes. Alternatively, another group employed visible light as the light source to simplify the requirements of instruments, but at the expense of lengthy exposure time (15-30 minutes)²⁶. In this study, the exposure time of the visible light was reduced to 5 min, which greatly shortened the overall assay time.

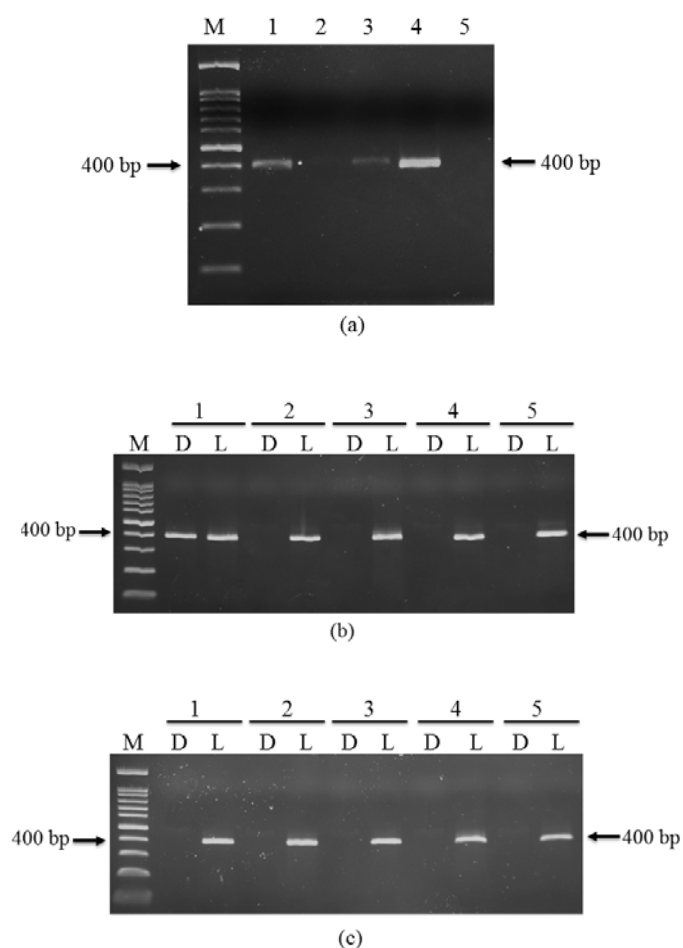


Figure 5. Optimization of EMA pre-treatment. (a) Optimization of the EMA concentration for preventing PCR from dead *E. coli* in un-infected joint fluid samples. M: 100-bp ladder; Lane 1: the PCR products from cloned plasmid without the EMA treatment as a positive control; Lane 2: the PCR products of dead *E. coli* treated with 1 mg/mL EMA; Lane 3: the PCR products of dead *E. coli* treated with 100 µg/mL EMA; Lane 4: the PCR products of dead *E. coli* treated with 10 µg/mL EMA; Lane 5: the PCR products of a negative control using distilled water. (b) Optimization of the visible light exposure time to trigger EMA interchelation. M: 100-bp ladder; D: dead *E. coli*; L: live *E. coli*; 1: 1-min exposure; 2: 5-min exposure; 3: 10-min exposure; 4: 20-min exposure; 5: 30-min exposure. (c) General applicability of using the EMA assay and PCR to detect live bacteria commonly cause PJI. M: 100-bp ladder; D: dead bacteria; L: live bacteria; 1: MRSA; 2: Coagulase negative Staphylococcus; 3: *Staphylococcus aureus*; 4: *Enterococcus* sp.; 5: *Pseudomonas syringae*.

The limit of detection of the proposed system

The limitation of detection of the proposed system was also investigated in this study. Here, 10^6 - 10^2 CFU/mL of *E.*

coli were spiked into un-infected joint fluid samples and used as the target of the proposed microfluidic system. The fluorescent results from the microfluidic system, as shown in Figure 6 (a), indicated that the proposed system could successfully detect 10^6 - 10^4 CFU/mL live *E. coli* from joint fluid samples. These results were confirmed by slab-gel electrophoresis. Although PCR products were observed from 10^3 CFU/mL of *E. coli* in the gel image, the band was very faint. Statistical analyses of the fluorescence between lane 5 (10^3 CFU/mL) and lane 1 (negative control) also indicated a lack of significant difference. Therefore, the detection limit of the proposed system was determined to be 10^4 CFU/mL. 10^4 CFU/mL is one order of magnitude worse than the detection limit of the previous study using the same PCR primer set¹⁶, which might be due to the fact that some lost *E. coli* during the vancomycin capturing process. On the other hand, another previous work using EMA-PCR to detect live *E. coli* in bacterial liquid culture by the conventional system showed a similar detection limit of 10^4 CFU/mL²⁴. PJI could be diagnosed if fluid culture has a bacteria number higher than 100 CFU²⁵. The detection sensitivity of the proposed system was found to be 200 CFU (10^4 CFU/mL, 20 µl), which is comparable to this PJI diagnosis criterion and is therefore useful for clinical purpose.

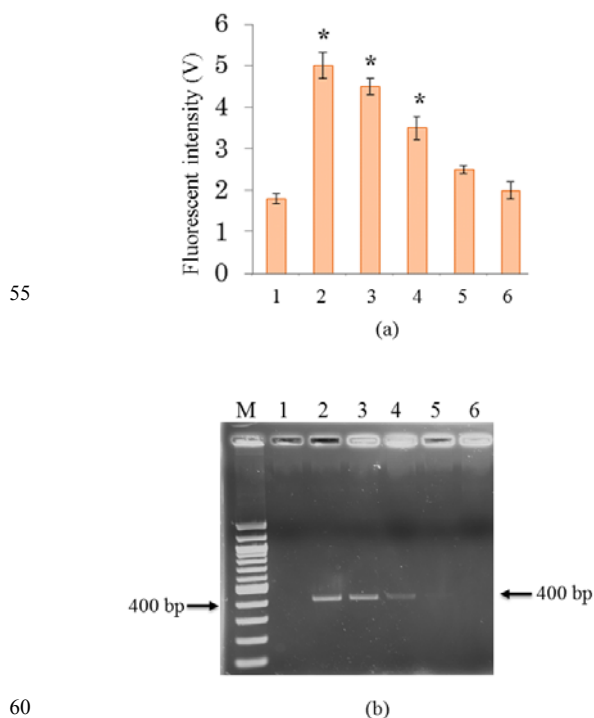


Figure 6. The limit of detection of the proposed system. (a) The fluorescent results from the integrated microfluidic system, (b) the results of slab gel electrophoresis used to confirm the fluorescent results. M: 100-bp ladder; Lane 1: the PCR products of the negative control using distilled

water; Lanes 2-6: the PCR products of 10^6 - 10^2 CFU/mL of *E. coli* spiked into joint fluid samples (* $p < 0.05$).

Clinical specimen tests

5 Nine clinical patient samples were tested with the
microfluidic assay to evaluate the occurrence of PJI.
According to the standard pathological diagnosis, six cases
were verified as PJI positive and another three cases were
classified as non-infective (Table 1). In the non-infection
10 cases, the microfluidic results were all negative for infection.
This indicated that the proposed system can avoid the false-
positive results of the conventional method. For the 6
confirmed PJI cases, the microfluidic results showed
positive for infection only when the joint fluid samples were
15 treated with EMA immediately after sampled from patients
(cases no. 8 and no. 9). This is presumably because live
bacteria can only stay alive for a short period of time after
sampled from patients, and it is therefore critical to perform
EMA treatment while they are still alive. Furthermore,
20 storage at -20°C without anti-freeze agents may disrupt the
cell membrane of bacteria so that the EMA treatment after
freezing will make no template for PCR. On the other hand,
when the joint fluid samples were treated with EMA
immediately after sampling from patients and before -20°C
25 storage, when the bacteria were still live, the microfluidic
assay can detect live bacteria from the joint fluid samples
successfully. This indicated that the microfluidic assay is
promising to be used at bed side in the near future to avoid
the false-negative results rising from sample processing and
30 treatment. Moreover, the microfluidic results are consistent

with that reported by using a quantitative RT-PCR assay²⁸
showing that the proposed system might be a potential tool
for PJI diagnosis in the near future.

Conclusions

35 Accurate diagnosis of PJI is crucial for two-stage
reimplantation procedure. The conventional methods for
PJI diagnosis are time-consuming, and lack sensitivity
and specificity, thus preventing them from assisting
clinicians in making critical decisions during surgeries.
40 In this study, a new microfluidic system was developed
for rapid and accurate diagnosis of PJI. With this
integrated microfluidic system, the diagnosis procedure,
which included live bacteria identification by EMA (5
minutes), bacteria isolation by magnetic beads coated
45 with vancomycin (10 minutes), PCR (about 35-40
minutes) and fluorescent signal detection, can
be completed within 55 minutes in an automatic manner.
The LOD of this developed system was measured to be
 10^4 CFU/mL, which is comparable to benchtop EMA-
50 PCR assays. The rapid diagnostic time makes it possible
to make more accurate surgical decisions during the
operation process. More importantly, this is the first
time that an integrated microfluidic system was applied
to detect live bacteria from joint infection samples
55 without using RNA so that complicated RNA handling
can be avoided. With the data of clinical samples, the
developed system can be a promising tool for PJI
diagnosis in the near future.

Table 1 Clinical data of patients with suspected infection status

Case	Age	Sex	Clinical data				PJI	Culture†	Sample		Molecular diagnosis	
			Fever	The erythrocyte sedimentation rate (mm/hr)	C-reactive protein (mg/mL)	Fistula			Processing	Storage	quantitative RT-PCR	Microfluidic
1	39	f	+	61	19.6	-	yes	Staph. lugdunensis	4°C overnight	-20°C	negative	negative
2	41	m	+	89	28.4	+	yes	Staph. lugdunensis	4°C overnight	-20°C	negative	negative
3	75	f	-	26	2.6	-	no	NG	4°C overnight	-20°C	negative	negative
4	67	f	-	67	5.9	-	no	NG	4°C overnight	-20°C	negative	negative
5	45	m	-	39	7.8	-	no	NG	4°C overnight	-20°C	negative	negative
6	61	m	+	67	56.5	-	yes	Staph. epidermidis	-20°C overnight	-20°C	negative	negative
7	35	m	+	57	25.7	-	yes	Staph. caprae	-20°C overnight	-20°C	negative	negative
CONS												
8	17	m	+	66	88.6	+	yes	Staph. epidermidis	Immediate	-20°C	positive	positive
										4°C	positive	positive
										RT‡	positive	positive
9	71	m	+	106	96.8	+	yes	Enterob. aerogenes	Immediate	-20°C	positive	positive
										Acinetob. pittii		

† Staph. = Staphylococcus; NG = No growth; CONS = Coagulase negative Staphylococcus; Enterob. = Enterobacter; Acinetob. = Acinetobacter

‡ RT = Room temperature

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