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ARTICLE

An Integrated Microfluidic Device Utilizing Dielectrophoresis and Multiplex Array PCR for Point-of-Care Detection of Pathogens†

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Early identification of the causative pathogens in physiological specimens that require no cultivation is essential for directing an evidence-based antimicrobial therapy in resource limited settings. Here, we describe an integrated microfluidic device for rapid identification of pathogens in complex physiological matrices such as blood. The device was designed and fabricated using SlipChip technologies, which integrated four channels processing independent samples and identifying up to twenty different pathogens. Briefly diluted whole human blood samples were directly loaded into the device for analysis. Pathogens were derived from blood by dielectrophoresis, retained in the array of grooves, and identified by multiplex array PCR in nanoliter volumes with end-point fluorescence detection. The universality of dielectrophoretic separation of pathogens from physiological fluids was evaluated with a panel of clinical isolates covering predominant bacterial and fungal species. Using this system, we simultaneously identified *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 within 3 hours. In addition to the prompt diagnosis of bloodstream infections, this method may be also utilized for differentiating microorganisms in other physiological and environmental samples.

Introduction

Bacteria and fungi can enter normally sterile human body sites such as peritoneum,[1] blood[2] and cerebrospinal fluid[3] and cause severe diseases.[4] For example, exogenous bacteria in blood can initiate sepsis, a life-threatening disease killing approximately 24,000 people each day.[2] The accurate and timely diagnosis is one of major challenges for saving lives from infection diseases. Currently, culturing samples from the infected sites remains the gold standard for identifying bacterial and fungal infections, and also brings the possibility of determining antibiotic susceptibility.[5] However, current culture-dependent tests are impaired due to the delay in diagnosis and high incidence of false-negative yields. In remote regions without access to clinical microbiology laboratories, storing and transporting clinical samples will further reduce or delay the yields with positive cultures. Therefore, it is essential to develop new approaches for point-of-care testing (POCT) of infectious diseases.

Extensive research interests and efforts have been aroused in molecular and other non-culture based methods for addressing the unmet needs in early diagnosis of infectious pathogens in clinical samples including blood.[5] Among various methods, Polymerase Chain Reaction (PCR) is the most sophisticated technique, which amplifies and detects specific nucleic acid sequences from cells, with potential to speed up the detection of infectious pathogens with very high specificity and sensitivity.[6] However, currently available PCR tests rely on rigorous sample preparation procedures, which prevent them to be used in resource limited settings.

Motivated by further improving portability and accessibility for POCT, various microfluidic devices which integrated miniaturized PCR reactors into miniaturized devices have emerged in recent years.[7-13] However, several important issues have to be addressed before we could practically use these devices. First, clinical samples can be very complex in terms of constituents, biochemical and rheological properties. For example, one microliter blood contains 4 to 6 billion erythrocytes and numerous kinds of proteins, but the concentration of bacteria in the early stage of sepsis can be as low as 1 to 100 CFU/mL. Therefore, integration of simple, high efficient and high throughput sample preparation methods on-chip is the key benefit of device-based system to compare with conventional PCR techniques.[7] Second, most devices have been designed for specific detection of single pathogen species from normally sterile clinical samples,[7, 14] which is not adequate since a broad range of bacteria and fungi may be present in

many infections. A realistic solution to this issue is using multiplex array PCR (maPCR) to identify multiple candidate pathogens simultaneously using an array of PCR reactors.[15] Finally, to develop a system with on-chip sample preparation and maPCR for pathogen identification, it requires even distribution of isolated pathogens from the sample to all reactors, which increases the complexity of device design and fluidic control.

Dielectrophoresis (DEP)[16] is one of the most widely used approaches among chip-based cell separation methods,[17] providing selective and label-free separation of microorganisms such as yeasts[18], bacteria[19] and viruses[20]. DEP separation of individual pathogen species from biological samples has recently been performed in microfluidic devices.[21-23] Previous research showed that DEP could be integrated with a PCR microreactor to detect *Listeria monocytogenes* in DI water.[24] Herein, we describe a microfluidic system integrating DEP with chip-based maPCR for fast separation and identification of pathogens for POC testing as shown in Fig. 1. Whole human Blood was used as the model sample to characterize the capability of the system to directly analyze complex physiological fluids. The utility of DEP for broad-spectrum pathogen separation directly from physiological samples, as wells as the integration of DEP with maPCR for identifying pathogens have been developed and validated with the help of recently introduced SlipChip technologies.[25]

Experimental Section

Fabrication of microfluidic devices. The microfluidic devices were fabricated with standard photolithography and wet etching techniques.[26] The top plate (2.5 cm × 3 cm) was made of a 0.7 mm thick glass with four parallel microchannels (800 μm wide, 15 μm deep), each with five PCR reaction microwells (800 μm × 800 μm in size, 135 μm in depth) evenly distributed on one side. Five additional microwells were made for the negative control as shown in Fig. S1. The bottom plate (2.5 cm × 4.5 cm) was made of a 1 mm thick ITO glass, with interdigitated ITO microelectrodes (Fig. 2a). The grooves (widths varied from 40 μm to 67 μm, 600 μm in length, 5 μm in depth) were fabricated by photolithography of a 5 μm thick SU-8 2005 coating on the bottom plate. The top plate was silanized with dichlorodimethylsilane, while the bottom plate with ITO electrodes and SU8 coating was silanized with dimethyldimethoxysilane to render the surface hydrophobic prior to use.

Preparation of samples. The original *Escherichia coli* (*E. coli*) strains (RP437 and RP1616) without exogenous plasmids were kindly provided by Professor J. S. Parkinson from the University of Utah. *E. coli* RP437 carrying plasmid DsRedT.4 suspended in DI water was used for optimizing capture efficiency. *E. coli* RP1616 carrying plasmid pAcGFP1 (Clontech) mixed with whole human blood was used for evaluating separation efficiency. Four American Type Culture Collection (ATCC) reference strains were used for evaluation of PCR, namely *E. coli* (ATCC 8739), *E. coli* O157:H7 (ATCC 35150), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 9027) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538p). All bacterial strains were grown in Luria-Bertani (LB) broth and plated on LB agar plate during log phase growth. Microbial cells were prepared by inoculating 5 mL of LB with a single colony and allowing it to grow overnight at 37 ºC. A subculture was prepared in 5 mL of fresh LB and shaken at 37 ºC for 5h. Cells were centrifuged at 8000 g for 5 min and suspended in DI water or diluted blood.

A set of bacterial and fungal clinical isolates from positive blood cultures including *E. coli*, *S. aureus*, *P. aeruginosa*, *Klebsiella pneumonia* (*K. pneumonia*), *Acinetobacter baumannii* (*A. baumannii*), *Enterococcus faecium* (*E. faecium*), *Enterococcus faecalis* (*E. faecalis*), *Enterobacter aerogenes* (*E. aerogenes*), *Candida glabrata* (*C. glabrata*), and *Candida albicans* (*C. albican*), and two ATCC reference fungal strains namely *Candida krusei* (*C. krusei*) and *Candida parapsilosis* (*C. parapsilosis*) were obtained from the Department of Clinical Laboratory, Peking Union Medical College Hospital (PUMCH, Beijing, China). The name and the serial number of these isolates were listed in the Electronic Supplementary Information (**ESI**).† All isolates were incubated on blood agar overnight, and then adjusted to 0.5 McFarland (approximately 1×10^8 CFU/mL) with DI water.[27]

Fresh whole human blood was obtained from healthy volunteers at the Hospital of Renmin University of China. Blood was diluted with DI water and added with microbial cells to different final concentrations. The addition of 10 mg/mL Bovine Albumin Serum (BSA) was used to prevent the adhesion of cells on the microchannel walls. To visualize blood cell ghosts (BCGs), we stained them with 0.4 mg/mL Rhodamine B. The concentration of BCGs in the original blood sample was roughly 4×10^9 cells/mL.

Assemble the device. The bottom plate was placed in a 9-cm Petri dish, with the patterned side facing up. 10 mL de-gassed mineral oil was poured into the Petri dish to cover the bottom plate. 50 nL of PCR mixture with different primer pairs was deposited into each microwell as described previously.[15, 25] The top plate was carefully placed onto the bottom plate, with the microchannels aligned with the grooves on the bottom plate (Fig. 1b). The two halves of the device were secured by two small binder clips.

DEP separation of microbial cells from blood. Four 250 μL syringes (Agilent, Santa Clara, CA) with 30-gauge Teflon tubing (Zeus Inc., Orangeburg, SC) were connected with the microchannels via access holes, and sealed with capillary wax (Hampton, Aliso Viejo, CA). Samples were fed into microchannels at a flow rate of 1 μL/min unless declared otherwise. Microbial cells were captured into grooves when a high frequency alternating current (HFAC) signal of 20 V_{pp} (Peak-to-Peak voltage) and 20 MHz was applied between interdigitated microelectrodes. The concentrations of microbial cells in the samples before and after DEP were determined by fluorescence imaging using an Eclipse Ti inversed microscope (Nikon, Japan) equipped with CoolSNAP HQ2 CCD camera (Photometrics, Tucson, AZ). After capturing, grooves with

captured microbial cells were flushed with DI water at a flow rate of 2 μL/min for 10 min to remove PCR inhibitors.

maPCR identification of pathogens. PCR was performed in an Eppendorf Mastercycler with an *in situ* Adapter (Hamburg, Germany). An initial step of 10 min at 95 °C was carried out to lyse bacteria and activate the enzyme for reaction. Next, a total of 30 cycles of amplification was performed as follows: a DNA denaturation step of 15 s at 95 °C; a primer annealing step and DNA extension step of 50 s at 65 °C. After the final cycle, the DNA extension step was performed for 5 min at 72 °C. A final hold temperature of 4 °C was utilized to deactivate the enzyme.

Ethics statement. The study was approved by the Hospital of Renmin University of China Ethics Board and the Committee and Research Ethics Board of PUMCH. All volunteer blood donors and all patients related to blood culture isolates provided informed consent.

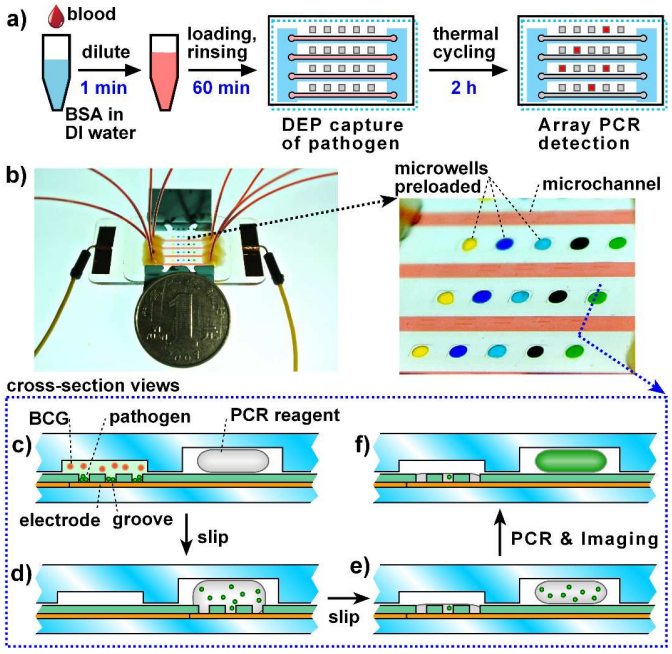

Figure 1. Device design and operation. a) Diagram depicting the workflow of capturing pathogens from blood and detecting it with multiplex array PCR (maPCR); b) Photograph of the device loaded with food dye solutions with a zoom-in view; c-e) Cross-section views of operation: The water-diluted blood sample containing blood cell ghosts (BCGs) and pathogens was loaded into the channel; pathogens (green dots) were retained in grooves by DEP, while BCGs were flushed to the outlet; the device was slipped to mix the captured pathogens with preloaded PCR reagents; the top plate was slipped back to its original position and away from contaminated surfaces; pathogens were identified by maPCR with fluorescence readout.

Results and discussion

Building of the microfluidic device. One of our primary objectives is to establish a broad-spectrum microbial isolation method compatible with physiological fluids, using blood as the model sample. To prevent false positive results due to the existence of circulating microbial DNA (DNAemia) and avoid DNA loss during extraction,[28] it is desirable to develop a device which directly captures pathogen cells from blood and detects them afterwards. DEP was previously used as a selective separation method for different microorganisms.[19] Here, by choosing appropriate parameters, DEP was validated as a broad-spectrum capture method for major bloodstream infections (BSI)-causing microbial species. Subsequently, our efforts were directed to incorporate DEP as the capture module for on-chip PCR, thus the DEP-captured microbial cells could be identified by PCR amplification with corresponding primer pairs. To identify causative pathogens in BSI, we introduced an array design of DEP-PCR integration in a 4 × 5 matrix, which was realized by SlipChip technologies.[25] As a difference from previously reported SlipChip devices which use pipette to inject microliter samples or reagents into the microwells, in this work, we designed the device which was able to process blood samples continuously to allow enrichment of pathogen cells from a large volume of samples. The operation of the device was shown in Fig. 1. Samples were injected through the channels at a constant flow rate. Facilitated by positive DEP force, microbial cells were captured into the grooves. Then DI water was loaded to rinse off PCR inhibitory as well as other interfering components in the samples. The device was then slipped to overlay the grooves containing microbial cells on the bottom plate with microwells preloaded with PCR reagents on the top plate. As the HFAC on the electrodes was removed, microbial cells were released and dispersed into PCR reagents. Slipped back the top plate, the device was placed on a standard thermal cycler for PCR amplification, and results were obtained by imaging with the microscope. It took approximately 3 hours from sample loading to detection.

Broad-spectrum pathogen capture with DEP. To evaluate if DEP could capture a broad-spectrum of pathogen species, we tested a variety of bacteria and fungi isolates from BSI with our DEP capture electrodes (Fig. 2). Interdigital electrodes (900μm long, 35 μm wide with 25 μm spacing) were designed for high efficient DEP capture as shown in Fig. 2a. During experiment, sample was deposited on the electrodes, and the HFAC was applied between interdigitated microelectrodes. 34 clinical isolates were selected based on the ranking of predominant species causing BSI (see Table 1 and **ESI**†).[29] According to Peking Union Medical College Hospital (PUMCH) surveillance data obtained from positive blood cultures from January 2008 to December 2012, these isolates covered 70.3 % of pathogen species other than coagulase-negative *staphylococci* (which were the most common contaminants of BC).[30] To further verify the applicability of our system in Candidemia diagnosis,[31] we tested another two reference fungal strains with high incidence of infection. As shown in Fig. 2b, all isolates, from 0.8 μm-sized *S. aureus* to 5 μm-sized *C. albican*, were attracted on the edge of electrodes under the same condition (20 V_{pp}, 20 MHz). Captured pathogens were visualized under the microscope for optical inspection of pathogen morphologies.[32] Our primary experiments also showed that it could also catch bacteria from milk or other physiological fluids after dilution (results not shown). In consideration of the representativeness of isolates we have tested, we believe that DEP can be qualified as a broad-spectrum enrichment method for pathogens in clinical samples.

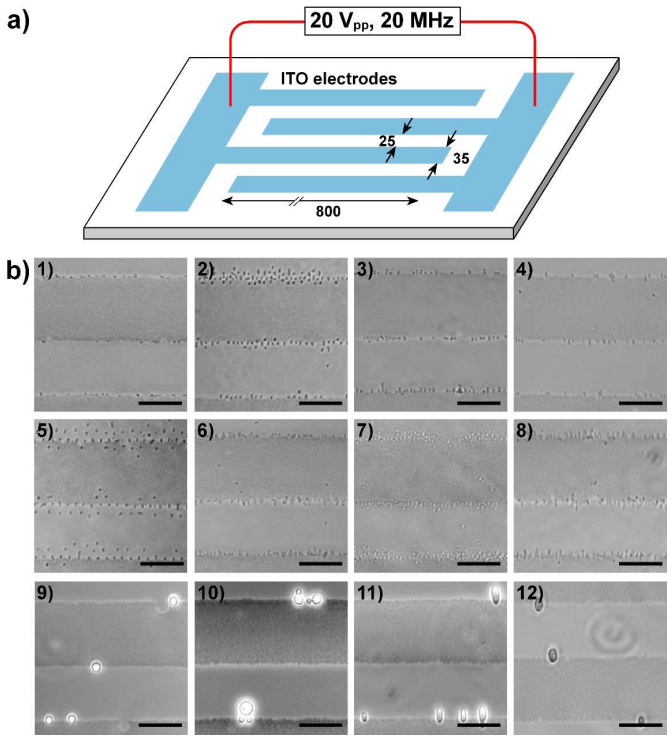

Figure 2. Dielectrophoresis of various septic pathogens. a) Schematic diagram of the interdigitated ITO microelectrodes; the isolates were deposited onto the electrodes for universality test; b) Microphotographs showed 12 species of pathogens were captured on the edge of electrodes by DEP. Isolates were obtained by blood cultures from patients at PUMCH. The scale bars are 20 μm.

Table 1. Pathogens captured by DEP shown in Figure 2.

	Name	Serial number	Category	Rate*
1	*S. aureus*	13B01049	Gram-positive	10%
2	*E. aerogenes*	13B02461	Gram-negative	N/A
3	*E. faecium*	13B01945	Gram-positive	4%
4	*E. faecalis*	13B00767	Gram-positive	4%
5	*K. pneumoniae*	13B00139	Gram-negative	9%
6	*E. coli*	13B00431	Gram-negative	18%
7	*A. baumannii*	13B02045	Gram-negative	7%
8	*P. aeruginosa*	13B00286	Gram-negative	3%
9	*C. glabrata*	13H04036	Fungi	N/A
10	*C. albicans*	13Z017401	Fungi	2%
11	*C. krusei*	ATCC 6258	Fungi	N/A
12	*C. parapsilosis*	ATCC22019	Fungi	1%

* The rates of infection were provided by PUMCH surveillance data obtained from positive blood cultures.

In this work, planar electrodes on the bottom plate were used for simplicity of fabrication. However, the DEP force acting on the microbial cells rapidly decreased when it moved away from the electrode's plane. This limited the depth of loading channel we could design (15 μm), and consequently affected the throughput of sample processing. To solve this problem, three dimensional dielectrophoretic electrodes which could provide better trapping efficiency and processing throughput could be used.[33, 34] This will enables us to further decrease the limit of detection for rare pathogens in clinical samples.

Design and optimization of the grooves. As shown in Fig. 3, three capture grooves were fabricated for each capture unit. The grooves were designed to be narrower than the loading channel, and were arranged in a ladder-like layout so that each unit only capture pathogen cells from a part of the sample flowing in the channel. Compared with designs with only one capture groove in each unit, dividing it into three in each unit and evenly distributing across the microchannel helped to reduce unequal capture ratio caused by flow rate difference of parabolic flow. The grooves had a depth of 5 μm for retaining bacterial or fungal cells during slipping.

The geometry of the grooves was optimized with RFP-tagged *E. coli* RP437 cells at a concentration of ~2×10^5 CFU/mL, at a flow rate of 1μL/min and capture time of 10 minutes. Under this condition, microbial cells captured in the grooves had no overlapping, and were countable with fluorescence imaging. We initially designed 15 grooves with individual width of 53.3 μm (800/15) in five units. As shown in Fig. S2d, the capture ratio of five units along the channel was around 4:2:2:2:1. The capture ratio of units 2, 3 and 4 was about 1/5, and unit 1 captured more bacteria than its width could cover, resulting in fewer cells captured by unit 5. This indicated that preceding grooves affected the capture efficiency of the latters. Therefore, we optimized the design. The width of grooves in Unit 1 was reduced. Units 2, 3 and 4 remained unchanged, and Unit 5 was increased. In the optimal design, the groove width of unit 1 was 40 μm and unit 5 was 67 μm. Uniform capture ratio of RFP-tagged *E. coli* RP437 for the five Units was obtained as shown in Fig. 3b. All of the experiments were repeated three times.

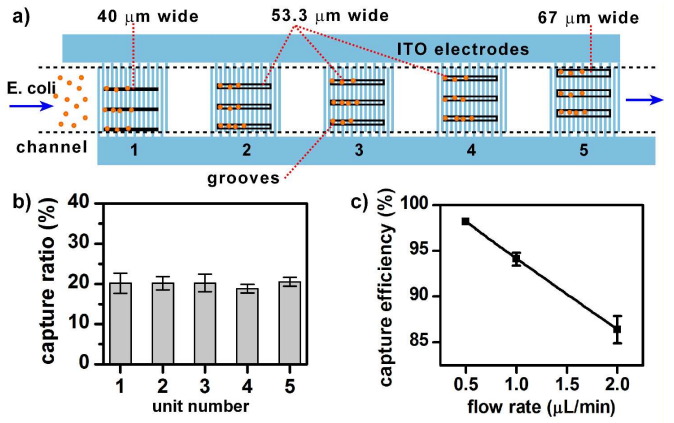

Figure 3. Efficiency and distribution of DEP-capture of bacteria (*E. coli*) with units of grooves. a) Schematic diagram of bacteria captured by DEP into the grooves with optimized widths; each capture unit had 3 capture grooves with the same width; the widths for unit 1, unit 2-4 and unit 5 were 40 μm, 53.3 μm and 67 μm, respectively; b) Equal distribution of captured *E. coli* cells in five units with optimized groove widths; c) The effect of flow rate on overall capture efficiency.

To evaluate the capture efficiency of the device, we monitored the concentration change of microbial cells in the samples before and after DEP capture. Before the grooves were saturated by *E. coli* cells, we took one microphotograph at the

upstream and another at the downstream, respectively (repeated 5 times). The cell number in each image was counted to calculate DEP capture efficiency. At a flow rate of 1 μL/min, a capture efficiency of 94.1% was attained.

Isolation of pathogens from blood samples. To demonstrate successful isolation of pathogens from complex physiological fluids, blood samples containing ~1.6×10^7 CFU/mL GFP-tagged *E. coli* RP1616 cells were used and diluted with DI water before being loaded into the channel. The fluorescence images shown in Fig 4 were obtained using 100-fold diluted blood. Before DEP capture, the overlay of green fluorescence and red fluorescence channel showed the coexistence of GFP-tagged *E. coli* with BCGs (Fig. 4b). By applying the HFAC on the ITO electrodes, *E. coli* cells were captured into the grooves by positive DEP force (Fig. 4c), and the BCGs which received negative DEP force were carried away by the flow to the outlets (Fig. 4d). Here, we found that the capture efficiency of DEP was affected by the conductivity of the sample. For 10-fold and 100-fold dilution, the conductivity of blood reduced to 1.07 mS/cm and 0.15 mS/cm (22 °C), while the capture efficiency increased to 70.9% and 91.5%, respectively (Fig. 4e). *E. coli* RP1616 suspended in DI water was used as a control sample, of which the highest capture efficiency 94.8% was obtained. All of the experiments were replicated three times.

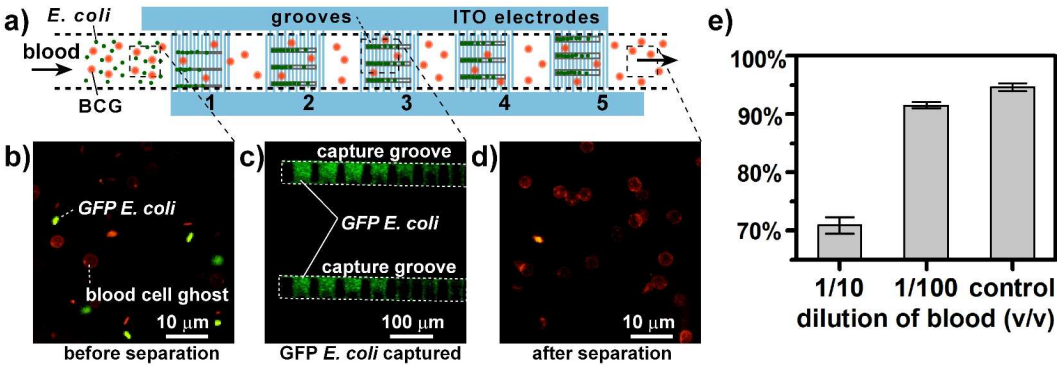

Figure 4. separation of *E. coli* cells from whole human blood by DEP. a) Schematic diagram showing one channel during separation; b) At the up-stream of the channel before DEP capture, BCGs (red) coexisted with a large amount of *E. coli* (green); c) DEP capture of *E. coli* from the sample into grooves (flow rate: 1 μL/min); d) At the down-stream of the channel after DEP capture, concentration of BCGs stayed the same before process, but *E. coli* were almost eliminated; e) Effect of blood dilution on capture efficiency of *E. coli* from blood.

Eliminate contaminants before PCR. To eliminate the contaminating components from whole human blood, we first rinsed captured microbial cells for 10 minutes at a flow rate of 2 μL/min. After DEP capture, we slipped the top plate to overlap PCR mixture with the grooves. We discovered that after PCR, the droplet shape was irregular and sometimes serious leakage of PCR reagents occurred. We speculated that the surfaces of grooves and ITO electrodes were contaminated during sample loading and made it hydrophilic. To overcome this problem, we simply slipped back the top plate to its original position, move the droplets of PCR mixture away from contaminated surfaces (Fig. 5a-d). This allowed us to get uniform droplet shapes and better PCR efficiency indicated by higher intensity of fluorescence shown in Fig. 5e and f.

Performance of maPCR. To evaluate the performance of array PCR on our device for fast identification of pathogens, we loaded a 100-fold diluted blood sample into the channel, containing 1.6×10^3 CFU/mL *E. coli* 8739. Meanwhile, every microwell on the top plate was pre-loaded with a 50 nL droplet composed of PCR master mixture and primers targeting the LacZ gene[38]. As shown in Fig. 5h, the control microwells overlaid with the grooves without *E. coli* showed no increase of fluorescence, while those overlaid with the grooves containing captured *E. coli* showed a significant increase of fluorescence intensity after thermal cycling. Line scans across the middle of individual microwells on the top and bottom panels of Fig. 5h showed the average fluorescence intensity of the microwells containing *E. coli* 8739 was more than 2-fold higher than that of the control ($p<0.0001$, $n=5$) (Fig. 5g). No cross-contamination occurred among adjacent channels and the results were reproducible.

Demonstration of potential application. To demonstrate the capability of the device for identifying candidate pathogens in multiple samples, we preloaded the microwell array with five different primer pairs targeting dominating pathogens of BSI, namely *Candida tropicalis* (*C. tropicalis*), *P. aeruginosa*, *E. coli* O157:H7, *S. aureus* and *Streptococcus mutans* (*S. mutans*) (See Table S1 in **ESI**† for primer design). It has been reported that more than 90% cases of BSI were infected by single pathogenic species.[35] Therefore, model blood samples containing one or two pathogens were tested. Sample 1 was loaded into channels 1 and 3, containing 1.8×10^3 CFU/mL *E. coli* O157:H7 only; Sample 2 was loaded into channels 2 and 4, containing the mixture of 1.0×10^3 CFU/mL *P. aeruginosa* (ATCC 9027) and 2.3×10^3 CFU/mL *S. aureus* (ATCC 6538p). After 50 minutes sampling, 10 minutes rinsing and 2 hours PCR amplification, the PCR results were shown in Fig. 5i. Only the microwells preloaded with primer pairs targeting corresponding pathogens showed significant increase of fluorescence intensity, indicating high specificity and reproducibility of the system.

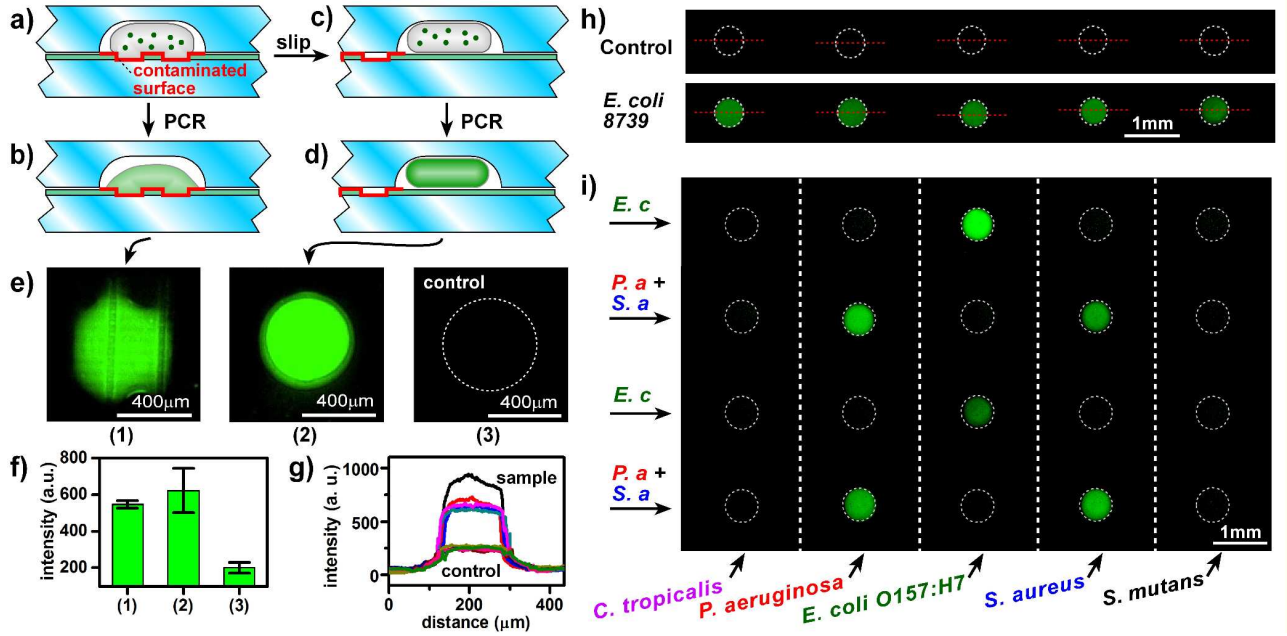

Figure 5. Microphotographs of droplets in the microwells after PCR amplification. a-d) Schematic diagraph of the slipping procedure before initiating PCR. The top plate was slipped back to avoid the microwell containing the PCR mixture and *E. coli* cells overlaying with the contaminated surface during PCR; e) Microphotographs of PCR droplets on/off capture electrodes and a control droplet without *E. coli*; f) The average fluorescence intensity of droplets in panel e; g) The average fluorescence intensity of droplets in panel h; h) Montages of fluorescence microphotographs showed the detection of DEP-captured *E. coli* by on-chip PCR, compared with the control with no *E. coli*; i) Montage of fluorescence microphotographs showed the identification of different pathogens in four samples with maPCR.

Conclusions

Direct detection of pathogens in physiological fluids is challenging for low concentration of pathogens with the vast diversity and complexity of physiological fluids. In this work, we developed a simple and portable microfluidic device for pathogen separation and identification in complex samples such as whole human blood. With grooves in a ladder-like layout over the DEP electrodes, the device efficiently and uniformly isolated pathogens from a model sample containing 40,000 times higher blood cells into an array. With simple slipping operations, the captured pathogens were mixed with an array of nanoliter reagents for PCR identification of up to twenty pathogens simultaneously. The device can detect pathogens with the concentration on the order of ~10^3 CFU/mL.

In comparison with conventional culture-based methods which take 24 to 72 hours, the device can detect pathogens within three hours from sample-in to answer-out. By optimizing the PCR conditions, the time might be further reduced to less than 1.5 hours.[7] Dielectrophoresis was found to be a universal sample preparation method for capturing broad-spectrum pathogens. The usefulness of DEP separation was manifested after we coupled it with the maPCR, enabling simultaneous identification of multiple candidate pathogens. Additionally, this design may be adopted for other assays based on preloaded reagents with nanoliter volumes. In an ongoing work, we are exploiting this array design with multiple antibiotics to screen the antibiotic resistance of captured pathogens. This DEP-maPCR system provided a convenient and accessible approach for early diagnosis of infectious disease caused by bacteria and fungi, greatly simplifying sample preparation procedures, and eliminating contamination during PCR due to preloaded reagents. We are convinced that this system can also be adopted for other applications such as food safety testing, water quality analysis and assessment of microbial communities in the environment.

Future work will be directed to improve the throughput of sample processing, expand the array size to increase the coverage, and optimize the performance of amplification using PCR or Loop-Mediated Isothermal Amplification (LAMP)[36] to further reduce the limit of detection. Moreover, portable DEP power supplies, thermal cyclers,[37, 38] self-containing microflow control systems,[39, 40] and smartphone-based imaging[41, 42] could be incorporated to make the system more compact and portable for application in resource limited conditions.

Acknowledgements

This study is supported by State key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences (SKLMR-20120604); the National Natural Science Foundation of China (21205134, 31230003 and 201402001) and the program of China Ocean Mineral Resources R&D Association (Grant DY125-15-R-02).

Notes and references

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† Electronic Supplementary Information (ESI) available: Details on materials and experiments, PCR primer pairs for detection of pathogens, and Figures on device design and optimization are available free of charge via the internet, See DOI: **10.1039/x0xx00000x**

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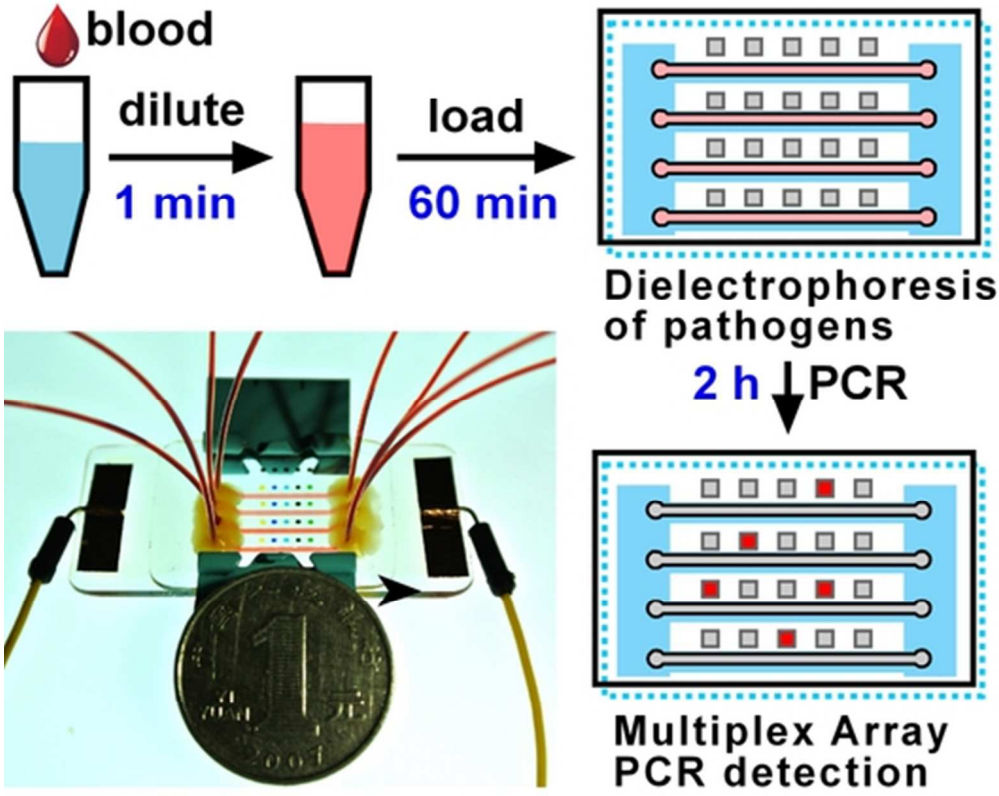

An integrated device utilizing dielectrophoresis and multiplex array PCR for rapid identification of pathogens in complex physiological matrices was developed, which is able to detect multiple pathogens in blood within 3 hours. It may be widely applied in point-of-care detection of microorganisms in physiological and environmental samples.
44x36mm (300 x 300 DPI)