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Segmented continuous-flow multiplex polymerase chain reaction microfluidics for high-throughput and rapid foodborne pathogen detection

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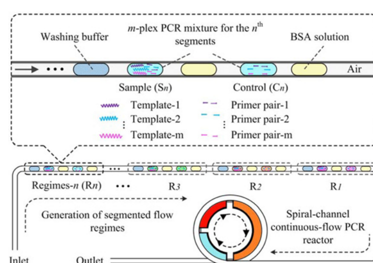
HIGHLIGHTS

- A high-throughput and rapid microfluidic method for pathogen detection is proposed.
- The method offers a simple and convenient way toward high-throughput DNA analysis.
- Important parameters of segmented continuous-flow multiplex PCR were investigated.
- The proposed method is suitable for high-throughput biomedical monitoring.

GRAPHICAL ABSTRACT

On a spiral-channel microfluidic platform, the high-throughput and rapid amplification for multiple foodborne bacterial pathogens was developed via the segmented continuous-flow multiplex PCR. Fig. 1 showed the design principle of spiral-channel segmented continuous-flow multiplex PCR amplification.



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ABSTRACT

High-throughput and rapid identification of multiple foodborne bacterial pathogens is vital in global public health and food industry. To fulfill this need, we propose a segmented continuous-flow multiplex polymerase chain reaction (SCF-MPCR) on a spiral-channel microfluidic device. The device consists of a disposable polytetrafluoroethylene (PTFE) capillary microchannel coiled on three isothermal blocks. Within the channel, n segmented flow regimes are sequentially generated, and m-plex PCR is individually performed in each regime when each mixture is driven to pass three temperature zones, thus providing a rapid analysis throughput of $m \times n$. To characterize the performance of the microfluidic device, continuous-flow multiplex PCR in a single segmented flow has been evaluated by investigating the effect of key reaction parameters, including annealing temperatures, flow rates, polymerase concentration and amount of input DNA. With the optimized parameters, the genomic DNAs from *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* could be amplified simultaneously in 19 min, and the limit of detection was low, down to 10^2 copies μL^{-1}. As proof of principle, the spiral-channel SCF-MPCR was applied to sequentially amplify four different bacterial pathogens from banana, milk, and sausage, displaying a throughput of 4×3 with no detectable cross-contamination.

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1. Introduction

Infectious diseases caused by foodborne pathogens have attracted considerable attention, due to the significant public health and global economic impact. Among thousands of the recognized

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foodborne pathogenic bacteria, about 20 of different bacteria including *Vibrio parahaemolyticus*, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 have been found to be responsible for most food-borne outbreaks [1]. These food-borne pathogens must be rapidly detected at all stages of food production, processing, transportation and marketing to ensure food safety along the food chain, thus requiring the rapid analysis of a large number of samples for possible contamination and then subjecting suspected samples to further confirmation [2]. However, the standard method for detection of bacteria mainly relies on specific microbiological identifications that are time-consuming, labor intensive and low throughput, which constrains the number of samples or targets that can be tested and limits the ability to detect putatively contaminated foods [3,4]. Therefore, it is of great importance to develop high-throughput multiplexed methods capable of simultaneously detecting a number of samples for multiple foodborne pathogens.

Recently, much effort has been devoted to developing high-throughput and multiplex systems that utilize various methods to detect bacterial pathogens [5–9]. Among them, the multiplex polymerase chain reaction (PCR), which enables simultaneous identification of several targets incorporation of multiple sets of primers, is promising in term of speed, reliability, high specificity and sensitivity. Furthermore, capitalizing on the advantages of microfluidic technology, such as shorter analysis time, lower reagent and power consumption, higher processing throughput and integration level, the multiplex PCR microfluidic systems have attracted a great deal of attention [10–14].

Generally, the developed microfluidic multiplex PCR are categorized into two types: static ones [10,11] and continuous-flow ones [12,13]. The continuous-flow PCR circumvent the need for repeated heating and cooling of the reaction chamber of the static ones by moving the sample through alternating temperature zones, thus providing much faster amplification speeds, simpler steady-state temperature control and lower fabrication cost. Up to now, several continuous-flow multiplex PCR systems have been demonstrated with different fluidic architectures: close-loop [12,13], bidirectional-flow [14–16], fixed-loop [17–19]. These systems are attractive in demonstrating the significant advantages of using continuous-flow microfluidic platform to perform multiplex PCR for simultaneous testing of multiple samples, including rapid thermal cycling speed and high potential of integrating other functionalities. However, these systems all only focus on the multiplex PCR amplification in a single reaction mixture per reaction channel and the analysis throughput are still limited, thus more reaction channels are needed for further enhancement of detection throughput, which may be at the price of the enlargement of footprint, complexity of fluid operation and fabrication cost increase [16,20,21]. As a high-throughput sample processing method, the segmented-flow technique involves the use of an immiscible phase such as oil or air to divide the aqueous flow stream into discrete slugs or droplets [22–25], and this technique has been implemented in microfluidic devices to perform high-throughput single-molecule and single-cell PCR [26–29]. However, the PCR amplifications in these microfluidic devices were intended for detection of one target per slug or droplet, which constrains the number of samples or targets to be analyzed in a single test.

To address the aforementioned issues, we introduce a segmented continuous-flow multiplex PCR (SCF-MPCR) on a spiral-channel microfluidic device for high-throughput and rapid DNA detection. In our prototype, four target genes in a single multiplex reaction solution were amplified for identification of four foodborne bacterial pathogens (including *Salmonella enterica*, *L. monocytogenes*, *E. coli* O157:H7 and *S. aureus*). In addition, three segmented continuous-flows were used to simultaneously amplify multiple bacterial pathogens in three different food samples. To our knowledge, we proposed for the first time a novel spiral-channel SCF-MPCR method for high-throughput and rapid detection of foodborne bacterial pathogens.

2. Experimental

2.1. Design principle of spiral-channel segmented continuous-flow multiplex PCR

Fig. 1 shows the schematic of design principle for the presented spiral-channel SCF-MPCR. The n segmented flow regimes are sequentially generated in a microchannel, while the channel is coiled around three heating zones those are maintained at constant temperatures for denaturation, annealing and extension. Within the multiplex PCR mixture in each sample segment, m pairs of primers, together with the m kinds of different DNA templates, are included. In order to ascertain whether the amplicons are products of residual contamination, a control segment, containing no DNA template but primers and all other reagents necessary for multiplex PCR, is injected before each sample segment. In addition, a BSA

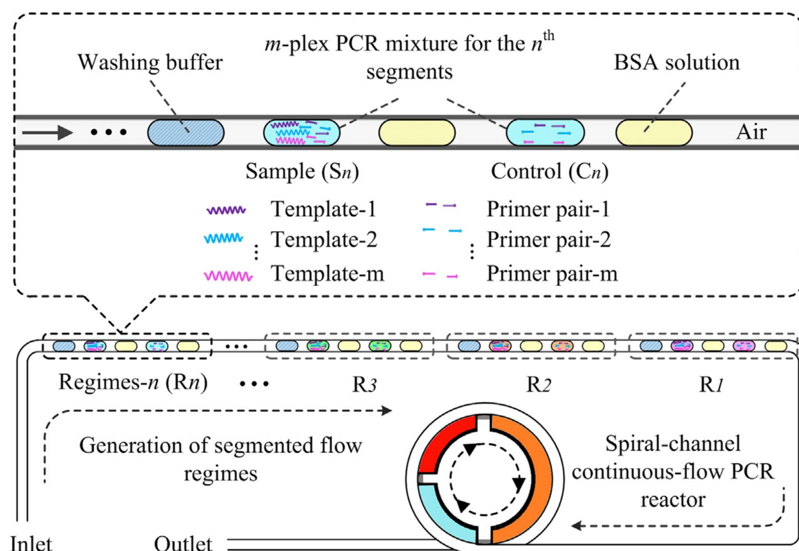

Fig. 1. Illustration of design principle of spiral-channel segmented continuous-flow multiplex PCR amplification.

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