AN INTEGRATED MICROFLUIDIC SYSTEM FOR AUTOMATING ON-CHIP SELEX PROCESS TO SCREEN TUMOR CELL-SPECIFIC APTAMERS

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ABSTRACT

A new microfluidic system involving a progressive selection of highly specific ligands by repeated rounds of partition and amplification from a large combinatorial nucleic acid library to screen tumor cell-specific aptamers was developed in this study. The systematic evolution of ligands by exponential enrichment (SELEX) process can be then automated. With this approach, an aptamer specific to lung cancer cells has been successfully screened. The entire process can be decreased from 2 weeks to only 3 days. The developed Cell-SELEX microsystem can be promising for fast screening of aptamers specific for tumor cells, which can be promising for early diagnosis of cancers and target therapy.

KEYWORDS: Cell-SELEX, microfluidics, cancer cell, cancer stem cell, aptamer

INTRODUCTION

The screening of tumor cell-specific molecular markers is crucial in cancer diagnostics and target therapy as well. Recently, the systematic evolution of ligands by exponential enrichment (SELEX) technology has been explored to screen specific ligands, usually referred as aptamers by performing reiterated cycles of enrichment and amplification of single-strain DNA (ssDNA) [1, 2]. The characteristics of the screened aptamers have led to their promising applications such as sample purification, target validation, drug development, diagnostics, and even therapy. Especially, a modification of the traditional SELEX process that uses living cells as targets was named Cell-SELEX [2] has attracted considerable consideration. For specific to cancer cells are in great demand for diagnosis and therapy. Aptamers generated from living cells are the optimal molecular probes to recognize target cells on a molecular level. Moreover, a small portion of cancer cells possess unlimited proliferation potential and are able to self-renew and to generate differentiated cancer cell progeny. These cells, usually referred as cancer stem cells are resistant to chemotherapy and radiotherapy. Therefore, biomarkers which can recognize the cancer stem cell are of great need. Aptamers have been recognized to be a promising candidate for cancer cells and cancer stem cells. Therefore, Cell-SELEX has been extensively investigated recently.

SELEX is a method to screen DNA or RNA ligands from a combinatorial library. This oligonucleotide library consists of millions of ssDNA with different sequences. At each cycle, the individual oligonucleotides with a high affinity for desired target cells are screened and those with affinity for control cells (non-cancer cells) are filtered out. However, Cell-SELEX is an iterative process requiring multiple rounds (typically 20-25) of extraction and polymerase chain reaction (PCR) amplification that requires significant samples (1 ml) and a lengthy process (240 min for a single round of Cell-SELEX process). In addition, a number of large-scale equipments such as PCR machines, high-speed centrifuges, shakers and pipettes are usually necessary. The entire operation process is thus labor-intensive and time-consuming.

Although Cell-SELEX has been successfully implemented in using different cancer cell lines [1-2], the automatic microfluidic platform has never been demonstrated. In this study, we present a new integrated microfluidic system to screen DNA aptamers using the Cell-SELEX scheme. An aptamer specific to lung cancer cells has been successfully screened within 3 days. With this approach, aptamers specific to cancer cells or even cancer stem cells can be screened and can be used for biomarkers for diagnostics and target therapy.

EXPERIMENTAL

The process for performing the Cell-SELEX can be described as follows. First, a combinatorial nucleic acid library (DNA or RNA) was synthesized. The library was then incubated with the desired target cells under conditions suitable for binding. Next, the unbound nucleic acids were partitioned from those bound specifically to the target cells, which are then eluted from the target cells and amplified by the following PCR process to amplify the nucleic acids with high affinity. This selection procedure was reiterated for several rounds until the resulting sequences were highly enriched. The selected nucleic acids were subjected to DNA sequencing and screened for potential binding affinity. The Cell-SELEX technology can therefore screen aptamers with high binding affinity and specificity. Their advantages have made them very promising in analytical, diagnostic and therapeutic applications.

A schematic illustration for the aptamer screening process performed on the microfluidic system is schematically shown in Fig. 1. An ssDNA library pool was first incubated with the target cells conjugated with magnetic beads (Fig. 1(a)). Non-bound DNA sequences were washed away (Fig. 1(b)) and bound DNA sequences were recovered from the target cells

by heating cell-DNA complexes at 95 °C, followed by magnetic isolation (Fig. 1(c) and Fig. 1(d)). The recovered pool was incubated with the control cells to filter out the sequences binding to common molecules on both the target and the control cells, leading to the enrichment of specific binders to the target cells (Fig. 1(e) and Fig. 1(f))). Binding sequences were finally amplified by PCR (Fig. 1(g)). After iterative screening and amplification processes, aptamers specific to the target cells can be screened. Figures 2 and 3 show a schematic illustration of the Cell-SELEX chip in both top- and cross-sectional views, respectively. Four modules, including a suction-type microfluidic control module, a magnetic bead-based aptamer extraction module, a PCR module and a cooling module for storing reagents were integrated on the chip to perform the entire process described previously. Figure 4(a) shows the photographs of the Cell-SELEX chip. The infrared image verified that the cooling module and the PCR module can be operated successfully (Fig. 4(b)). Cell-SELEX has been performed using cultured cancer cell lines, and aptamers have been screened between two different cancer cells or between cancer and normal cells. Table 1 lists two examples of target and control cell lines using the microfluidic system to screen specific aptamers.

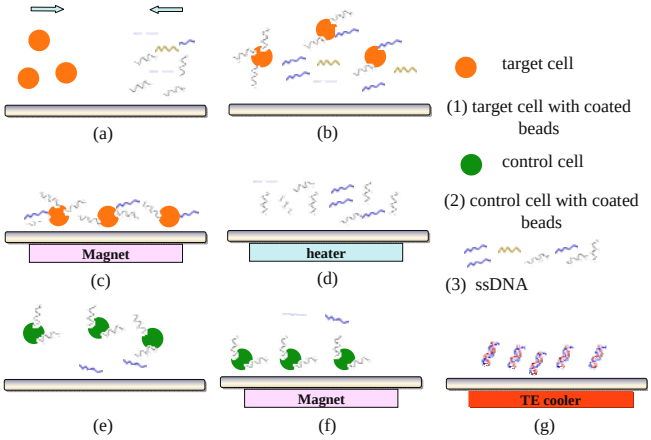


Figure 1: The Cell-SELEX process including incubation, separation and enrichment were automatically performed.

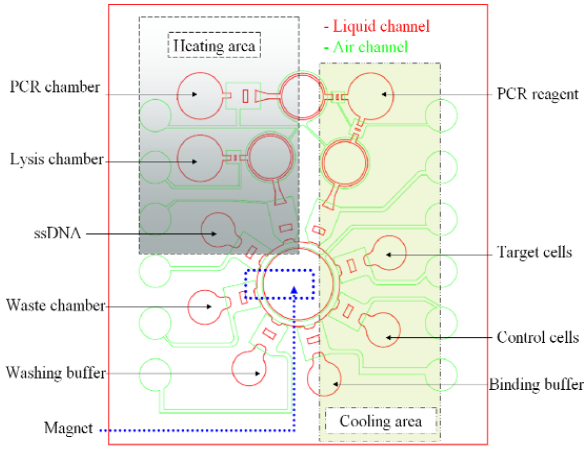


Figure 2: The schematic illustration of the Cell- SELEX chip.

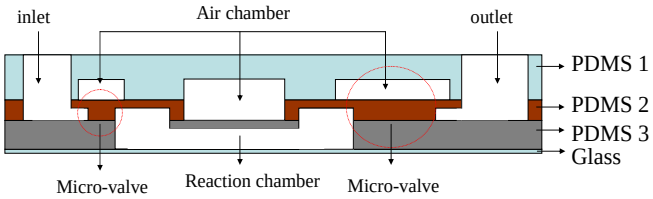


Figure 3: The cross-section view of the Cell- SELEX chip.

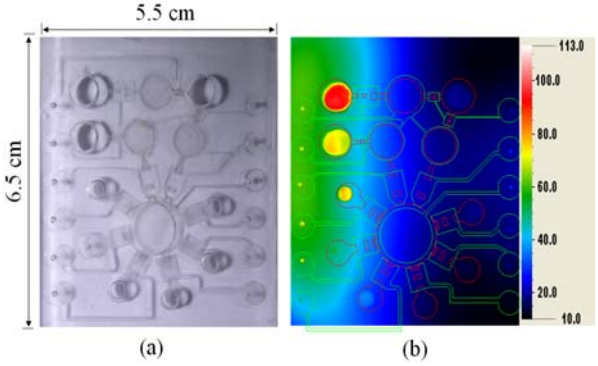


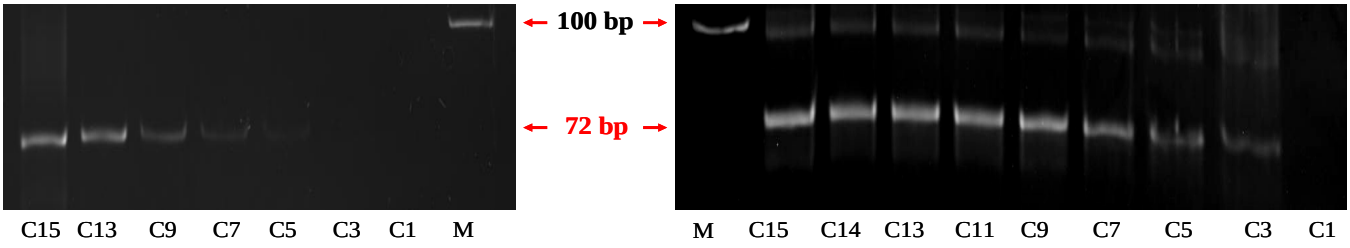
Figure 4: Photographs of (a) the Cell- SELEX chip and (b) the infrared image.

Table 1 Two Examples of target and control cell lines

Cell-SELEX	Target cell	Description	Control cells	Description
1.	H1650	Human lung cancer cells	BG1	Human ovarian cancer cells
2.	$A549^{ShEcad}$	Lung cancer stem cell-like; Human lung cancer cells with shRNA targeting E-cadherin (shEcad)	$A549^{ShLuc}$	Lung cancer cell; Human lung cancer cells with shRNA targeting Luciferase (shLuc)

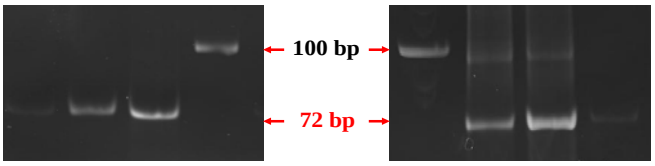
RESULTS AND DISCUSSION

During the Cell-SELEX process, the extracted and amplified ssDNA produced after each round was examined by gel electrophoresis (Figs. 5(a) and 5(b)). Experimental results showed that the lung cancer cell-specific DNA sequences (with a length of 72 bps) can be successfully separated and enriched by using the developed Cell-SELEX chip. After the TA cloning process, one of the individual DNA sequence were confirmed with high binding affinity and specificity (Fig. 6(a) and 6(b)) after a specificity test process schematically shown in Fig. 7. With this approach, the screened ssDNA can be verified to have high sensitivity and selectivity.



(a) (b)

Figure 5: Experimental results showing that the DNA-aptamers with a length of 72 bps can be successfully extracted and amplified by the developed system. Agarose gel electrophoresis image shows the products after various cycles of selected DNA library amplification; (a) Cell-SELEX for H1650 after 1, 3, 5, 7, 9, 13, and 15 cycles, respectively. (b) Cell-SELEX for A549^{ShEcad} after 1, 3, 5, 7, 9, 11, 13, 14 and 15 cycles, respectively. M is DNA ladder.



(a) (b)

Figure 6: Binding affinity test for the screened aptamers from the specificity test. M, A, P, and N are for ladder, positive control, positive selection, and negative selection, respectively. (a) The screened ssDNA specific to H1650 lung cancer cell. (b) The screened ssDNA specific to A549ShEcad cancer stem cell-like.

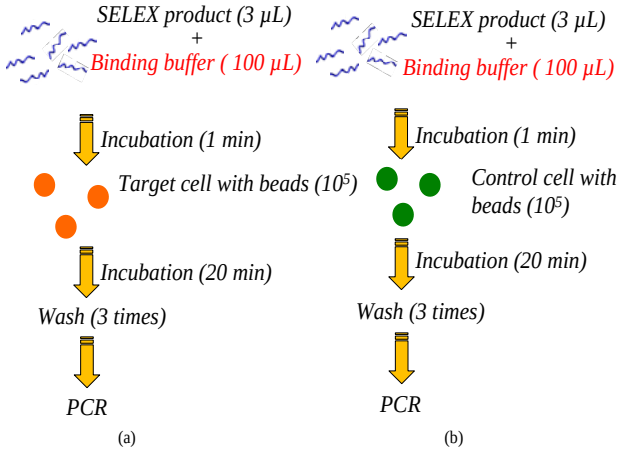


Figure 7: Schematic illustration of the specificity test process for (a) positive selection (P), (b) negative selection (N), which uses cell-conjugated magnetic beads.

CONCLUSION

This study demonstrated a new magnetic bead-based microfluidic system for rapid screening of tumor cell-specific aptamers. When compared to the traditional Cell-SELEX process, the new microfluidic Cell-SELEX system is more compact in size, consumes fewer samples (100 µl), and much faster (50 min for a single round of SELEX process). It may be promising to screen aptamers for diagnosis and target therapy of cancers.

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