A RAPID SCREENING FOR HEMOGLOBIN-SPECIFIC APTAMERS BY USING A CONTINUOUS MICROFLUIDIC SYSTEM

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ABSTRACT

Systematic evolution of ligands by exponential enrichment (SELEX) is a method to screen specific DNA- or RNA-based aptamers by repeated rounds of partition and amplification from random pool of oligonucleotides. The screening products have high binding affinity for the target molecule, which can be an excellent biomarker for diagnosis and therapeutics. This study reports a new continuous microfluidic system to perform SELEX in an automatic mode, which is an excellent tool for fast selection of aptamers. As a demonstration, hemoglomin-specific aptamer was screened. The screened aptamer can be used for fast diagnosis of hemoglobin, which is a crucial indicator for diabetes mellitus.

KEYWORDS

SELEX, aptamers, microfluidics, SELEX, hemoglobin

INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases worldwide and has become one of the most challenging health problems in the 21st century [1]. Therefore, the detection of DM is crucial with a high economic value. The ratio of hemoglobin A1c (HbA1c) and hemoglobin (Hb) provides a crucial index of DM patients, which is more reproducible than blood glucose measurement [2]. Thus, precise and accurate measurement of HbA1c is critical and essential for proper diabetic care. Recently, aptamers with a high binding affinity and a high selectivity for specific targets have shown great potential as a recognition molecule in diagnostic assays. The aptamers are selected repeatedly by the SELEX process which allows extraction of aptamers with desired binding affinity for a target molecule from an initially random pool of oligonucleotides [3]. However, this SELEX process is relatively labor-intensive and time-consuming. Furthermore, the consumption of samples/reagents is relatively high. More importantly, the dead-volume issue may result in low yield of screening [3].

In the past two decades, microfluidics and micro-electro-mechanical-systems (MEMS) technology have enabled the miniaturization of biomedical and chemical analysis systems. Micro-scale bio-systems can provide even superior performance compared with their large-scale counterparts. This study therefore presents an automatic, magnetic bead-based microfluidic system which integrates a random ssDNA extraction device and an on-chip nucleic acid amplification device for fast screening of aptamers. In this study, we developed a continuous microfluidic system which can automatically perform the SELEX rounds on a single chip for screening of Hb-specific aptamers. Comparing to the traditional process, the developed microfluidic system is more compact in size and consumes fewer samples and reagents. More importantly, the entire process can be shortened from 2 weeks to 3 days.

EXPERIMENTAL

In this work, we aim to extend our efforts to pursue the continuous platform to automate the entire SELEX process for screening an important biomarker, which is Hb. The continuous system for screening of Hb-specific aptamers utilizing SELEX would be implemented. It would be even more useful if rapid screening of aptamers can be automatically performed on a single miniature platform, which integrates devices for extraction and amplification of nucleic acids.

Figure 1 shows a schematic illustration of the experimental procedure implemented on the integrated microfluidic system. The magnetic beads pre-coated with target proteins (Hb) and blocked by bovine serum albumin (BSA) were first loaded into the SELEX chip. Then, the ssDNA library was loaded into the chip. The SELEX process including incubation, partition and enrichment was automatically performed. After several selection cycles, the high-affinity and high-specificity DNA-aptamers were screened. The individual DNA sequences were then cloned to execute a competitive test, which could confirm the binding affinity and specificity of the screened aptamers. Three kinds of experiments, including a positive selection, a competitive selection and a negative selection, were performed to make sure the specificity of the screened aptamers. Briefly, positive selection (P) used a solution containing selected ssDNA, Hb-coated beads and 1% BSA buffer. Competitive selection (C) used a solution containing selected ssDNA, Hb, Hb-coated beads and 1% BSA buffer. Negative selection (N) used a solution containing ssDNA, BSA-coated beads and 1% BSA buffer. After incubation and washing process, the result on the slab-gel electropherograms can be used to verify if the screened aptamers have strong affinity and specificity. Finally, the screened ssDNA would be sequenced.

Figure 2 shows a schematic diagram of the SELEX chip, which can automate the entire process by using micropumps, microvalves, micromixers and a polymerase chain reaction (PCR) module. Figure 3(a) shows an exploded view of the microfluidic chip, which consists of a liquid layer, a pneumatic layer and a glass plate. The dimensions of the chip were measured to be 59 cm x 53 cm (Fig. 3(b)). These microfluidic components have been characterized. For instance, Fig. 4 shows a cross-sectional concentration profile inside the micromixer. The

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micromixer can achieve an efficient mixing within 3 seconds.

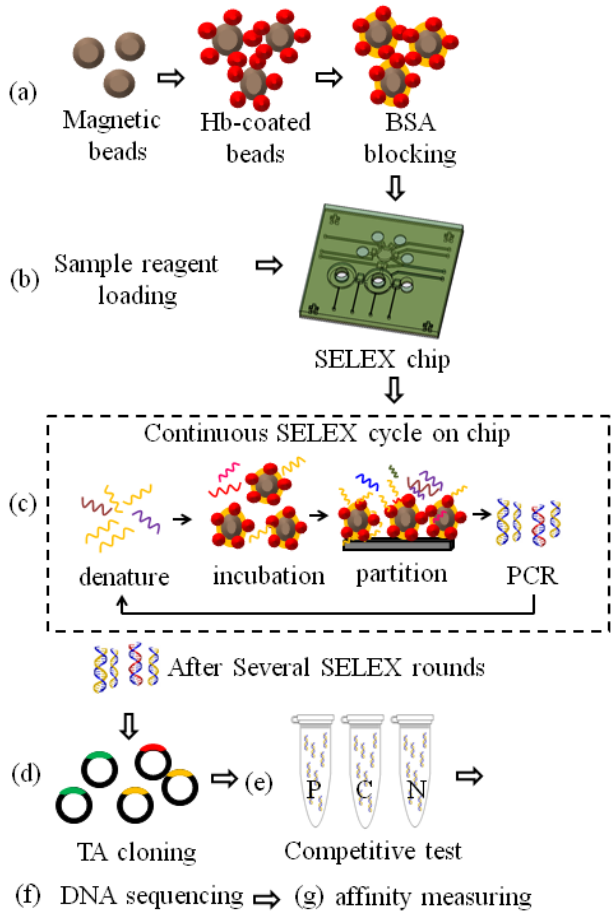

Figure 1: Schematic illustration of the experimental procedure for screening of Hb-specific aptamer (a) the beads coated with Hb and BSA (b) beads and sample reagent load into the SELEX chip (c) continuous SELEX cycle on chip, including denature, incubation, partition and amplification (d) after several SELEX rounds, the high-affinity aptamer can be purified (d) TA cloning (e) competitive test to make sure the specific of aptamers (f) DNA sequencing to analysis the structure (g) measure to certify the affinity

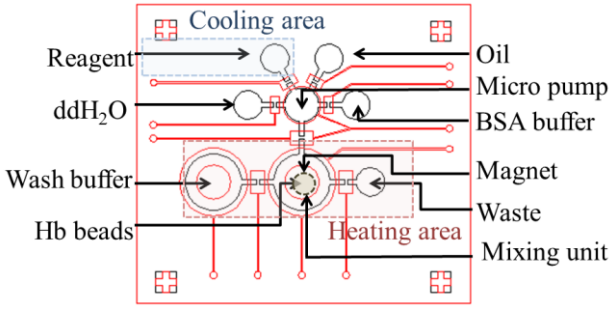

Figure 2: Schematic illustration of the continuous SELEX chip.

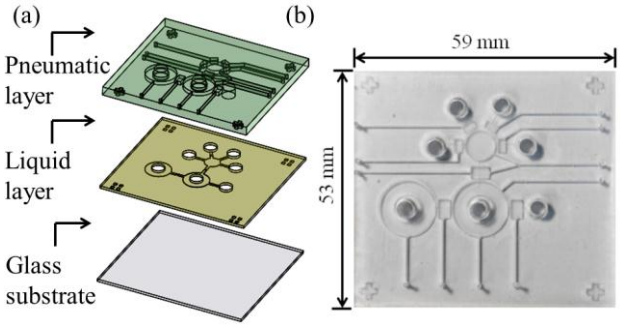

Figure 3: (a) Exploded view and (b) a photograph of the SELEX chip.

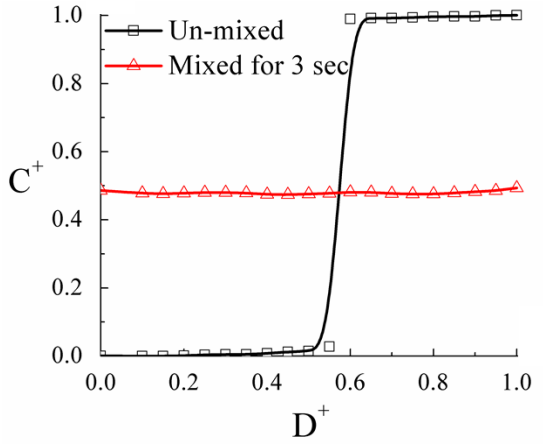

Figure 4: The normalized concentration profile across the mixing chamber.

RESULTS AND DISCUSSION

The target Hb-specific DNA-aptamers can be successfully isolated and enriched by the developed system, as shown in Figure 5(a). After five consecutive selections, the concentrations of the aptamers can be significantly enriched. In order to reduce the absorption of the screened aptamers on proteins, the enriched ssDNA was incubated with BSA-coated beads to obtain highly specific aptamers. Figure 5(b) shows that the non-specific DNA was successfully reduced and more specific aptamers with high affinity can be obtained by using negative selection. Figure 6 shows the competitive test results. The screened ssDNA can be verified to have high sensitivity and selectivity. Hb-specific aptamer with the strongest affinity was then sequenced. A secondary structure analysis of the aptamer was performed with MFOLD software (version 3.2). The predicted structure has five loops and low free energy, which makes it a promising biomarker for Hb. The affinity test for measurement of dissociation constant is undergoing.

The entire SELEX process for screening Hb-specific aptamers can be automatically performed on the developed system. The entire time for one round of SELEX only takes 70 min. When compared to the traditional method, samples and reagents consumption can be significantly reduced and has better washing efficiency by using the

magnetic beads in the microfluidic system. It is therefore promising for fast screening of aptamers, which are excellent candidates for diagnosis or even therapeutics.

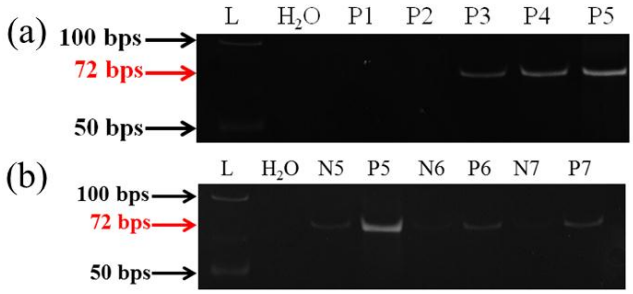

Figure 5: Slab-gel electropherograms for selected aptamers from the SELEX chip. (a) After five consecutive selections, the concentrations of the aptamers can be significantly enriched. (b) After several rounds of negative selection (using BSA-beads), the non-specific binding materials significantly decrease.

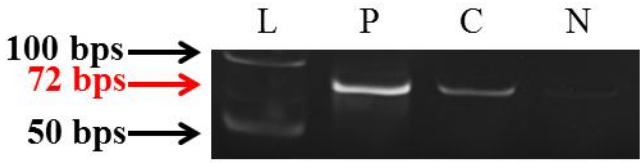

Figure 6: Binding affinity test for the screened aptamers from the competitive test, P, C and N are for positive selection, competitive control and negative selection, respectively.

CONCLUSION

The Hb-specific aptamers can be successfully isolated and enriched by utilizing a microfluidic system and can be performed in a shorter period of time. The developed microsystem may provide a powerful tool for fast screening of aptamers, which are excellent candidates for biomarkers.

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